

**PHARMACOLOGICAL EVALUATION OF SELECTED
PHYTOCONSTITUENTS FOR ALZHEIMER'S DISEASE
USING ANIMAL MODEL**

Thesis Submitted For the Award of the Degree of

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LIST OF TABLES

Table No.	Title of table
2.1	The stages and symptoms of Alzheimer's disease
2.2	Diagnostic procedure of Alzheimer's disease
2.3	Transgenic mice and rats models for Alzheimer's disease
2.4	Non-transgenic animal models for Alzheimer's disease
2.5	<i>In vitro</i> models for Alzheimer's disease
2.6	Reported activities of Bromelain
2.7	Reported activities of Myrcene
4.1	Grouping of all mice
4.2	Bromelain treatment schedule
4.3	Myrcene treatment schedule
4.4	Combination of Bromelain and Myrcene treatment schedule
5.1a	Effect of Bromelain on spatial reference memory of AD induced mice in MWM
5.1b	Effect of Bromelain on time spent in target quadrant (Probe trial) of AD induced mice in MWM
5.2a	Effect of Bromelain on spatial working memory (Acquisition trial) of AD induced mice in MWM
5.2b	Effect of Bromelain on spatial working memory (Retrieval trial) of AD induced mice in MWM
5.3	Effect of Bromelain on transfer latency of AD induced mice in EPM
5.4	Effect of Bromelain on locomotor activity of AD induced mice
5.5	Effect of Bromelain on AChE of AD induced mice
5.6	Effect of Bromelain on MDA of AD induced mice
5.7	Effect of Bromelain on GSH of AD induced mice
5.8	Effect of Bromelain on CAT of AD induced mice
5.9	Effect of Bromelain on SOD of AD induced mice
5.10	Effect of Bromelain on Nitrite of AD induced mice
5.11	Effect of Bromelain on BDNF of AD induced mice
5.12	Effect of Bromelain on β - secretase level of AD induced mice
5.13	Effect of Bromelain on amyloid-beta peptide ₁₋₄₂ level of AD induced mice
5.14	Effect of Bromelain on TNF- α level of AD induced mice
5.15	Effect of Bromelain on IL-6 level of AD induced mice
5.16a	Effect of Myrcene on spatial reference memory of AD induced mice in MWM
5.16b	Effect of Myrcene on time spent in the target quadrant (Probe trial) of AD induced mice in MWM
5.17a	Effect of Myrcene on the spatial working memory (acquisition trial) of AD induced mice in MWM
5.17b	Effect of Myrcene on the spatial working memory (retrieval trial) of AD induced mice in MWM
5.18	Effect of Myrcene on transfer latency of AD induced mice in EPM
5.19	Effect of Myrcene on locomotor activity of AD induced mice
5.20	Effect of Myrcene on acetylcholinesterase levels of AD induced mice
5.21	Effect of Myrcene on MDA levels of AD induced mice
5.22	Effect of Myrcene on GSH levels of AD induced mice
5.23	Effect of Myrcene on catalase levels of AD induced mice

5.24	Effect of Myrcene on SOD levels of AD induced mice
5.25	Effect of Myrcene on Nitrite levels of AD induced mice
5.26	Effect of Myrcene on BDNF levels of AD induced mice
5.27	Effect of Myrcene on β -secretase levels of AD induced mice
5.28	Effect of Myrcene on amyloid-beta peptide ₁₋₄₂ levels of AD induced mice
5.29	Effect of Myrcene on TNF- α levels of AD induced mice
5.30	Effect of Myrcene on IL-6 levels of AD induced mice
5.31a	Effect of Bromelain and Myrcene on spatial reference memory of AD induced mice in MWM
5.31b	Effect of bromelain and Myrcene on time spent in the target quadrant (Probe trial) of AD induced mice in MWM
5.31c	Effect of Bromelain and Myrcene on the spatial working memory (acquisition trial) of AD induced mice in MWM
5.31d	Effect of Bromelain and Myrcene on the spatial working memory (retrieval trial) of AD induced mice in MWM
5.31e	Effect of Bromelain and Myrcene on the transfer latency of AD induced mice in EPM
5.32	Effect of Bromelain and Myrcene on locomotor activity of AD induced mice
5.33	Effect of Bromelain and Myrcene on acetylcholinesterase levels of AD induced mice
5.34	Effect of Bromelain and Myrcene on MDA levels of AD induced mice
5.35	Effect of Bromelain and Myrcene on GSH levels of AD induced mice
5.36	Effect of Bromelain and Myrcene on catalase levels of AD induced mice
5.37	Effect of Bromelain and Myrcene on SOD levels of AD induced mice
5.38	Effect of Bromelain and Myrcene on nitrite levels of AD induced mice
5.39	Effect of Bromelain and Myrcene on BDNF levels of AD induced mice
5.40	Effect of Bromelain and Myrcene on β -secretase levels of AD induced mice
5.41	Effect of Bromelain and Myrcene on amyloid-beta peptide ₁₋₄₂ levels of AD induced mice
5.42	Effect of Bromelain and Myrcene on TNF- α level of AD induced mice
5.43	Effect of Bromelain and Myrcene on IL - 6 level of AD induced mice

LIST OF FIGURES

Figure No.	Title of figure
2.1	Histopathological evaluation of Alzheimer's disease
2.2	The pathogenesis of Alzheimer's disease
2.3	Etiology of Alzheimer's disease
2.4	The various stages of Alzheimer's disease with symptoms
2.5	Classification of drugs used for Alzheimer's disease
2.6	Mechanism action of donepezil, rivastigmine and galantamine inhibit AChE enzyme, rivastigmine also inhibit the BuChE enzyme and galantamine potentiate the ChR
2.7	NMDA receptor antagonist effect of memantine
4.1	Schematic study protocol timeline
4.2	Protocol for intraperitoneal injection (i.p.) route of administration for Swiss albino mouse
4.3	Protocol for intragastric (p.o.) route of administration for Swiss albino mouse
4.4	Protocol for isolation of hippocampus and cerebral cortex from the brain of Swiss albino mouse
4.5	Protocol for homogenization of cerebral cortex and hippocampus of Swiss albino mouse brain
4.6	Calibration curve of standard sodium nitrite
4.7	Calibration curve of standard GSH
4.8	Calibration curve of standard protein (BSA)
4.9	Schematic diagram indicates illustration of ELISA tests
4.10	Calibration curve of mouse β -secretase
4.11	Calibration curve of mouse amyloid β_{1-42} peptide
4.12	Calibration curve of mouse brain-derived neurotrophic factor
4.13	Calibration curve of mouse tumor necrosis factor- α
4.14	Calibration curve of mouse interleukin-6
5.1a	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on spatial reference memory of (AlCl_3 + D - galactose) administered mice during training days 81, 82 and 83
5.1b	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on time spent in target quadrant (Probe trial) of (AlCl_3 + D - galactose) administered mice in MWM test
5.2a	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on spatial working memory of (AlCl_3 + D - galactose) administered mice at days 87, 88, 89 and 90, acquisition trial
5.2b	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on spatial working memory of (AlCl_3 + D - galactose) administered mice at days 87, 88, 89 and 90, retrieval trial
5.3	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on

	transfer latency of (AlCl ₃ + D - galactose) administered mice in elevated plus maze test
5.4	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on AChE level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.5	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on MDA level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.6	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on GSH level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.7	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on CAT level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.8	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on SOD level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.9	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on nitrite level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.10	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on BDNF level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.11	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on β - secretase level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.12	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on amyloid beta peptide ₁₋₄₂ level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.13	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on TNF - α level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.14	Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on IL - 6 level in cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.15	Analysis of histopathological data; (40X) of transverse section of the cerebral cortex (A) and the hippocampus (B) using hematoxylin and eosin staining
5.16a	: Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.),

	Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on spatial reference memory of (AlCl ₃ + D - galactose) administered mice during training days 81, 82 and 83
5.16b	Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on time spent in the target quadrant (Probe trial) of (AlCl ₃ + D - galactose) administered mice in MWM test
5.17a	Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on the spatial working memory of (AlCl ₃ + D - galactose) administered mice at days 87, 88, 89 and 90, acquisition trial
5.17b	Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on the spatial working memory of (AlCl ₃ + D - galactose) administered mice at days 87, 88, 89 and 90, retrieval trial
5.18	Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on transfer latency of (AlCl ₃ + D - galactose) administered mice in the elevated plus-maze test
5.19	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on AChE level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.20	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on MDA level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.21	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on GSH level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.22	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on catalase level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.23	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on SOD level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose) administered mice
5.24	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on nitrite level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose) administered mice
5.25	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on brain-derived neurotrophic factor level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose) administered mice
5.26	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on β - secretase level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose) administered mice

5.27	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on amyloid-beta peptide _{1 - 42} level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.28	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on tumour necrosis factor - α level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.29	Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on interleukin – 6 level in the cerebral cortex and hippocampus of (AlCl ₃ +D - galactose)administered mice
5.30	Analysis of histopathological data; (40X) of transverse section of the cerebral cortex (A) and hippocampus (B) using hematoxylin and eosin staining
5.31a	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on spatial reference memory of (AlCl ₃ + D - galactose) administered mice during training days 81, 82 and 83
5.31b	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on time spent in the target quadrant (Probe trial) of (AlCl ₃ + D - galactose) administered mice in MWM test
5.31c	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on the spatial working memory of (AlCl ₃ + D - galactose) administered mice at days 87, 88, 89 and 90, acquisition trial
5.31d	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on the spatial working memory of (AlCl ₃ + D - galactose) administered mice at days 87, 88, 89 and 90, retrieval trial
5.31e	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on the transfer latency of (AlCl ₃ + D - galactose) administered mice in the elevated plus-maze test
5.32	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on AChE levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)given mice
5.33	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on MDA levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)administered mice
5.34	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on GSH levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)induced mice
5.35	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on CAT levels in the cerebral cortex, as well as the hippocampus of (AlCl ₃ +D - galactose)administered mice
5.36	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on SOD levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)induced mice

5.37	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on nitrite levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)administered mice
5.38	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on BDNF levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)administered mice
5.39	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on β -secretase levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)administered mice
5.40	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on amyloid beta peptide ₁₋₄₂ levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)administered mice
5.41	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on TNF- α levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)administered mice
5.42	Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on IL – 6 levels in the cerebral cortex and the hippocampus of (AlCl ₃ +D - galactose)administered mice
5.43	Histopathological analysis (40X) of transverse section of the cerebral cortex (A) and the hippocampus (B) using hematoxylin and eosin staining
7.1	Schematic diagram indicates the possible mechanism of Bromelain alone and in combination with donepezil
7.2	Schematic diagram indicates the possible mechanism hypothesis of Myrcene alone and in combination with donepezil

LIST OF PUBLICATIONS

S. No.	Thesis work publications	Annexure No.
1	Ph.D. research work publication	
1.1	Rakesh Kumar, Rajan Kumar , Neha Sharma , Navneet Khurana, Sachin Kumar Singh , Saurabh Satija , Meenu Mehta , Manish Vyas. <i>Pharmacological evaluation of bromelain in mouse model of Alzheimer's disease</i> . Neurotoxicology, 2022. IF – 4.29.	X
1.2	Rakesh Kumar, Rajan Kumar, Neha Sharma, Navneet Khurana. <i>Ameliorative effect of myrcene in mouse model of Alzheimer's disease</i> . European Journal of Pharmacology, 2021. IF - 4.42.	XI
1.3	Rakesh Kumar, Rajan Kumar, Abhinav Anand, Rishi Mahajan, Gopal Lal Khatik, Navneet Duggal, Meenu Mehta, Saurabh Satija, Neha Sharma, Navneet Khurana. <i>Potential of Prediction of Activity Spectra of Substances Software to Justify 3Rs Ethics for In Vivo Anti-Alzheimer's Studies of Phytochemicals</i> . International Journal of Green Pharmacy (IJGP), Original Article, LPU Conference Apr-June, 2018:66-72.	XII
2	Book chapter publication	
2.1	Rakesh Kumar, Rajan Kumar, Abhinav Anand, Neha Sharma and Navneet Khurana. <i>Pharmacological Management of Alzheimer's disease</i> . Advances in Neuropharmacology and drug therapeutics, CRC Taylor & Francis group, Apple academic press Inc. USA, Edition-1, 2020: 133-150.	XIII
3	Allied publications (Other than thesis work)	
3.1	Rajan Kumar, Rakesh Kumar, Navneet Khurana, Sachin Kumar Singh, Shelly Khurana, Surajpal Verma, Neha Sharma, Manish Vyas, Kamal Dua, Rubiya Khursheed, Ankit Awasthi, Sukriti Vishwas. <i>Improved neuroprotective activity of Fisetin through SNEDDS in ameliorating the behavioral alterations produced in rotenone-induced Parkinson's model</i> . Environmental Science and Pollution Research, 2022. IF – 4.22.	XIV
3.2	Bhupinder Kapoor, Monica Gulati, Sachin K Singh, Gopal L Khatik, Reena Gupta, Rakesh Kumar, Rajan Kumar, K Gowthamarajan, Sanjeev Mahajan, Som Gupta. <i>Fail-safe nano-formulation of prodrug of sulfapyridine: Preparation and evaluation for treatment of rheumatoid arthritis</i> . Materials Science and Engineering: C, 2021. IF – 7.3.	XV
3.3	Mukta Gupta, Monica Gulati, Bhupinder Kapoor, Bimlesh Kumar, Rakesh Kumar, Rajan Kumar, Navneet Khurana, Reena Gupta, Naresh Singh. <i>Anti-ulcerogenic effect of methanolic extract of Elaeagnus conferta Roxb. seeds in Wistar rats</i> . Journal of Ethnopharmacology, 2021. IF – 4.3.	XVI
3.4	Rajan Kumar, Rakesh Kumar, Rubiya Khursheed, Ankit Awasthi, Navneet Khurana, Sachin Kumar Singh, Shelly Khurana, Neha Sharma, Pradnya Gunjal, Jaskiran Kaur, Leander Corrie. <i>Development and validation of RP-HPLC method for estimation of fisetin in rat plasma</i> . South African Journal of Botany, 2021. IF – 2.29.	XVII
3.5	Rajan Kumar, Rakesh Kumar, Navneet Khurana, Sachin Kumar	XVIII

	Singh, Shelly Khurana, Surajpal Verma, Neha Sharma, Bhupinder Kapoor, Manish Vyas, Rubiya Khursheed, Ankit Awasthi, Jaskiran Kaur, Leander Corrie. <i>Enhanced oral bioavailability and neuroprotective effect of fisetin through its SNEDDS against rotenone-induced Parkinson's disease rat model</i> . Food and Chemical Toxicology, 2020. IF – 4.4.	
3.6	Paranjeet Kaur, Zahid Rafiq Bhat, Sana Bhat, Rakesh Kumar, Rajan Kumar, Kulbhushan Tikoo, Jeena Gupta, Navneet Khurana, Jaskiran Kaur, Gopal L. Khatik. <i>Synthesis and evaluation of New 1,2,4-oxadiazole based trans- acrylic acid derivatives as potential PPAR-alpha/gamma dual agonist</i> . Bioorganic Chemistry, 2020. IF – 5.03.	XIX
3.7	Yogita Kumari, Sachin Kumar Singh, Rajesh Kumar, Bimlesh Kumar, Gurmandeep Kaur, Monica Gulati, Devesh Tewari, K. Gowthamarajan, V.V.S. Narayana Reddy Karri, Clarisse Ayinkamiye, Rubiya Khursheed, Ankit Awasthi, Narendra Kumar Pandey, Souvik Mohanta, Saurabh Gupta, Leander Corrie, Pooja Patni, Rajan Kumar, Rakesh Kumar. <i>Modified apple polysaccharide capped gold nanoparticles for oral delivery of insulin</i> . International Journal of Biological Macromolecules, 2020. IF – 6.78.	XX
3.8	Rajan Kumar, Rakesh Kumar, Rubiya Khursheed, Ankit Awasthi, Arya Kadukkattil Ramanunny, Jaskiran Kaur, Navneet Khurana, Sachin Kumar Singh, Shelly Khurana, Narendra Kumar Pandey, Bhupinder Kapoor, Neha Sharma. <i>Validated Reverse Phase-High-Performance Liquid Chromatography Method for Estimation of Fisetin in Self-Nanoemulsifying Drug Delivery System</i> . ASSAY and Drug Development Technologies, 2020. IF – 1.7.	XXI
3.9	Rajan Kumar, Rakesh Kumar, Rubiya Khursheed, Bhupinder Kapoor, Neha Sharma, Shelly Khurana, Navneet Khurana, Sachin Kumar Singh, Manish Vyas. <i>Development and Validation of UV Spectroscopic Method for Estimation of Fisetin in Self Nanoemulsifying Drug Delivery System</i> . Research Journal of Pharmacy and Technology, 2020. SJR – 1.2.	XXII
3.10	Rajan Kumar, Rubiya Khursheed, Rakesh Kumar, Ankit Awasthi, Neha Sharma, Shelly Khurana, Bhupinder Kapoor, Navneet Khurana, Sachin Kumar Singh, Kuppusamy Gowthamarajan, Ashish Wadhwani. <i>Self-nanoemulsifying drug delivery system of fisetin: Formulation, optimization, characterization and cytotoxicity assessment</i> . Journal of Drug Delivery Science and Technology, 2019. IF – 2.9.	XXIII
3.11	Rajan Kumar, Rakesh Kumar, Neha Sharma, Manish Vyas, Sanchit Mahajan, Saurabh Satija, Sachin Kumar Singh, Rubiya Khursheed, Meenu Mehta, Shelly Khurana, Navneet Khurana. <i>Fisetin: A phytochemical with various pharmacological activities</i> . Plant Archives, 2019. SJR – 0.27.	XXIV

LIST OF CONFERENCES

S. No.	Presentation in conferences	Annexure No.
1	Oral presentation	
1.1	Rakesh Kumar*, Rajan Kumar, Neha Sharma, Navneet Khurana. <i>Potential of PASS Software to Justify 3Rs Ethics for in vivo Anti-Alzheimer's studies of Phytochemicals</i> . International Conference on, Commercialization of Medicinal Plant Product: Lab Techniques to Trade, At School of Pharmaceutical Sciences, Lovely Professional University, Phagwara Punjab, India, Phytocon, April 14,(2018).	XXV
2	Poster presentation	
2.1	Rakesh Kumar*, Rajan Kumar, Neha Sharma, Navneet Khurana. <i>Effects of Biomolecules on adaptation of β-secretase and β-amyloid in $AlCl_3$ and D-galactose induced mouse model of Alzheimer's disease</i> . International Conference (IC-2020) on “Emerging Techniques in Drug Discovery and Drug Delivery: Current Challenges and Future Prospects”, organized by Chandigarh Group of Pharmacy, Landran, Mohali, February 10-11, (2020).	XXVI
2.2	Rakesh Kumar*, Rajan Kumar, Navneet Khurana, Neha Sharma. <i>Recent Advancement in the Management of Alzheimer's disease</i> . International Conference of Pharmacy (ICP-2019) on Pharmacy; Realigning Focus on Health” organized by LPU, Phagwara, Punjab, India, in collaboration with Indian Pharmacy Graduate Association (IPGA), September 13-14, (2019).	XXVII
2.3	Rakesh Kumar*, Rajan Kumar, Navneet Khurana, Neha Sharma. <i>PASS Software Prediction of Phytoconstituents for Anti-Alzheimer's disease Activities</i> . Graduation Project Expo, organized by Division of Student welfare, lovely Professional University, Phagwara, 144411, Punjab, India, April 26-27, (2019).	XXVIII
2.4	Rakesh Kumar*, Rajan Kumar, Neha Sharma, Navneet Khurana. <i>Potential Effects of Phytoconstituents in Symptomatic Relief of Alzheimer's disease</i> . National Conference on RAPSCON sponsored by Association of Pharmaceutical Teachers of India (APTI), at SSGI, Manawala, Amritsar, Punjab, India, March 27, (2019).	XXIX
2.5	Rakesh Kumar*, Rajan Kumar, Neha Sharma, Navneet Khurana. <i>Potential Effects of Biomolecules in Pathogenesis of Alzheimer's disease</i> . National seminar on Recent advancement in Pharmaceutical Research and Future Prospects, at Radharaman Institute of Pharmaceutical Sciences, Bhopal, India, February 22, (2019).	XXX
2.6	Rakesh Kumar*, Rajan Kumar, Navneet Khurana, Neha Sharma. <i>In vivo and In vitro Pharmacological Activities of Naturally Occuring Proleolytic Enzyme, Bromelain</i> . 106 th Indian Science Congress (ISC), at LPU, Phagwara, Punjab, India, PP 22, January 3-7, (2019).	XXXI

2.7	Rakesh Kumar*, Rajan Kumar, Neha Sharma, Navneet Khurana. <i>To Predict the Anti-Alzheimer's Activities of Phytochemicals by using PASS Software.</i> 69 th Indian Pharmaceutical Congress (IPC) Association, at Chitkara university, Rajpura, Punjab, India, Poster No., D-314, December 23, (2017).	XXXII
2.8	Rakesh Kumar*, Rajan Kumar, Neha Sharma, Navneet Khurana. <i>Pharmacological Activities of Hesperidin: An overview of in vivo model.</i> National Conference on Challenge and Future Vision in Phytomedicine – An alternative to Modern Medicine, at GHG Khalsa College of Pharmacy, Gurusar Sadhar, Ludhiana, Punjab, India, PC11, Page 70, September 22, (2017).	XXXIII

LIST OF WORKSHOPS

S. No.	Participation in workshops	Annexure No.
1	SERB – Scientific Social Responsibility Research Facility Training workshop on immunofluorescence, organized by Amity Institute of Physiology and Allied Sciences, Amity University, Noida, U.P. India, 29 March 2022.	XXXIV
2	Training on the care use of laboratory animals in accordance with the ethical guideline, organized by Lovely Professional University, Phagwara, Punjab, India, 11 March to 12 March 2022.	XXXV
3	GUJCOST sponsored two days online national workshop on In vitro and In vivo models in neuropsychological screening, organized by Ganpat University, Shree S.K. Patel College of Pharmaceutical Education and Research, Gujarat, India, 7 August to 8 August 2021.	XXXVI
4	National Pharmacovigilance week – 2021, Pharmacovigilance: A step towards patient safety, organized by adverse drug reaction monitoring centre (AMC) and medical device adverse event monitoring centre (MDMC), at School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, 12 November 2021.	XXXVII
5	National workshop on advanced instrumentation, organized by Central Instrumentation Facility, Division of Research and Development, 20 April 2019.	XXXVIII
6	One day Author Workshop By Springer Nature on “ <i>How to Write and Publish Scientific Articles and Manuscripts</i> ”, Organized at Lovely Professional University, Phagwara, Punjab, India, 15 April 2018.	XXXIX
7	Obtained ‘O’ Grade in two days workshop, “ <i>Hands on Training to Improve Quality of Research Conducted on Experimental Animals</i> ”, School of Pharmaceutical Sciences, L.P.U. Phagwara, Punjab, India, 23 March 2018.	XXXX
8	Design of Experiments, jointly organized by Qsutra & School of Pharmaceutical Sciences, Lovely Professional University, Punjab, India, 13 July to 14 July 2018.	XXXXI
9	How to write and Publish Scientific Article and Manuscript, organized by Lovely Professional University, Punjab, India, 15 April 2018.	XXXXII
10	Pre-conference workshop on “ <i>Pharmacokinetics and Toxicokinetics</i> ” under the aegis of Society of Toxicology (STOX), Organized at Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, 17 November 2017.	XXXXIII

LIST OF ABBREVIATIONS

S. No.	Abbreviations	Full form
1	AD	Alzheimer's disease
2	APP	Amyloid precursor protein
3	ApoE	Apolipoprotein
4	AlCl ₃	Aluminium trichloride
5	A β ₁₋₄₂	Amyloid beta peptide ₁₋₄₂
6	AChE	Acetylcholinesterase
7	ANOVA	Analysis of variance
8	AMPA	A-amino 3-hydroxy 4-isoxazole propionic acid
9	Brm L	Bromelain low dose
10	Brm H	Bromelain high dose
11	b.w.	Bodyweight
12	BDNF	Brain-derived neurotrophic factor
13	BBB	Blood-brain barrier
14	BACE	β -secretase
15	CAT	Catalase
16	ChAT	Choline acetyl transferase
17	CMC	Carboxymethyl cellulose
18	CPCSEA	Committee for the purpose of control and supervision of experiments on animals
19	CYP	Cytochrome
20	Dnpz	Donepezil
21	DTNB	5, 5'-dithiobis (2-nitrobenzoic acid)
22	ELISA	Enzyme-linked immunosorbent assay
23	EPM	Elevated plus-maze
24	EDTA	Ethylene diamine tetraacetic acid
25	FDA	Food drug administration
26	GSH	Reduced glutathione
27	GPx	Glutathione peroxidase
28	GR	Glutathione reductase
29	IAEC	Institutional animal ethics committee
30	i.p.	Intraperitoneal
31	IL-6	Interleukin-6
32	MAO	Monoamine oxidase
33	MWM	Morris water maze
34	MDA	Malondialdehyde
35	Myr H	Myrcene high dose
36	Myr L	Myrcene low dose
37	NO	Nitrite
38	NFTs	Neurofibrillary tangles
39	NFB	Nuclear factor B
40	NBT	Nitro blue tetrazolium
41	NMDA	N-methyl D-aspartate
42	p.o.	Per oral
43	PTZ	Pentylenetetrazol
44	ROS	Reactive oxygen species

45	SOD	Superoxide dismutase
46	SRM	Spatial reference memory
47	SWM	Spatial working memory
48	TNF- α	Tumour necrosis factor- α
49	TfR	Transferrin
50	TL	Transfer latency
51	TCA	Trichloroacetic acid
52	TBA	Thiobarbituric acid
53	TrkB	Tropomyosin related kinase B
54	TK	Tyrosin kinase
55	TBARS	Thiobarbituric acid reactive substances
56	TLR	Toll like receptor

TABLE OF CONTENTS

S. No.	Title	Page No.
Chapter - 1		
Introduction		
1.	Introduction	1 - 4
Chapter - 2		
Literature review		
2.	Literature review	5 - 34
2.1	Epidemiology of Alzheimer's disease	5
2.2	Pathophysiology	5
2.3	Etiology	6
2.3.1	Age factors	8
2.3.2	Genetic factors	8
2.3.3	Environmental factors	9
2.3.4	Drug as well as chemical induced Alzheimer's disease	9
2.4	Stages and symptoms	10
2.5	Diagnosis	12
2.6	Drug used in Alzheimer's disease	12
2.6.1	Approved drugs	14
2.6.1.1	Cholinesterase inhibitors	14
2.6.1.1.1	Donepezil	14
2.6.1.1.1.1	Pharmacokinetic	15
2.6.1.1.1.2	Mechanism of action	15
2.6.1.1.1.3	Adverse effects	16
2.6.1.1.2	Rivastigmine	16
2.6.1.1.2.1	Pharmacokinetic	16
2.6.1.1.2.2	Mechanism of action	16
2.6.1.1.2.3	Adverse effects	17
2.6.1.1.3	Galantamine	17
2.6.1.1.3.1	Pharmacokinetic	17
2.6.1.1.3.2	Mechanism of action	17
2.6.1.1.3.3	Adverse effects	17
2.6.1.2	N-methyl-D-aspartate receptor antagonists	18
2.6.1.2.1	Memantine	18
2.6.1.2.1.1	Pharmacokinetic	18
2.6.1.2.1.2	Mechanism of action	18
2.6.1.2.1.3	Adverse effects	19
2.6.2	Investigational drugs	20
2.6.2.1	Antioxidants	20
2.6.2.1.1	Ginkgo biloba	20
2.6.2.1.1.1	Pharmacokinetic	20
2.6.2.1.1.2	Mechanism of action	20
2.6.2.1.1.3	Medicinal uses	20
2.6.2.1.1.4	Adverse effects	21
2.6.2.1.2	Vitamin E	21
2.6.2.1.2.1	Pharmacokinetic	21

2.6.2.1.2.2	Medicinal uses	21
2.6.2.1.3	Melatonin	22
2.6.2.1.3.1	Medicinal uses	22
2.6.2.1.3.2	Adverse effects	22
2.6.2.2	Peroxisome proliferators activated receptor γ agonist	22
2.6.2.2.1	Pioglitazone	22
2.6.2.2.1.1	Pharmacokinetic	22
2.6.2.2.1.2	Therapeutic uses	23
2.6.2.3	γ -secretase inhibitor	23
2.6.2.3.1	Semagacestat	23
2.6.2.3.1.1	Pharmacokinetic	23
2.6.2.3.1.2	Therapeutic uses	23
2.6.2.4	5-HT ₆ antagonist	23
2.6.2.4.1	SB-271046	23
2.6.2.5	Statins	24
2.6.2.5.1	Simvastatin and Pravastatin	24
2.6.2.5.1.1	Pharmacokinetic	24
2.6.2.5.1.2	Therapeutic uses	24
2.6.2.5.1.3	Adverse effects	24
2.6.2.6	Curcumin	25
2.6.2.6.1	Pharmacokinetic	25
2.6.2.6.2	Medicinal uses	25
2.6.3	Recent drugs	25
2.6.3.1	Cholinesterase inhibitors	25
2.6.3.1.1	Phenserine	25
2.6.3.1.1.1	Pharmacokinetic	25
2.6.3.1.1.2	Mechanism of action	26
2.6.3.1.1.3	Therapeutic uses	26
2.6.3.1.1.4	Adverse effects	26
2.6.3.1.2	Latrepidine	26
2.6.3.1.3	Huperizine A	26
2.6.3.1.3.1	Pharmacokinetic	26
2.6.3.1.3.2	Mechanism of action	27
2.6.3.1.3.3	Adverse effects	27
2.6.3.1.4	Ladostigil (TV3326)	27
2.6.3.1.4.1	Mechanism of action	28
2.6.3.2	Immunotherapy	28
2.6.3.2.1	Bapineuzumab	28
2.6.3.2.2	Solanezumab	28
2.6.3.2.3	Ponezumab (PF4360365)	29
2.7	Models for Alzheimer's disease	29
2.8	Selected phytoconstituents and its reported pharmacological activities	29
2.8.1	Bromelain	29
2.8.2	Myrcene	29
Chapter - 3		
Research hypothesis and objectives		
3.	Research hypothesis and objectives	35

3.1	Hypothesis of research	35
3.2	Aim	35
3.3	Brief objectives of the research	35
Chapter - 4		
Materials and methods		
4.	Materials and methods	36 - 58
4.1	Drugs, ELISA kits, and reagents	36
4.2	Animals	36
4.3	Drug treatment schedule	37
4.4	Behavioral parameters	44
4.4.1	Morris water maze task	44
4.4.2	Elevated plus-maze task	45
4.4.3	Assessment of locomotor activity	45
4.5	Biochemical parameters	46
4.5.1	Homogenization of mice brain	46
4.5.2	Estimation of acetylcholinesterase activity	46
4.5.3	Estimation of lipid peroxidation	48
4.5.4	Assessment of nitrite	49
4.5.5	Detection of reduced glutathione	50
4.5.6	Determination of catalase activity	51
4.5.7	Determination of superoxide dismutase activity	51
4.5.8	Determination of protein	52
4.5.9	Enzyme-linked immunosorbent assay	54
4.5.9.1	Mouse β -secretase and Amyloid β_{1-42} peptide ELISA	54
4.5.9.2	Mouse brain-derived neurotrophic factor ELISA	56
4.5.9.3	Mouse tumor necrosis factor- α and interleukin-6 ELISA	57
4.6	Statistical analysis	58
4.7	Histopathological assessment	58
Chapter - 5		
Results		
5.	Results	59 - 140
5.1	Bromelain	59
5.1.1	Behavioral analysis	59
5.1.1.1	Bromelain alone and in combination with donepezil improve SRM and SWM in $AlCl_3$ and D - galactose induced AD mice in MWM test	59
5.1.1.2	Bromelain alone and in combination with donepezil improve the learning memory of $AlCl_3$ and D - galactose induced AD mice in the EPM test	65
5.1.1.3	Bromelain alone and in combination with donepezil show no locomotor activity alteration of $AlCl_3$ and D - galactose induced AD mice	65
5.1.2	Biochemical analysis	68
5.1.2.1	Bromelain alone and in combination with donepezil inhibit the acetylcholinesterase activity in different brain regions of $AlCl_3$ and D - galactose induced AD mice	68
5.1.2.2	Bromelain alone and in combination with donepezil decrease the MDA level in different brain regions of $AlCl_3$ and D - galactose induced AD mice	70

5.1.2.3	Bromelain alone and in combination with donepezil increase the reduced glutathione level in different brain regions of AlCl ₃ and D - galactose induced AD mice	70
5.1.2.4	Bromelain alone and in combination with donepezil increase the catalase level in different brain regions of AlCl ₃ and D - galactose induced AD mice	73
5.1.2.5	Bromelain alone and in combination with donepezil increase the superoxide dismutase level in different brain regions of AlCl ₃ and D - galactose induced AD mice	73
5.1.2.6	Bromelain alone and in combination with donepezil reduce the nitrite level in different brain regions of AlCl ₃ and D - galactose induced AD mice	76
5.1.2.7	Bromelain alone and in combination with donepezil increase the brain-derived neurotrophic factor level in different brain regions of AlCl ₃ and D - galactose induced AD mice	76
5.1.2.8	Bromelain alone and in combination with donepezil decrease the β -secretase level in different brain regions of AlCl ₃ and D - galactose induced AD mice	79
5.1.2.9	Bromelain alone and in combination with donepezil decrease the amyloid-beta peptide ₁₋₄₂ level in different brain regions of AlCl ₃ and D - galactose induced AD mice	79
5.1.2.10	Bromelain alone and in combination with donepezil decrease the tumour necrosis factor- α level in different brain regions of AlCl ₃ and D - galactose induced AD mice	82
5.1.2.11	Bromelain alone and in combination with donepezil decrease the interleukin-6 level in different brain regions of AlCl ₃ and D - galactose induced AD mice	82
5.1.3	Histopathological analysis	85
5.2	Myrcene	87
5.2.1	Behavioral analysis	87
5.2.1.1	Myrcene alone and in combination with donepezil improve the spatial reference memory and spatial working memory in AlCl ₃ and D-galactose induced AD mice in MWM test	87
5.2.1.2	Myrcene alone and in combination with donepezil improve the learning memory of AlCl ₃ and D-galactose induced AD mice in EPM test	93
5.2.1.3	Myrcene alone and in combination with donepezil show no locomotor activity alteration of AlCl ₃ and D-galactose induced AD mice	93
5.2.2	Biochemical analysis	96
5.2.2.1	Myrcene alone and in combination with donepezil inhibit the acetylcholinesterase activity in different brain regions of AlCl ₃ and D-galactose induced AD mice	96
5.2.2.2	Myrcene alone and in combination with donepezil decrease the MDA level in different brain regions of	96

	AlCl ₃ and D-galactose induced AD mice	
5.2.2.3	Myrcene alone and in combination with donepezil increase the reduced glutathione level in different brain regions of AlCl ₃ and D-galactose induced AD mice	99
5.2.2.4	Myrcene alone and in combination with donepezil increase the catalase level in different brain regions of AlCl ₃ and D-galactose induced AD mice	99
5.2.2.5	Myrcene alone and in combination with donepezil increase the superoxide dismutase level in different brain regions of AlCl ₃ and D-galactose induced AD mice	102
5.2.2.6	Myrcene alone and in combination with donepezil reduce the nitrite level in different brain regions of AlCl ₃ and D-galactose induced AD mice	102
5.2.2.7	Myrcene alone and in combination with donepezil increase the brain derived neurotrophic factor level in different brain regions of AlCl ₃ and D-galactose induced AD mice	105
5.2.2.8	Myrcene alone and in combination with donepezil decrease the β -secretase level in different brain regions of AlCl ₃ and D-galactose induced AD mice	105
5.2.2.9	Myrcene alone and in combination with donepezil decrease the amyloid beta peptide ₁₋₄₂ level in different brain regions of AlCl ₃ and D-galactose induced AD mice	108
5.2.2.10	Myrcene alone and in combination with donepezil decrease the tumor necrosis factor- α level in different brain regions of AlCl ₃ and D-galactose induced AD mice	108
5.2.2.11	Myrcene alone and in combination with donepezil decrease the interleukin-6 level in different brain regions of AlCl ₃ and D-galactose induced AD mice	111
5.2.3	Histopathological analysis	111
5.3	Combination of bromelain and Myrcene	114
5.3.1	Behavioral analysis	114
5.3.1.1	Bromelain and Myrcene improve the spatial reference memory and spatial working memory in AlCl ₃ and D - galactose induced AD mice in the MWM test	114
5.3.1.2	Bromelain and Myrcene improve the learning memory of AlCl ₃ and D - galactose induced AD mice in the EPM test	119
5.3.1.3	Bromelain alone and in combination with donepezil show no locomotor activity alteration of AlCl ₃ and D - galactose induced AD mice	122
5.3.2	Biochemical analysis	123
5.3.2.1	Bromelain and Myrcene inhibit the acetylcholinesterase activity in different brain regions of AlCl ₃ and D - galactose induced mice	123
5.3.2.2	Bromelain and Myrcene decrease the MDA level in different brain regions of AlCl ₃ and D - galactose induced mice	123
5.3.2.3	Bromelain and Myrcene increase the reduced glutathione	126

	level in different brain regions of AlCl ₃ and D - galactose induced mice	
5.3.2.4	Bromelain and Myrcene increase the catalase level in different brain regions of AlCl ₃ and D - galactose induced mice	126
5.3.2.5	Bromelain and Myrcene increase the superoxide dismutase level in different brain regions of AlCl ₃ and D - galactose induced mice	129
5.3.2.6	Bromelain and Myrcene reduce the nitrite level in different brain regions of AlCl ₃ and D - galactose induced mice	129
5.3.2.7	Bromelain and Myrcene increase the brain-derived neurotrophic factor level in different brain regions of AlCl ₃ and D - galactose induced mice	132
5.3.2.8	Bromelain and Myrcene decrease the β -secretase level in different brain regions of AlCl ₃ and D - galactose induced mice	132
5.3.2.9	Bromelain and Myrcene decrease the amyloid-beta peptide ₁₋₄₂ level in different brain regions of AlCl ₃ and D - galactose induced mice	135
5.3.2.10	Bromelain and Myrcene decrease the tumour necrosis factor- α level in different brain regions of AlCl ₃ and D - galactose induced mice	135
5.3.2.11	Bromelain and Myrcene decrease the interleukin-6 level in different brain regions of AlCl ₃ and D - galactose induced mice	138
5.3.3	Histopathological analysis	138
Chapter - 6		
Discussion		
6.	Discussion	141 - 147
Chapter - 7		
Summary and conclusion		
7.	Summary and conclusion	148 - 153
7.1	Summary	148
7.2	Conclusion	152
Bibliography		154 - 180

ABSTRACT

Alzheimer's disease (AD) is an age-related neuronal degenerative disease characterized by the occurrence of three major pathological hallmarks such as loss of synapses, extracellular accumulation of amyloid-beta ($A\beta$) peptides, and intracellular neurofibrillary tangles (NFTs). Cholinesterase inhibitors, which are currently prescribed for the treatment of AD, only give symptomatic relief and do not protect against neurodegeneration. The discovery of therapeutic techniques as well as molecules that not only improve brain functions but also prevent neurodegeneration appears to be an urgent need. One of the key causes of AD is oxidative stress. A variety of antioxidants are being studied to see if they can help prevent neurodegeneration in people with AD.

In this study, we investigated the Bromelain (Brm), Myrcene (Myr), and Brm + Myr effect on neurobehavioural, and neuropathological alteration in mice induced by Aluminium trichloride ($AlCl_3$) and D - galactose. In mice, co-administration of $AlCl_3$ at a dose of 5 mg/kg b.w., via the oral route, and D - galactose at a dose of 60 mg/kg b.w., via intraperitoneal route for 90 days resulted in cognitive impairment, spatial learning, and memory deficits, as well as neurotoxicity. However, 30 consecutive days, treatments via an intraperitoneal route with donepezil (Dnpz) at dose 2 mg/kg b.w., Brm low dose (Brm L) at dose 10 mg/kg b.w., Brm high dose (Brm H) at dose 20 mg/kg b.w., Brm L + Dnpz at doses 10, 2 mg/kg b.w., Myr low dose (Myr L) at dose 100 mg/kg b. w., Myr high dose (Myr H) at dose 200 mg/kg b. w., Myr L + Dnpz at doses 100, 2 mg/kg b. w., Brm L + Myr L at doses 10, 100 mg/kg b. w., and Brm L + Myr L + Dnpz at doses 10, 100, 2 mg/kg b. w. were considerably reversed the effect of $AlCl_3$ and D - galactose induced AD mice. The consequences of behavioral tests such as Morris water maze, elevated plus maze, and locomotor; biochemical analysis such as malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), nitrite, and acetylcholinesterase (AChE); and ELISA tests such as mouse β - secretase (BACE), amyloid-beta peptide₁₋₄₂ ($A\beta_{1-42}$), tumor necrosis factor - α (TNF- α), interleukin - 6 (IL-6), and brain-derived neurotrophic factor (BDNF) demonstrated a significant ($p < 0.05$) neuroprotective effect of Dnpz, Brm L, Brm H, Brm L + Dnpz, Myr L, Myr H, Myr L + Dnpz, Brm L + Myr L, and Brm L + Myr L + Dnpz treatments. In addition, hematoxylin and eosin staining of the cerebral cortex and hippocampus revealed eosinophilic lesions and hyperchromatic nuclei in Alzheimer's disease mice, but treatments with Dnpz, Brm L, Brm H, Brm L + Dnpz, Myr L, Myr H, Myr L + Dnpz, Brm L + Myr L, and Brm L + Myr L + Dnpz reversed these neurodegenerative effects. Thus, Brm and Myr individually; the

combination of Brm + Myr, and in co-administration with donepezil prevent AlCl_3 and D - galactose induced spatial learning and memory deficits, as well as cognitive impairment, by increasing cholinergic activity and synaptic plasticity, as well as reducing oxidative damage, neuroinflammation, $\text{A}\beta_{1-42}$ aggregations, and histopathological damage, according to our findings. The present study consequences indicate that Brm alone; Myr alone; the combination of Brm + Myr, and co-administration with donepezil appear to have neuroprotective properties. Henceforward, Brm alone, Myr alone, the combination of Brm + Myr, and in combination with donepezil may serve as a potential candidate for the treatment of Alzheimer's disease.

Keywords: Bromelain, Myrcene, Aluminium trichloride, D-galactose, Donepezil, Alzheimer's disease, Oxidative stress, Histopathology.

CHAPTER - 1

INTRODUCTION

1. Introduction

Neurodegeneration is an ameliorative depletion of structure, function of neurons, or death of neurons that occur in the central nervous system. Neurodegenerative disorders are defined as the effect of neurons in the brain. Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic lateral sclerosis are the major neurodegenerative disorders (**Chen et al., 2016; Gitler et al., 2017**). Aging is the highest risk factor for neurodegenerative disorders. Mitochondrial DNA mutation and oxidative stress subscribe to aging (**Lin and Beal, 2006**).

The word dementia, obtained from the Latin word “de” and “mente” which means “out (de) of one's mind (mente).” Dementia is a common term that is characterized by deterioration, of cognitive function, involving loss of memory, impairment of language, and disorientation as well as a degree of mental ability in the patient to interfere with daily life (**Boller and Forbes, 1998**). AD is the general type of dementia. AD accounts for about 80% of cases more than other all types of dementia such as 25% vascular dementia, 15% Lewy body dementia, and frontotemporal (FT) dementia (**Boller and Forbes, 1998; Alzheimer's Association, 2015**).

Alzheimer's disease (AD) was described first by the neuropathologist, and the German psychiatrist, Alois Alzheimer in 1906. AD is an insidious and progressive neurodegenerative disease (**Wenk 2003; Burns 2009**). AD is characterized by three major hallmarks such as synapse loss (**Priller et al., 2006; Lacor et al., 2007**), amyloid-beta ($A\beta$) accumulation extracellularly, and neurofibrillary tangles (NFTs) intracellularly (**Risacher and Saykin 2013; Bloom 2014**). $A\beta_{1-42}$ or $A\beta_{40-42}$ peptide monomer is derived from proteolytic cleavage of amyloid precursor protein in presence of the enzyme β -secreta along with γ -secretase (**DeKosky 2001; Hampel 2012**). Familial AD occur due to mutations in APP, PS1 and PS2 (**Hardy and Selkoe, 2002; Brunden et al., 2011**). In AD, as per the cholinergic hypothesis, the level of acetylcholine decreases in the brain (**Martorana et al., 2010**). Neuroinflammation and brain trauma is observed as the risk factor for AD (**Aisen, 2002; Candore et al., 2006; Giunta et al., 2008**). Potentiate of free radical initiated oxidative stress enhances with age, attenuates endogenous anti-oxidant enzymes (**Rahman et al., 2007**), and influences the pathophysiology of AD as a result, deposition of extracellular $A\beta$ and intracellular NFTs (**Huang et al., 2016**). The aggregation of $A\beta$ has been shown to activate microglia and increase the development of tumor necrosis factor- α (TNF- α) as well as interleukin-6 (IL-6) (**Xiong et al., 2014**). The

rich antioxidant diet lowers the incidence rate of AD by preventing oxidation and mitochondrial dysfunction (**Gibson and GE., 2007**). During AD, neuronal destruction and deterioration of neural connections in the cerebral cortex region of the brain further lead to substantial loss of brain mass (**Perl, 2010**). The mitochondrial dysfunction for the pathogenesis of AD proposes amyloidosis. This also leads to cell cycle re-entry, and tau phosphorylation (**Moreira, 2018**).

For many years, researchers have been using a combination of AlCl_3 and D-galactose to investigate Alzheimer's disease mechanisms and evaluate molecules (**Wei et al., 2005**). As a result, the combination of AlCl_3 and D-galactose has been studied as a model animal for improving cognitive dysfunction and memory impairment, oxidative stress, neuroinflammatory responses, cholinesterase enzyme variation, and changes in pathological markers associated with Alzheimer's disease (**Li et al., 2016; Wei et al., 2017; Chiroma et al., 2018; Haider et al., 2020**).

Aluminium (Al) is a neurotoxic agent (**Shati et al., 2011**) as well as the third most basic rich element on the earth (**Farina et al., 2002**). The regular consumption of Al from various items used in our daily life like food additives, deodorants, and a few antacid medicines enhanced the Al concentration in the brain. Al enters the brain by binding with high-affinity receptors for transferrin (TfR) in the blood-brain barrier (BBB) (**Roskams et al., 1990**). In the brain, Al affects fast and slow axonal transport and induces inflammatory responses (**Amjad et al., 2015; Campbell et al., 2004**). Long-term potentiation is inhibited by Al, resulting in synaptic and structural abnormalities. The result was significant neuronal and memory loss (**Amjad et al., 2015; Kawahara et al., 2005**). The enhanced Al concentration in Alzheimer's disease brain causes neuritic aggregate, senile plaques, and neurofibrillary tangles (NFTs) (**McLachlan et al., 1991**). "Al is a potent cholinergic toxin" (**Gulya et al., 1990**). This causes neuron apoptotic loss as an associated symptom of AD (**Ghribi et al., 2001**). In a molecular study, Al affects cellular energy metabolism, gene transcription, and DNA topology (**Lukiw et al., 1998**). Aluminium was reported to induce the misfolding, and self-accumulation of highly phosphorylated cytoskeleton proteins like microtubules associated proteins thereby implicating in Alzheimer's disease (**Kawahara, 2003**).

D - galactose is obtained from the lactose present in milk. Galactose and monosaccharide glucose are formed later through hydrolysis of lactose in the intestine (**Lu et al., 2006**). D - galactose is metabolized in presence of D-galactokinase enzyme as

well as galactose -1- phosphate uridylyltransferase enzyme, while D - galactose supply leads consequences to abnormal metabolism (Lu et al., 2006; Kaplan et al., 1996).“As aforementioned, D - galactose is converted into galactitol. But, D-galactokinase and galactose-1-phosphate uridylyltransferase enzymes do not metabolize it. This clumping in the cell creates an imbalance between antioxidants and the production of reactive oxygen species (ROS)” (Hsieh et al., 2009). Furthermore, D - galactose is a reducing sugar and a physiological nutrient that reacts through free amines in proteins along with peptides to form advanced glycation end products through non-enzymatic glycation (Hsieh et al., 2009; Chen et al., 2006; Vlassara et al., 1994). It leads to the activation of the receptor for AGE and causes oxidative stress, as well as cellular injury (Song et al., 1999; Baynes 2001; Lu et al., 2006). D - galactose chronic administration has been reported to influence age-related cognitive decline as well as accelerate aging in mice (Lei et al., 2008).

In various studies, it has been reported that the present drug therapy for AD bestows only symptomatic relief, but is not able to nurse the underline pathological condition. That is why, in the present study, the potential of selected compounds such as Bromelain and Myrcene could be evaluated to treat the underline pathological etiology.

Bromelain is obtained from *Ananas comosus* stem and belongs to the family of sulfhydryl proteolytic enzymes. Bromelain is reported to possess various medicinal values such as anti-inflammatory (Eric et al., 2005), anti-rheumatoid (Cohen and Goldman 1964), anticarcinogenic (Baez et al., 2007; Kalra et al., 2008), immunomodulatory (Hale 2004), cardioprotective (Bhattacharya and Barun 2008), and anti-adipogenic (Dave et al., 2012) effect. Bromelain is reported to attenuate oxidative stress and improve cholinergic deficits in dichlorvos-induced mice brain (Chaudhary et al., 2018). Moreover, Bromelain was observed to enhance the permeability of BBB to nutrients (Laurer et al., 2001). This evidence could represent the therapeutic potential use in AD. Further, *in vitro* study, Bromelain was found to degrade A β ₁₋₄₂ monomer and soluble aggregate in cerebrospinal fluids of AD patients (Sancesario et al., 2018) which can be potential support for further evaluation of Bromelain effects on A β ₁₋₄₂ monomer and soluble aggregate in an animal model of AD.

Myrcene or β -Myrcene (Myr) is a monoterpene that occurs naturally in various plants such as lemongrass, hops, cardamom, mangoes, cannabis, and Myrcia (Bonamin et

al., 2014). Myr is extensively used for flavoring additives in the production of food, and soft beverages, and in the manufacturing of cosmetic products (**Cesta et al., 2013**). Myr has been reported to show various pharmacological actions such as analgesic, anti-inflammatory, and anti-oxidant effects (**Osman et al., 2011; Islam et al., 2020**). Myr has been reported to enhance antigen-specific total IgG immune responses against ovalbumin and Ag85B in mice (**Uyeda et al., 2016**). Besides all these, Myr improved the oxidative damage and histopathological neuronal damage in mice brain tissue induced by global cerebral ischemia/ reperfusion in C57BL/J6 mice (**Osman et al., 2014**). Myr's neuroprotective effect could be further explored to investigate the neuroprotective effect and effect of Myr on AD pathological alteration of markers induced by AlCl₃ and D - galactose mouse model of AD.

Based on these reported activities of Bromelain and Myrcene, these phytoconstituents can be expected to show a beneficial effect in AD. Thus, the proposed study was conducted to evaluate the ameliorative effect of Bromelain and Myrcene individually, as well as in combination, against the AD model.

CHAPTER – 2
LITERATURE REVIEW

2. Literature review

2.1. Epidemiology of Alzheimer's disease

In AD, the prevalence and incidences occur as per the standard of living in the country. The numbers of AD cases are more in developed countries rather than in developing countries. Prevalence is defined as the total number of people diagnosed with dementia or AD in a group of people at a particular period. While, incidence define as the total number of new cases of dementia or AD during a fixed time period (**Kalaria et al., 2008**). In India, epidemiological transition leads to an aging population, greater prevalence, and incidences of non-communicable diseases like cardiovascular disorders, stroke, and stress of routine lifestyle (**Das et al., 2012**). At the beginning of the 21st century, it was acknowledged the highly pervasive dementia type in greater than 60 years old people (**World Health Organization, 2012**). In 2016, worldwide people suffering from dementia were evaluated by more than 47.5 million. By 2030, it is being hypothesized globally escalation up to 75.6 million people with dementia (**World Health Organization, 2016**). This is a loss of neuron disorder with a progressive decline in memory and cognitive function in the brain that usually appears in late adulthood. In this disease, continuous neuronal damage occurs in the hippocampus and cerebral cortex area of the brain which leads to reduce the mass of the brain (**Uddin and Amran, 2018; Perl, 2010**). AD is the five most basic causes of mortality in the United States (US) (**Berríos-Torres et al., 2017**). As, it is a disorder above the 60 years of age, but, at 40 years and 50 years age of people, appears rarely. As per the clinical and epidemiology studies, about two lakhs people of age 65 years are suffering from this disorder. By 2050, as per speculations, a new case of AD is supposed to develop every 33 seconds (**Alzheimer's Association, 2014; Prince et al., 2016**).

2.2. Pathophysiology

The extracellular senile plaques composed mainly of amyloid β ($A\beta$) and neurofibrillary tangles (NFTs) composed of tau protein are represented as the major pathological hallmarks of the AD as shown in **Figure 2.1**. The relatively less prominent familial form of the AD which may present in the early years of one's life is the consequence of a mutation in any of three genes namely *APP*, *PS-1*, or *PS-2*. The sporadic variant appears generally after 65 years of age which oversees a large majority of the cases (**Blennow and Zetterberg, 2018**). Mostly, it occurs from an association of both the genetic and the environmental factors (**Aprahamian et al., 2013**).

The only definite risk factors for the sporadic are the E4 allele of apolipoprotein E and senescence. The plaques are constituted chiefly of the neurotoxic peptide. The A β is the by-product of the cleavage of a large precursor protein (APP) by enzymes (β -secretase and γ -secretase) (Allen et al., 2011). However, the A β is invisible in the case of APP which is cleaved by the enzyme α -secretase. NFTs mainly comprise the tau protein which is a microtubule-associated protein. The function of tau protein is to facilitate the neuronal transport system by binding with microtubules in cells. In the case of the AD, tau is uncoupled microtubules and aggregates into tangles by inhibiting transport and resulting in microtubule disassembly. It also depends on the phosphorylation of tau (Allen et al., 2011; Ravi et al., 2018). In a hypothesis, it has been reported that the mitochondrial dysfunction leads to amyloidosis, alters the kinase and tau phosphatase enzyme, and cell cycle re-entry (Moreira, 2018; Shoshan-Barmatz et al., 2018). The Pathogenesis of Alzheimer's disease is shown in **Figure 2.2**.

2.3. Etiology

The strategy behind the formation of the AD is not yet explained properly. But, certain factors are such as variation in tau phosphorylation, oxidative stress, calcium metabolism alteration, neuroinflammation, protein processing, and atypical energy metabolism. It is an accumulation of A β observed to be an essential pathological mark of AD (Munoz and Feldman, 2000). The cause of AD is based on aging, genetic factors, environmental factors, drug-induced factors, mitochondrial dysfunction, vascular factors, infectious agents, and the decline of the immune system. This disorder has been proposed as a multi-factorial disorder in which external and internal factors act together to ameliorate the rate of normal aging 'allostatic load'. The progressive neurodegeneration and blood vessels result in the formation of A β and tau protein clusters which spread from cell to cell, a transfer from the medial temporal lobe to affect association areas of the brain and then the primary sensory areas. The heterogeneity characteristic of the AD is due to the differentiation in the routes (Armstrong, 2013). Hippocampal sulcus cavities are estimated on 1.5 T magnetic resonance imaging (MRI) which suggest that, apart from the marker of pathology, Hippocampal sulcus cavities, patient with a history of vascular disease and initial attendees are the portion of normal anatomic differentiation of human hippocampus (Blom et al., 2018). The various etiology of Alzheimer's disease are shown in **Figure 2.3**.

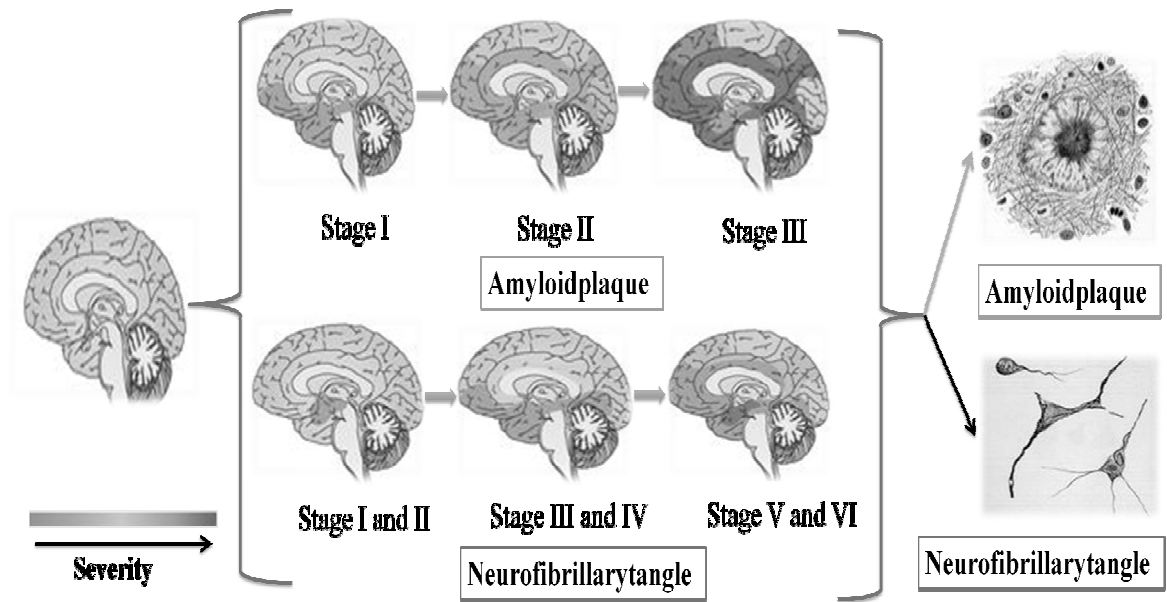


Figure 2.1: Histopathological evaluation of Alzheimer's disease (Masters et al., 2015)

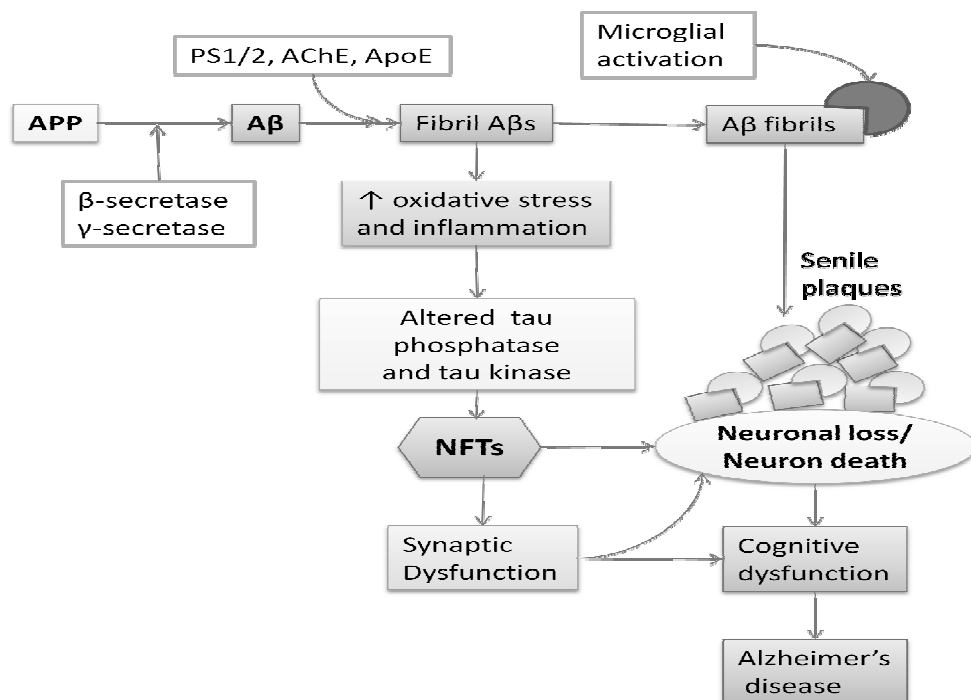


Figure 2.2: The pathogenesis of Alzheimer's disease. Where: APP; amyloid precursor protein, Aβ: β-amyloid, AChE: acetyl-cholinesterase, ApoE; apolipoprotein E, PS; presenilin and NFTs; neurofibrillary tangles.

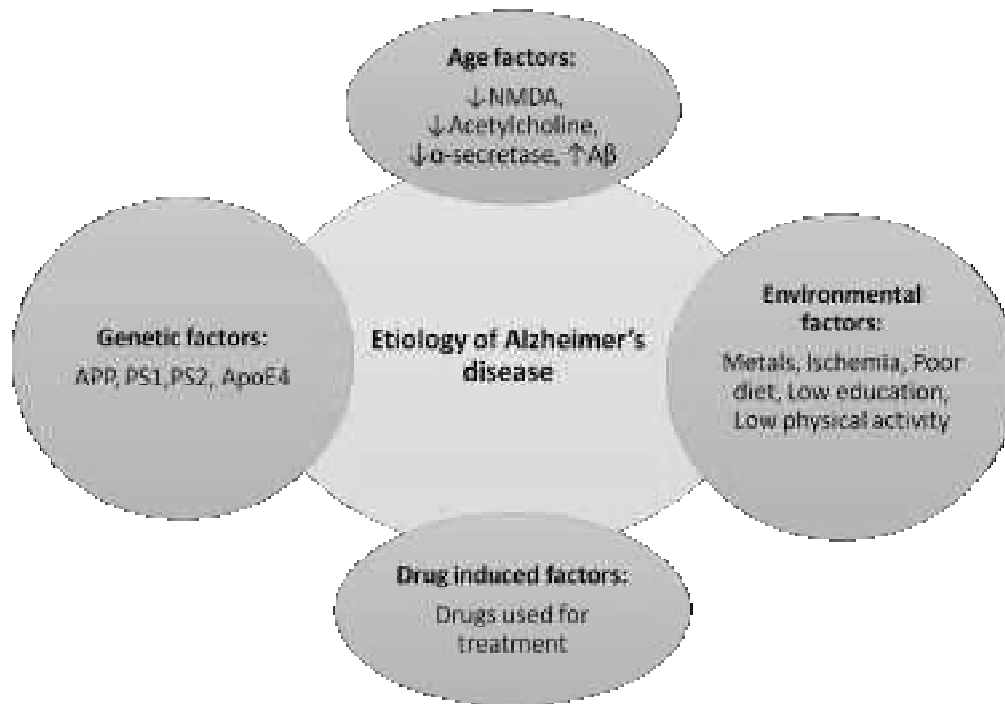


Figure 2.3: Etiology of Alzheimer's disease

2.3.1. Age factors

Age is the major risk factor in AD. In the genome study, it has been identified that chromosome 17 is related to age at onset. Association occurs due to the main variant in CCL11. The association of protective halo-type with 10 years late in age at onset of AD and acknowledgment of CCL11 variant can be a growth of immune modulators in the future (**Guerreiro and Bras, 2015**). Age-associated AD risk has genes that act in inflammation and the immune response (**Jones et al., 2015**).

2.3.2. Genetic factors

Genetics is another risk factor in AD. APP (amyloid precursor protein), PS1 (presenilin 1), and PS2 (presenilin 2) are the types of autosomal dominant in early-onset familial AD that are associated with mutations in genes (**Bird, 2009; Schu et al., 2012**). The $\epsilon 4$ allele of APoE (Apolipoprotein E) was known to have a risk factor in AD. Transcriptional regulation of AD genes can play important role in the neurodegenerative process and implication of variable susceptibility of AD (**Theuns and Van Broeckhoven, 2000**). The AD risk factor can be formed due to changed transcription of long ncRNAs, endogenous, ncRNAs longer than 200 nucleotides (**Zusso et al., 2018**). The association study has acknowledged the top 20 AD risk genes in the brain, but the mechanism is unclear (**Apostolova et al., 2018**).

2.3.3. Environmental factors

Environmental factors are involved in the AD risk that affects AD pathogenesis (Yadav, 2018). The essential transition metals (iron, copper, manganese, zinc, selenium, and cobalt) control metabolic and signaling pathways. The AD patients were diagnosed with altered metals homeostasis in plasma/serum and brain. The increased level of zinc and copper in cerebrospinal fluid facilitates the β -amyloid aggregation (Hozumi et al., 2011). The essential transition metals enhance the senile plaques and neurofibrillary tangles (NFTs) (Ayton et al., 2013). The β -amyloid forms fibril in presence of zinc and copper forms oligomers (Tōugu et al., 2009).

Aluminium (Al) is a neurotoxic agent (Shati et al., 2011) as well as the third most basic rich element on the earth (Farina et al., 2002). The regular consumption of Al from various items used in our daily life like food additives, deodorants, and a few antacid medicines enhanced the Al concentration in the brain. Al enters the brain by binding with high-affinity receptors for transferrin (TfR) in the blood-brain barrier (BBB) (Roskams et al., 1990). In the brain, Al affects fast and slow axonal transport and induces inflammatory responses (Amjad et al., 2015; Campbell et al., 2004). Long-term potentiation is inhibited by Al, resulting in synaptic and structural abnormalities. The result was significant neuronal and memory loss (Amjad et al., 2015; Kawahara et al., 2005). The enhanced Al concentration in Alzheimer's disease brain causes neuritic aggregate, senile plaques, and neurofibrillary tangles (NFTs) (McLachlan et al., 1991). "Al is a potent cholinergic toxin" (Gulya et al., 1990). This causes neuron apoptotic loss as an associated symptom of AD (Ghribi et al., 2001). In a molecular study, Al affects cellular energy metabolism, gene transcription, and DNA topology (Lukiw et al., 1998). Aluminium was reported to induce the misfolding, and self-accumulation of highly phosphorylated cytoskeleton proteins like microtubules associated proteins thereby implicating in Alzheimer's disease (Kawahara, 2003).

2.3.4. Drug as well as chemical-induced Alzheimer's disease

AD can be developed by using certain drugs which are recommended for the treatment of other diseases. Such types of drugs are used to induce AD *in vitro* and *in vivo* models. A drug such as scopolamine is used for a muscarinic cholinergic receptor but induces cognitive dysfunction *in vivo*, colchicine is used to treat gout arthritis but used as a neurotoxin, okadaic acid is as selectively serine phosphatase antagonist in rat brain while inducing neurotoxicity in rat brain, and streptozotocin (STZ) is used as antibiotic

but later used as an anticancer drug to treat neuroendocrine tumors, on other hand, STZ is used popular AD model by enhancing β -amyloid aggregation and Tau level of the hippocampus (Alagiakrishnan and Wiens, 2004; Malekzadeh et al., 2017).

D - galactose is obtained from the lactose present in milk. Galactose and monosaccharide glucose are formed later through hydrolysis of lactose in the intestine (Lu et al., 2006). D - galactose is metabolized in presence of D-galactokinase enzyme as well as galactose -1- phosphate uridyltransferase enzyme, while D - galactose supply leads consequences to abnormal metabolism (Lu et al., 2006; Kaplan et al., 1996).“As aforementioned, D - galactose is converted into galactitol. But, D-galactokinase and galactose-1-phosphate uridyltransferase enzymes do not metabolize it. This clumping in the cell creates an imbalance between antioxidants and the production of reactive oxygen species (ROS)” (Hsieh et al., 2009). Furthermore, D - galactose is a reducing sugar and a physiological nutrient that reacts through free amines in proteins along with peptides to form advanced glycation end products through non-enzymatic glycation (Hsieh et al., 2009; Chen et al., 2006; Vlassara et al., 1994). It leads to the activation of the receptor for AGE and causes oxidative stress, as well as cellular injury (Song et al., 1999; Baynes 2001; Lu et al., 2006). D - galactose chronic administration has been reported to influence age-related cognitive decline as well as accelerate aging in mice (Lei et al., 2008).

2.4. Stages and symptoms

The stages and symptoms of AD progress are variables that get worse with time. Changes in the brain are the symptoms of AD that began years before. The period of time over a few years is called a preclinical AD. AD occurs slowly in form of three stages, namely mild AD (early-stage), moderate AD (intermediate-stage), and severe AD (late-stage) (Alzheimer’s Association, 2017; Förstl et al., 1993). The symptoms are associated with each stage, as shown in Table 2.1 and Figure 2.4.

Table 2.1: The stages and symptoms of Alzheimer’s disease (Alzheimer’s Association, 2017)

Stage	Symptoms
Preclinical AD	The brain alteration occurs with no one symptom.
Mild cognitive impairment due to AD	That is considered as a decrease in memory and decline in thinking skills, but no harm to function.
Mild AD	Unable to recognize the objects and not able to perform a normal task.
Moderate AD	Increase the risk of diverging, behavioral and personality changes, forgetting historical events, moody, confusion, and the sleep patterns change.
Severe AD	Decreasing current experiences awareness, Failing to judge, enhancing speaking problems, facing trouble during walking, sitting, and engulfing, trouble to the patient who needs around the clock for daily activities.

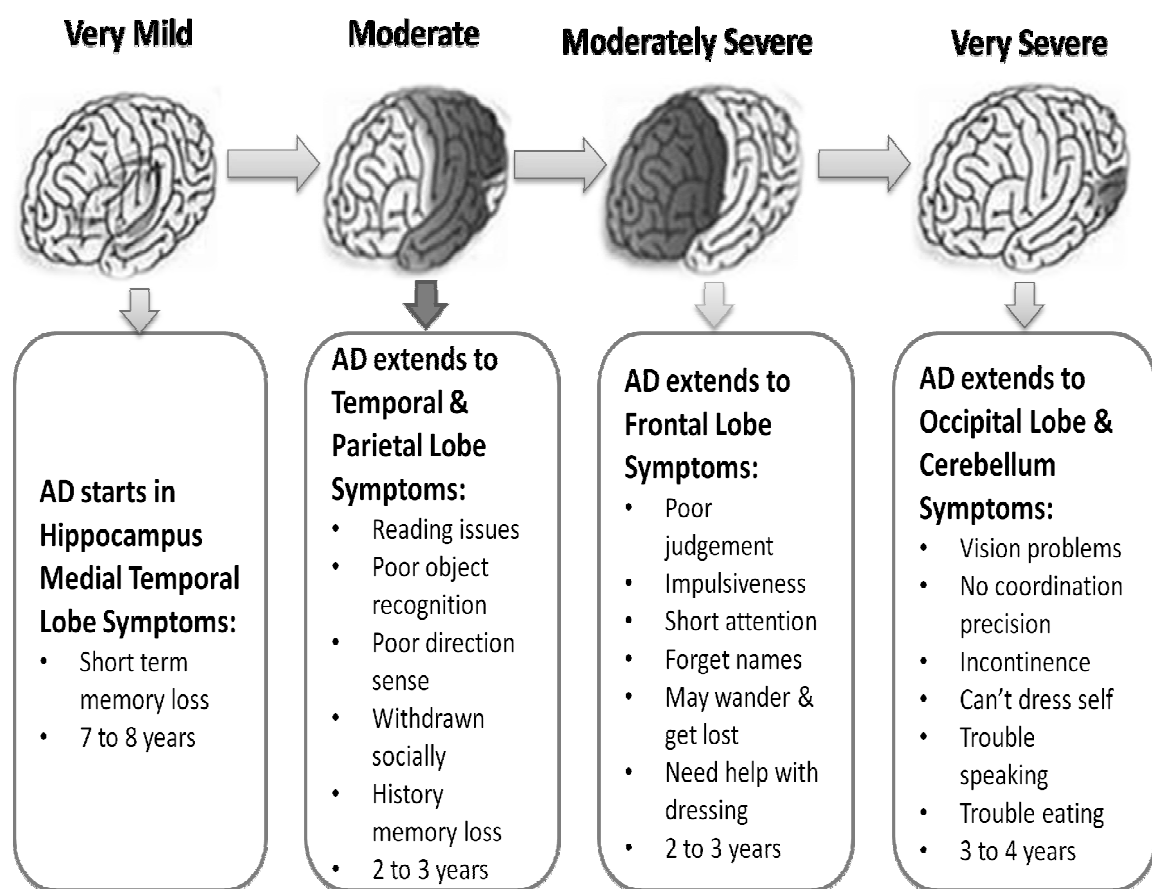


Figure 2.4: The various stages of Alzheimer’s disease with symptoms (Penttilä et al., 1985; Yang et al., 2003)

2.5. Diagnosis

Diagnosis involves knowing some questions from the patient and their friends, colleague, and relatives. The patient diagnosis is done by scanning and imaging techniques such as positron emission tomography, magnetic resonance imaging, and computerized tomography scan of the brain temporal lobe. This can be carried out depending upon the need, severity of symptoms, and the gravity of the situation (**Armstrong, 2013**). In fact, as per the presence of other severe diseases in geriatric people, several cases of AD and other types of dementia fall under the line. The new technologies come in research studies on AD that is placed toward diagnosis at the earlier stages when symptoms are mild or even absent. Although the drugs developing for AD have demonstrated a monstrous challenge, according to the experts to treat AD at early stages will stand the best opportunity (**Munoz and Feldman, 2000**). In **Table 2.2**, the very common diagnostic procedures along with their purposes are shown.

2.6. Drugs used in Alzheimer's disease

The treatment approaches are included using cholinergic agents with the expectation of enhancing brain acetylcholine levels. Cholinesterase inhibitors block the enzyme-induced metabolism of intra-synaptic acetylcholine. The barriers to muscarinic agonists include straining in finding a medicine with a clear M₁ subtype agonist, not affecting other muscarinic receptor subtypes, and some adverse events (**Schneider, 2013**). The drugs for the treatment of AD are represented in **Figure 2.5**.

Table 2.2: Diagnostic procedure of Alzheimer's disease

Procedure	Purposes
Medical history	Gives an image of cognitive and behavioral alterations over time. This helps doctors to search that the cognitive decrement is due to medication.
Family history	To know about the disease history of the family members and individuals at higher risk of forming the AD.
Physical and neurological exam	To screen the physical medical monitoring such as blood pressure, hearing, vision, sensation, reflexes, and muscle strength, and to find an abnormality that reflects causes of cognitive loss other than the AD.
Lab tests (glucose, hormones, vitamin deficiencies, electrolytes, etc.)	Diagnosing metabolic disorders can guide cognitive symptoms after lab tests.
Tests for cognitive, functional, and psychological status	To assess mental and function of daily routine, to check depression, that can cause issues of memory, assess cognitive symptoms and other types of dementia which can manifest in various ways.
MRI or CT scan	Look for brain structural lesions, atrophy, and stroke.
PET scan	PET may be used to diagnose certain forms of dementia including AD.
Cerebrospinal fluid analysis	Cerebrospinal fluid analysis is used to know the free-floating proteins in the brain that form A β plaques and NFTs of AD.

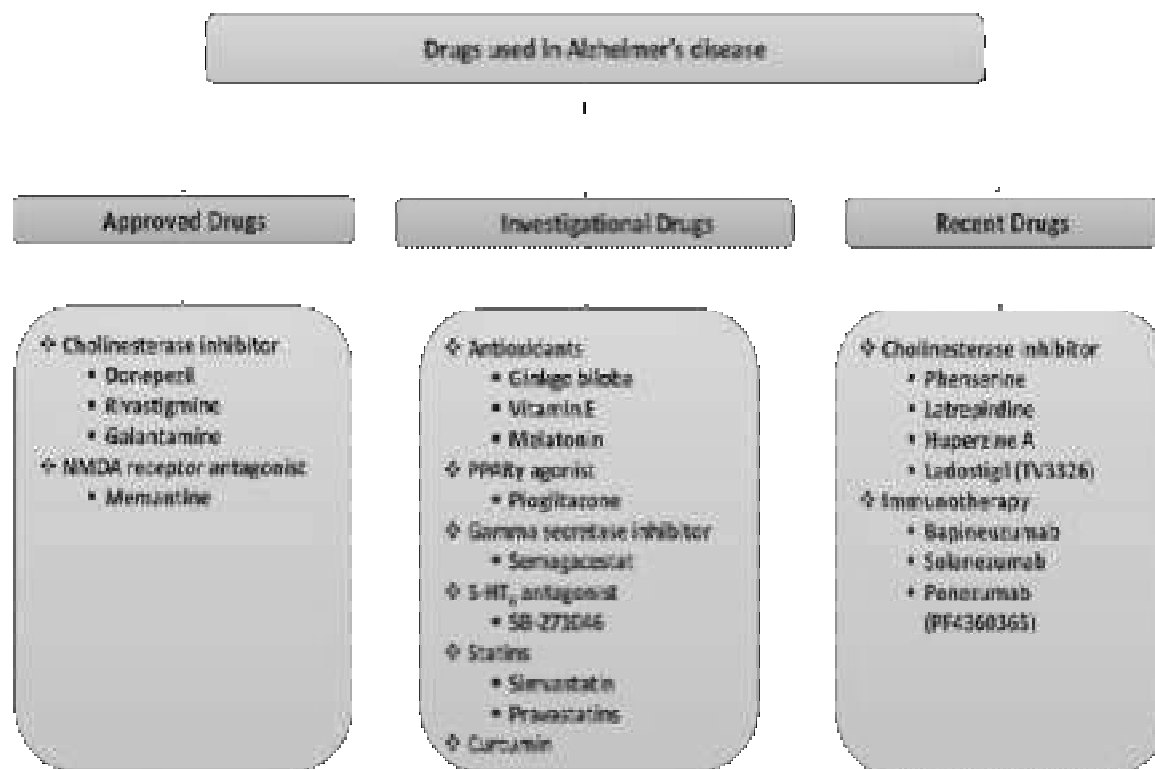


Figure 2.5: Classification of drugs used for Alzheimer's disease

2.6.1 Approved drugs

2.6.1.1 Cholinesterase inhibitors

2.6.1.1.1 Donepezil

Donepezil is an extended-performing reversible acetylcholinesterase inhibitor. It shows selectivity for cholinesterase enzyme within the brain. Once a day administration, Donepezil has protracted long-time inhibition of cholinesterase enzyme. Donepezil is certified for the symptomatic remedy of dementia in mild to moderately excessive AD (Jones, 2002). The preliminary recommended 5 mg dose of Donepezil should be given in the evening once a day for a minimum of one month to maintain the therapeutic response. Then after, the dose may be also enhanced to 10 mg once daily. The two-phase III clinical trials demonstrated proof of efficacy to get FDA approval. The six and twelve months duration randomized trials were conducted in both chronic impaired and nursing home patients. In the industry-sponsored trial, randomized, placebo-controlled trials were followed by the patients over many years that reported the modest cognitive effects over two years, however, no important effects on loss of performance, health economic, or nursing home placement measures (Jones, 2010).

2.6.1.1.1 Pharmacokinetic

The relative oral bioavailability of Donepezil is about 100% with 3 hours to 4 hours peak concentration. Donepezil is metabolized largely in the liver and eliminated in unchanged form in the urine. It has a long elimination half-life of up to 70 hours, and steady-state occurs in approximately two weeks. 10 mg per day dose was maintained for the patients with intermediate to chronic AD and might benefit from an enhanced dose (Perry et al., 1982; Davies and Maloney, 1976).

2.6.1.1.2 Mechanism of action

Donepezil is an acetylcholinesterase (AChE) inhibitor (**Figure 2.6**) with dose-dependent activity. Donepezil binds to inhibit the hydrolysis of acetylcholine that reversibly inactivates cholinesterases by enhancing acetylcholine concentration at cholinergic synapses. The actual mode of action of Donepezil in the AD patient is not fully understood (Perry et al., 1982). The AD is accepted that the symptoms of AD are related to the cholinergic deficit, mainly in the brain (cerebral cortex region) and other areas of the brain. AD implicates a substantial decline in the elements of the cholinergic system (Davies and Maloney, 1976).

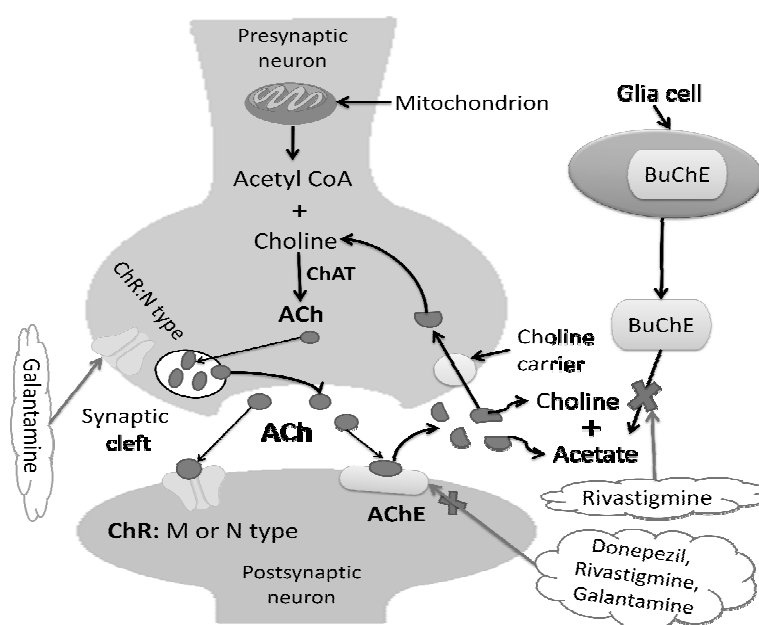


Figure 2.6: Mechanism action of Donepezil, rivastigmine, and galantamine inhibit AChE enzyme, rivastigmine also inhibits the BuChE enzyme and galantamine potentiates the ChR. Where, ChR: N-type channel; ChAT: Choline acetyltransferase; ACh: Acetylcholine; ChR: Cholinergic receptor; AChE: Acetylcholinesterase; BuChE: Butyrylcholinesterase; M: Muscarinic type receptor; N: Nicotinic type receptor

2.6.1.1.3 Adverse effects

During a clinical trial, the general side effects were observed such as nausea, vomiting, and diarrhea. Loss of appetite, difficulty in sleeping, and muscle cramps were also observed as per the marketed brand Aricept[®] (Davies and Maloney, 1976; Perry et al., 1982).

2.6.1.1.2 Rivastigmine

Rivastigmine is used for the mild to intermediate stage of AD treatment. The pseudo-irreversible mechanism results in prolonged inhibition of AChE. Rivastigmine, primarily 1.5 mg twice daily dose is assigned treatment for the AD patient which would be initiated by the experienced physician in diagnosis (Winblad et al., 2007). This therapy will be given to regularly monitor patients with AD. If it is tolerated well, the dose can be enhanced with a maintenance dose of 3-6 mg twice daily. In two published clinical trials, it was showing efficacy, and doses were titrated weekly over seven weeks with one of two dosage ranges, 1 mg per day to 4 mg per day or 6 mg per day and 12 mg per day. The low dose of rivastigmine was not accepted, possibly contributing to less tolerability and considerably high adverse effects. The clinical study was conducted on 1195 patients with a moderately severe AD and based on a placebo-controlled study differentiating the rivastigmine dose of 17.4 mg, 9.5 mg, and 6 mg administered orally twice daily with mini-mental state examination scores of 10 to 20 over six months. All formulations were demonstrated to have efficacy, however, fewer side effects were obtained with the patch formulations (Winblad et al., 2007).

2.6.1.1.2.1 Pharmacokinetic

Rivastigmine is administered orally, it has 40% plasma protein binding and is well absorbed in a dose of 3 mg two times a day. The elimination half-life is less than two hours, and enzyme inhibition last about nine hours. This drug is unmetabolized by the hepatic. Rivastigmine is slowly hydrolyzed and then excreted by the renal. The peak of plasma concentration is 1 hour, and the peak of cerebrospinal fluid concentration at 1.4-3.8 hours. Its extrahepatic metabolism is not likely to have significant pharmacokinetic interactions (Cummings et al., 2007; Jann et al., 2002).

2.6.1.1.2.2 Mechanism of action

Rivastigmine is selective for AChE and BChE (**Figure 2.6**) within the brain. But, the precise mechanism of rivastigmine is unknown (Winblad et al., 2007).

2.6.1.1.2.3 Adverse effects

The common side effects were observed during clinical studies such as loss of weight, loss of appetite, nausea, and vomiting (**Winblad et al., 2007; Cummings et al., 2007; Jann et al., 2002**).

2.6.1.1.3 Galantamine

Galantamine is used in cognitive decline in mild to moderate AD. Galantamine acts on nicotinic receptors as an allosteric modulator. It is possibly increasing cholinergic transmission by presynaptic nicotinic stimulation (**Birks, 2012**). At the 8 mg and 16 mg dose twice daily, the efficacy of galantamine was demonstrated with some side effects at the decreased dose in at least four randomized trials for three to six months. As per the Cochrane review, it was concluded that galantamine showed affirmative effects for three to six months study with no additional changes at the overdose of 16 mg per day (**Birks, 2012; Andrea et al., 2013**).

2.6.1.1.3.1 Pharmacokinetic

Galantamine has approximately 90% bioavailability with a peak plasma concentration of approximately one hour and a half-life of about 7 hours. Galantamine is metabolized by hepatic and excreted by renal. Galantamine has a minimum effect on the metabolism of other drugs. However, the drug paroxetine and ketoconazole are the inhibitors of CYP 2D6 or 3A4 that may decrease clearance; enhance the bioavailability and plasma levels of galantamine (**Birks, 2012; Andrea et al., 2013**).

2.6.1.1.3.2 Mechanism of action

Galantamine is a reversible competitive inhibitor of the enzyme AChE (**Figure 2.6**). The competitive inhibitors are potentially less active within brain areas having high acetylcholine levels and more active in other brain areas (**Andrea et al., 2013**).

2.6.1.1.3.3 Adverse effects

Nausea, vomiting, diarrhea, weight loss, and anorexia are the most common side effect due to cholinesterase inhibitors which are cholinergic mediated. The early

cholinergic effects are very common at the primary dosing. Decreasing the dose temporarily may decline the re-emergence of acute cholinergic adverse effects. But, several patients may tend to become tolerant of the adverse effects (**Andrea et al., 2013**).

2.6.1.2 N-methyl-D-aspartate receptor antagonists

2.6.1.2.1 Memantine

In late 2003, memantine was approved by the FDA for intermediate to severe AD. Memantine is usually prescribed to ameliorate memory, attention, language, and the ability to perform common tasks. It was the first drug for AD that is approved in the USA as an NMDA receptor antagonist. Memantine is particularly given to treat moderate-to-severe AD. In 2005, the FDA has declined the approval of memantine for mild AD. Memantine is characterized as a moderate-affinity, and uncompetitive NMDA receptor antagonist. The rationale for use of memantine is that it may protect against overstimulation of NMDA receptors. It may also occur in the AD as well as consequent glutamate and calcium-mediated neurotoxicity. For the approval, it was shown the positive outcomes during six months of placebo-controlled clinical trials. In one clinical trial, cholinesterase inhibitors were not permitted, but in a second clinical trial, Donepezil was received by all patients for at least six months duration before being randomized to memantine or the placebo. The third moderate to severe AD clinical trial, it was not shown the significant effects for memantine, however, the trials were similar to the design of cholinesterase inhibitors trials that included only patients with mini-mental state examination (**Mount and Downton, 2006; McShane et al., 2006**).

2.6.1.2.1.1 Pharmacokinetic

Memantine has a better rate of absorption to approach 100% bioavailability. The maximum plasma protein binding concentration is three to seven hours with a 60 to 80 hours elimination half-life. It has no food interaction and is broadly distributed throughout the body. Memantine has minimum metabolism that occurs in the liver and is excreted unchanged in the urine by the kidneys (**Mount and Downton, 2006; McShane et al., 2006**).

2.6.1.2.1.2 Mechanism of action

Memantine is known as an NMDA receptor antagonist (**Figure 2.7**). But, the therapeutic mode of action is not known properly. Memantine did not show apparent

pharmacological activity. At the higher level of glutamate triggers the receptor and potentiates the ion channel opening. It is hypothesized that memantine inhibits NMDA receptors and prevents calcium influx, depolarization, and high activation of the neuron. The glutamatergic activity of memantine could be seen. In six months of the clinical trial, it was not observed to indicate a short-term and symptomatic effect (Mount and Downton, 2006; McShane et al., 2006).

2.6.1.2.1.3 Adverse effects

The common side effects are included confusion, diarrhea, headache, dizziness, infrequent hallucinations, and somnolence. Memantine may decrease the cholinergic effects of Donepezil. It is observed to have no interaction with cholinesterase inhibitors (Mount and Downton, 2006; McShane et al., 2006).

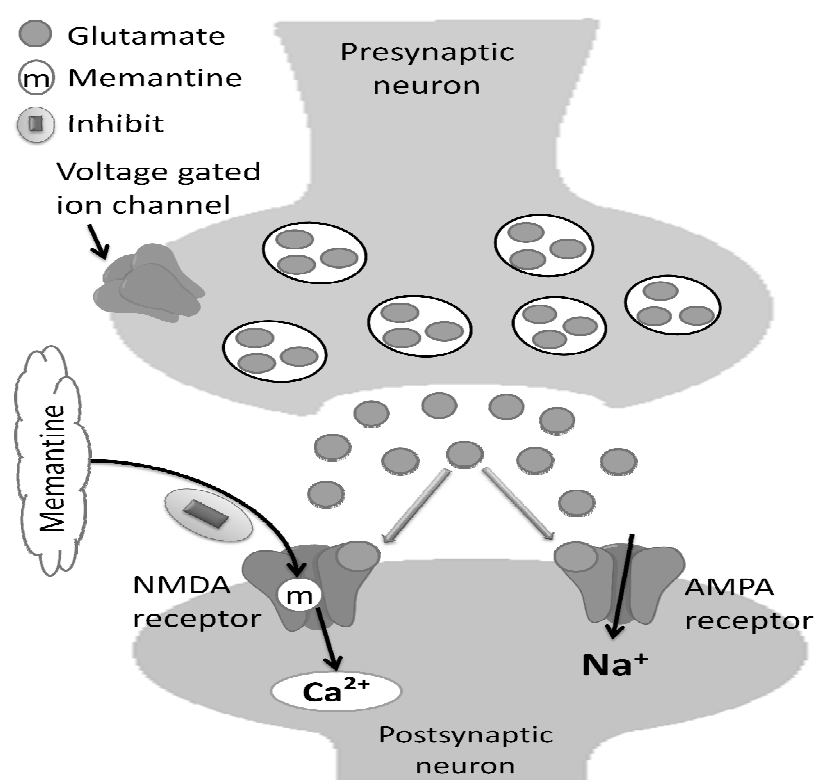


Figure 2.7: NMDA receptor antagonist effect of memantine. Where NMDA: N-methyl-D-aspartate; AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

2.6.2 Investigational drugs

2.6.2.1 Antioxidants

2.6.2.1.1 Ginkgo biloba

Ginkgo biloba belongs to the family Ginkgoaceae, a member of the gymnosperms. The rest plants of the Ginkgoaceae family are now extinct and found as a fossil. *Ginkgo biloba* is a small tree that originates in China but is now planted worldwide as an ornamental plant. It is also cultivated in Korea, France, and the United States (US). The major chemical constituents present in *Ginkgo biloba* as the mixture of flavonoids and terpenoids. The dried leaves of *Ginkgo biloba* contain ginkgolides (A, B, C, J, and M), bilobalide and 0.1 to 0.25% terpene, and lactones. 30 to 40% of the mixture is bilobalide whereas ginkgolide A is present in a maximum amount and accounts for about 30% of the mixture. The nature of ginkgolides is diterpenoid while bilobalide is sesquiterpenoid (Pereira, 2013).

2.6.2.1.1.1 Pharmacokinetic

After oral intake, the intestinal microflora hydrolyzes the flavonoid glycoside into sugar moiety and aglycone which further get absorbed. In humans, a minor part of the flavonoid is absorbed without hydrolysis. The major metabolic pathway is glucuronidation in the intestine (Hakk and Larsen G, 2002; Hollman et al., 1997; Mauri et al., 1999).

2.6.2.1.1.2 Mechanism of action

The mode of action includes antioxidant effect, radical-scavenging, alteration of the membrane fluidity, and inhibition of the glucocorticoid synthesis and platelet-activating factors (Diamond and Bailey, 2013; Pereira et al., 2013).

2.6.2.1.1.3 Medicinal uses

The extract of the leaves of *Ginkgo biloba* has a long history and in today's world, it is the most commonly used herbal medicinal product in Europe and USA. The extract of this plant is used in cerebral vascular disease and senile dementia. It is reported to provide positive results in the treatment of AD. As it acts as an antioxidant and also improves cerebral blood circulation. The bilobalide is not able to control the platelet-activating factors but still, it provides a neuroprotective effect. EGb761[®] is the

standardized extract of the plant. The extract is available in suitable forms such as film-coated tablets, oral liquids, and parenteral solutions (**Heinonen and Gaus, 2015**).

2.6.2.1.1.4 Adverse effects

Mostly it is considered that the natural herbs don't have any side effects, but it is not true. As they all have chemicals, different chemicals have different kind of action with the targets. So, *Ginkgo biloba* also reported having certain adverse effects such as gastrointestinal upset, headache, and dizziness. In the animal study, it is reported to have carcinogenic at a high dose level (**Diamond and Bailey, 2013; Ulbricht et al., 2008**).

2.6.2.1.2 Vitamin E

Vitamin E is a fat-soluble vitamin that is chemically known as tocopherol. Vitamin E was discovered by Evans and Bishop in 1922. There are a total of eight forms of vitamin E namely, alfa, beta, gamma, and delta form of tocopherol and tocotrienol. It is present in fatty food materials and it can get stored in the fat tissue of the body. So, taking vitamin E on daily basis is not required. The best source of vitamin E is vegetable oils (**Rizvi et al., 2014**).

2.6.2.1.2.1 Pharmacokinetic

Plasma concentration of vitamin E directly proportions to its absorption. As it is fat-soluble so it is directly absorbed from a diet in the intestinal lumen. Then it gets incorporated into chylomicrons and distributed in the body (**Boccardi et al., 2016; Yap et al., 2001**).

2.6.2.1.2.2 Medicinal uses

Vitamin E is highly beneficial for the body as it has good antioxidant property, help to maintain the integrity of the cell membrane, regulate platelet aggregation and also control the activity of protein kinase C (Burton et al., 1983; Howard et al., 2011). Now, it is reported that tocopherol can be helpful as a remedy for AD. As in the pathology of AD oxidative stress is the main cause and vitamin E has a good antioxidant that is free radical scavenging activity. So it will help to cure AD and other diseases also which are related to oxidative stress (**Boccardi et al., 2016; Rizvi et al., 2014**).

2.6.2.1.3 Melatonin

Melatonin is an indole hormone, and neurotransmitter as the compound produced by the pineal gland, and a small amount is also produced from the gastrointestinal tract. This compound involves various kinds of biological activities of the body. The sources of melatonin are human milk, banana, beets, cucumbers, and tomatoes (**Boccardi et al., 2016; Yap et al., 2001**).

2.6.2.1.3.1 Medicinal uses

The main physiological role of melatonin is to control the circadian rhythm. The role of melatonin in some other disease conditions is also reported as it is helpful in the treatment of breast cancer, fibrocystic breast disease, and colon cancer. It is also reported that melatonin modifies the immunity, stress responses and also helps to reduce the aging effect. The antioxidant effect of melatonin is well established. As it was reported, that administration of the melatonin enhances the expression of antioxidant enzyme superoxide dismutase and glutathione peroxidase. In the animal study, it was found that melatonin helps to reduce the symptoms of AD, as it reduces the plasma homocysteine and lipid level which is due to its antioxidant property. It was also found that the level of melatonin was reduced in cerebrospinal fluid of the patients with AD (**Baydas et al., 2003; Malhotra et al., 2012**).

2.6.2.1.3.2 Adverse effects

Melatonin has very low toxicity in animals and humans. But, headache, insomnia, rash, stomach upset, and nightmares are the common side effect of melatonin (**Boccardi et al., 2016; Yap et al., 2001**).

2.6.2.2 Peroxisome proliferators activated receptor γ agonist

2.6.2.2.1 Pioglitazone

Pioglitazone is a compound of the category thiazolidinediones (TZDs). It is a specific agonist of the receptor nuclear peroxisome-proliferator-activated receptor γ (PPAR γ) (**Galimberti and Scarpini, 2017**).

2.6.2.2.1.1 Pharmacokinetic

Pioglitazone is shown to have a better absorption rate with an absolute bioavailability of 83%. Approximately at 1.5 hours, pioglitazone is observed to reach a

maximum concentration and metabolized by the liver cytochrome P450 (CYP). But, only 15 to 30 % of pioglitazone is recovered after oral administration from the urine. The negligible elimination of pioglitazone occurs through the kidneys. The drug is excreted initially as metabolites and their conjugates. Most of the oral dose is excreted into the bile either unchanged or as metabolites and eliminated in the feces(Harrington et al., 2011).

2.6.2.2.1.2 Therapeutic uses

The main use of pioglitazone is the remedy for type 2 diabetes. It acts via receptor nuclear $PPAR\gamma$. It attaches and forms a heterodimer with retinoid X receptor (RXR), which further leads to the alteration of the transcription of the gene and proteins related to glucose and lipid metabolism. Now it was reported that $PPAR\gamma$ is also involved in the neuroinflammation of AD. Rosiglitazone is one of the first $PPAR\gamma$ compounds which was tested in the AD and entered phase III of the clinical trials but was later abandoned. Later on, pioglitazone was reported to reduce the glial cell inflammation and $A\beta$ level in transgenic mice (Harrington et al., 2011).

2.6.2.3 γ -secretase inhibitor

2.6.2.3.1 Semagacestat

Semagacestat is an azepine class of gamma-secretase inhibitor. This is the only one from other gamma-secretase which entered into clinical trials for the treatment of AD (Olson and Albright, 2008).

2.6.2.3.1.1 Pharmacokinetic

It is quickly absorbed and has a maximum concentration in plasma between 0.5 to 1 hour (York et al., 2010).

2.6.2.3.1.2 Therapeutic uses

Semagacestat is developed to reduce the $A\beta$ peptide production by inhibiting intramembranous cleavage of APP. Initially, it was founded a beneficial compound in AD, but later on, it was found that it aggravates cognitive dysfunction and produces certain other side effects (Tagami et al., 2017).

2.6.2.4 5-HT₆ antagonist

2.6.2.4.1 SB-271046

It is developed as a potential cognition enhancer and 5HT6 receptor antagonist. Blockade of the 5-HT6 receptor is found to improve cognitive function. Cognitive dysfunction is recognized as a key symptom of AD; SB-271046 can be useful as therapeutic potential in the treatment of AD. In various research articles, it has been found to improve cognitive function. Still, there is a lack of facts available regarding SB-271046 (Upton et al., 2008).

2.6.2.5 Statins

2.6.2.5.1 Simvastatin and Pravastatin

HMG-CoA reductase inhibitors (commonly known as statins) have been widely prescribed groups of drugs in the world since their introduction to the market (Ramkumar et al., 2016).

2.6.2.5.1.1 Pharmacokinetic

Statins have low bioavailability due to high first-pass metabolism except for pravastatin which is an active metabolite. Mostly statins vary in the extent of metabolism, and active and inactive metabolites. The therapeutic activity of pravastatin is based on the kinetic profile of both the parent compound and active metabolite. Pravastatin has the lowest plasma protein binding profile. The metabolism and excretion occur in the liver and kidneys respectively. Atorvastatin showed to have the longest terminal half-life (Flint-Garcia et al., 2003).

2.6.2.5.1.2 Therapeutic uses

Statins are used to down the increased lipid profile. The statins have a therapeutic effect by inhibiting HMG-CoA which is a rate-limiting step in cholesterol biosynthesis. Statin therapy was observed to be effective in lowering low-density lipoprotein cholesterol levels by 20-50%, as well as lowering triglyceride levels by 10-20% and causing a possible rise in serum high-density lipoprotein cholesterol levels (5-10%). It is reported to lower the level of cholesterol which can be supposed to have a significance of statins in the treatment of AD (Taylor et al., 2014).

2.6.2.5.1.3 Adverse effects

Mostly statins are associated with certain side effects like musculoskeletal, hepatic, renal dysfunction, and malignancy (Ramkumar et al., 2016).

2.6.2.6 Curcumin

Curcumin is a polyphenolic compound, obtained from turmeric. Turmeric is a spice that has received much interest in the medical and scientific fields worldwide. Turmeric is a rhizomatous herbaceous perennial plant (*Curcuma longa*) of the ginger family, Zingiberaceae (Hewlings and Kalman, 2017).

2.6.2.6.1 Pharmacokinetic

Curcumin was reported to have low systemic availability after oral administration due to high first-pass metabolism and metabolism (glucuronidation and sulfation of curcumin) in the intestine (Hewlings and Kalman, 2017).

2.6.2.6.2 Medicinal uses

Curcumin was reported to have various pharmacological properties. It provides benefits for inflammation, pain, and metabolic disturbance. In many studies, curcumin as an antioxidant was reported to have a positive effect on various kinds of diseases with oxidative stress. Curcumin can be beneficial therapeutic potential in the treatment of AD (Hewlings and Kalman, 2017).

2.6.3 Recent drugs

2.6.3.1 Cholinesterase inhibitors

2.6.3.1.1 Phenserine

Phenserine is a phenyl carbamate derivative, well known as the backbone of physostigmine which is a natural product obtained from the calabar bean of West Africa. Phenserine was synthesized and prepared as a crystalline compound, selected from preclinical evaluation among the various novel carbamates (Greig et al., 2005).

2.6.3.1.1.1 Pharmacokinetic

As per Phase II of clinical studies, phenserine showed safety and pharmacological profile at the dose (5-10 mg b.i.d) in patients with AD. It produced significant amelioration in cognitive function. Phenserine showed a low pharmacokinetic half-life, decreases the dosing frequency, reduces drug exposure to the body, and declines the dependence on drug metabolism in the elderly (Greig et al., 2005).

2.6.3.1.1.2 Mechanism of action

Phenserine has two modes of action. At first, it showed a selective, non-competitive inhibitor of AChE and in second. it showed the depletion secretion of A β (Greig et al., 2005).

2.6.3.1.1.3 Therapeutic uses

Phenserine is used as therapy for AD by ameliorating cognitive, behavioral, and functional impairments in patients with AD (Greig et al., 2005).

2.6.3.1.1.4 Adverse effects

During the II Phase of clinical studies, basic adverse events such as nausea and vomiting were observed (Greig et al., 2005).

2.6.3.1.2 Latrepirdine

Dimebon is a trade name of latrepirdine which is also known as dimebolin. In 1983, it was used as an antihistamine drug in Russia. But as per clinical research, latrepirdine improved cognitive function in AD patients while destroying the psychopathic symptoms. Dimebon is administered orally to show cholinesterase inhibition and NMDA-antagonist activities. It has been predicted that brain cell death inhibition in the animal model of AD. Due to a non-reactive result in a human trial, it couldn't show a positive response in AD patients. That is why, dimebon was unlicensed for neurodegenerative conditions (Doody et al., 2008; Jones, 2010).

2.6.3.1.3 Huperzine A

Huperzine A is a phytochemical from Chinese herbs, a novel lycopodium alkaloid that is isolated from herbs *Huperzia serrata*. In China, traditionally, it has been used for strains, confusion swelling, and schizophrenia for centuries, but now it is used for AD and vascular dementia (Qian and Ke, 2014).

2.6.3.1.3.1 Pharmacokinetic

Huperzine A has greater oral bioavailability, better penetration through the blood-brain barrier (BBB), and longer duration inhibition of AChE. In pharmacokinetic studies, huperzine A was absorbed rapidly in rats, canines, and healthy human volunteers. It has

been manifested to have better distribution and elimination at a moderate rate of administration (Zhang et al., 2008).

2.6.3.1.3.2 Mechanism of action

Huperzine A is a reversible inhibitor of acetylcholinesterase and improves cognitive deficits of animal models with AD. Huperzine A has also cell protection against A β protein, H₂O₂, and glutamate. It possesses to prevent cells from ischemia, staurosporine-induced cytotoxicity, and apoptosis. Such protective effects protect mitochondria, regulate the expression of apoptotic proteins Bcl-2, Bax, P53, and caspase-3, attenuates oxidative stress, and upregulates nerve growth factor. Huperzine A interferes with APP metabolism. It shows antagonizing effects for NMDA receptors and potassium currents that can also provide neuroprotection (Wang et al., 2006).

2.6.3.1.3.3 Adverse effects

Huperzine-A showed the normal adverse effect of nausea during the clinical study, but apart from this, it has no serious adverse events during the study (Rafii et al., 2011).

2.6.3.1.4 Ladostigil (TV3326)

Ladostigil has both neuroprotective effects cholinesterase inhibitory and monoamine oxidase (MAO)-A and MAO-B activities in one molecule. Ladostigil has observed about 25 to 40% cholinesterase inhibitor in rats. When ladostigil was administered orally at doses ranging from 12 to 35 mg per kg that antagonize scopolamine-induced spatial memory impairments. Ladostigil has completed a preclinical study and entered clinical trial phase II. The researchers hypothesized that an increased level of synaptic AChE induced by ladostigil might be one of the modes of action involved in cognitive function improvement. However, salivation, diarrhea, and muscle weakness are the symptoms that arise due to access to cholinergic stimulation in the periphery until the high dose of 139 mg per kg was administered orally, which inhibited 50 to 60% cholinesterase. Such a wide therapeutic ratio was probably observed due to the relatively enhanced small rate in cholinesterase inhibition with increasing drug doses range over the ranges of 17 to 69 mg per kg. This increase in cholinesterase inhibition with oral administration of ladostigil at doses of 17 to 69 mg per kg was markedly lower and compared to subcutaneous injection of ladostigil at the doses range of 8.6 to 17 mg/kg; a 40% increase in cholinesterase inhibition. Ladostigil was observed to inhibit

both AChE and BuChE in an *In vitro* study. In which, the inhibitory effect was 100 times more potent against AChE than BuChE (Weinstock et al., 2000).

2.6.3.1.4.1 Mechanism of action

Ladostigil showed both cholinesterase inhibition and MAO-A and MAO-B activities in a single molecule (Weinreb et al., 2012). The APP can be processed in two alternative ways, one is mutually exclusive posttranslational pathways and another is the amyloidogenic pathway. The APP is cleaved sequentially by β -secretase and γ -secretase in the N-terminal and C-terminal of the $A\beta$ domain respectively to release $A\beta$. Similarly, in the non-amyloidogenic pathway, APP is cleaved by α -secretase and γ -secretase within the $A\beta$ sequence, and forms a soluble APP, hence, it is precluding the formation of the $A\beta$ (Vetrivel and Thinakaran, 2006).

2.6.3.2 Immunotherapy

The monoclonal antibodies are administered intravenously in the AD against $A\beta$ peptide, to ameliorate cognitive function and the slight improvement in functional scores (Salloway et al., 2014).

2.6.3.2.1 Bapineuzumab

Bapineuzumab, a humanized monoclonal antibody investigated by Elan/Johnson & Johnson can have the potential therapeutic effect on AD. It was found to acknowledge the N-terminal 5 residues of $A\beta$ peptide in a helical conformation. This is stabilized by internal hydrogen bonds involving the first three amino acids. It is speculated to underlie AD neuropathology (Miles et al., 2013). After getting an active immunization, the AD patients were found the positive outcomes with the reduction of plaques, but 6% of subjects were developed aseptic meningitis and the clinical trial was stopped (Woodhouse et al., 2007). Bapineuzumab is in clinical development for the remedy the AD (Salloway et al., 2014).

2.6.3.2.2 Solanezumab

Solanezumab is an anti- $A\beta$ monoclonal antibody and neuroprotector that is under the clinical trial for the remedy AD (McCartney, 2015). Solanezumab is investigated by Eli Lilly. In a clinical study of 24 months old PDAPP, transgenic mice observed the antibody 'm266' with rapid normalization of the cognitive deficits (Dodart et al., 2002).

2.6.3.2.3 Ponezumab (PF4360365)

Ponezumab is an anti-A β monoclonal antibody investigated by Pfizer. Ponezumab was in phase II of the clinical trial the remedy the AD and the cerebral amyloid angiopathy, but it has been discontinued in 2011 and 2016 respectively (Salloway et al., 2014; Dodart et al., 2002).

2.7. Models for Alzheimer's disease

The reported transgenic and non-transgenic models for Alzheimer's disease are shown in **Table 2.3** and **Table 2.4** respectively. The *in vitro* models for Alzheimer's disease are shown in **Table 2.5**.

2. 8. Selected phytoconstituents and their reported pharmacological activities

2.8.1. Bromelain

Bromelain is obtained from *Ananas comosus* stem and belongs to the family of sulfhydryl proteolytic enzymes. Bromelain is reported to possess various medicinal as shown in **Table 2.6**. Moreover, Bromelain was observed to enhance the permeability of BBB to nutrients (Laurer et al., 2001). This evidence could represent the therapeutic potential use in AD. Further, *In vitro* study, Bromelain was found to degrade A β ₁₋₄₂ monomer and soluble aggregate in cerebrospinal fluids of AD patients (Sancesario et al., 2018).

2.8.2. Myrcene

Myrcene or β -Myrcene (Myr) is a monoterpene that occurs naturally in various plants such as lemongrass, hops, cardamom, mangoes, cannabis, and Myrcia (Bonamin et al., 2014). Myr is extensively used for flavoring additives in the production of food, soft beverages, and the manufacturing of cosmetic products (Cesta et al., 2013). Myr has been reported to show various pharmacological actions as shown in **Table 2.7**. Myr improved the oxidative damage and histopathological neuronal damage in mice brain tissue induced by global cerebral ischemia/ reperfusion in C57BL/J6 mice (Osman et al., 2014).

Table 2.3: Transgenic mice and rats models for Alzheimer's disease

Models	Transgene	Pathological changes	Neurological deficits	References
APP transgenic mice	PDAPP	Happ695(Ind), PDGF pr.	A β plaques, synaptic loss, astrogliosis, no NFTs	Cognitive deficits at six months (Games et al., 1995)
	APP23	Happ751(Swe), Thy-1 pr.	Plaques, amyloid $A\beta$ deposits, synaptic loss, inflammation,	Cognitive deficits at three months (Calhoun et al., 1998; German, 2007)
	Tg2576	hAPP695(swe), HamPrP pr.	Plaques in cortical & limbic parts, synaptic loss	Cognitive deficits at nine months (German, 2007)
	PS2APP	hAPP(swe), PS2(N141I), Thy-1.2 pr.	Severe plaques, synaptic loss, inflammation, glial activation,	Behavioural & cognitive loss at eight months (Richards et al., 2003)
	PrP-tau	hTau(P301L), murinePr.	Age & gene dependent development of NFTs and spinal cord	Behavioural & motor deficits at six months (Götz et al., 2001)
Tau transgenic mice	Thy-Tau22	hTau (P301S), Thy-1.2 pr.	NFTs, mild astrogliosis, synaptic loss	↓ in learning at three months, Memory loss at six months (German, 2007)
	GFAP-Tau	hTau (P301L), GFAP pr.	Astrocytic tau, mild BBB disruption, focal neuron damage	Motor deficits (Forman, 2005)
APP and Tau double transgenic mice	APP/Tau	hAPP695(swe), hTau(P301L), PrP pr.	A β deposits, NFTs, nerve loss	Motor disturbance at eight to fifteen months (Ribé et al., 2005)
Triple transgenic mice	3xTg-AD	hAPP695(swe), Thy-1 pr., hTau(P301L), Thy-1 pr., PSEN1(M146V) mPS1 pr.	A β deposits, NFTs, nerve loss, synaptic loss, microglial activation, inflammation	Age-related behavior deficits (Oddo et al., 2003)
Five transgenic mice	5xTg-AD	hAPP695(Swe, Lon, Flo), PS1(M146L, L28V), Thy-1 pr.	A β 12, A β plaques, gliosis, synaptic loss, no NFTs	Memory & cognitive loss (Oakley et al., 2006)
Transgenic rats	APP ^{Swe}	hAPP751(Swe), PDGF pr.	↓ A β 42 and A β 40 peptide, plaques	↓ learning and memory (German, 2007)
	PS1APP	hAPP695(Swe), PS1(M146V), synapsin I pr.	A β deposits, astrocytic and microglial activation, ↑p-tau reactivity, no NFTs	Behavioral deficits (Flood and E. Morley, 1997; German, 2007)
	Thy-Tau	hTau truncated(151-391), Thy-1 pr.	↑p-tau, tangles in cortex, no neuron loss	↓ lifespan (Filipczik et al., 2012)

Table 2.4: Non-transgenic animal models for Alzheimer’s disease

Models		Pathophysiological changes	Advantages	Limitations	Mimicking AD for	References
Natural AD model	Rodents	A β memory and learning deficits	Low cost, small size, short life span, assessable behaviors	Different A β sequence and profile, A β initiate in neocortex	APP processing, A β impact, pathophysiological alters after therapies	(German, 2007)
	SAMP8 mice, OXYS rats	A β memory and learning deficits	assessable behaviors, age-related cognitive deficits	Different A β sequence and profile, A β initiate in neocortex	Genetics and proteomic changes under age-related memory and learning deficits	(Flood and E. Morley, 1997; Li et al., 2016)
	Dog	A β deposits, cognitive deficits	Correlates between A β s and cognitive deficits	No NFTs, require to predict which dog will develop AD	Preclinical testing of drugs, new diagnostic tools	(Russell, 2012)
	Non-human primates	A β deposits, NFTs, cognitive deficits, brain atrophy	Genetic proximity, similar AD pathology to human	Low output, High costs, ethical constraints, long development period	Testing safety and effectiveness of new drugs, diagnostic markers	(Darusman et al., 2014)
High-fat diet-induced model	Rodents	A β deposits, cognitive deficits	Abnormalities in genes and proteins, easy manipulation	No NFTs, Costs time and vigor, devoid other metabolic abnormalities	The role and mechanism of different dietary components in AD	(Herculano et al., 2013)
	Chemically induced model	Cognitive deficits, specific neurotransmitter pathway damage	Similar cognitive deficits to humans, rapid, easy to gain	No neurodegenerative process	Testing symptomatic treatments, the complex mechanisms of dementia	(Zhang et al., 2013)
Intervention model	Lesion model	Learning and memory deficits	Similar cognitive deficits to humans, rapid, easy to gain	No NFTs and plaques	Testing symptomatic treatments, the complex mechanisms of dementia	(Li et al., 2016)

Table 2.5: *In vitro* models for Alzheimer's disease

Models		Identified aspects AD	Advantages	Limitations	References
Cell models	iPSC	Amyloidosis, biochemical alterations, p-Tau, Neuron degeneration	Similar genetics to humans	No neurological and pathological alterations	(Israel et al., 2012)
	Neuroblastoma cell	Specific molecular pathways like APP processing	Direct and rapid	No neurological and pathological alterations	(Mitchell and King, 2014)
Tissue models	Brain slices	Neuron degeneration, p-Tau, amyloidosis, biochemical alterations	The natural physiological environment, show pathology biochemically and histologically	No NFTs and plaques, no neurological alterations	(Deng et al., 2007; Li et al., 2016)
	Cultured tissues	Neuron degeneration, p-Tau, amyloidosis, biochemical alterations	A controllable environment, show the AD mechanism at the molecular and cellular level	No NFTs and plaques, no neurological alterations	(Li et al., 2016)
Molecular stimulation model	Anti-bubble biomachinery	Neuronal death, Inflammatory reactivation	Dissect molecular functions of endogenous regulatory AD pathway	No neurological and pathological alterations	(Minicozzi et al., 2014)

Table 2.6: Reported activities of Bromelain

Reported activities	Study model	Dose	Mechanistic Activities	Results	References
Anti-inflammatory	<i>In vitro</i> and <i>In vivo</i>	20µg/ml and 100mg/kg b.wt/day	↓ inflammatory cytokine IL8 and TNFα	↓ activation of TLR4 restricts NFB activation to improve colitis inflammation	(Verma et al., 2017)
Anti-tumour	<i>In vivo</i>	12.5mg to 25mg/kg	Tumour improvement in metastatic	Acts as anti-metastatic	(Bééz et al., 2007)
Anti-microbial	<i>In vitro</i>	1.8mg/ml, 1mg to 4mg/ml	↓ E.coli, B.subtilis, S.pyogenes	Effective against gram positive microbial	(Abdulrahman Ali, 2015)
Anti-oxidant	<i>In vitro</i> (BNC loaded)	30mg/ml	↓ free radicals	Inhibits oxidation	(Ataide et al., 2017)
Anti-Adipogenic	<i>In vitro</i> (SBM)	50µg/ml	↓ adipogenic gene expression	Inhibits 3T3-L1 adipocyte	(Dave et al., 2012)
Anti-inflammatory and Neuroprotective Cognitive function	<i>In vitro</i> (Microglial cells) and <i>In vivo</i> (Scopolamine induced)	40µg, 50µg/ml and 50mg, 75mg and 100mg/kg b.wt., Pineapple extract	↓ Nitric oxide level	Acts as anti-inflammatory, neuroprotection	(Habashi et al., 2012)
Antioxidant	<i>In vivo</i> (Dichlorvos induced)	40mg to 70mg/kg body weight	↓ oxidative stress and Cholinesterase	Acts as neuroprotective by ↓ oxidative stress and Cholinesterase	(Chaudhary et al., 2018a)
Anti-AD	<i>In vitro</i>	3 to 6mU/ml	Degrades Aβ-1-42	Acts as a neuroprotection	(Sancesario et al., 2018)
Anti-AD and improved Aβ catabolism	<i>Ex-Vivo</i>	90mg thrice daily	Monomers ~ Aβ catabolism	Preventing Aβ cumulation in the brain and vasculature	(Lauer et al., 2001b)

Note: b.wt.; body weight, TNF-α, Tumor necrosis factor-α, IL8; interleukin8, TLR4, toll-like receptor, NFB; nuclear factor B, Aβ; β-amyloid, MAO; Monoamine oxidase A, AD; Alzheimer's disease

Table 2.7: Reported activities of Myrcene

Reported activities	Study model	Dose	Mechanism Activities	Results	References
Anti-inflammatory	<i>In vitro</i>	50µg/ml	↓ IL-1β	↓ inflammation	(Rufino et al., 2015)
Anti-oxidant	<i>In vivo</i>	200mg/kg b.wt.	↑ GSH, GPx, CAT, ↓ SOD and ↓ TBARS	Prevents oxidative and histological damage in heart tissue after ischemic stroke	(Burcu et al., 2016)
Anticonvulsant	<i>In vivo</i>	200mg/kg b.wt.	↑ latency in PTZ induced convulsion	Reported to have anticonvulsive effects	(Almeida et al., 2011; Sousa et al., 2006)
Neuroprotective effect	<i>In vivo</i>	200mg/kg b.wt.	↓ oxidative stress, ↓ TBARS level in brain	Acts as neuroprotective	(Ciftci et al., 2014)
Anti-photoaging	<i>In vitro</i> (cell)	1-10µM	Suppressed ROS production under	Improve human skin extrinsic aging	(Hwang et al., 2017)
Anti-ulcer	<i>In vivo</i>	7.50mg/kg b.wt.	↑ MDA, ↓ SOD, ↓ GPx, ↓ GR	Prevents peptic ulcer	(Bonamin et al., 2014)
Anti-oxidant	<i>In vivo</i>	200mg/kg b.wt.	↑ GSH, GPx, CAT, ↓ SOD and ↓ TBARS	Acts as ↓ oxidative stress in liver	(Ciftci et al., 2011)
Anti-AChE	<i>In vitro</i>	0.7-44.75 µg/ml	↓ cortical levels of neurotransmitter acetylcholine	<i>Artemisia maderaspatana</i> extract contains β-Myrcene and other chemicals to have AChE inhibition activity	(Jyotshna et al., 2015)

Note: b.wt. : body weight, IL-1β, Interleukin-1β, GSH: Glutathione, CAT: Catalase, TBARS: Thiobarbituric acid reactive substances, ROS: Reactive oxygen species, PTZ: Pentylenetetrazol, MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxide, GR: Glutathione reductase.

CHAPTER – 3

**RESEARCH HYPOTHESIS AND
OBJECTIVES**

3. Research hypothesis and objectives

3.1. Hypothesis of research

In various studies, it has been reported that the present drug therapy for AD provides only symptomatic relief, but it is unable to ameliorate the underline pathological condition. That is why, in the present study, the potential of selected phytoconstituents (Bromelain and Myrcene) were evaluated to treat the underline pathological condition.

Bromelain is a proteolytic enzyme that occurs naturally and is known to attenuate the A β ₁₋₄₂ monomer aggregation in previously reported *in vitro* study (Lauer et al., 2001; Sancesario, et al., 2018). Bromelain is also reported to have *in vivo* antioxidant and anti-inflammatory properties (Verma et al., 2017; Chaudhary et al., 2018). Extract of pineapple (the main source of Bromelain) is also reported to enhance memory in an *in vivo* study (Momtazi-borojeni et al., 2017).

On the other hand, Myrcene showed anti-AChE and anti-inflammatory activities in a previously reported *in vitro* study (Jyotshna et al., 2015; Rufino et al., 2015). Myrcene is also reported to have *in vivo* antioxidant and neuroprotective effects (Burcu et al., 2016; Ciftci et al., 2014).

Based on *in vitro* reported activities of Bromelain and Myrcene, can be expected to show to attenuate the A β ₁₋₄₂ monomer aggregation and anti-AChE *in vivo* AD model.

Thus, the proposed study conducted to evaluate the ameliorative effect of selected phytoconstituents individually as well as in combination against the AD model.

The Bromelain and Myrcene are hypothesized to bestow better therapeutic potential for AD, and can add therapeutic value to the existing treatment of AD.

3.2. Aim: Pharmacological Evaluation of Selected Phytoconstituents for Alzheimer's disease using Animal Model

3.3. Brief objectives of the research

1. To evaluate and compare neuroprotective effect of selected phytoconstituents in Alzheimer's disease using animal model.
2. To assess the synergistic effect of selected phytoconstituents with standard drug in Alzheimer's disease.

CHAPTER – 4
MATERIALS AND METHODS

4. Materials and methods

4.1. Drugs, ELISA kits, and reagents

Bromelain (Brm), Myrcene (Myr), Donepezil hydrochloride, aluminium trichloride (AlCl_3), acetylthiocholine iodide, and D - galactose were procured from **Sigma – Aldrich (Saint Louis, USA)**. The mouse β - secretase (BACE), mouse amyloid beta peptide₁₋₄₂ ($\text{A}\beta_{1-42}$ peptide), and mouse brain-derived neurotrophic factor (BDNF) ELISA kits were procured from **MyBiosource Inc. USA**. The mouse ELISA kits of interleukin – 6 (IL – 6), and tumor necrosis factor - α ($\text{TNF} - \alpha$) were obtained from **Raybiotech USA**. **Loba Chemie. Pvt. Ltd., Mumbai, India**, provided reduced glutathione, N-1-naphthyl ethylene di-amine di-hydrochloride, Disodium hydrogen phosphate, acetone, acetic acid, sodium di-hydrogen phosphate, thiobarbituric acid (TBA), trichloroacetic acid, sodium chloride, hydrochloric acid, orthophosphoric acid, nitro blue tetrazolium (NBT), methanol, and tween 80. **Himedia Laboratories, Pvt. Ltd., Mumbai, India**, provided ethylenediaminetetraacetic acid (EDTA), hydroxylamine hydrochloride, and 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB). **Central Drug House, Mumbai, India**, provided pyridine, sodium nitrite, carboxymethyl cellulose (CMC), n-butanol, sodium citrate, tris-hydrochloric buffer, sulphanilamide, and sodium carbonate.

4.2. Animals

Healthy female Swiss albino mice (approx 4 months old) weighing 20g - 35g were collected from the “National Institute of Pharmaceutical Education and Research (NIPER), Ministry of Chemicals and Fertilizers, Government of India, Mohali, Punjab, India”. The mice were certified pathogen-free. The mice were divided into different experimental groups. The mice were placed in sterilized cages and acclimatized at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 45% - 55% relative humidity (RH) with free access to water and food in the departmental animal house. The mice were exposed to 12 h light/dark cycle before using them for experiments. “Institutional Animal Ethics Committee (IAEC), Lovely Professional University, Phagwara, Punjab, India” approved the experimental protocol (**LPU/IAEC/2019/Protocol No.51**). The treatment of the animals and experimental protocol were confirmed to be in accordance with the rule as per the “Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India”.

4.3. Drug treatment schedule

All mice (n = 66) were assigned into 11 groups (each group, n = 6), as shown in **Table 4.1**. AlCl_3 (5mg/kg/day, with 0.9% normal saline, p.o.), (**Peng et al., 2015; Gao et al., 2015**); D -galactose (60 mg/kg/day, with 0.9% normal saline, i.p.), (**Peng et al., 2015; Gao et al., 2015**); Donepezil HCl (2mg/kg/day, with 0.9% normal saline), (**Yu et al., 2015; Wang et al., 2019**); Brm (10, 20 mg/kg/day, with 0.9% normal saline, i.p.), (**Baez R et al., 2007**); and Myr (100, 200 mg/kg/day, with 0.1% CMC, i.p.) was suspended with 0.1% Carboxy methyl cellulose (CMC) (**Ciftci et al., 2014; Burcu et al., 2016**).

Treatments with Bromelain (Brm), Myrcene (Myr), and Bromelain + Myrcene were given to mice which were divided into six different groups (each group, n = 6), shown in **Table 4.2, Table 4.3, and Table 4.4** respectively. Co-administration of AlCl_3 with D - galactose was given for 90 consecutive days to all groups except the control group. The treatments were started on 61 days till 90 days (30 consecutive days). All the drugs were freshly prepared in 0.9% normal saline except Myr (prepared 0.1% CMC) before administration in mice.

The duration and selection of dose were based on previous research studies (**Peng et al., 2015; Wang et al., 2019; Baez R et al., 2007; Osman et al., 2014; Burcu et al., 2016; Zeiger, 1997**). Later 20 days of treatment of drugs; mice (24 h after the last dose of drugs) were subjected to behavioral analysis, as shown in **Figure 4.1**.

3.3.1. Route of administration: The drugs were administered through the intragastric route (p.o.) and intraperitoneal route (i.p.). The materials used such as disposable gloves, syringe (1mL), hypodermic needle (22 ga) isopropyl alcohol, gavage needle (20 ga), and gauze was required before drug administration as per guidelines, as shown in **Figure 4.2** and **Figure 4.3**.

Table 4.1: Grouping of all mice

Groups	Treatment	Dose and route	No. of animals
Group 1 (Normal group)	Normal saline	5 mL/kg, i.p.	6
Group 2 (Negative control/DC)	AlCl ₃ + D-galactose (for consecutive 90 days)	5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 3 (Positive control)	Donepezil + AlCl ₃ + D-galactose (for consecutive 30 days)	2 mg/kg, i.p + 5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 4 (Test-1 Low dose)	Bromelain Ld + AlCl ₃ + D-galactose (for consecutive 30 days)	10 mg/kg, i.p. + 5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 5 (Test-1 High dose)	Bromelain Hd + AlCl ₃ + D-galactose (for consecutive 30 days)	20 mg/kg, i.p. + 5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 6 (Test-1 Low dose + Std)	Bromelain Ld + Donepezil+ AlCl ₃ + D-galactose (for consecutive 30 days)	10 mg/kg, i.p. + 2 mg/kg, i.p. + 5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 7 (Test-2 Low dose)	Myrcene Ld + AlCl ₃ + D-galactose (for consecutive 30 days)	100 mg/kg, i.p. + 5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 8 (Test-2 High dose)	Myrcene Hd + AlCl ₃ + D-galactose (for consecutive 30 days)	200 mg/kg, i.p. + 5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 9 (Test-2 Low dose + Std)	Myrcene Ld + Donepezil + AlCl ₃ + D-galactose (for consecutive 30 days)	100 mg/kg, i.p. + 2 mg/kg, i.p. + 5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 10 Combination: (Test-1 + Test-2) Low dose	Bromelain Ld + Myrcene Ld + AlCl ₃ + D-galactose (for consecutive 30 days)	10 mg/kg, i.p. + 100 mg/kg, i.p. + 5 mg/kg, p.o. + 60 mg/kg, i.p.	6
Group 11 Combination: (Test-1 + Test-2) Low dose + Std	Bromelain Ld + Myrcene Ld + Donepezil + AlCl ₃ + D-galactose (for consecutive 30 days)	10 mg/kg, i.p. + 100 mg/kg, i.p. + 2 mg/kg, i.p. + 5mg/kg, p.o. + 60 mg/kg, i.p.	6

Note: For consecutive 30 days: from 61th day to 90th day of treatment protocol; DC: Disease control; CMC: Carboxymethylcellulose; Std: Standard; Ld: Low dose; Hd: High dose.

Table 4.2: Bromelain treatment schedule

Groups	(n)	Treatment	Drug dose and routes
1	6	Control	0.9 % normal saline (5 mL/kg b. w., i. p.)
2	6	M	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.)
3	6	M + Dnpz	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.) + Dnpz (2 mg/kg b. w., i. p.)
4	6	M + Brm L	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.) + Brm L (10 mg/kg b. w., i. p.)
5	6	M + Brm H	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.) + Brm H (20 mg/kg b. w., i. p.)
6	6	M + Brm L + Dnpz	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.) + Brm L (10 mg/kg b. w., i. p.) + Dnpz (2 mg/kg b. w., i. p.)

Note: M = Model (AlCl₃ + D – galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; b. w. = Body weight; p. o. = per oral; i. p. = Intraperitoneal; (n) = Number of animals per group.

Table 4.3: Myrcene treatment schedule

Groups	(n)	Treatment	Drug dose and routes
1	6	Control	0.9 % normal saline (5 mL/kg b. w., i. p.)
2	6	M	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.)
3	6	M + Dnpz	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.) + Dnpz (2 mg/kg b. w., i. p.)
7	6	M + Myr L	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.) + Myr L (100 mg/kg b. w., i. p.)
8	6	M + Myr H	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.) + Myr H (200 mg/kg b. w., i. p.)
9	6	M + Myr L + Dnpz	AlCl ₃ (5 mg/kg b. w., p. o.) + D - galactose (60 mg/kg b. w., i. p.) + Myr L (100 mg/kg b. w., i. p.) + Dnpz (2 mg/kg b. w., i. p.)

Note: M = Model (AlCl₃ + D – galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; b. w. = Body weight; p. o. = per oral; i. p. = Intraperitoneal; (n) = Number of animals per group.

Table 4.4: Combination of Bromelain and Myrcene treatment schedule

Groups	Treatment	Drug dose and routes
1	Control	0.9 % normal saline (5 mL/kg, i. p.)
2	M	AlCl ₃ (5 mg/kg, p. o.) + D - galactose (60 mg/kg, i. p.)
3	M + Dnpz	AlCl ₃ (5 mg/kg, p. o.) + D - galactose (60 mg/kg, i. p.) + Dnpz (2 mg/kg, i. p.)
4	M + Brm	AlCl ₃ (5 mg/kg, p. o.) + D - galactose (60 mg/kg, i. p.) + Brm (10 mg/kg, i. p.)
7	M + Myr	AlCl ₃ (5 mg/kg, p. o.) + D - galactose (60 mg/kg, i. p.) + Myr (100 mg/kg, i. p.)
10	M + Brm + Myr	AlCl ₃ (5 mg/kg, p. o.) + D - galactose (60 mg/kg, i. p.) + Brm (10 mg/kg, i. p.) + Myr (100 mg/kg, i. p.)
11	M + Brm + Myr + Dnpz	AlCl ₃ (5 mg/kg, p. o.) + D - galactose (60 mg/kg, i. p.) + Brm (10 mg/kg, i. p.) + Myr (100 mg/kg, i. p.) + Dnpz (2 mg/kg, i. p.)

Note: M = Model (AlCl₃ + D – galactose); Dnpz = Donepezil; Brm = Bromelain; Myr =Myrcene; p. o. = per oral; i. p. = Intraperitoneal; Number of animals per group (n) = 6.

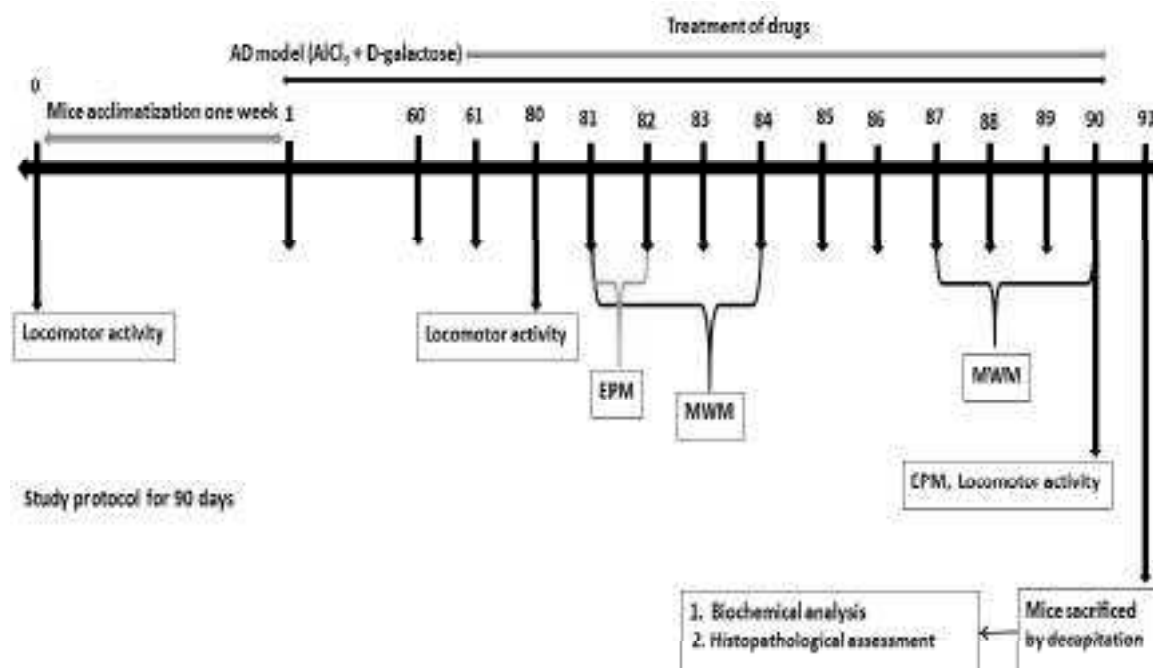


Figure 4.1: Schematic study protocol timeline; after one-week acclimatization of mice, the AD model (AlCl_3 + D - galactose) was induced for 90 consecutive days. On day 61, treatment of drugs (Myrcene, and Donepezil) was given for the last 30 consecutive days followed by behavioral tests such as Morris water maze (MWM), elevated plus maze (EPM), and locomotor activity. At the end of the tests, all the mice were sacrificed by decapitation to collect the cerebral cortex and hippocampus regions of the mice's brains for biochemical analysis and histopathological assessment.

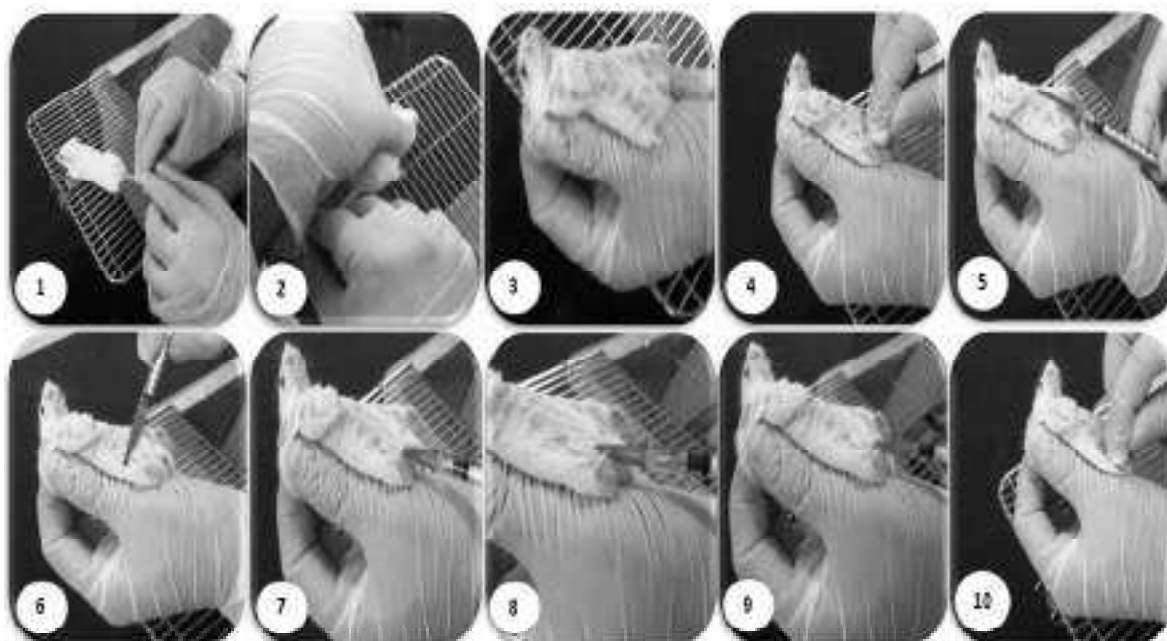


Figure 4.2: Protocol for intraperitoneal injection (i.p.) route of administration for Swiss albino mouse; **1.** Restrain mouse; **2 & 3.** Gently grip the forearms of the mouse with for finger, hold the tail under the little finger, and tilt the body at 45° angle with head down; **4.** Alcohol swab area; **5 & 6.** Identify an appropriate area for injection by measuring the abdomen manually; **7.** Insert the needle into the mouse's lower left quadrant of abdomen at 30° angle; **8.** Aspirate needle to ensure proper insertion of the needle. If, an air bubble appears after aspirating represents a needle being inserted into the proper intraperitoneal cavity, but any fluid or blood that appears in the syringe represents improper insert of the needle. To prevent inducing peritonitis remove the syringe discard needle, use a new syringe and needle; **9.** Now, administer the drug in steady fluid motion; **10.** Withdraw the syringe gently, put alcohol swab cotton for a few seconds, and finally, the mouse was placed in its respective cage.

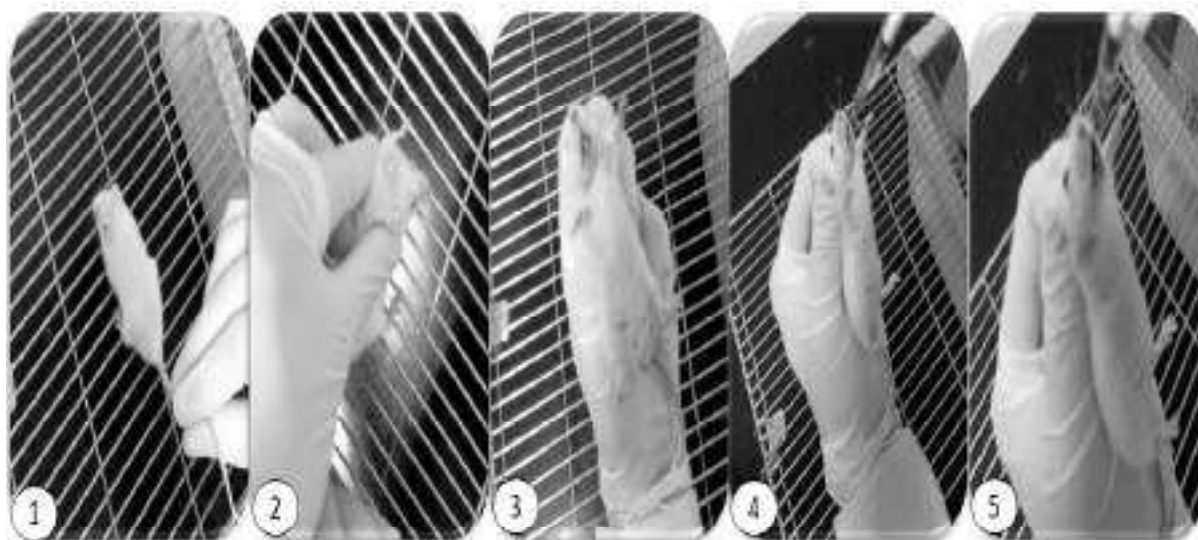


Figure 4.3: Protocol for intragastric (p.o.) route of administration for Swiss albino mouse; **1.** Restrain mouse; **2 & 3.** Gently grip the forearms of the mouse with for finger; **4.** Place the gavage tip near of mouse mouth to induce swallowing, slowly insert the gavage in the mouse mouth to extend the neck, align the mouth and esophagus; **5.** Once the gavage was placed properly, the drug was administered.

4.4. Behavioral parameters

4.4.1. Morris water maze task

MWM was developed by **Morris (1982)**, consisted of a height of 0.5 m and a 1.2 m diameter of circular pool tank. The circular pool tank was filled with opaque water (0.3 m depth) at $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The floor of the circular tank was marked off into four equal quadrants namely the east, west, north, and south quadrant. The spatial cues were fixed in each quadrant. 1 cm under the surface of the water at the center of one quadrant, 4.5 cm diameter of the escape platform was placed. The spatial reference learning and working memory of all mice were performed (**Ojha et al., 2010; Wei et al., 2017**).

A 24 h before beginning training, all mice (n=36) were released to the circular water pool by allowing them freely swim for 90 s (cut-off time) to get habituated to this water pool. All mice were taken out from the water pool, gently ringed water on their body with sterilized soft fabric cloth, and placed into their respective cages.

Spatial reference memory (SRM) was begun on day 81 after 1h of the last dose of treatment. Individual mice from all groups were kept in a water pool facing the wall of the circular water pool. Mice were allowed for 90 s i.e. cut-off time to search secret platform. To see the spatial cues, 20 s were given to stay on the hidden platform. If mice failed to find the platform guided properly. All mice had four trials per day (each trial gap of 10 min) for three consecutive days i.e. day 81 to day 83. Escape latency i.e. time engaged by mice to get onto the secret platform within 90 s was recorded. A 24 h later successful completion of the three days training; probe trial (without hidden platform) was performed on day 84. The mice were sanctioned to free swim for 90 s along the time spent in the targeted quadrant was recorded to assess the reference memory of mice by a live video tracking system (VJI all maze Pvt. Ltd., Mumbai).

Spatial working memory (SWM) was performed two days later of the pre-training reference memory. On day 87, 1h later the last dose; the navigation task for working memory was begun with two trials each day for four consecutive days i.e. day 87 to day 90. The first trial was the acquisition trial in which the platform was placed into a new quadrant at the center. Mice were released to the water pool to search the hidden platform, allowed to rest onto platform 20 s, and returned to their respective cages. While the second trial is the retrieval trial in which the platform was kept in its previous quadrant at the center. 75 min later of the first trial, mice were released into the water pool to find the platform and stayed on the platform for 20 s. Escape latency was recorded

for assessing the SWM of mice by using a live video tracking system (VJI all maze Pvt. Ltd., Mumbai).

4.4.2. Elevated plus-maze task

EPM was used to measure the learning and memory development by recording the transfer latency of mice (**Kulkarni et al., 2010**). EPM comprised of two open arms of size 16 cm x 5 cm as well as two shut arms of size 16 cm x 5 cm x 12 cm, stretched out from the central platform (5 cm x 5 cm). From the floor, the maze raised 25 m in height. Acquisition memory was tested on day 81; 30 min after the last dose of treatment, individual mice were kept at the end of the open arm facing away from the middle platform. However, TL indicates the time engaged by mice to reach into one of the closed arms from open arms with all four legs within 60 s that was recorded by a live video tracking system (VJI all maze Pvt. Ltd., Mumbai). If the mice failed to reach into one of the closed arms within 60 s were guided to reach into the closed arm. The mouse was placed in the closed arm for 10 s and then kept in their respective cages. The first retention transfer latency was tested on day 82; the same procedure was followed and the transfer latency of individual mice was recorded. Correspondingly later one-week interval, second retention transfer latency was performed on day 90. Individual mice's transfer latency was assessed to test long-term memory.

4.4.3. Assessment of locomotor activity

Locomotor activity was performed on days 0, 80, and 90. This was assessed by using a digital actophotometer (INCO. Pvt. Ltd., India). Actophotometer was kept in a darkened, attenuated sound and ventilated room and consisted of a standard plastic cage encircled by a stainless steel frame. The frame was comprised of an infrared photocell beam across the frame axis which was raised 2 cm above the floor. Individual mice were placed in the chamber of a digital actophotometer to assess locomotor activity counts per 5 min and measured the number of photocell beams crossed by mice (**Khurana et al., 2011; Kulkarni et al., 2010**).

4.5. Biochemical parameters

4.5.1. Homogenization of mice brain

At the end of the study, mice were sacrificed by cervical dislocation. The brains were extracted, as shown in **Figure 4.4**, and ringed with 4⁰ C stored isotonic saline to separate the cerebral cortex and hippocampus, which were weighted correctly to make tissue homogenate (10% w/v) in 4⁰ C stored 0.1 M phosphate buffer (pH 7.4). The pH meter (Phan. Lab., Mumbai, India) was used to change the pH. To homogenize the cerebral cortex along with hippocampus tissues, they were put in an ice-cooled glass with having 0.1M phosphate buffer (pH 7.4) homogenizer (RQ-127, REMI, India). Further, the homogenate was centrifuged at 10,000g for 15 min at 4⁰ C using a centrifuge (CM-12 Plus, REMI, India) and the supernatant was collected in sterilized Eppendorf tubes which were stored at -20⁰C as shown in **Figure 4.5**, to estimate the level of the various biochemical studies such as oxidative stress maker, cholinesterase enzymes, inflammatory markers Elisa kits and BDNF, β - secretase. and A β ₁₋₄₂ peptide Elisa kits. For histopathological studies, the cerebral cortex and hippocampus tissues were also stored in 10% formaldehyde at room temperature.

4.5.2. Estimation of acetylcholinesterase activity

Acetylcholinesterase (AChE) level was estimated in the supernatant of the cerebral cortex and hippocampus as per **Ellman et al., (1961)**.

Assay procedure:

Test tubes mixture were enclosed supernatant (0.05 mL), 0.1M phosphate buffer pH 8 (3 mL), 0.075 M acetylthiocholine iodide (0.1 mL), and 0.01 M Ellman's reagent (0.1 mL) i.e. [5, 5'-dithiobis (2-nitrobenzoic acid)], mixed 15 mg of sodium bicarbonate properly. The change in absorbance was calculated at 412 nm for 2 min, using a "UV-spectrophotometer, (UV-1800, Shimadzu, Japan)". The consequences were measured using a molar extinction coefficient ($1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$). This unit was articulated as n moles per mg protein.

Calculation of Acetylcholinesterase concentration:

Formula: $(3 \times \Delta\text{O.D.}) / \epsilon \times \text{volume of sample (mL)} \times \text{mg of protein}$; **Unit:** n moles / mg of protein

Note: 1. $\Delta\text{O.D.}$ = Change in absorbance per minute; 2. ϵ = Molar extinction coefficient ($1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) 3. Sample volume = 0.05 mL 4. 3 = It is reaction volume in cuvette

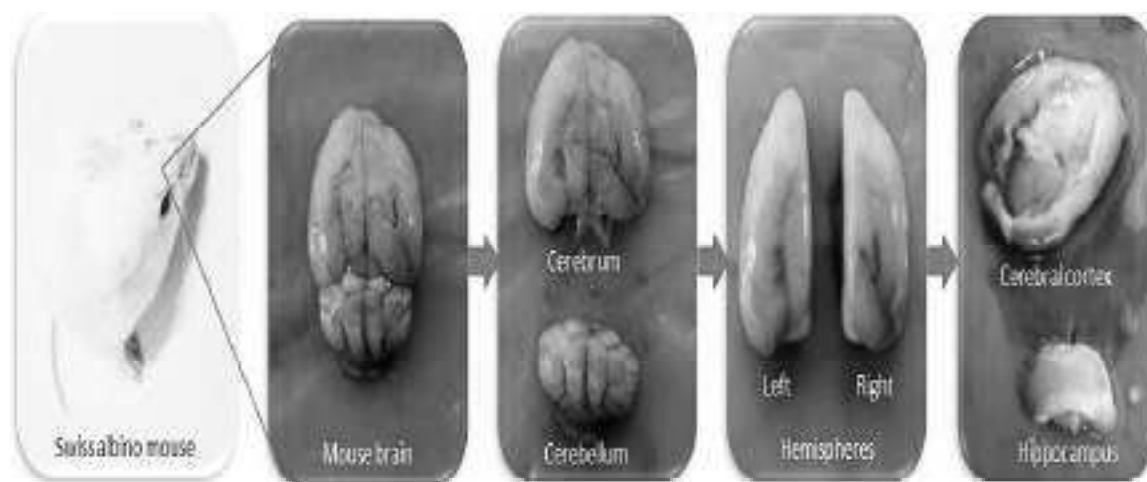


Figure 4.4: Protocol for isolation of hippocampus and cerebral cortex from the brain of Swiss albino mouse.



Figure 4.5: Protocol for homogenization of cerebral cortex and hippocampus of Swiss albino mouse brain. **A.** the AlCl_3 and D - galactose induced or treatment mouse, **B.** Isolation of brain **C.** Separation of cerebral cortex and hippocampus from the isolated brain, **D.** Cerebral cortex and hippocampus placed in a sterilized container, at 4°C , 0.1M PBS, 7.4 pH, **E.** Homogenate at, 4°C , 0.1M PBS, 7.4pH, **F.** Collected homogenate in sterilized Eppendorf, **G.** Centrifuge at 10000 g, 15 min, 4°C , **H.** Discarded Eppendorf with pellet, **I.** Saved supernatant in sterilized Eppendorf and **J.** Stored at -20°C for biochemical estimation.

4.5.3. Estimation of lipid peroxidation

Malondialdehyde (MDA) quantity was calculated by reaction of thiobarbituric acid in the degree of lipid peroxidation in the supernatant of the cerebral cortex and hippocampus as per **Will (1966)**.

Assay procedure:

Briefly, in sterilized Eppendorf tubes, supernatant (0.2 mL) was added with Tris HCl pH 7.4 (0.2 mL) and incubated at 37⁰ C for 2 h. Later incubation, 10% ice-cold trichloroacetic acid (TCA) (1 mL) was added. Then, for 10 min, eppendorf tubes were centrifuged at 1000g. The supernatant (1 mL) was aliquoted into test tubes in which 0.67% thiobarbituric acid (TBA) (1 mL) was added. For 10 min, the test tubes having samples were kept in a boiling water bath. Later cooling test tubes having samples were added with distilled water (1 mL). The absorbance was estimated at wavelength 532 nm by using a “UV- spectrophotometer (UV-1800, Shimadzu, Japan)”. Therefore, the calculation was done using the extinction coefficient of MDA ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$). This unit was articulated as n moles of MDA per mg protein.

Calculation of lipid peroxidation:

Formula: $(3 \times \Delta \text{O.D.}) / \epsilon \times \text{volume of sample (mL)} \times \text{mg of protein}$

Unit: n moles of MDA / mg of protein

Note:

1. $\Delta \text{O.D.}$ = Change in absorbance per minute
2. ϵ = Molar extinction coefficient ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$)
3. Sample volume = 0.2 mL
4. 3 = It is reaction volume in cuvette

4.5.4. Assessment of nitrite

Nitrite amount in the supernatant of the cerebral cortex and hippocampus was estimated as per described colorimetric method with Griess reagent **Green et al., (1982)**.

Assay procedure:

In sterilized Eppendorf tubes, Griess reagent i.e. 5% phosphoric acid w/v in water, 1 gm of sulphanilamide, and 0.1g of N-1-naphthyl ethylenediamine dihydrochloride, along with an equivalent volume of supernatant were taken and mixed properly. For 10 min, the solution was held at room temperature in the dark. Using a “UV-spectrophotometer (UV-1800, Shimadzu, Japan)”, the absorbance was measured at a wavelength of 540 nm. The concentration of Nitrite was measured using a standard plot of sodium nitrite (10 μ M – 100 μ M). This unit was expressed in μ M per mg protein as shown in **Figure 4.6**.

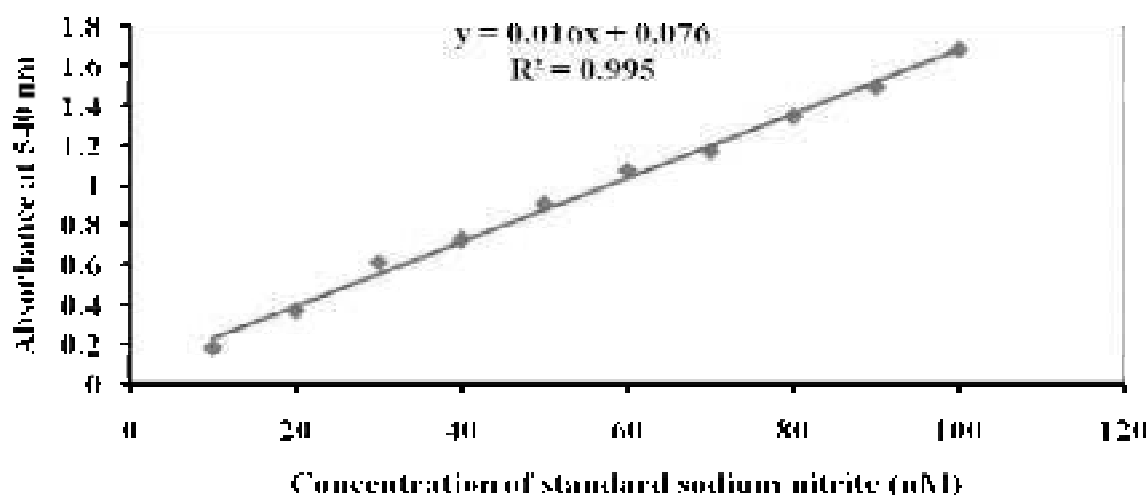


Figure 4.6: Calibration curve of standard sodium nitrite

Calculation of nitrite concentration:

Formula:

1. Nitrite concentration in sample = [(X/sample volume)* Dilution factor]
2. Nitrite concentration in homogenate = [Nitrite concentration in sample (n mole /mL) / Total protein conce. (mg/mL)]

Unit: n moles per mg protein

Note:

1. 1 μ M = 1 nano mole / mL

2. X = It is sample concentration obtained from regression equation ($y = mx + c$) of standard plot

4.5.5. Detection of reduced glutathione

Glutathione (GSH) level was determined in supernatant of the cerebral cortex and hippocampus as per **Jollow et al., (1974)**.

Assay procedure:

In sterilized Eppendorf tubes, supernatant (1 mL) was added in 10% trichloroacetic acid (TCA) (1 mL) w/v in distilled water. The Eppendorf tubes with samples were centrifuged at 1000g, 4°C for 10 min. Later centrifugation, supernatant (0.5 mL) was aspirated and added into 0.3M phosphate buffer pH 7.4 (2 mL). In samples, freshly prepared 0.001 M 5, 5'- dithiobis (2-nitrobenzoic acid) (0.001M DTNB in 1% w/v sodium citrate) (0.25 mL) was put. As a result, the absorbance was measured using a UV- spectrophotometer at a wavelength of 412 nm. Reduced glutathione (10 µM – 100 µM) was used to map the standard curve. The standard plot was used to measure the GSH concentration. This unit was articulated as n moles of GSH per mg protein as shown in **Figure 4.7**.

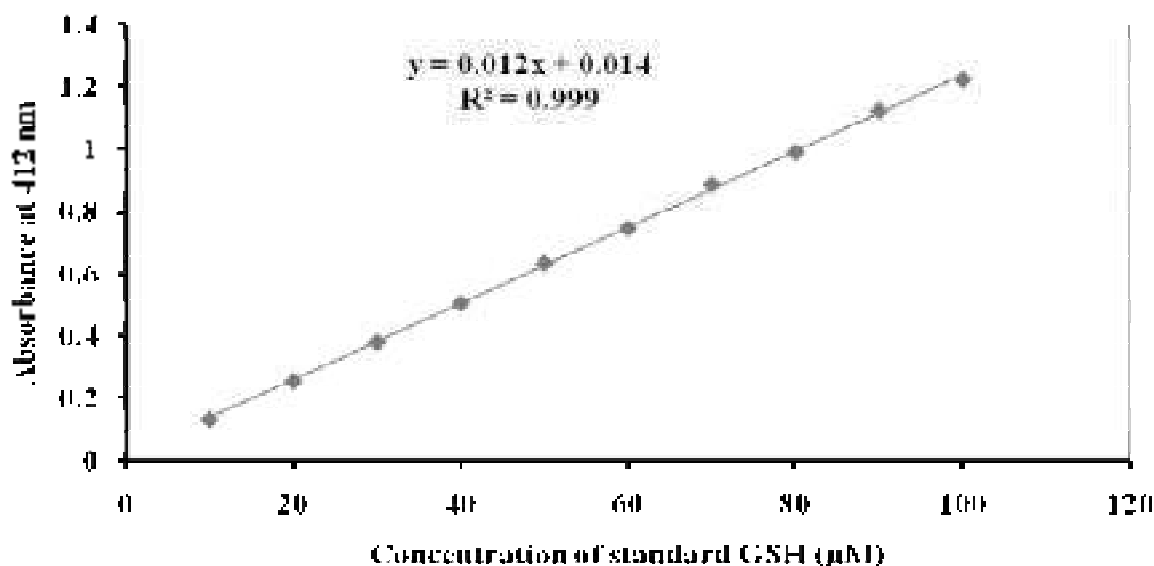


Figure 4.7: Calibration curve of standard GSH

Calculation of GSH concentration:

Formula:

1. GSH concentration in sample = $[(X/\text{Sample volume}) \times \text{Dilution factor}]$

2. GSH concentration in homogenate = (GSH concentration in sample / Total protein concentration)

Unit: n moles per mg protein

Note:

1. $1 \mu\text{M} = 1 \text{ nano mole / mL}$
2. X = It is sample concentration obtained from regression equation ($y = mx + c$) of standard plot

4.5.6. Determination of catalase activity

Catalase (CAT) activity was measured in the supernatant of the cerebral cortex and hippocampus as per described method in **Luck (1965)**.

Assay procedure:

The supernatant (0.5 mL) was added to cuvette enclosed 50 mM phosphate buffer (pH 7) (1.95 mL). Into the tubes with samples, 30 mM hydrogen peroxide (H_2O_2) (1 mL) was added and mixed properly. As a result, the changes in absorbance were analyzed using UV-spectrophotometer for 30 s at 15 s intervals at wavelength 240 nm, UV-1800, Shimadzu, Japan, and calculated the catalase amount. This unit was expressed in catalase units per mg protein.

Calculation of catalase concentration:

Formula:

Step 1: $k = (2.3)/(\Delta t) \times \log A_1 / A_2$

Step 2: 1 Unit = $(k)/(6.93) \times 1000$

Step 3: CAT units / Total Protein

Unit: CAT (Units per mg protein)

Note:

Δt : Change in time interval

A_1 : Initial absorbance

A_2 : Final absorbance

k = It can be converted to any catalase units

2.3 = Factor to convert from ln to log

4.5.7. Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined in the supernatant of mice brain regions as per the described method in **Kono (1978)**.

Assay procedure:

In cuvette, 2 mL of a solution (0.1 mM EDTA, 96 μ M nitro blue tetrazolium, 50 mM sodium carbonate, pH 10.2) was put. The supernatant (0.1 mL) and 1 mM hydroxylamine hydrochloride (0.1 mL) (pH 6 modified with sodium hydroxide) were correctly applied to the cuvette containing the solution above 2 mL. Using UV-spectrophotometer, the alter in absorbance was calculated for 2 min at 60 s intervals at wavelength 560 nm (UV-1800, Shimadzu, Japan). The SOD activity was estimated. This unit was expressed in SOD units per mg protein.

Calculation of SOD concentration:

Formula:

SOD activity (X) = [(Control – Δ O.D. / min) / (Control)*(100%)/(50%)] / [(volume of sample) / mg protein]

Unit: SOD units per mg protein

Note:

Control = [(Final absorbance (A_2) – Initial absorbance (A_1)]

Δ O.D. / min = Change in absorbance

SOD = Super oxide dismutase

Sample volume = 0.1 mL

4.5.8. Determination of protein

The amount of total protein in the cerebral cortex along with the hippocampus was measured by the Lowry method. In this method, the phenolic group of tryptophan and tyrosine residues (amino acid) in a protein produce a purple-blue color at maximum absorption in the region of 550 nm wavelength. Folin- Ciocalteau reagent consists of sodium tungstate molybdate and phosphate. The intensity of color depends on the amount of these aromatic amino acids present and varies for various proteins. Bovine Serum Albumin (BSA) is used in most protein estimation techniques universally as a standard protein, because of its low cost, high purity, and ready availability (**Lowry et al. 1951**).

Assay procedure:

- Various dilutions of BSA (range 0.05-1mg/mL) were prepared by mixing stock solution (1mg/mL) and distilled water in test tubes with 5mL as the final volume. Distilled water (1mL) is used as a blank.
- From these different dilutions, 0.2 mL protein solution was pipetted out to different test tubes and added 2 mL of alkaline copper sulphate analytical reagent (A. 50 mL of 2% sodium carbonate mixed with 50 mL of 0.1 N NaOH solution (0.4 gm in 100 mL distilled water). B. 10 mL of 1.56% copper sulphate solution mixed with 10 mL of 2.37% sodium potassium tartrate solution and mixed well A and B solutions). Mixed the solutions properly.
- The solution was incubated at room temperature for 10 mins.
- Then added 0.2 mL of reagent FolinCiocalteau solution (reagent solutions) to each tube and incubated for 30 min.
- Zero the colorimeter with blank and take the measure the absorbance at 550 nm. Plot the absorbance against protein concentration to get a standard calibration curve.
- The absorbance of the hippocampus and cerebral cortex samples was taken and calculated the amount of total protein in the cerebral cortex along with the hippocampus samples using the bovine serum albumin (BSA) standard plot as shown in **Figure 4.8**.

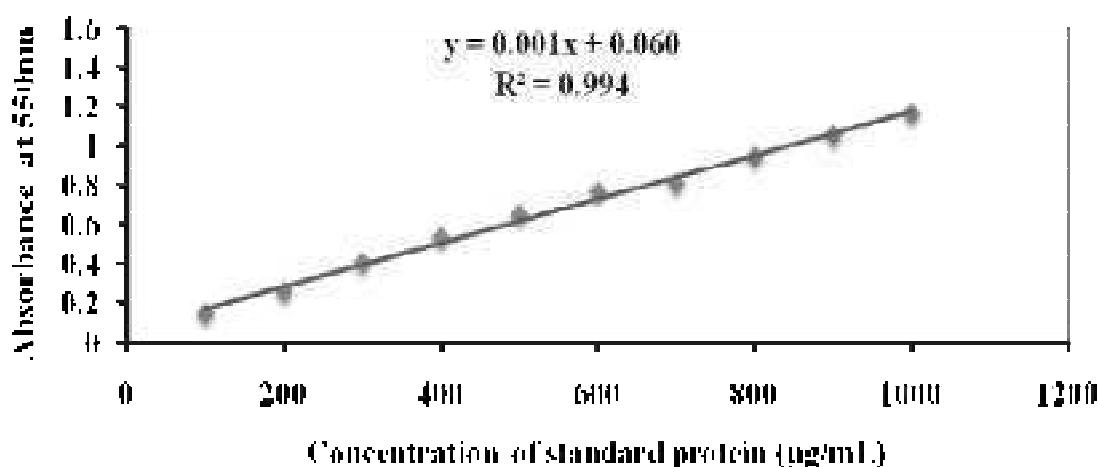


Figure 4.8: Calibration curve of standard protein (BSA)

Calculation of protein concentration:

Formula:

Protein concentration = [(X/sample volume)* dilution factor]

Unit: n moles per mg protein

Note:

1. 1 µg = 1 nano mole / mL
2. X = It is sample concentration obtained from regression equation ($y = mx + c$) of standard plot

4.5.9. Enzyme-linked immunosorbent assay

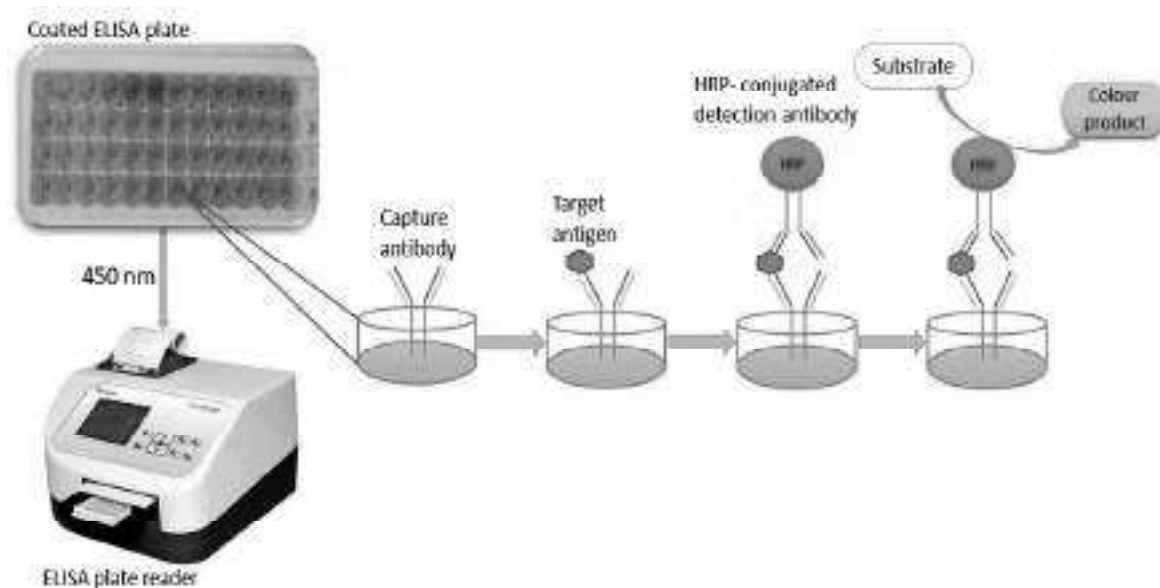


Figure 4.9: Schematic diagram indicates illustration of ELISA tests; β -secretase (BACE), amyloid beta-peptide 1-42 ($A\beta_{1-42}$), brain-derived neurotrophic factor (BDNF), $TNF-\alpha$, and IL - 6 tests were performed by ELISA kits.

4.5.9.1. Mouse β -secretase and Amyloid β_{1-42} peptide ELISA

The level of mouse β -secretase (BACE) and mouse amyloid betapeptide₁₋₄₂ was determined in the supernatant of mice brain regions according to the procedure given by MyBiosource Inc. USA mouse BACE and mouse $A\beta_{1-42}$ peptide ELISA kits, as shown in **Figure 4.9**. The assay was based on double antibody sandwich technology of enzyme-linked immunosorbent assay.

Assay procedure:

The wells of the microplate were precoated with a monoclonal antibody specific for mouse BACE or mouse $A\beta_{1-42}$ peptide. A 10 μ L standard was added to the standard well of mouse BACE or mouse $A\beta_{1-42}$ peptide microplate. 10 μ L supernatant of cerebral cortex and hippocampus was added in testing well and followed by adding 40 μ L diluents to testing well. Pipette 100 μ L horse redox peroxidase conjugate reagent to standard and testing wells. Then seal plate membrane was used to cover the microplate and placed in an incubator (REMI, India) for 1h at 37°C with gently shaking. After 1h of incubation, the sealing membrane was removed, the wells liquid was drained, the microplate was gently washed 5 times with 350 μ L washing buffer to extract unbound antibody enzymes, and the microplate was properly dried. Pipetted 50 μ L chromogen A and 50 μ L chromogen B solution into each well, mixed thoroughly, and incubate for 15 min at 37°C. Further, a 50

μL stop solution was put to all wells to stop the reaction in which the blue color changed into yellow color. As a result, the intensity of the yellow color was measured using an ELISA plate reader at a wavelength of 450 nm (iMark Microplate Reader, BIORAD, USA). The concentration of mouse BACE and mouse $\text{A}\beta_{1-42}$ peptide was calculated using standard curve plots of mouse BACEas shown in Figure 4.10, and mouse $\text{A}\beta_{1-42}$ peptide as shown in Figure 4.11, and expressed as β -secretase U/L and amyloid-beta peptide₁₋₄₂ ng/mL respectively.

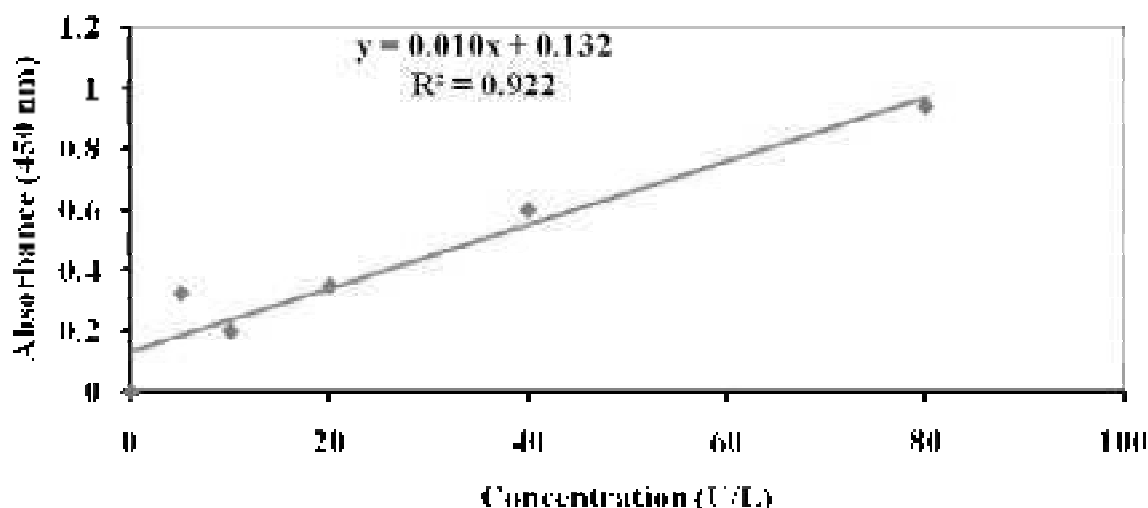


Figure 4.10: Calibration curve of mouse β -secretase

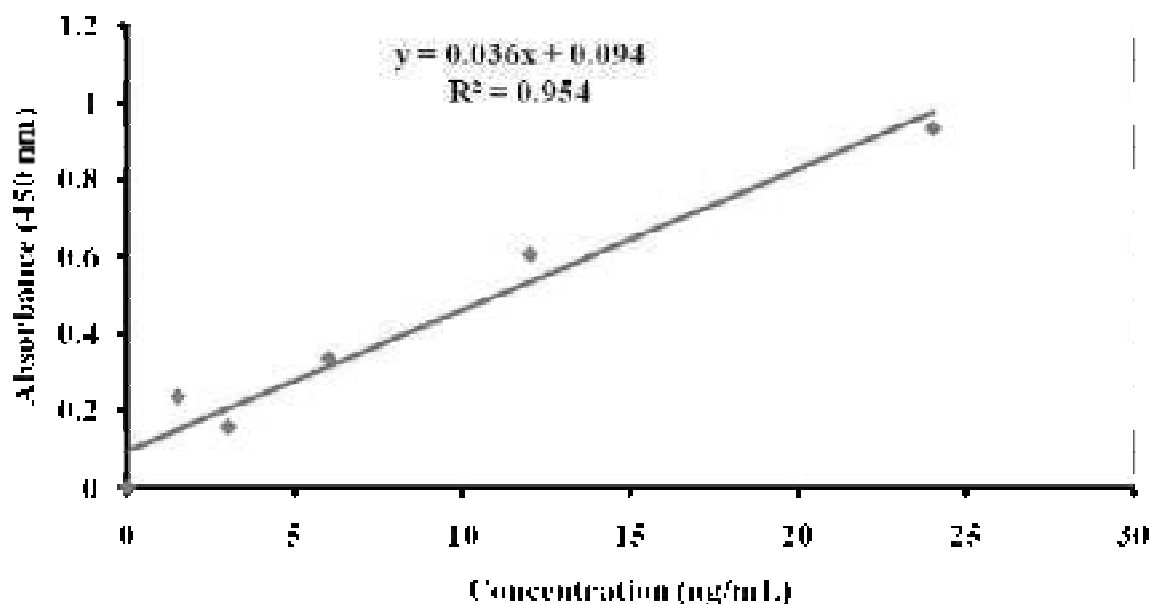


Figure 4.11: Calibration curve of mouse amyloid β_{1-42} peptide

4.5.9.2. Mouse brain-derived neurotrophic factor ELISA

The level of BDNF level was assessed in the supernatant of the cerebral cortex, and hippocampus. The assay was performed as per the instructions given by MyBiosource Inc. USA mouse BDNF ELISA kit.

Assay procedure:

A 10 μ L standard in standard wells furthermore 10 μ L supernatant of cerebral, as well as hippocampus of the mice, brain in test wells was added followed by the addition of a 40 μ L sample diluent. Then, 100 μ L HRP-conjugate reagents were added to each well, covered microplate with plate membrane, and placed in a 37°C incubator for 1h. Further, 5 times washed was done to remove unbound antibody-enzyme with 350 μ L washing buffer and dried properly. A chromogen and B chromogen pipetted 50 μ L of each solution into each well, mixed thoroughly, and then apply the stop solution of 50 μ L to stop the reaction by changing the blue color to yellow color. Using an ELISA plate reader, the absorbance reading was measured at a wavelength of 450 nm (iMark Microplate Reader, BIORAD, USA). The concentration of BDNF was measured using a mouse BDNF standard plot as shown in Figure 4.12. This unit was expressed as BDNF pg/mL.

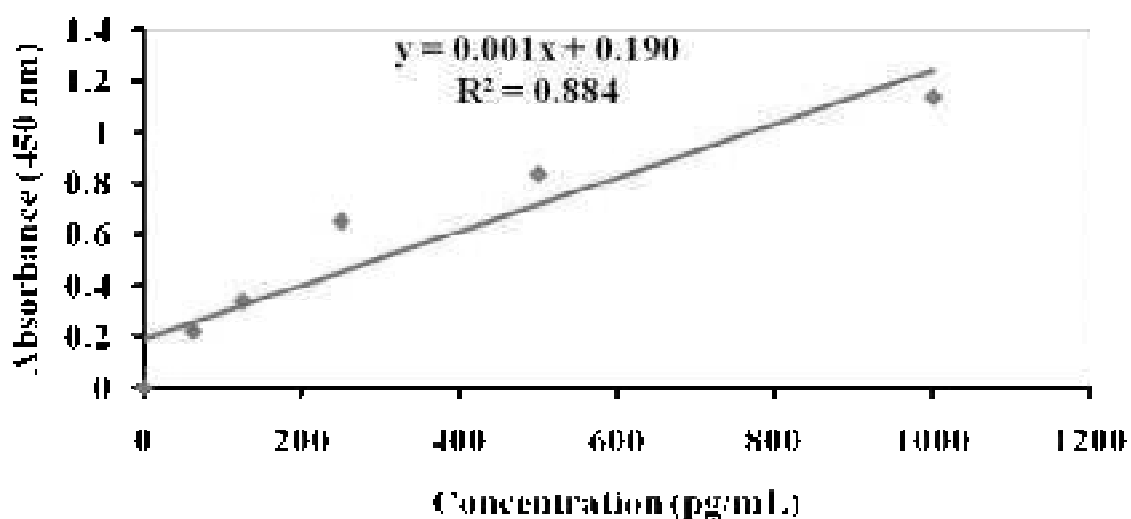


Figure 4.12: Calibration curve of mouse brain-derived neurotrophic factor

4.5.9.3. Mouse tumor necrosis factor- α and interleukin-6 ELISA

Estimation of the mouse TNF- α along with IL-6 determined in supernatant of the mice brain regions according to the instructions provided by Raybiotech USA.

Assay procedure:

The mouse TNF- α monoclonal antibody and IL-6 monoclonal antibody were pre-coated to all wells of the microplate respectively. All reagents were kept at room temperature (25°C) before using them. A 100 μ L standard and 100 μ L supernatant of cerebral cortex and hippocampus were pipetted into standard and test wells, respectively. It was placed for 2.5 h at room temperature (25°C) with gentle shaking. Later 2.5 h, the solution was removed and washed 4 times with 300 μ L washing buffer to remove unbound antibody-enzyme using the pipette and dried properly by inverting microplate on paper. 100 μ L of 1X biotinylated antibody was added to all wells of a microplate, placed for 1 h at 25°C with gentle shaking, and repeated above washing step. Streptavidin solution 100 μ L was added to all wells, kept at 25°C for 45 min with gentle shaking, and repeated the above washing step. Further, 100 μ L TMB one-step substrate reagent was pipette to all wells and placed for 30 min at 25°C. The stop solution of 50 μ L was put to each well. Immediately, using an ELISA plate reader, the absorbance was measured at a wavelength of 450 nm. The mouse TNF- α and IL-6 concentrations were calculated using the standard plot of mouse TNF- α as shown in Figure 4.13, along with IL-6 as shown in Figure 4.14. This unit was expressed as mouse TNF- α (pg/mL), and IL-6 (pg/mL).

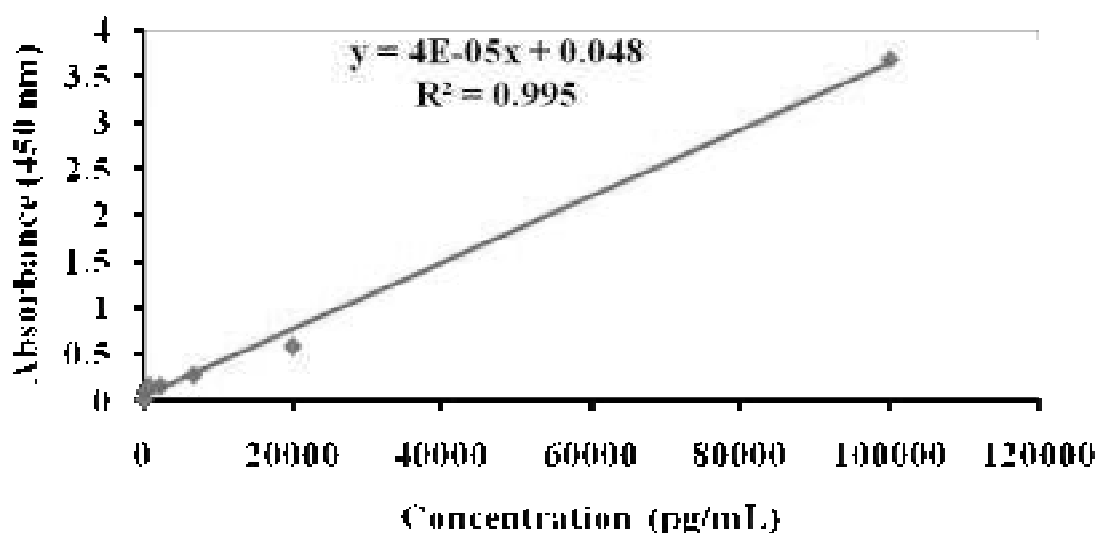


Figure 4.13: Calibration curve of mouse tumor necrosis factor- α

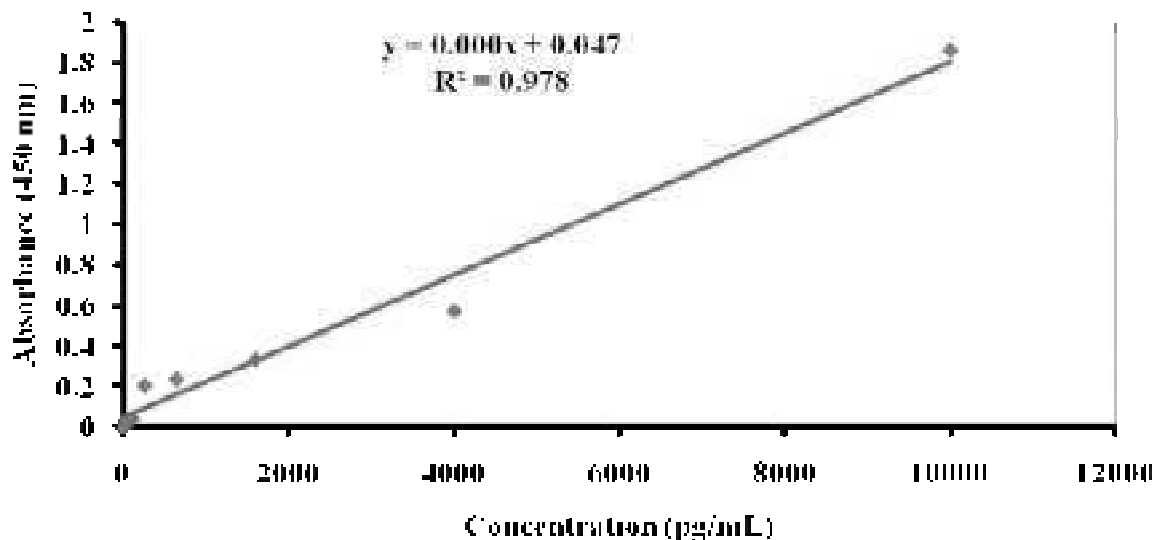


Figure 4.14: Calibration curve of mouse interleukin-6

4.6. Statistical analysis

The observations of all experiments were expressed as mean $\pm$ S.E.M. (standard error mean). All data were analyzed using one-way analysis of variance (ANOVA) and two-way ANOVA followed by the Tukey test (Sigma Stat. Software version 3.5). The values $p < 0.05$ were considered a significant difference in the obtained data results.

4.7. Histopathological assessment

Histopathological assessment of cerebral cortex and hippocampus regions of the mice brain was performed with hematoxylin and eosin staining (**Humanson, 1962**).

Assay procedure:

Transverse sections (7-9 mm thickness) of the cerebral cortex and hippocampus were fixed in formal calcium and embedded in paraffin wax. Then, it was cut and dewaxed in xylene, hydrated in reducing alcohol percentage, stained with hematoxylin, dehydrated in enhancing alcohol percentage to 70%, and stained with 1 % alcoholic eosin. Further, differentiated in 90% alcohol and cleared in xylene. The stained transverse sections were observed and took images under the microscope (Zeiss Primo star) for histopathological assessment.

CHAPTER – 5

RESULTS

5. Results

5.1. Bromelain

5.1.1. Behavioral analysis

5.1.1.1. Bromelain alone and in combination with Donepezil improve SRM and SWM in AlCl_3 and D - galactose induced AD mice in MWM test

In the MWM task, SRM data analysis by two-way ANOVA was found considerable differences between treatments ($F_{(5, 90)} = 84.037$, $P < 0.001$) as well as the test days ($F_{(2, 90)} = 7.718$, $P < 0.001$). There was no significant interaction between treatments, and days ($F_{(10, 90)} = 0.131$, $P = 0.999$) **Table 5.1a and Figure 5.1a**. Data analysis has shown that on training day 81, 82, and 83; the model (AlCl_3 + D - galactose treated) group was found to significantly enhance escape latency as compared to control, representing increased cognitive dysfunction and decline in the reference memory of mice. The treatments with Dnpz (2mg/kg), Brm L (10mg/kg), Brm H (20mg/kg), and Brm L + Dnpz to induced AD mice were observed significantly attenuation in escape latency as compared to the model. The concomitant treatment with Brm L + Dnpz to AD-induced mice was shown to significantly reduce escape latency as compared to Dnpz and Brm L treated mice, signifying decreased cognitive dysfunction and enhance reference memory of mice. However, no significant variation was observed among the day 81, 82, and 83.

On day 84, a probe trial task was conducted for evaluating the retention of memory. The time passed in the target quadrant, the significant differences were observed among treatments ($F_{(5, 30)} = 68.109$, $P < 0.001$) **Table 5.1b and Figure 5.1b**, according to a one-way ANOVA study. The model was observed to significantly reduce the time passed in the target quadrant as compared to the control. The treatments with Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz to induced AD mice were shown to significantly enhance the time passed in the target quadrant than the model. The concomitant treatment with BrmL + Dnpz to AD-induced mice was found to significantly increase the time passed in the target quadrant than Dnpz and Brm L treated mice, representing memory improvement in mice.

Table 5.1a: Effect of Bromelain on spatial reference memory of AD-induced mice in MWM

Day	Control	M	M + Dnpz	M + Brm L	M + Brm H	M + Brm L + Dnpz
Day-81	14.208 ± 4.343	67.041 ± 3.515***	38.125 ± 1.700###	45.000 ± 3.985###	40.833 ± 2.264###	16.541 ± 1.697####,^^,\$\$\$
Day-82	9.500 ± 1.200	64.375 ± 5.791***	32.291 ± 1.901###	39.041 ± 3.764###	34.083 ± 3.760###	11.458 ± 2.540####,^^,\$\$\$
Day-83	7.250 ± 0.642	59.125 ± 7.938***	29.041 ± 1.619###	34.250 ± 2.317###	31.000 ± 2.889###	8.875 ± 0.960####,^^,\$\$\$

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from control group; ###p<0.001 significant from M treated group; p<0.05, ^^p<0.01 and ^^^p<0.001 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm L treated group (two way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group; AD: Alzheimer's disease; MWM: Morris water maze.

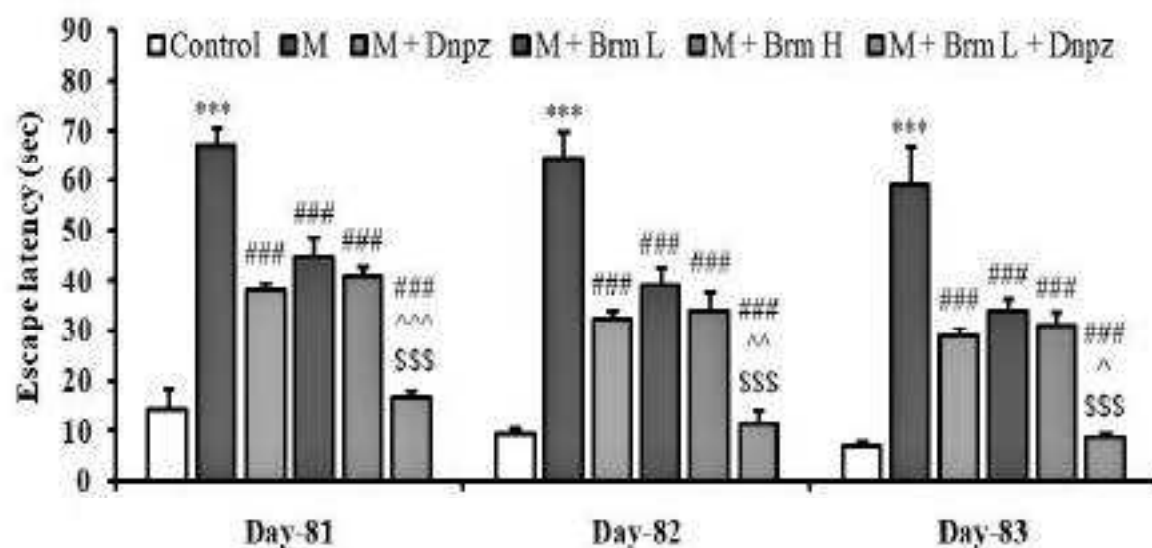


Figure 5.1a: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on spatial reference memory of (AlCl₃ + D - galactose) administered mice during training days 81, 82 and 83. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; ###p<0.001 significant from M treated group; p<0.05, ^^p<0.01 and ^^^p<0.001 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm L treated group (two way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

Table 5.1b: Effect of Bromelain on time spent in target quadrant (Probe trial) of AD-induced mice in MWM

Day	Control	M	M + Dnpz	M + Brm L	M + Brm H	M + Brm L + Dnpz
Day-84	49.125 ± 2.739	6.458 ± 0.748 ^{***}	23.583 ± 1.452 ^{###}	16.625 ± 1.936 [#]	20.041 ± 2.276 ^{###}	42.708 ± 2.053 ^{###,^^,\$\$\$}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***} p<0.001 significant from control group; [#] p<0.05 and ^{###} p<0.001 significant from M treated group; ^{^^} p<0.001 significant from Dnpz treated group; ^{\$\$\$} p<0.001 significant from Brm L treated group (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group; AD: Alzheimer's disease; MWM: Morris water maze.

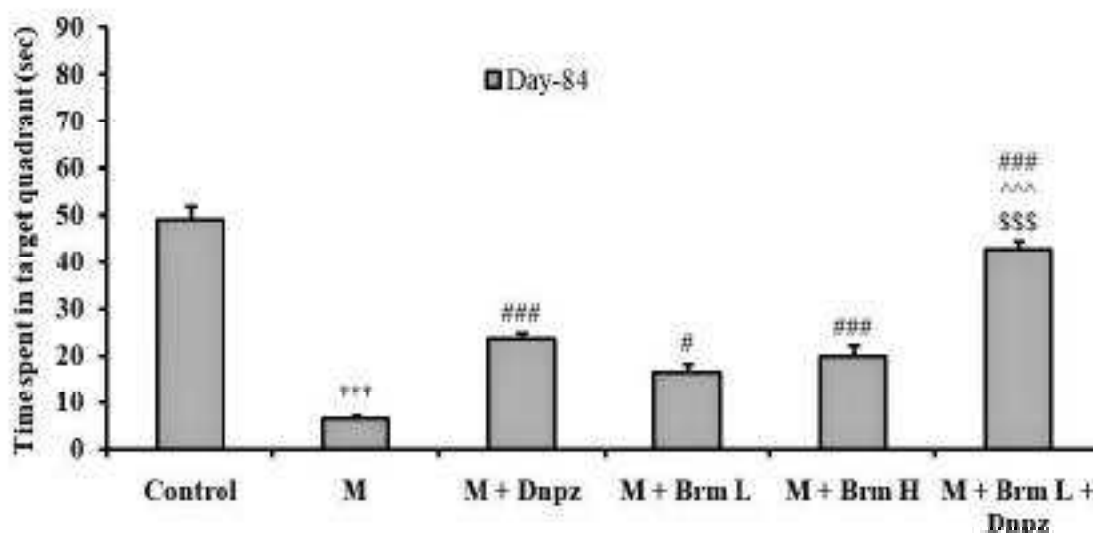


Figure 5.1b: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on time spent in target quadrant (Probe trial) of (AlCl₃ + D - galactose) administered mice in MWM test. Bars in chart represent the mean ± S.E.M. of each group (n = 6). ^{***} p<0.001 significant from control group; [#] p<0.05 and ^{###} p<0.001 significant from M treated group; ^{^^} p<0.001 significant from Dnpz treated group; ^{\$\$\$} p<0.001 significant from Brm L treated group (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

In the SWM task; an acquisition and retrieval trial was performed to evaluate the spatial working memory of mice on days 87, 88, 89, and 90. The acquisition and retrieval trial data analysis by using two-way ANOVA has shown considerable variations in escape latency among the treatments respectively. In the acquisition trial; two-way ANOVA analysis was found significant changes among treatments ($F_{(5, 120)} = 104.401$, $P < 0.001$), and test days ($F_{(3, 120)} = 5.200$, $P = 0.002$). No significant interaction between treatments and days was obtained ($F_{(15, 120)} = 0.037$, $P = 1.00$), **Table 5.2a and Figure 5.2a**. The model was found to significantly increase escape latency as compared to control demonstrating enhance cognitive dysfunction and a decrease in the working memory of mice. The treatments with Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz to induced AD mice were shown a significant reduction in escape latency as compared to the model. The concomitant treatment with BrmL + Dnpz to AD-induced mice was found to significantly decrease escape latency as compared to Dnpz and Brm L treated mice, indicating reduce cognitive dysfunction and increase working memory of mice. Similarly, in the retrieval trial; two-way ANOVA analysis were found considerable changes among treatments ($F_{(5, 120)} = 104.390$, $P < 0.001$), and test days ($F_{(3, 120)} = 3.535$, $P = 0.017$). No significant changes between treatments and days was obtained ($F_{(15, 120)} = 0.0912$, $P = 1.000$) **Table 5.2b and Figure 5.2b**. A significant increase and decrease in escape latency were observed in the model and treatments respectively. However, no significant differences were observed among the days 87, 88, 89, and 90 in the acquisition and retrieval trial.

Table 5.2a: Effect of Bromelain on spatial working memory (Acquisition trial) of AD-induced mice in MWM

Day	Control	M	M + Dnpz	M + Brm L	M + Brm H	M + Brm L + Dnpz
Day-87	13.541 ± 2.447	81.583 ± 2.887 ^{***}	44.625 ± 3.848 ^{###}	51.458 ± 8.033 ^{###}	46.458 ± 2.677 ^{###}	15.791 ± 3.406 ^{###, ^^, \$\$\$}
Day-88	11.041 ± 2.805	76.166 ± 5.581 ^{***}	41.291 ± 3.586 ^{###}	46.541 ± 9.373 ^{###}	42.875 ± 3.765 ^{###}	12.500 ± 2.967 ^{###, ^^, \$\$\$}
Day-89	9.500 ± 3.032	75.500 ± 4.096 ^{***}	39.291 ± 2.979 ^{###}	44.833 ± 11.181 ^{###}	41.416 ± 5.163 ^{###}	10.416 ± 2.734 ^{###, ^^, \$\$\$}
Day-90	5.708 ± 0.350	67.916 ± 5.389 ^{***}	34.833 ± 3.279 ^{###}	38.583 ± 5.306 ^{###}	36.208 ± 2.734 ^{###}	5.958 ± 0.876 ^{###, ^^, \$\$\$}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p < 0.001 significant from control group; ^{###}p < 0.001 significant from M treated group; ^{^^}p < 0.001 significant from Dnpz treated group; ^{\$\$\$}p < 0.001 significant from Brm L treated group; (two way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group; AD: Alzheimer's disease; MWM: Morris water maze.

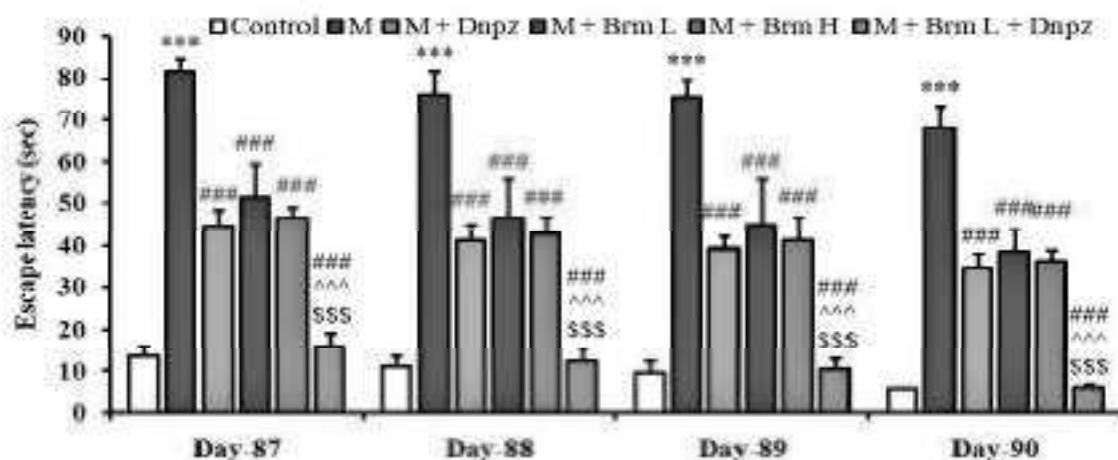


Figure 5.2a: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on spatial working memory of (AlCl₃ + D - galactose) administered mice at days 87, 88, 89 and 90, acquisition trial. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p < 0.001 significant from the control group; ^{###}p < 0.001 significant from M treated group; ^{^^}p < 0.001 significant from Dnpz treated group; ^{\$\$\$}p < 0.001 significant from Brm L treated group; (two way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animals in each group.

Table 5.2b: Effect of Bromelain on spatial working memory (Retrieval trial) of AD-induced mice in MWM

Day	Control	M	M + Dnpz	M + Brm L	M + Brm H	M + Brm L + Dnpz
Day-87	12.875 ± 3.579	80.916 ± 1.227***	43.791 ± 1.951###	48.833 ± 6.218###	44.416 ± 3.258###	14.375 ± 3.006###, ^^^, \$\$\$
Day-88	10.458 ± 1.975	79.333 ± 2.413***	41.875 ± 5.939###	45.666 ± 9.744###	42.125 ± 3.730###	12.708 ± 2.787###, ^^^, \$\$\$
Day-89	7.416 ± 0.554	73.500 ± 5.569***	39.541 ± 4.596###	42.958 ± 7.818###	40.750 ± 7.084###	10.500 ± 1.738###, ^^^, \$\$\$
Day-90	6.291 ± 0.705	67.041 ± 6.446***	36.125 ± 5.312###	39.750 ± 7.167###	37.750 ± 4.391###	7.583 ± 0.902###, ^^^, \$\$\$

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from control group; ##p<0.01 and ###p<0.001 significant from M treated group; ^^p<0.01 and ^^^p<0.001 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm L treated group; (two way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group; AD: Alzheimer's disease; MWM: Morris water maze.

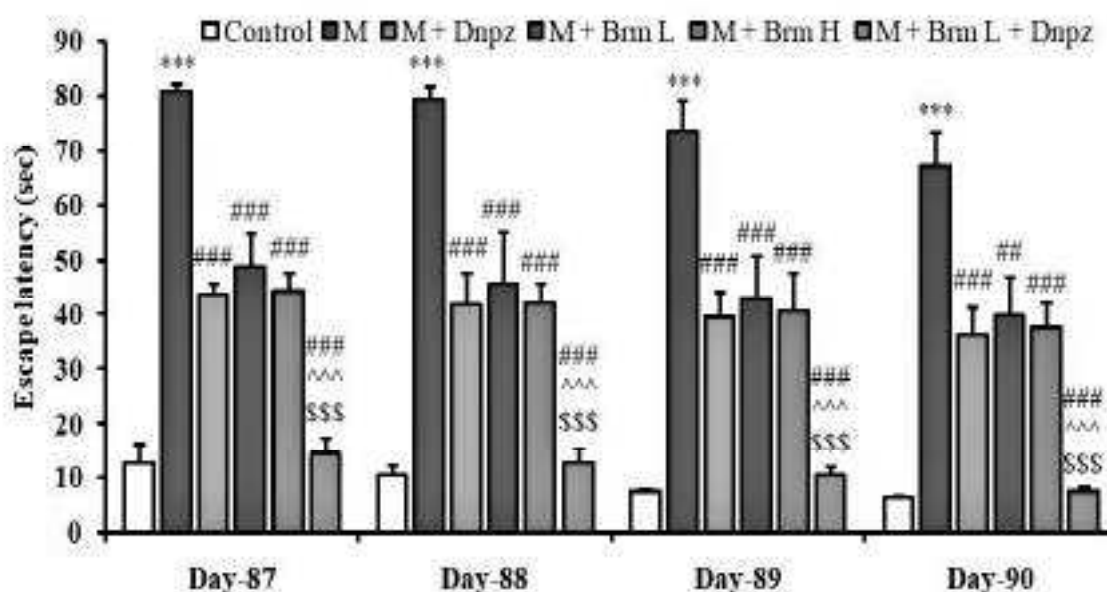


Figure 5.2b: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on spatial working memory of (AlCl₃ + D - galactose) administered mice at days 87, 88, 89 and 90, retrieval trial. Bars in chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from control group; ##p<0.01 and ###p<0.001 significant from M treated group; ^^p<0.01 and ^^^p<0.001 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm L treated group; (two way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

5.1.1.2. Bromelain alone and in combination with Donepezil improve the learning memory of AlCl_3 and D - galactose induced AD mice in the EPM test

Two way ANOVA analysis were found significant variations among treatments ($F_{(5, 90)} = 43.536$, $P < 0.001$) as well as test days ($F_{(2, 90)} = 109.780$, $P < 0.001$). A significant interaction between treatments and days were obtained ($F_{(10, 90)} = 7.314$, $P < 0.001$) **Table 5.3 and Figure 5.3**. Thus, it was observed that on day 81; in any treatment, transfer latency did not differ significantly. On day 82 and 90; the Model (AlCl_3 + D - galactose treated) group was found to significantly increase transfer latency than the control (vehicle-treated) group. The treatments with Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz to induced AD mice were found significantly attenuation in transfer latency as compared to the model. The concomitant treatment with BrmL + Dnpz to AD-induced mice was observed to significantly decrease transfer latency as compared to Dnpz and Brm L treated mice, demonstrating improvement in learning along with the memory of mice. However, significant differences in transfer latency were observed on day 82 and 90 as compared to day 81.

5.1.1.3. Bromelain alone and in combination with Donepezil show no locomotor activity alteration of AlCl_3 and D - galactose induced AD mice

Locomotor activity was performed on day 0, 80, and 90 to assess the effect of treatments on spontaneous locomotion of mice of all groups. Data analysis by one-way ANOVA showed that the model (AlCl_3 + D-galactose) group was not found significantly than the control (vehicle-treated) group. The treatments with Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz to induced AD mice were not observed significantly as compared to the model. The concomitant treatment with BrmL + Dnpz to AD-induced mice was not found significantly as compared to Dnpz and Brm L treated mice, signifying no alteration in locomotor activity of mice throughout the study as shown in **Table 5.4**.

Table 5.3: Effect of Bromelain on transfer latency of AD-induced mice in EPM

Day	Control	M	M + Dnpz	M + Brm L	M + Brm H	M + Brm L + Dnpz
Day-81	45.500 ± 3.956	51.666 ± 3.062	46.500 ± 3.712	48.500 ± 3.452	47.166 ± 4.206	46.166 ± 4.840
Day-82	10.333 ± 1.475 ^a	52.166 ± 2.937 ^{***}	29.500 ± 1.056 ^{###, a}	37.666 ± 2.333 ^{#, a}	33.166 ± 1.833 ^{###, a}	11.833 ± 2.468 ^{###, ^^^, \$\$\$, a}
Day-90	5.666 ± 0.954 ^a	47.333 ± 4.835 ^{***}	23.000 ± 2.236 ^{###, a}	31.166 ± 2.650 ^{###, a}	26.500 ± 1.408 ^{###, a}	5.833 ± 1.301 ^{###, ^^^, \$\$\$, a}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; ^{^^^}p<0.001 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm L treated group; ^a represent significant from day 81 (two way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group; AD: Alzheimer's disease; EPM: Elevated Plus maze.

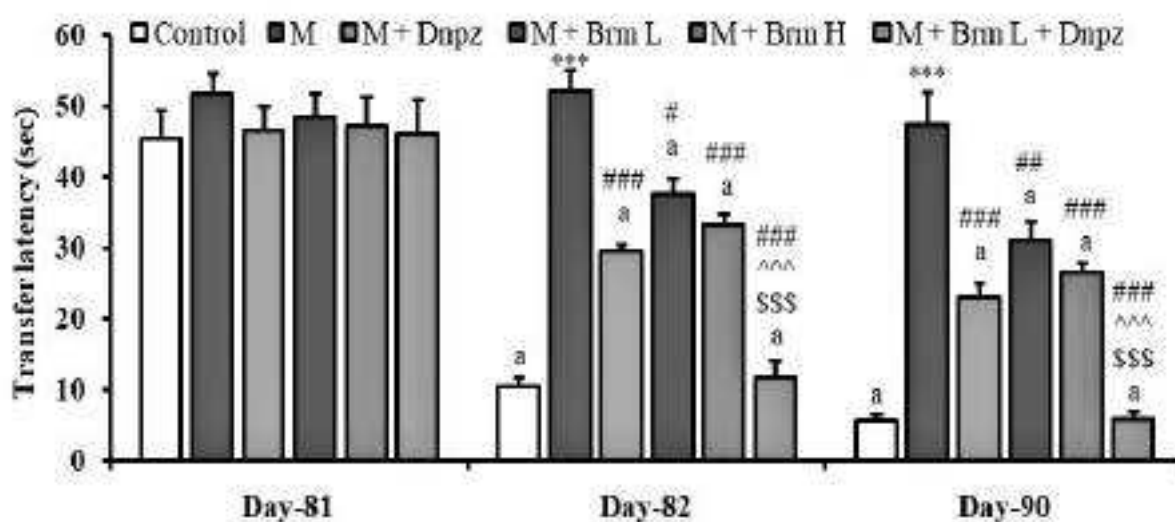


Figure 5.3: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on transfer latency of (AlCl₃ + D - galactose) administered mice in elevated plus maze test. Bars in chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; ^{^^^}p<0.001 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm L treated group; ^a represent significant from day 81 (two way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

Table 5.4: Effect of Bromelain on locomotor activity of AD-induced mice

Groups	Day 0		Day 80		Day 90	
	Before dose	After dose	Before dose	After dose	Before dose	After dose
Control	388.33 ± 31.43	367.67 ± 22.16	361.17 ± 17.43	355.17 ± 13.95	376.33 ± 10.41	364.50 ± 8.86
M	355.50 ± 21.92	328.17 ± 18.11	348.33 ± 10.22	342.67 ± 8.41	362.00 ± 6.60	349.67 ± 10.73
M + Dnpz	379.17 ± 20.93	351.00 ± 15.46	359.00 ± 8.33	355.00 ± 7.02	358.00 ± 10.55	340.33 ± 4.48
M + Brm L	361.50 ± 15.74	367.33 ± 14.52	334.33 ± 15.06	333.17 ± 14.03	366.17 ± 25.04	329.50 ± 22.44
M + Brm H	338.50 ± 11.31	337.00 ± 9.43	349.50 ± 17.17	344.83 ± 15.79	366.33 ± 11.49	358.33 ± 10.43
M + Brm L + Dnpz	350.00 ± 5.92	345.00 ± 7.63	352.50 ± 6.26	348.17 ± 7.28	347.83 ± 14.74	342.50 ± 13.57

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz) treatment on locomotion of (AlCl_3 + D - galactose) administered mice at day 0; day 80; and day 90. Value expressed as the mean $\pm$ S.E.M. of each group (n=6), (one way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

5.1.2. Biochemical analysis

5.1.2.1. Bromelain alone and in combination with Donepezil inhibit the acetylcholinesterase activity in different brain regions of AlCl_3 and D - galactose induced AD mice

AChE activity was considered at the end of the study in the both region of mice brain for assessing the effect of treatments on cholinergic function. The one-way ANOVA study exposed that the treatments had a significant impact on levels of AChE enzyme in the cortex ($F_{(5, 30)} = 33.010$, $P < 0.001$) as well as the hippocampus ($F_{(5, 30)} = 20.580$, $P < 0.001$) of the mice brain. When compared to the control, the model (AlCl_3 + D - galactose treated) showed a significant increase in AChE amount in the cerebral cortex as well as the hippocampus. However, when Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz were given to AD-induced mice, the AChE amount in the cerebral cortex along with the hippocampus was found to be significantly lower than in the model. In comparison to Dnpz and Brm L treated mice, concomitant treatment with BrmL + Dnpz considerably reduced the AChE levels in the cerebral cortex along with the hippocampus. In the hippocampus, the higher dose of Brm showed a better effect ($p < 0.01$) than the lower dose of Brm ($p < 0.05$) as depicted by the increased significant level. The best effect was observed in Brm + Dnpz combination group with the highest level of significance ($p < 0.001$), as shown in **Table 5.5 and Figure 5.4**. Therefore, the treatment of drugs efficiently inhibited AChE activity and subsequently restored the cerebral cortex and the hippocampus acetylcholine homeostasis which led to enhancement in learning and memory in AD model mice.

Table 5.5: Effect of Bromelain on AChE of AD-induced mice

Treatment	Mice brain regions	AChE (n moles per mg protein)
Control	Cerebral cortex	2.093 ± 0.284
	Hippocampus	2.026 ± 0.592
M	Cerebral cortex	8.372 ± 0.465 ^{***}
	Hippocampus	8.154 ± 0.666 ^{***}
M + Dnpz	Cerebral cortex	5.161 ± 0.378 ^{###}
	Hippocampus	5.001 ± 0.368 ^{###}
M + Brm L	Cerebral cortex	6.234 ± 0.393 ^{##}
	Hippocampus	5.751 ± 0.337 [#]
M + Brm H	Cerebral cortex	5.780 ± 0.515 ^{###}
	Hippocampus	5.403 ± 0.459 ^{##}
M + Brm L + Dnpz	Cerebral cortex	3.274 ± 0.199 ^{###, ^, \$\$\$}
	Hippocampus	2.918 ± 0.359 ^{###, ^, \$\$}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; AChE: Acetylcholinesterase; n = Number of animal in each group.

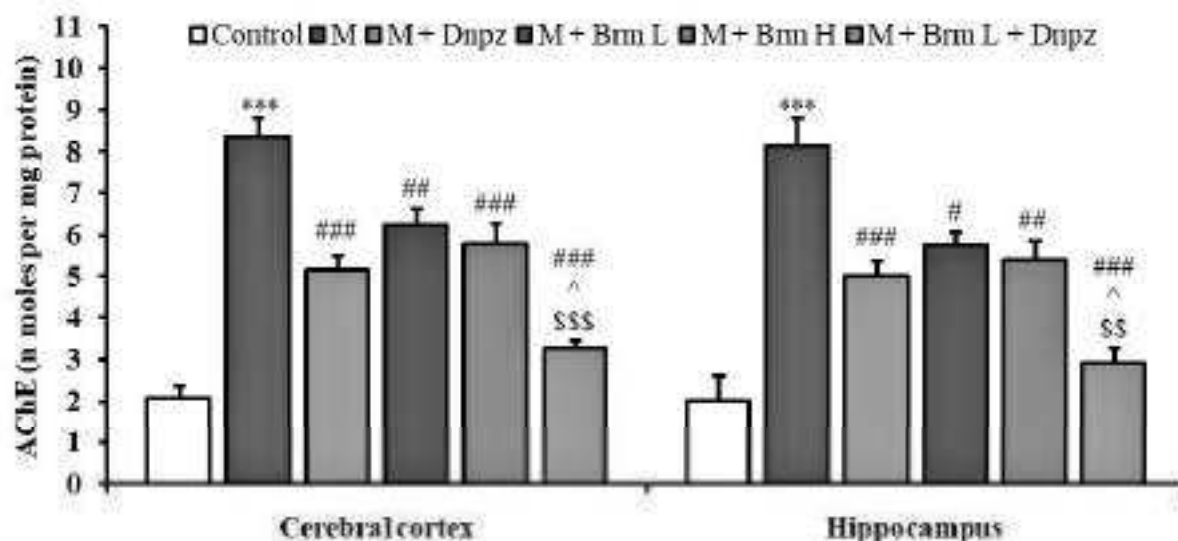


Figure 5.4: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on AChE level in cerebral cortex and hippocampus of (AlCl₃ + D - galactose)administered mice. Bars in chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M= Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; AChE = Acetylcholinesterase; n = Number of animal in each group.

5.1.2.2. Bromelain alone and in combination with Donepezil decrease the MDA level in different brain regions of AlCl₃ and D - galactose induced AD mice

Lipid peroxidation measured the MDA amount in the cerebral cortex along with the hippocampus to consider the treatments effect on antioxidant. The treatments had a significant impact on MDA level in the cortex ($F_{(5, 30)} = 17.592$, $P < 0.001$) as well as the hippocampus ($F_{(5, 30)} = 14.426$, $P < 0.001$) of the mice brain, according to one-way ANOVA study. When compared to the control group, the model (AlCl₃ + D-galactose) group showed a significantly enhance MDA amount in the cerebral cortex along with the hippocampus. Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz treatments to induced AD mice were found to significantly reduce MDA level in the cerebral cortex along with the hippocampus than the model. In comparison to Dnpz and Brm L treated mice, concomitant treatment with BrmL + Dnpz resulted in a significantly decrease MDA level in the cerebral cortex as well as the hippocampus. In cerebral cortex and hippocampus, a higher dose of Brm was observed to have a better significant effect ($p < 0.001$ and $p < 0.001$ respectively) as compare to the effect ($p < 0.001$ and $p < 0.05$ respectively) of lower dose of Brm, as shown in **Table 5.6** and **Figure 5.5**.

5.1.2.3. Bromelain alone and in combination with Donepezil increase the reduced glutathione level in different brain regions of AlCl₃ and D - galactose induced AD mice

GSH level was evaluated in the cerebral cortex along with the hippocampus for assessing treatments effect on antioxidant. In one way ANOVA study, the treatments had a significant impact on the level of GSH in the cerebral cortex ($F_{(5, 30)} = 16.862$, $P < 0.001$) and the hippocampus ($F_{(5, 30)} = 21.198$, $P < 0.001$) regions of the mice brain. In comparison to the control group, the model (AlCl₃ + D - galactose treated) group had significantly attenuated GSH in the cerebral cortex along with the hippocampus. Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz treatments to induced AD mice significantly enhanced GSH level in the cerebral cortex along with the with the hippocampus than the model. In comparison to Dnpz and Brm L treated mice, concomitant treatment with BrmL + Dnpz significantly increase GSH in the cerebral cortex along with the hippocampus, as shown in **Table 5.7** and **Figure 5.6**.

Table 5.6: Effect of Bromelain on MDA of AD-induced mice

Treatment	Mice brain regions	MDA (n moles per mg protein)
Control	Cerebral cortex	0.843 ± 0.079
	Hippocampus	0.753 ± 0.127
M	Cerebral cortex	4.251 ± 0.389 ^{***}
	Hippocampus	3.743 ± 0.300 ^{***}
M + Dnpz	Cerebral cortex	2.404 ± 0.262 ^{##}
	Hippocampus	2.285 ± 0.483 [#]
M + Brm L	Cerebral cortex	2.651 ± 0.314 ^{##}
	Hippocampus	2.269 ± 0.330 [#]
M + Brm H	Cerebral cortex	2.044 ± 0.436 ^{###}
	Hippocampus	1.399 ± 0.203 ^{###}
M + Brm L + Dnpz	Cerebral cortex	0.959 ± 0.145 ^{###, ^, \$\$}
	Hippocampus	0.888 ± 0.180 ^{###, ^, \$}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05, and ^{\$\$}p<0.01 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; MDA = Malondialdehyde; n = Number of animal in each group; AD: Alzheimer's disease.

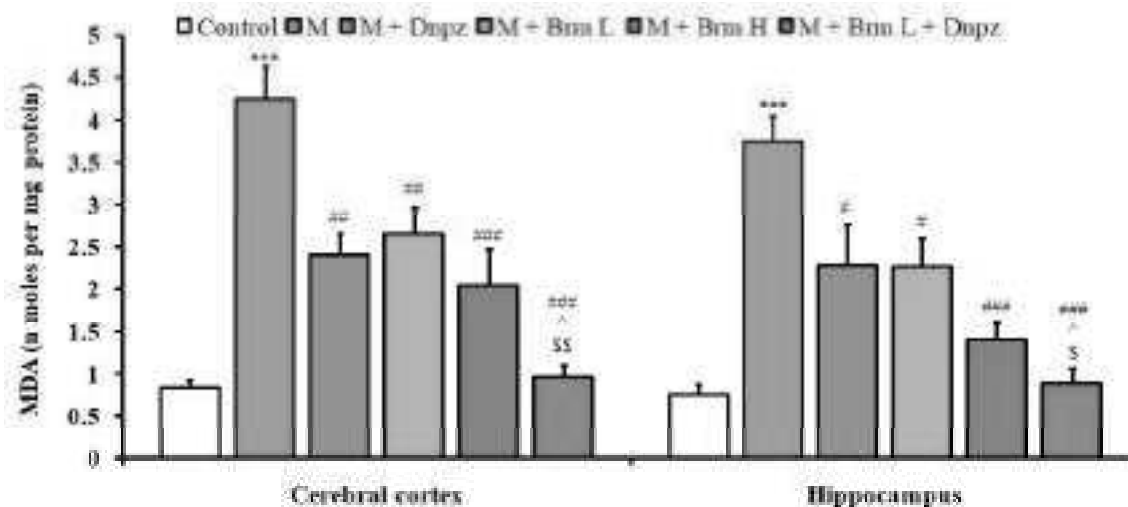


Figure 5.5: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on MDA level in cerebral cortex and hippocampus of (AlCl₃ + D - galactose)administered mice. Bars in chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05, and ^{\$\$}p<0.01 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; MDA = Malondialdehyde; n = Number of animal in each group.

Table 5.7: Effect of Bromelain on GSH of AD-induced mice

Treatment	Mice brain regions	GSH (n moles per mg protein)
Control	Cerebral cortex	3.255 ± 0.210
	Hippocampus	3.056 ± 0.274
M	Cerebral cortex	1.130 ± 0.099 ^{***}
	Hippocampus	0.750 ± 0.166 ^{***}
M + Dnpz	Cerebral cortex	2.101 ± 0.288 ^{##}
	Hippocampus	1.629 ± 0.126 [#]
M + Brm L	Cerebral cortex	1.950 ± 0.113 [#]
	Hippocampus	1.641 ± 0.179 [#]
M + Brm H	Cerebral cortex	2.153 ± 0.226 ^{##}
	Hippocampus	1.717 ± 0.135 ^{##}
M + Brm L + Dnpz	Cerebral cortex	2.969 ± 0.075 ^{###, ^, \$\$}
	Hippocampus	2.641 ± 0.149 ^{###, ^^, \$\$}

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ^{***} p<0.001 significant from control group; [#] p<0.05, ^{##} p<0.01 and ^{###} p<0.001 significant from M treated group; [^] p<0.05 and ^{^^} p<0.01 significant from Dnpz treated group; ^{\$\$} p<0.01 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; GSH = Reduced glutathione; n = Number of animal in each group; AD: Alzheimer's disease.

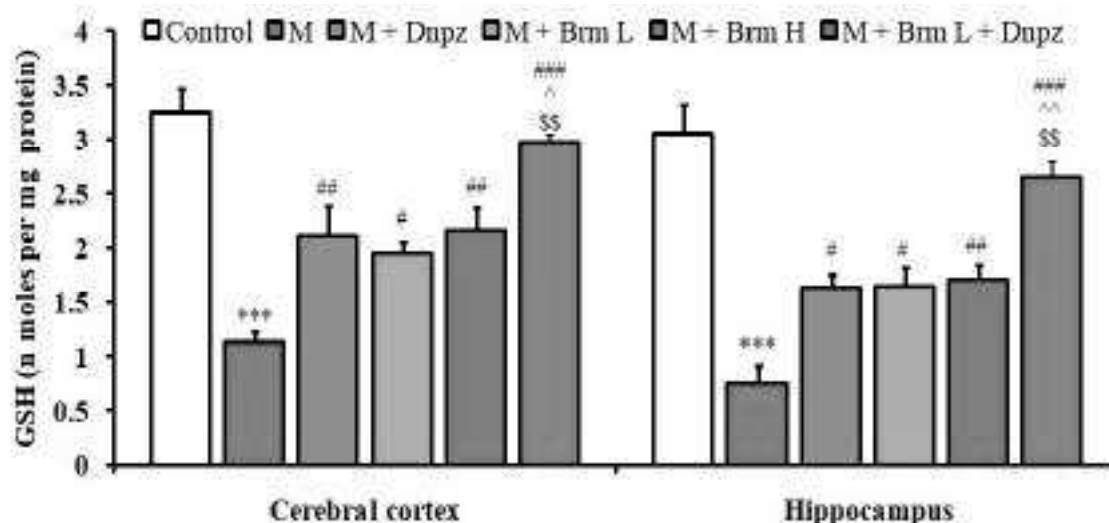


Figure 5.6: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on GSH level in cerebral cortex and hippocampus of (AlCl_3 + D - galactose)administered mice. Bars in chart represent the mean $\pm$ S.E.M. of each group (n = 6). ^{***} p<0.001 significant from control group; [#] p<0.05, ^{##} p<0.01 and ^{###} p<0.001 significant from M treated group; [^] p<0.05 and ^{^^} p<0.01 significant from Dnpz treated group; ^{\$\$} p<0.01 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; GSH = Reduced glutathione; n = Number of animal in each group.

5.1.2.4. Bromelain alone and in combination with Donepezil increase the catalase level in different brain regions of AlCl₃ and D - galactose induced AD mice

CAT level was estimated in the cerebral cortex along with the hippocampus to calculate the effect of the treatment. In one way ANOVA study, the treatments had a significant effect on the level of CAT in the cortex ($F_{(5, 30)} = 23.998$, $P < 0.001$) as well as the hippocampus ($F_{(5, 30)} = 19.678$, $P < 0.001$) of the mice brain. In comparison to the control (vehicle-treated) group, the model (AlCl₃ + D - galactose treated) group had significantly attenuated CAT amount in the cerebral cortex along with the hippocampus. When induced AD mice were given Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz, CAT amount in the cerebral cortex along with the hippocampus was found to be significantly higher than the model. The concomitant treatment with BrmL + Dnpz to AD-induced mice was found to significantly increase CAT amount in cerebral cortex along with the hippocampus than Dnpz and Brm L treated mice, as shown in **Table 5.8** and **Figure 5.7**.

5.1.2.5. Bromelain alone and in combination with Donepezil increase the superoxide dismutase level in different brain regions of AlCl₃ and D - galactose induced AD mice

SOD level was calculated in the cerebral cortex as well as the hippocampus to assess treatments effect on antioxidant. The results of the one-way ANOVA showed the treatments had a significant impact on the level of SOD in the cortex ($F_{(5, 30)} = 25.122$, $P < 0.001$) as well as the hippocampus ($F_{(5, 30)} = 16.651$, $P < 0.001$) of the mice brain. In comparison to the control (vehicle-treated) group, the model (AlCl₃ + D - galactose treated) group had significantly decreased SOD level in the cerebral cortex along with the hippocampus. When Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz were given to AD mice the SOD amount in the cerebral cortex along with the hippocampus were considerably higher than in the model. In comparison to Dnpz and BrmL treated mice, concomitant treatment with BrmL + Dnpz significantly enhanced the SOD level in the cerebral cortex as well as the hippocampus, as shown in **Table 5.9** and **Figure 5.8**.

Table 5.8: Effect of Bromelain on CAT of AD-induced mice

Treatment	Mice brain regions	CAT (Units per mg protein)
Control	Cerebral cortex	0.975 ± 0.064
	Hippocampus	0.929 ± 0.101
M	Cerebral cortex	0.328 ± 0.052***
	Hippocampus	0.248 ± 0.021***
M + Dnpz	Cerebral cortex	0.645 ± 0.032###
	Hippocampus	0.560 ± 0.034##
M + Brm L	Cerebral cortex	0.612 ± 0.031##
	Hippocampus	0.542 ± 0.020##
M + Brm H	Cerebral cortex	0.668 ± 0.047###
	Hippocampus	0.618 ± 0.056###
M + Brm L + Dnpz	Cerebral cortex	0.907 ± 0.048###, ^, \$\$
	Hippocampus	0.877 ± 0.058###, ^, \$\$

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from control group; ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.01 significant from Dnpz treated group; \$\$p<0.01 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; CAT =Catalase; n = Number of animal in each group; AD: Alzheimer's disease.

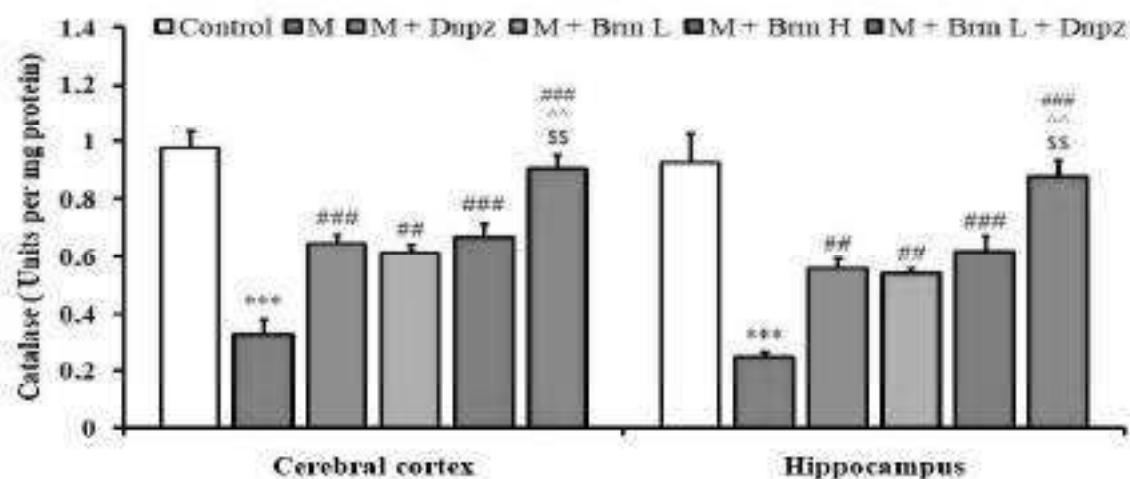


Figure 5.7: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on CAT level in cerebral cortex and hippocampus of (AlCl₃ + D - galactose)administered mice. Bars in chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from control group; ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.01 significant from Dnpz treated group; \$\$p<0.01 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; CAT =Catalase; n = Number of animal in each group.

Table 5.9: Effect of Bromelain on SOD of AD-induced mice

Treatment	Mice brain regions	SOD (Units per mg protein)
Control	Cerebral cortex	2.184 ± 0.167
	Hippocampus	2.105 ± 0.196
M	Cerebral cortex	0.610 ± 0.055 ^{***}
	Hippocampus	0.406 ± 0.110 ^{***}
M + Dnpz	Cerebral cortex	1.382 ± 0.047 ^{###}
	Hippocampus	1.137 ± 0.122 [#]
M + Brm L	Cerebral cortex	1.163 ± 0.077 [#]
	Hippocampus	1.102 ± 0.135 [#]
M + Brm H	Cerebral cortex	1.359 ± 0.079 ^{###}
	Hippocampus	1.290 ± 0.155 ^{##}
M + Brm L + Dnpz	Cerebral cortex	1.975 ± 0.179 ^{###, ^, \$\$\$}
	Hippocampus	1.907 ± 0.165 ^{###, ^, \$\$}

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; SOD = Superoxide dismutase; n = Number of animal in each group; AD: Alzheimer's disease.

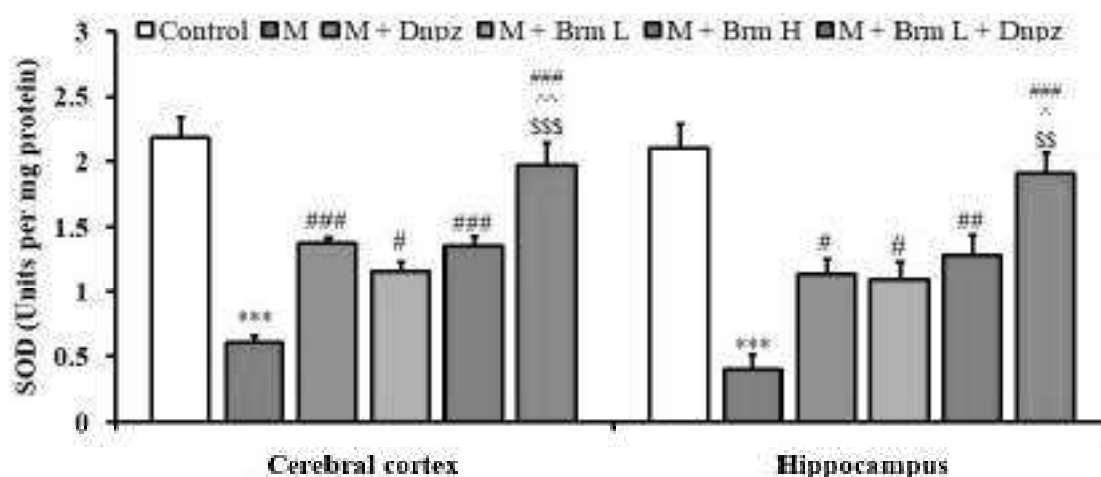


Figure 5.8: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on SOD level in cerebral cortex and hippocampus of (AlCl_3 + D - galactose)administered mice. Bars in chart represent the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; SOD = Superoxide dismutase; n = Number of animal in each group.

5.1.2.6. Bromelain alone and in combination with Donepezil reduce the nitrite level in different brain regions of AlCl₃ and D - galactose induced AD mice

The level of NO in the cortex ($F_{(5, 30)} = 19.971$, $P < 0.001$) as well as the hippocampus ($F_{(5, 30)} = 21.374$, $P < 0.001$) of the mice brain was significantly affected by the treatments, according to one way ANOVA study. In comparison to the control group, the model (AlCl₃ + D - galactose treated) showed a significantly enhance NO amount in the cerebral cortex along with the hippocampus. Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz treatments to induced AD mice resulted in a considerable reduction level of NO in the cerebral cortex along with the hippocampus than the model. In comparison to Dnpz and Brm L treated mice, concomitant treatment with BrmL + Dnpz significantly reduced NO amount in the cerebral cortex along with the hippocampus, as shown in **Table 5.10** and **Figure 5.9**.

5.1.2.7. Bromelain alone and in combination with Donepezil increase the brain-derived neurotrophic factor level in different brain regions of AlCl₃ and D - galactose induced AD mice

BDNF level in the cerebral cortex along with the hippocampus was examined to assess treatments effect on neurogenesis in AD mice. BDNF level was analyzed by ELISA. The treatments had a significant impact on the level of BDNF in the cortex ($F(5, 30) = 24.537$, $P < 0.001$) as well as the hippocampus ($F(5, 30) = 23.537$, $P < 0.001$) of the mice brain, according to one way ANOVA study. In comparison to the control group, the model (AlCl₃ + D-galactose) group showed a significantly lower BDNF amount in the cerebral cortex along with the hippocampus. Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz treatments significantly increase BDNF level in the cerebral cortex along with the hippocampus in induced AD mice than the model. In comparison to Dnpz and Brm L treated mice, concomitant treatment with BrmL + Dnpz significantly elevated BDNF level in the cerebral cortex as well as the hippocampus, as shown in **Table 5.11** and **Figure 5.10**.

Table 5.10: Effect of Bromelain on Nitrite of AD-induced mice

Treatment	Mice brain regions	Nitrite (μM per mg protein)
Control	Cerebral cortex	0.961 ± 0.223
	Hippocampus	0.772 ± 0.205
M	Cerebral cortex	$4.215 \pm 0.385^{***}$
	Hippocampus	$4.014 \pm 0.180^{***}$
M + Dnpz	Cerebral cortex	$2.454 \pm 0.351^{###}$
	Hippocampus	$2.214 \pm 0.279^{###}$
M + Brm L	Cerebral cortex	$2.918 \pm 0.207^{\#}$
	Hippocampus	$2.702 \pm 0.273^{\#}$
M + Brm H	Cerebral cortex	$2.376 \pm 0.214^{###}$
	Hippocampus	$2.201 \pm 0.354^{###}$
M + Brm L + Dnpz	Cerebral cortex	$1.094 \pm 0.170^{###,^{\wedge},\$ \$ \$}$
	Hippocampus	$0.906 \pm 0.230^{###,^{\wedge},\$ \$ \$}$

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). *** $p < 0.001$ significant from control group; $^{\#}p < 0.05$ and $^{###}p < 0.001$ significant from M treated group; $^{\wedge}p < 0.05$ significant from Dnpz treated group; $^{\$ \$ \$}p < 0.001$ significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group; AD: Alzheimer's disease.

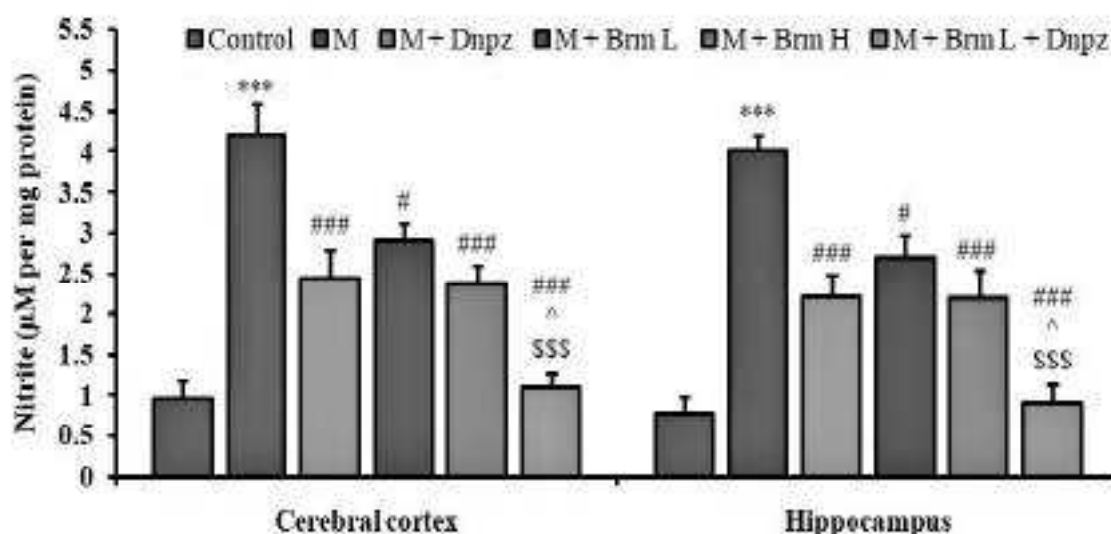


Figure 5.9: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on nitrite level in cerebral cortex and hippocampus of (AlCl_3 + D - galactose) administered mice. Bars in the chart represent the mean $\pm$ S.E.M. of each group (n = 6). *** $p < 0.001$ significant from the control group; $^{\#}p < 0.05$ and $^{###}p < 0.001$ significant from M treated group; $^{\wedge}p < 0.05$ significant from Dnpz treated group; $^{\$ \$ \$}p < 0.001$ significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D-galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

Table 5.11: Effect of Bromelain on BDNF of AD-induced mice

Treatment	Mice brain regions	BDNF (pg/mL)
Control	Cerebral cortex	559.833 ± 12.892
	Hippocampus	523.833 ± 16.924
M	Cerebral cortex	121.166 ± 31.834***
	Hippocampus	110.166 ± 12.752***
M + Dnpz	Cerebral cortex	346.833 ± 39.797###
	Hippocampus	327.500 ± 29.722###
M + Brm L	Cerebral cortex	273.500 ± 37.501 [#]
	Hippocampus	250.000 ± 49.890 [#]
M + Brm H	Cerebral cortex	332.833 ± 22.626###
	Hippocampus	312.833 ± 27.248###
M + Brm L + Dnpz	Cerebral cortex	482.166 ± 34.433###, ^, \$\$\$
	Hippocampus	461.833 ± 32.292###, ^, \$\$\$

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from control group; [#]p<0.05 and ###p<0.001 significant from M treated group; ^p<0.05 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; BDNF = Brain Derived Neurotrophic Factor; n = Number of animal in each group; AD: Alzheimer's disease.

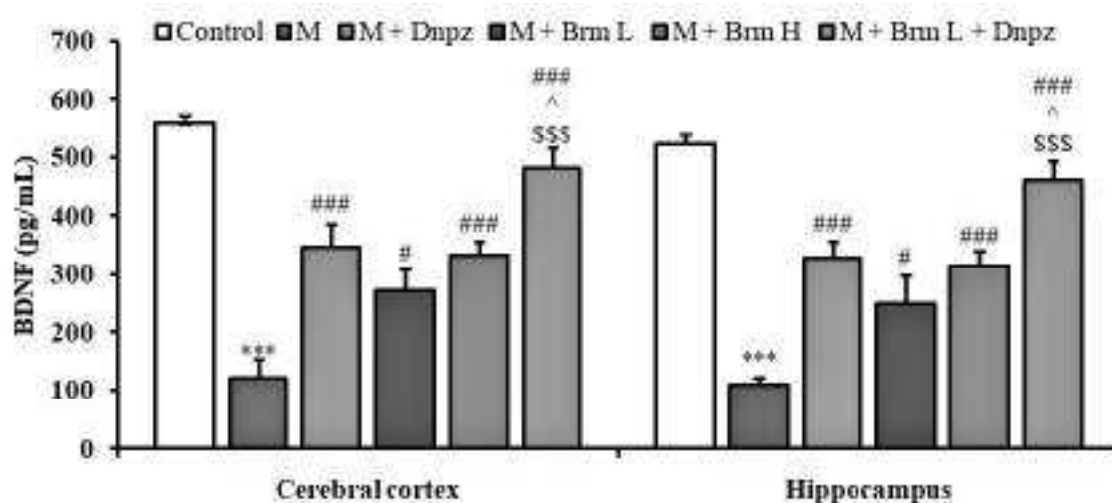


Figure 5.10: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on BDNF level in cerebral cortex and hippocampus of (AlCl₃ + D - galactose)administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; [#]p<0.05 and ###p<0.001 significant from M treated group; ^p<0.05 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; BDNF = Brain Derived Neurotrophic Factor; n = Number of animal in each group.

5.1.2.8. Bromelain alone and in combination with Donepezil decrease the β -secretase level in different brain regions of AlCl_3 and D - galactose induced AD mice

β -secretase level alteration affects the amyloidogenic process of AD as a consequence of fluctuation of amyloid beta-peptide in the cerebral cortex along with the hippocampus. β -secretase level in the cerebral cortex along with the hippocampus was examined to measure treatments effect on the amyloidogenic process of AD. The treatments showed a significant impact on the level of β -secretase in the cortex ($F_{(5, 30)} = 36.550$, $P < 0.001$) as well as the hippocampus ($F_{(5, 30)} = 41.279$, $P < 0.001$) of the mice brain, according to a one way ANOVA study. In contrast to the control (vehicle-treated) group, the model (AlCl_3 + D-galactose) group revealed a considerably higher β -secretase level in the cerebral cortex along with the hippocampus. When induced AD mice were given Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz amount of β -secretase in the cerebral cortex along with the hippocampus was found to be considerably lower than the model. In comparison to Dnpz and Brm L treated AD mice, concomitant treatment with Brm L + Dnpz significantly reduced β -secretase level in the cerebral cortex along with the hippocampus, as shown in **Table 5.12** and **Figure 5.11**.

5.1.2.9. Bromelain alone and in combination with Donepezil decrease the amyloid-beta peptide₁₋₄₂ level in different brain regions of AlCl_3 and D - galactose induced AD mice

The level of amyloid-beta peptide₁₋₄₂ in the cerebral cortex along with the hippocampus was analyzed by ELISA to assess the effect of the treatment on amyloidopathy of AD mice. The treatments showed a significant impact on the amyloid-beta peptide₁₋₄₂ level in the cortex ($F_{(5, 30)} = 16.299$, $P < 0.001$) as well as the hippocampus ($F_{(5, 30)} = 14.825$, $P < 0.001$) of the mice brain, accordance to one way ANOVA study. In comparison to the control (vehicle-treated) group, the model (AlCl_3 + D - galactose treated) significantly enhanced the level of amyloid-beta peptide₁₋₄₂ in the cerebral cortex along with the hippocampus. When induced AD mice were given Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz, levels of amyloid beta peptide₁₋₄₂ in the cerebral cortex along with the hippocampus were considerably reduced than the model. In comparison to Dnpz and Brm L treated mice, concomitant treatment with Brm L + Dnpz significantly reduced amyloid beta peptide₁₋₄₂ level in the cerebral cortex along with the hippocampus, as shown in **Table 5.13** and **Figure 5.12**. Consequently, the treatment of drugs efficiently attenuated the amyloid-beta peptide₁₋₄₂ level which leads to improvement in learning and memory in AD model mice.

Table 5.12: Effect of Bromelain on β - secretase level of AD-induced mice

Treatment	Mice brain regions	β -Secretase (U/L)
Control	Cerebral cortex	11.516 $\pm$ 0.839
	Hippocampus	9.450 $\pm$ 0.852
M	Cerebral cortex	41.366 $\pm$ 1.467 ^{***}
	Hippocampus	38.283 $\pm$ 1.726 ^{***}
M + Dnpz	Cerebral cortex	21.183 $\pm$ 2.543 ^{###}
	Hippocampus	18.416 $\pm$ 2.856 ^{###}
M + Brm L	Cerebral cortex	25.550 $\pm$ 0.933 ^{###}
	Hippocampus	18.983 $\pm$ 1.008 ^{###}
M + Brm H	Cerebral cortex	22.216 $\pm$ 2.743 ^{###}
	Hippocampus	14.283 $\pm$ 1.007 ^{###}
M + Brm L + Dnpz	Cerebral cortex	13.383 $\pm$ 1.007 ^{###, ^, \$\$\$}
	Hippocampus	11.283 $\pm$ 1.321 ^{###, ^, \$\$\$}

Note: Control (Normal saline), Model (AlCl₃ +D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group; AD: Alzheimer's disease.

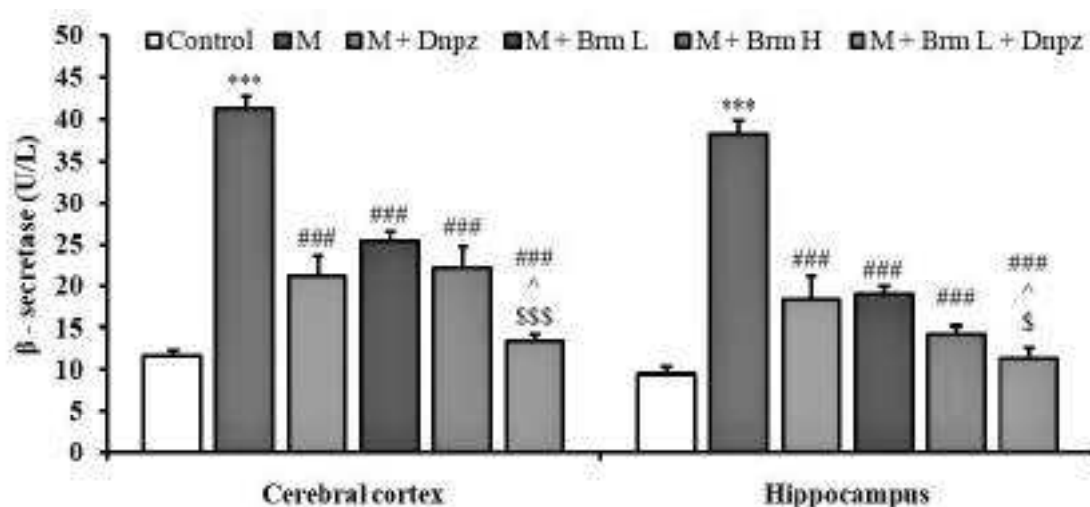


Figure 5.11: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on β - secretase level in cerebral cortex and hippocampus of (AlCl₃ +D - galactose)administered mice. Bars in the chart represent the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

Table 5.13: Effect of Bromelain on amyloid-beta peptide₁₋₄₂ level of AD induced mice

Treatment	Mice brain regions	Amyloid beta peptide ₁₋₄₂ (ng/mL)
Control	Cerebral cortex	2.125 ± 0.591
	Hippocampus	1.810 ± 0.589
M	Cerebral cortex	12.351 ± 0.692 ^{***}
	Hippocampus	11.615 ± 1.003 ^{***}
M + Dnpz	Cerebral cortex	6.648 ± 1.718 ^{##}
	Hippocampus	6.333 ± 1.675 ^{##}
M + Brm L	Cerebral cortex	7.796 ± 0.350 [#]
	Hippocampus	7.004 ± 0.710 [#]
M + Brm H	Cerebral cortex	5.476 ± 1.083 ^{###}
	Hippocampus	4.194 ± 0.616 ^{###}
M + Brm L + Dnpz	Cerebral cortex	2.606 ± 0.349 ^{###,^,\$}
	Hippocampus	2.236 ± 0.567 ^{###,^,\$}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$\$}p<0.01 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group; AD: Alzheimer's disease.

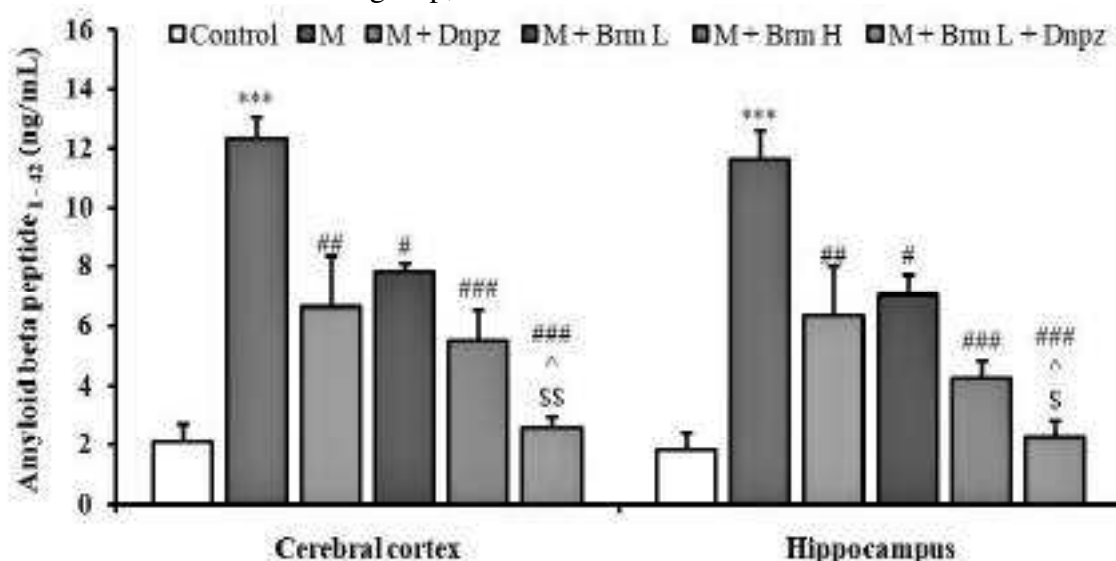


Figure 5.12: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on amyloid beta peptide₁₋₄₂ level in cerebral cortex and hippocampus of (AlCl₃ + D - galactose)administered mice. Bars in chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$\$}p<0.01 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

5.1.2.10. Bromelain alone and in combination with Donepezil decrease the tumor necrosis factor- α level in different brain regions of $AlCl_3$ and D - galactose induced AD mice

TNF- α was found at elevated levels in both plasma and brain of AD patients. The TNF- α level was examined in the cerebral cortex, and the hippocampus to assess treatments effect on neuroinflammation. The treatments showed a significant effect on the level of TNF- α in the cortex ($F_{(5, 30)} = 55.668$, $P < 0.001$) along with the hippocampus ($F_{(5, 30)} = 44.575$, $P < 0.001$) of the mice brain, according to one way ANOVA study. In comparison to the control group, the model ($AlCl_3$ + D-galactose) group significantly enhanced TNF- α level in the cerebral cortex along with the hippocampus. When induced AD mice were given Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz, TNF- α level in the cerebral cortex along with the hippocampus were obtained to be significantly lower than the model. In comparison to Dnpz and Brm L treated mice, concomitant treatment with Brm L + Dnpz significantly decreased the level of TNF- α in the cerebral cortex as well as the hippocampus, as shown in **Table 5.14** and **Figure 5.13**. Therefore, the treatment of drugs efficiently decreased the TNF- α level which leads to reduce neuroinflammation in AD-induced mice.

5.1.2.11. Bromelain alone and in combination with Donepezil decrease the interleukin-6 level in different brain regions of $AlCl_3$ and D - galactose induced AD mice

The level of IL-6 was estimated in the cerebral cortex along with the hippocampus to assess the effect of treatments on neuroinflammation. The treatments had a significant effect on IL-6 level in the cortex ($F_{(5, 30)} = 71.622$, $P < 0.001$) as well as the hippocampus ($F_{(5, 30)} = 53.824$, $P < 0.001$) of the mice brain, according to one way ANOVA study. In comparison to control, the model ($AlCl_3$ + D - galactose treated) showed a significant increase level of IL-6 in the cerebral cortex along with the hippocampus. When induced AD mice were received Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz, levels of IL-6 in the cerebral cortex along with the hippocampus were found to be significantly attenuated than the model. In comparison to Dnpz and Brm L treated AD mice, concomitant treatment with Brm L + Dnpz significantly decreased the level of IL-6 in the cerebral cortex along with the hippocampus, as shown in **Table 5.15** and **Figure 5.14**. Thus, the treatment of drugs efficiently decreased the IL-6 level which leads to attenuate neuroinflammation in AD-induced mice.

Table 5.14: Effect of Bromelain on TNF- α level of AD-induced mice

Treatment	Mice brain regions	TNF - α (pg/mL)
Control	Cerebral cortex	963.333 $\pm$ 70.019
	Hippocampus	884.166 $\pm$ 69.646
M	Cerebral cortex	3738.333 $\pm$ 141.370 ^{***}
	Hippocampus	3313.333 $\pm$ 209.230 ^{***}
M + Dnpz	Cerebral cortex	1817.500 $\pm$ 177.570 ^{###}
	Hippocampus	1688.333 $\pm$ 178.380 ^{###}
M + Brm L	Cerebral cortex	2534.166 $\pm$ 154.301 ^{###}
	Hippocampus	2180.000 $\pm$ 104.880 ^{###}
M + Brm H	Cerebral cortex	2150.833 $\pm$ 161.169 ^{###}
	Hippocampus	1938.333 $\pm$ 90.292 ^{###}
M + Brm L + Dnpz	Cerebral cortex	1159.166 $\pm$ 65.005 ^{###, ^, \$\$\$}
	Hippocampus	921.666 $\pm$ 100.346 ^{###, ^, \$\$\$}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; TNF - α = Tumor Necrosis Factor - α ; n = Number of animal in each group.

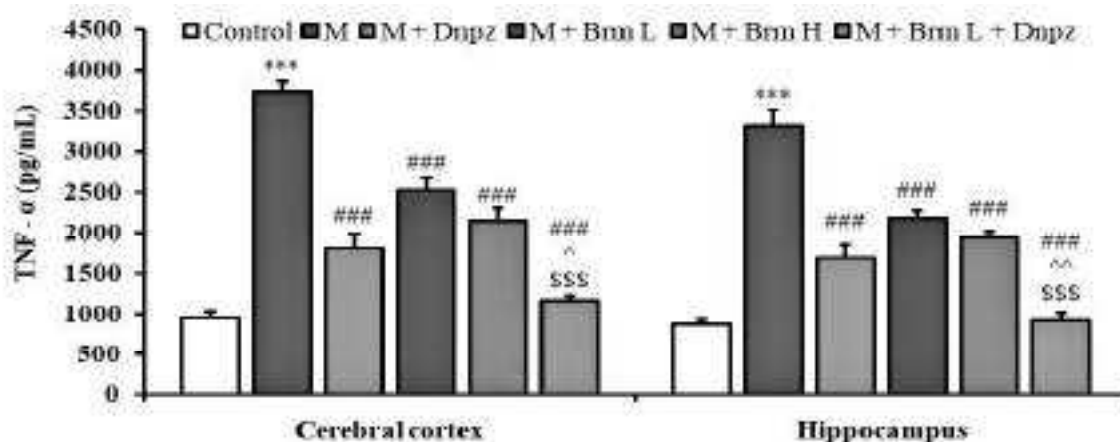


Figure 5.13: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on TNF - α level in cerebral cortex and hippocampus of (AlCl₃ + D - galactose)administered mice. Bars in chart represent the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; TNF - α = Tumor Necrosis Factor - α ; n = Number of animal in each group.

Table 5.15: Effect of Bromelain on IL-6 level of AD-induced mice

Treatment	Mice brain regions	IL - 6 (pg/mL)
Control	Cerebral cortex	215.583 ± 28.884
	Hippocampus	198.500 ± 36.600
M	Cerebral cortex	1163.500 ± 23.013 ^{***}
	Hippocampus	1081.833 ± 32.925 ^{***}
M + Dnpz	Cerebral cortex	511.000 ± 29.467 ^{###}
	Hippocampus	471.833 ± 24.098 ^{###}
M + Brm L	Cerebral cortex	711.000 ± 63.888 ^{###}
	Hippocampus	628.500 ± 64.688 ^{###}
M + Brm H	Cerebral cortex	577.666 ± 49.911 ^{###}
	Hippocampus	481.833 ± 48.104 ^{###}
M + Brm L + Dnpz	Cerebral cortex	321.833 ± 24.644 ^{###, ^, \$\$\$}
	Hippocampus	243.500 ± 43.469 ^{###, ^, \$\$\$}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz). Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; IL - 6 = Interleukin - 6; n = Number of animal in each group.

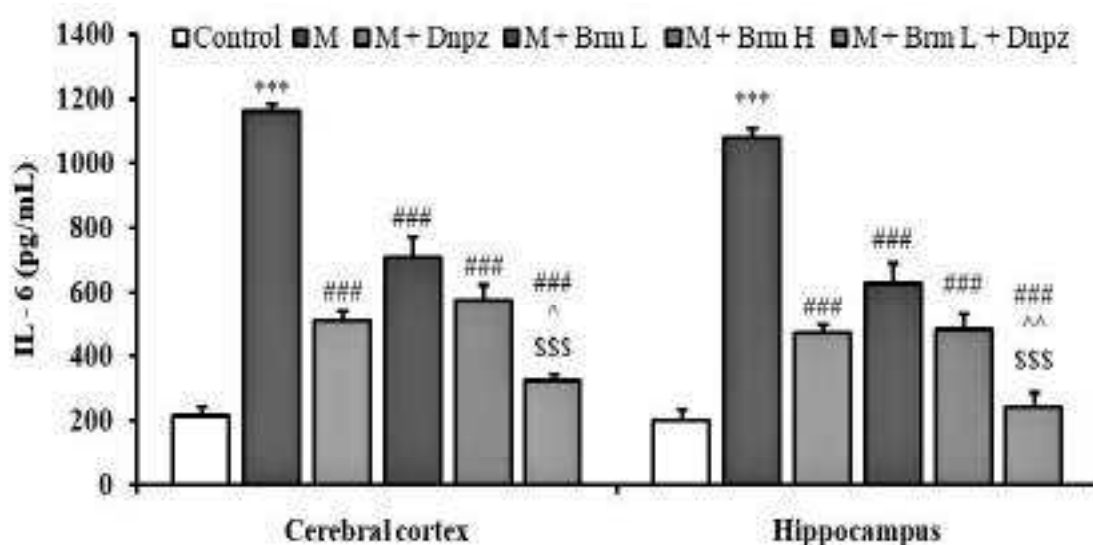


Figure 5.14: Effect of Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and co-administration (Brm L + Dnpz) treatment on IL - 6 level in cerebral cortex and hippocampus of (AlCl₃ + D - galactose) administered mice. Bars in chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm L treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight; IL - 6 = Interleukin - 6; n = Number of animal in each group.

5.1.3. Histopathological analysis

The analysis of histopathology was shown no damage signs to the cerebral cortex along with the hippocampus of control (vehicle-treated) mice. The model (AlCl_3 + D - galactose treated) group showed the abnormal neuron cells, eosinophilic lesions, and hyperchromatic nuclei in the cerebral cortex as well as the hippocampus. The treatments with Dnpz (2 mg/kg), Brm L (10 mg/kg), Brm H (20 mg/kg), and Brm L + Dnpz to induced AD mice showed the improvement in normal neuron cells, eosinophilic lesions, and hyperchromatic nuclei in the cerebral cortex as well as the hippocampus than the model group. The concomitant treatment with Brm L + Dnpz to AD-induced mice showed the maximum protection in dispersedly arranged neurons, eosinophilic lesions, and hyperchromatic nuclei in the cerebral cortex along with the hippocampus than Dnpz and Brm L treated mice, as shown in **Figure 5.15**.

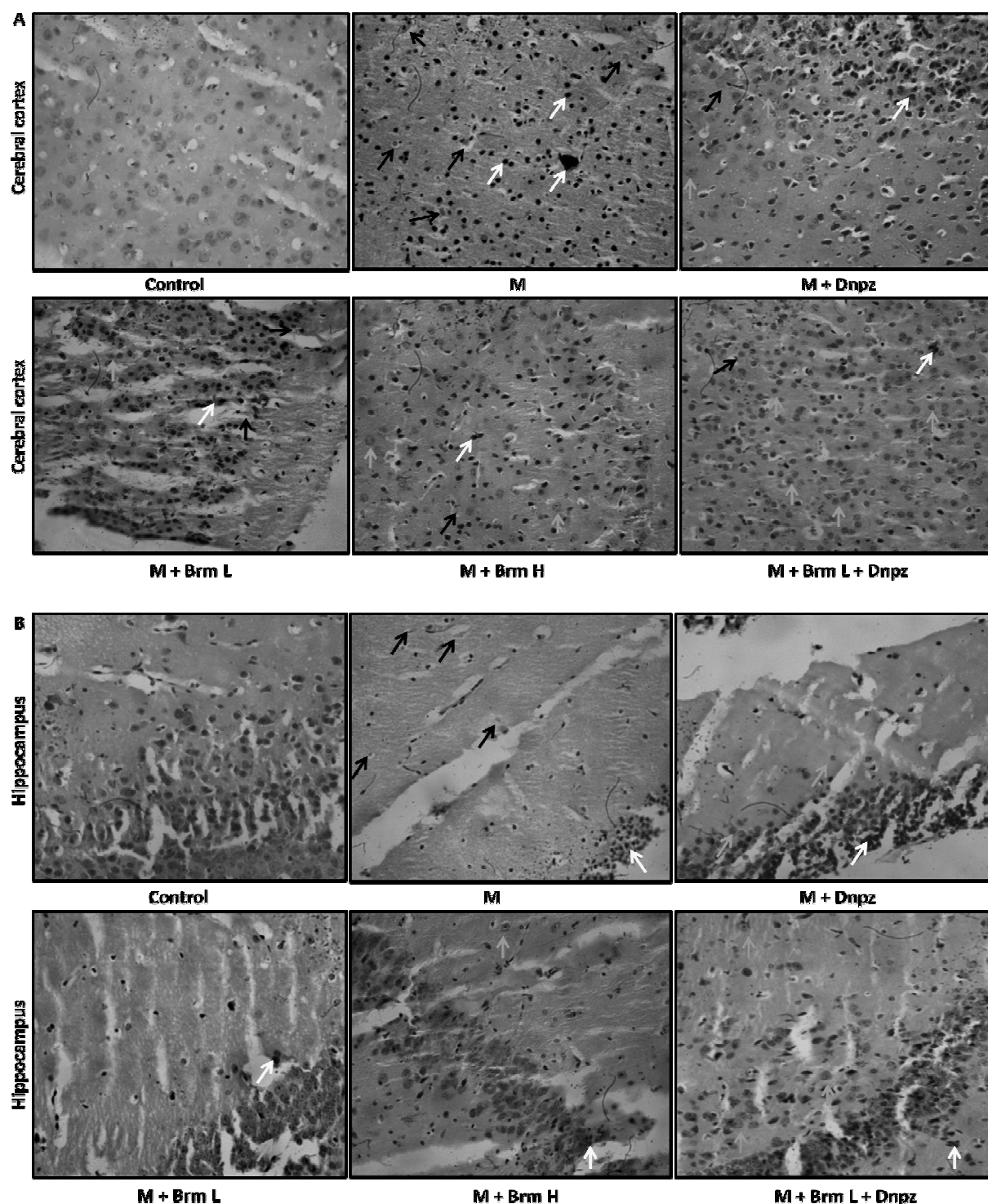


Figure 5.15: Analysis of histopathological data; (40X) of transverse section of the cerebral cortex (A) and the hippocampus (B) using hematoxylin and eosin staining; Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm L (10 mg/kg b. w.; i. p.), Brm H (20 mg/kg b. w.; i. p.), and co-administration (Brm L + Dnpz) of mice of all groups; Black, Yellow and Green arrows express the eosinophilic lesions, hyperchromatic nuclei, and normal neuron cells respectively. Where: M = Model (AlCl_3 + D-galactose); Dnpz = Donepezil; Brm L = Bromelain low dose; Brm H = Bromelain high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

5.2. Myrcene

5.2.1. Behavioral analysis

5.2.1.1. Myrcene alone and in combination with Donepezil improve the spatial reference memory and spatial working memory in AlCl_3 and D-galactose induced AD mice in MWM test

In MWM task, analysis of SRM data by two-way ANOVA was found significant differences between treatments ($F_{(5, 90)} = 70.501$, $P < 0.001$) as well as test days ($F_{(2, 90)} = 5.764$, $P = 0.004$). There was no significant interaction between treatments and days ($F_{(10, 90)} = 0.0439$, $P = 1.0$) **Table 5.16 a** and **Figure 5.16 a**. Data analysis shown that on training day 81, 82, and 83; model (AlCl_3 + D-galactose treated) group was found significantly enhance escape latency as compared to the control, and representing increase cognitive dysfunction and decline the reference memory of mice. The treatments with Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz to induced AD mice were observed significantly attenuation in escape latency as compared to the model. The concomitant treatment with Myr L + Dnpz to AD-induced mice was shown significantly reduce escape latency as compared to Dnpz and Myr L treated mice, signifying decrease cognitive dysfunction and enhance reference memory of mice. However, no significant variation was observed among the day 81, 82, and 83.

Probe trial was conducted on day 84 for measuring retention of memory. In one way ANOVA study, the time spent in the target quadrant revealed significant variations among treatments ($F_{(5, 30)} = 55.867$, $P < 0.001$) **Table 5.16 b** and **Figure 5.16 b**. The model was found considerably reduce time spent in the target quadrant than the control. The treatments with Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz to induced AD mice were shown considerably augment the time spent in the target quadrant than the model. The concomitant treatment with MyrL + Dnpz to AD-induced mice was observed significantly raise the time spent in the target quadrant than Dnpz and Myr L treated mice, representing memory improvement in mice.

Table 5.16 a: Effect of Myrcene on spatial reference memory of AD-induced mice in MWM

Day	Control	M	M + Dnpz	M + Myr L	M + Myr H	M + Myr L + Dnpz
Day-81	14.208 ± 4.343	67.041 ± 3.515***	38.125 ± 1.700###	42.416 ± 5.549###	40.208 ± 3.215###	15.916 ± 3.522###, ^, &&&
Day-82	9.500 ± 1.200	64.375 ± 5.791***	32.291 ± 1.901###	40.250 ± 6.033###	35.541 ± 3.720###	10.416 ± 1.422###, ^, &&&
Day-83	7.250 ± 0.642	59.125 ± 7.938***	29.041 ± 1.619###	35.250 ± 5.224###	31.625 ± 2.671###	7.625 ± 0.955###, ^, &&&

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; ###p<0.001 significant from M treated group; ^p<0.01 significant from Dnpz treated group; &&&p<0.001 significant as compared to Myr L treated group (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

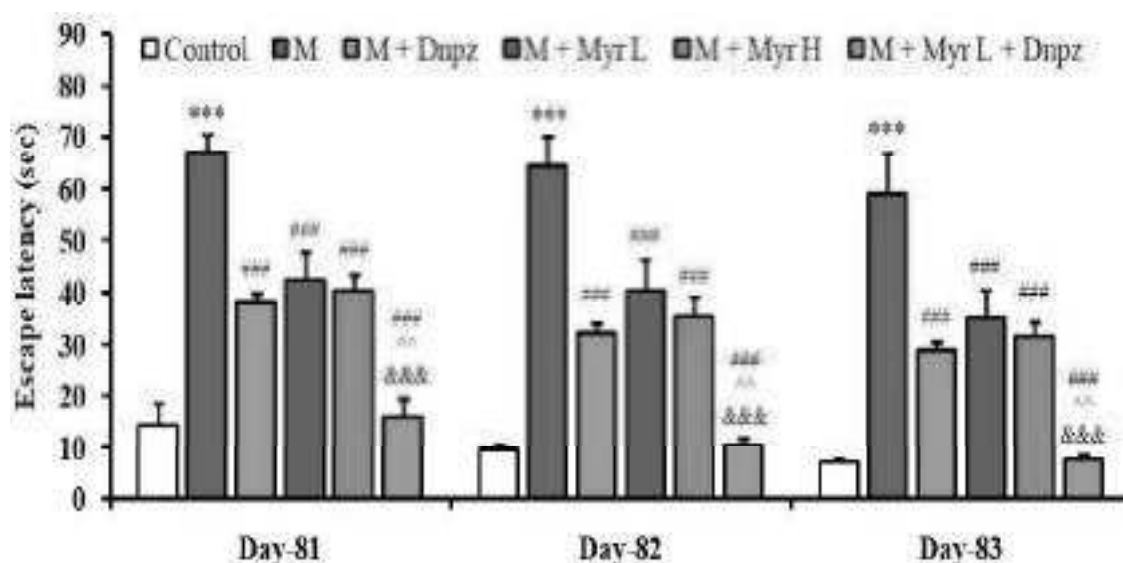


Figure 5.16 a: Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on spatial reference memory of (AlCl_3 + D - galactose) administered mice during training days 81, 82 and 83. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; ###p<0.001 significant from M treated group; ^p<0.01 significant from Dnpz treated group; &&&p<0.001 significant as compared to Myr L treated group (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

Table 5.16 b: Effect of Myrcene on time spent in the target quadrant (Probe trial) of AD induced mice in MWM

Day	Control	M	M + Dnpz	M + Myr L	M + Myr H	M + Myr L + Dnpz
Day-84	49.125 ± 2.739	6.458 ± 0.748***	23.583 ± 1.452###	17.166 ± 2.917 [#]	19.791 ± 2.392###	40.416 ± 1.539###,^^,&&&

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; [#]p<0.05, and ###p<0.001 significant from M treated group; ^^p<0.001 significant from Dnpz treated group; &&&p<0.001 significant from Myr L treated group; (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

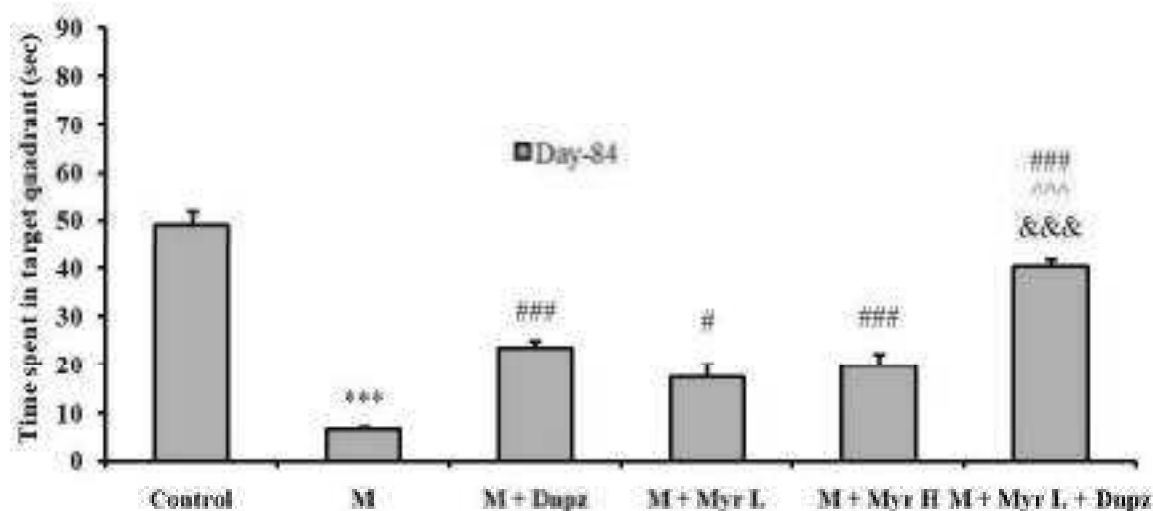


Figure 5.16 b: Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on time spent in the target quadrant (Probe trial) of (AlCl₃ + D - galactose) administered mice in MWM test. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; [#]p<0.05, and ###p<0.001 significant from M treated group; ^^p<0.001 significant from Dnpz treated group; &&&p<0.001 significant from Myr L treated group; (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

In SWM task; acquisition, as well as retrieval trial, were conducted to evaluate the spatial working memory of mice on day 87, 88, 89, and 90. The acquisition, as well as retrieval trial data analysis by two-way ANOVA, showed significant changes in escape latency among the treatments respectively. In acquisition trial; two way ANOVA analysis were found considerable changes among treatments ($F_{(5, 120)} = 71.757$, $P < 0.001$) and test days ($F_{(3, 120)} = 3.862$, $P = 0.011$). There was no significant interaction between treatments and days ($F_{(15, 120)} = 0.0305$, $P = 1.00$) **Table 5.17 a** and **Figure 5.17 a**. Model was found significantly increase escape latency as compared to control demonstrating enhance cognitive dysfunction and decrease the working memory of mice. The treatments with Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz to induced AD mice were shown significantly attenuation in escape latency than the model. The concomitant treatment with Myr L + Dnpz to AD-induced mice was found significantly reduce escape latency as compared to Dnpz and Myr L treated mice, indicating reduce cognitive dysfunction and increase working memory of mice. Similarly, in retrieval trial; two way ANOVA analysis shown considerable differences among treatments ($F_{(5, 120)} = 96.599$, $P < 0.001$) and test days ($F_{(3, 120)} = 3.379$, $P = 0.021$). No significant interaction between treatments and days was obtained ($F_{(15, 120)} = 0.0835$, $P = 1.000$) **Table 5.17 b** and **Figure 5.17 b**. The considerable enhancement and decrease in escape latency were observed in the model and treatments respectively. However, no significant differences were observed among the day 87, 88, 89, and 90 in acquisition and retrieval trial.

Table 5.17 a: Effect of Myrcene on the spatial working memory (acquisition trial) of AD-induced mice in MWM

Day	Control	M	M + Dnpz	M + Myr L	M + Myr H	M + Myr L + Dnpz
Day-87	13.541 ± 2.447	81.583 ± 2.887***	44.625 ± 3.848###	49.083 ± 8.560###	45.458 ± 6.755###	16.250 ± 6.075###,^,&&&
Day-88	11.041 ± 2.805	76.166 ± 5.581***	41.291 ± 3.586###	46.250 ± 11.938##	42.750 ± 5.239###	12.916 ± 3.109###,^,&&&
Day-89	9.500 ± 3.032	75.500 ± 4.096***	39.291 ± 2.979###	44.041 ± 12.352##	41.166 ± 7.287###	10.916 ± 1.388###,^,&&&
Day-90	5.708 ± 0.350	67.916 ± 5.389***	34.833 ± 3.279###	36.875 ± 8.244##	33.541 ± 3.751###	6.041 ± 0.462###,^,&&

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; ##p<0.01, and ###p<0.001 significant from M treated group; ^^p<0.01 significant from Dnpz treated group; &&p<0.01 and &&&p<0.001 significant from Myr L treated group; (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

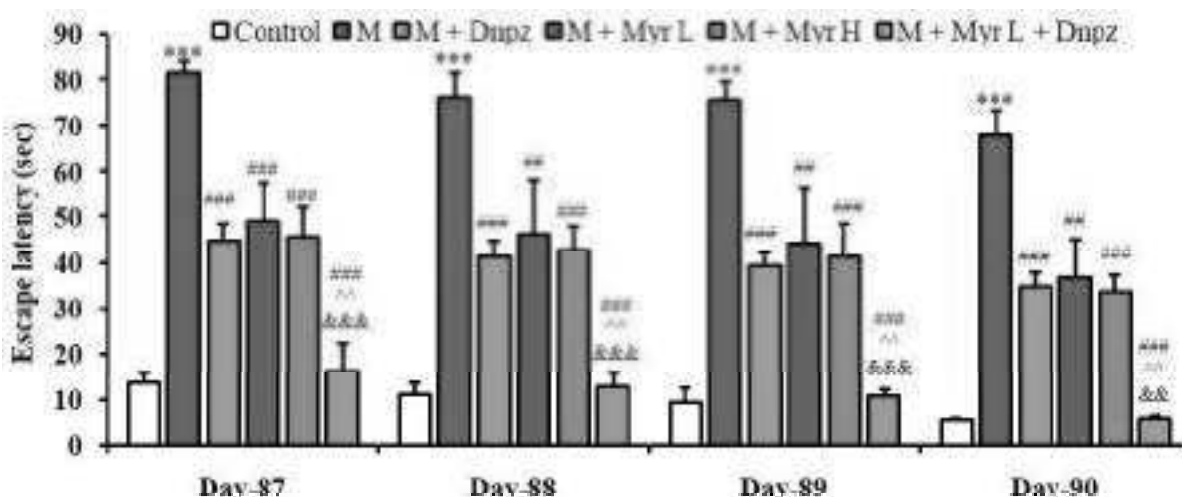


Figure 5.17 a: Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on the spatial working memory of (AlCl₃ + D - galactose) administered mice at days 87, 88, 89 and 90, acquisition trial. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; ##p<0.01, and ###p<0.001 significant from M treated group; ^^p<0.01 significant from Dnpz treated group; &&p<0.01 and &&&p<0.001 significant from Myr L treated group; (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

Table 5.17 b: Effect of Myrcene on the spatial working memory (retrieval trial) of AD induced mice in MWM

Day	Control	M	M + Dnpz	M + Myr L	M + Myr H	M + Myr L + Dnpz
Day-87	12.875 ± 3.579	80.916 ± 1.227***	43.791 ± 1.951###	47.791 ± 7.674###	46.541 ± 7.392###	14.250 ± 2.658####,^^,&&&
Day-88	10.458 ± 1.975	79.333 ± 2.413***	41.875 ± 5.939###	44.583 ± 5.173###	43.958 ± 3.972###	12.958 ± 3.701####,^^,&&&
Day-89	7.416 ± 0.554	73.500 ± 5.569***	39.541 ± 4.596###	44.625 ± 11.595##	41.625 ± 4.742###	10.375 ± 1.687####,^^,&&&
Day-90	6.291 ± 0.705	67.041 ± 6.446***	36.125 ± 5.312###	39.791 ± 6.628##	38.166 ± 5.274##	6.708 ± 0.969####,^^,&&&

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; ##p<0.01, and ###p<0.001 significant from M treated group; ^^p<0.01 and ^^p<0.001 significant from Dnpz treated group; &&p<0.001 significant from Myr L treated group; (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

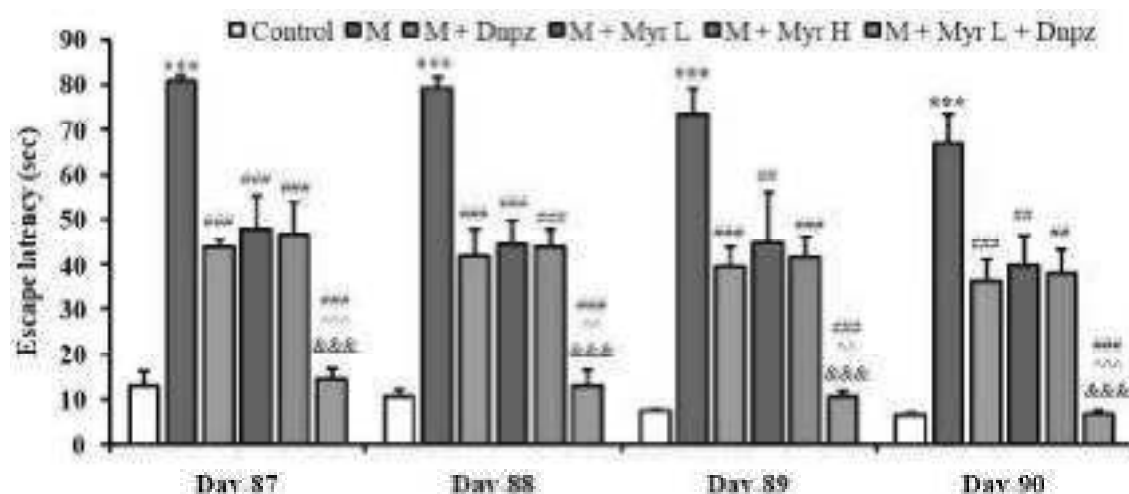


Figure 5.17 b: Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on the spatial working memory of (AlCl₃ + D - galactose) administered mice at days 87, 88, 89 and 90, retrieval trial. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; ##p<0.01, and ###p<0.001 significant from M treated group; ^^p<0.01 and ^^p<0.001 significant from Dnpz treated group; &&p<0.001 significant from Myr L treated group; (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

5.2.1.2. Myrcene alone and in combination with Donepezil improve the learning memory of AlCl_3 and D-galactose induced AD mice in EPM test

Two-way ANOVA analysis has shown significant differences among treatments ($F_{(5, 90)} = 41.285$, $P < 0.001$) as well as test days ($F_{(2, 90)} = 104.528$, $P < 0.001$). A significant interaction between treatments and days was obtained ($F_{(10, 90)} = 6.833$, $P < 0.001$) **Table 5.18** and **Figure 5.18**. As a result, it was observed that on day 81; transfer latency did not differ considerably in any treatment. On day 82 and 90; model (AlCl_3 + D-galactose treated) group was found significantly enhance transfer latency than the control (vehicle-treated) group. The treatments with Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz to induced AD mice were found significantly attenuation in transfer latency than the model. The concomitant treatment with Myr L + Dnpz to AD-induced mice observed a significant decline in transfer latency as compared to Dnpz and Myr L treated mice, demonstrating improvement in learning and memory of mice. Further, significant differences in transfer latency were observed on day 82 and 90 as compared to day 81.

5.2.1.3. Myrcene alone and in combination with Donepezil show no locomotor activity alteration of AlCl_3 and D-galactose induced AD mice

Locomotor activity was conducted on day 0, 80, and 90 to assess the effect of treatments on spontaneous locomotion of mice of all groups. Data analysis by one-way ANOVA shown that the model (AlCl_3 + D-galactose treated) group was not obtained significantly as compared to the control (vehicle-treated) group. The treatments with Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz to induced AD mice were not observed significantly than the model. The concomitant treatment with Myr L + Dnpz to AD-induced mice was not found significantly as compared to Dnpz and Myr L treated mice, signifying no alteration in locomotor activity of mice throughout the study, as shown in **Table 5.19**.

Table 5.18: Effect of Myrcene on transfer latency of AD induced mice in EPM

Day	Control	M	M + Dnpz	M + Myr L	M + Myr H	M + Myr L + Dnpz
Day-81	45.500 ± 3.956	51.666 ± 3.062	46.500 ± 3.712	49.166 ± 3.646	48.166 ± 2.809	46.333 ± 4.695
Day-82	10.333 ± 1.475	52.166 ± 2.937 ^{***}	29.500 ± 1.056 ^{###, a}	36.333 ± 2.043 ^{##, a}	31.500 ± 2.526 ^{###, a}	12.333 ± 3.675 ^{###, ^, &&&, a}
Day-90	5.666 ± 0.954	47.333 ± 4.835 ^{***}	23.000 ± 2.236 ^{###, a}	33.666 ± 2.996 ^{#, a}	27.166 ± 2.749 ^{###, a}	6.166 ± 1.815 ^{###, ^, &&&, a}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6).
^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.01 significant from Dnpz treated group; ^{&&&}p<0.001 significant from Myr L treated group; ^a represent significant from day 81(two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

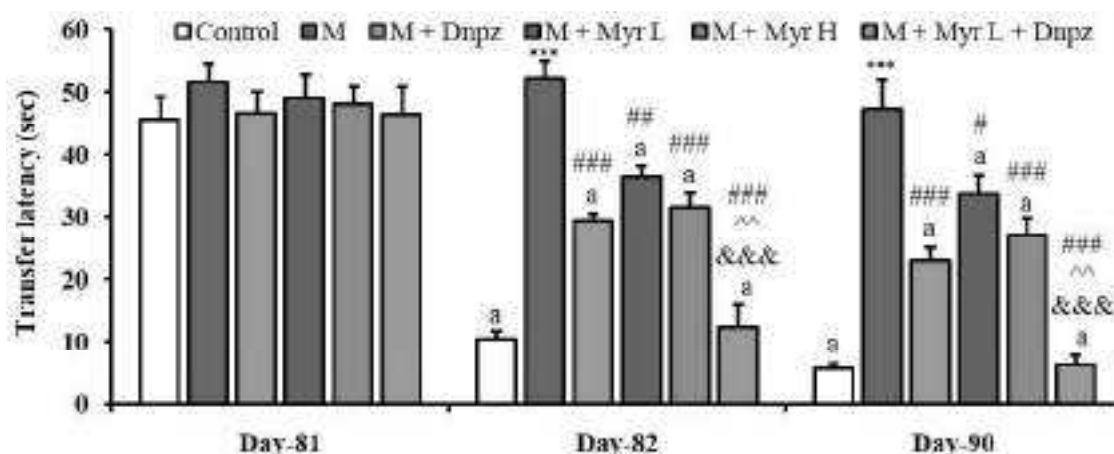


Figure 5.18: Effect of Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) treatment on transfer latency of (AlCl₃ + D - galactose) administered mice in the elevated plus-maze test. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.01 significant from Dnpz treated group; ^{&&&}p<0.001 significant from Myr L treated group; ^a represent significant from day 81(two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

Table 5.19: Effect of Myrcene on locomotor activity of AD-induced mice

Groups	Day 0		Day 80		Day 90	
	Before dose	After dose	Before dose	After dose	Before dose	After dose
Control	388.33 ± 31.43	367.67 ± 22.16	361.17 ± 17.43	355.17 ± 13.95	376.33 ± 10.41	364.50 ± 08.86
M	355.50 ± 21.92	328.17 ± 18.11	348.33 ± 10.22	342.67 ± 08.41	362.00 ± 06.60	349.67 ± 10.73
M + Dnpz	379.17 ± 20.93	351.00 ± 15.46	359.00 ± 08.33	355.00 ± 07.02	358.00 ± 10.55	340.33 ± 04.48
M + Myr L	332.17 ± 22.67	332.83 ± 19.40	311.50 ± 16.60	310.00 ± 15.49	329.50 ± 14.71	328.67 ± 14.41
M + Myr H	342.50 ± 06.49	339.67 ± 06.41	319.83 ± 08.20	321.83 ± 06.08	342.33 ± 18.52	336.50 ± 17.83
M + Myr L+ Dnpz	333.50 ± 18.86	345.83 ± 10.08	349.83 ± 12.17	346.83 ± 11.33	331.67 ± 10.81	331.67 ± 10.81

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment on locomotion of (AlCl_3 + D - galactose) administered mice at day 0; day 80; and day 90. Value expressed as the mean ± S.E.M. of each group (n = 6), (one way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

5.2.2. Biochemical analysis

5.2.2.1. Myrcene alone and in combination with Donepezil inhibit the acetylcholinesterase activity in different brain regions of AlCl₃ and D-galactose induced AD mice

AChE activity was considered at the end of the study in the cerebral cortex along with hippocampus to assess the impact of treatments on the function of cholinergic system. One way ANOVA study has shown a significant impact of the treatments on AChE enzyme activity in the cortex ($F_{(5, 30)} = 30.481$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 20.736$, $P < 0.001$) of the mice brain. In comparison to the control, the model (AlCl₃ + D-galactose treated) observed significant increase AChE levels in the cerebral cortex as well as hippocampus. When, Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatment were given to AD mice, AChE levels in the cerebral cortex along with hippocampus were obtained significantly reduction than the model. In comparison to Dnpz and Myr L treated mice, concomitant treatment with Myr L + Dnpz considerably decreased the AChE levels in the cerebral cortex along with hippocampus, as shown in **Table 5.20 and Figure 5.19**. Therefore, the treatment of drugs efficiently inhibited AChE activity and subsequently restored the cerebral cortex along with hippocampus acetylcholine homeostasis which led to enhancement in learning and memory in AD model mice.

5.2.2.2. Myrcene alone and in combination with Donepezil decrease the MDA level in different brain regions of AlCl₃ and D-galactose induced AD mice

Lipid peroxidation determined the MDA level in the cerebral cortex along with hippocampus to consider the impact of treatments on antioxidant. The treatments had a significant impact on MDA level in the cortex ($F_{(5, 30)} = 15.872$, $P < 0.001$) along with hippocampus ($F_{(5, 30)} = 16.472$, $P < 0.001$) of the mice brain, according to one way ANOVA study. The model (AlCl₃ + D-galactose treated) showed a significant increase MDA level in the cerebral cortex along with hippocampus than control group. When, Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatment were given to AD mice significantly attenuate MDA level in the cerebral cortex as well as hippocampus than the model. In comparison to Dnpz and Myr L treated mice, concomitant treatment with Myr L + Dnpz resulted in a significantly decrease MDA level in the cerebral cortex as well as hippocampus, as shown in **Table 5.21 and Figure 5.20**.

Table 5.20: Effect of Myrcene on acetylcholinesterase levels of AD-induced mice

Treatment	Mice brain regions	AChE (n moles per mg protein)
Control	Cerebral cortex	2.093 ± 0.284
	Hippocampus	2.026 ± 0.592
M	Cerebral cortex	8.372 ± 0.465***
	Hippocampus	8.154 ± 0.666***
M + Dnpz	Cerebral cortex	5.161 ± 0.378###
	Hippocampus	5.001 ± 0.368###
M + Myr L	Cerebral cortex	6.174 ± 0.573##
	Hippocampus	5.949 ± 0.353#
M + Myr H	Cerebral cortex	5.831 ± 0.464##
	Hippocampus	5.546 ± 0.534##
M + Myr L + Dnpz	Cerebral cortex	2.951 ± 0.199###, ^, &&&
	Hippocampus	2.685 ± 0.347###, ^, &&&

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.05 and ^^p<0.01 significant from Dnpz treated group; &&&p<0.001 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; AChE = Acetylcholinesterase.

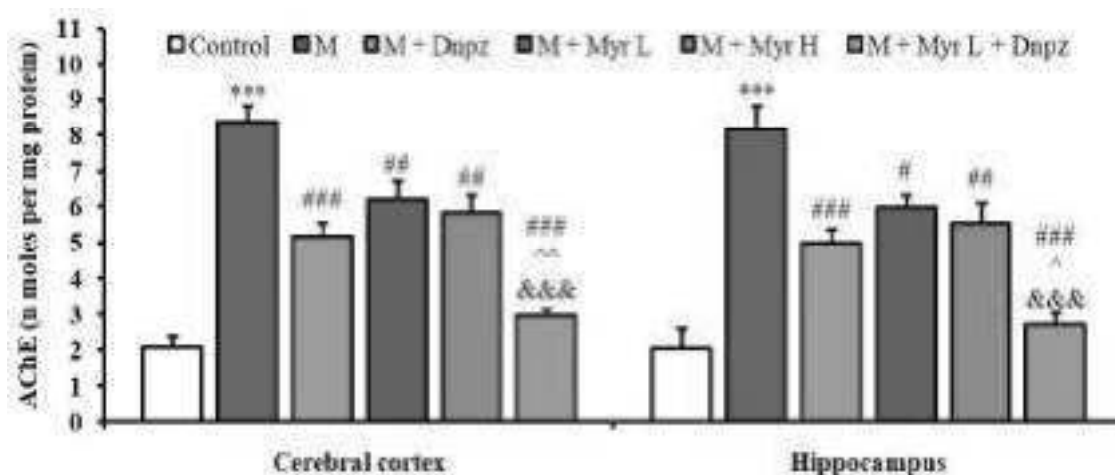


Figure 5.19: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on AChE level in the cerebral cortex and hippocampus of (AlCl_3 + D - galactose) administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.05 and ^^p<0.01 significant from Dnpz treated group; &&&p<0.001 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; AChE = Acetylcholinesterase.

Table 5.21: Effect of Myrcene on MDA levels of AD-induced mice

Treatment	Mice brain regions	MDA (n moles per mg protein)
Control	Cerebral cortex	0.843 ± 0.079
	Hippocampus	0.753 ± 0.127
M	Cerebral cortex	4.251 ± 0.389 ^{***}
	Hippocampus	3.743 ± 0.300 ^{***}
M + Dnpz	Cerebral cortex	2.404 ± 0.262 ^{##}
	Hippocampus	2.285 ± 0.483 ^{##}
M + Myr L	Cerebral cortex	2.792 ± 0.494 [#]
	Hippocampus	2.314 ± 0.289 ^{##}
M + Myr H	Cerebral cortex	1.956 ± 0.320 ^{###}
	Hippocampus	1.511 ± 0.146 ^{###}
M + Myr L + Dnpz	Cerebral cortex	0.981 ± 0.176 ^{###,^,&&}
	Hippocampus	0.915 ± 0.061 ^{###,^,&}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; [&]p<0.05 and ^{&&}p<0.01 significant from Myr L treated group. (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; MDA = Malondialdehyde.

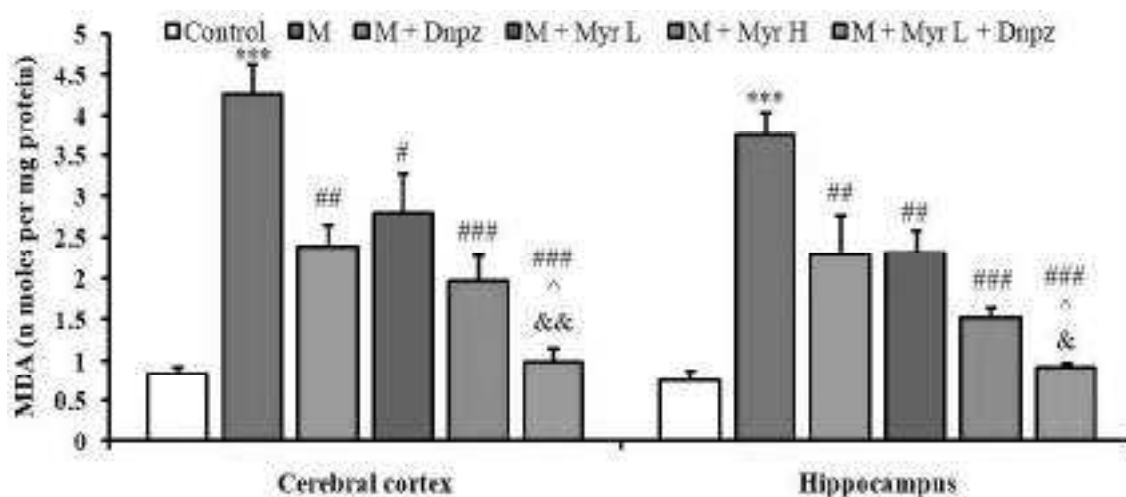


Figure 5.20: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on MDA level in the cerebral cortex and hippocampus of (AlCl₃ + D - galactose) administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; [&]p<0.05 and ^{&&}p<0.01 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; MDA = Malondialdehyde.

5.2.2.3. Myrcene alone and in combination with Donepezil increase the reduced glutathione level in different brain regions of AlCl₃ and D-galactose induced AD mice

Reduced glutathione level was estimated in the cerebral cortex along with hippocampus to assess the impact of treatments on antioxidant. The treatments had a considerable effect on GSH level in cortex ($F_{(5, 30)} = 20.519$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 21.269$, $P < 0.001$) of the mice brain, according to one way ANOVA study. In comparison to the control group, the model (AlCl₃ + D-galactose treated) group had a significantly decrease GSH in the cerebral cortex along with hippocampus. When, Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatments were given to AD mice significantly raised GSH level in the cerebral cortex along with hippocampus than the model. In comparison to Dnpz and Myr L treated mice, concomitant treatment with Myr L + Dnpz significantly raise GSH in the cerebral cortex along with hippocampus, as shown in **Table 5.22** and **Figure 5.21**.

5.2.2.4. Myrcene alone and in combination with Donepezil increase the catalase level in different brain regions of AlCl₃ and D-galactose induced AD mice

Catalase level was measured in the cerebral cortex along with hippocampus to assess the effect of treatments on antioxidant. In one way ANOVA study, the treatments had a significant impact on the level of CAT in the cortex ($F_{(5, 30)} = 14.700$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 19.220$, $P < 0.001$) of the mice brain. When compared to the control group, the model (AlCl₃ + D-galactose treated) group showed a significantly reduce CAT amount in the cerebral cortex along with hippocampus. As a result, Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatment were given to AD mice significantly increase CAT level in the cerebral cortex along with hippocampus than the model. The concomitant treatment with Myr L + Dnpz to AD-induced mice was found significantly increase CAT amount in cerebral cortex along with hippocampus than Dnpz and Myr L treated mice, as shown in **Table 5.23** and **Figure 5.22**.

Table 5.22: Effect of Myrcene on GSH levels of AD-induced mice

Treatment	Mice brain regions	GSH (n moles per mg protein)
Control	Cerebral cortex	3.255 ± 0.210
	Hippocampus	3.056 ± 0.274
M	Cerebral cortex	1.130 ± 0.099***
	Hippocampus	0.750 ± 0.166***
M + Dnpz	Cerebral cortex	2.101 ± 0.288##
	Hippocampus	1.629 ± 0.126#
M + Myr L	Cerebral cortex	1.912 ± 0.070#
	Hippocampus	1.601 ± 0.118#
M + Myr H	Cerebral cortex	2.059 ± 0.143##
	Hippocampus	1.732 ± 0.136##
M + Myr L + Dnpz	Cerebral cortex	2.932 ± 0.082###,^,&&
	Hippocampus	2.642 ± 0.202###,^,&&

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.05 and ^^p<0.01 significant from Dnpz treated group; &&p<0.01 significant from Myr L treated group. (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; GSH = Glutathione.

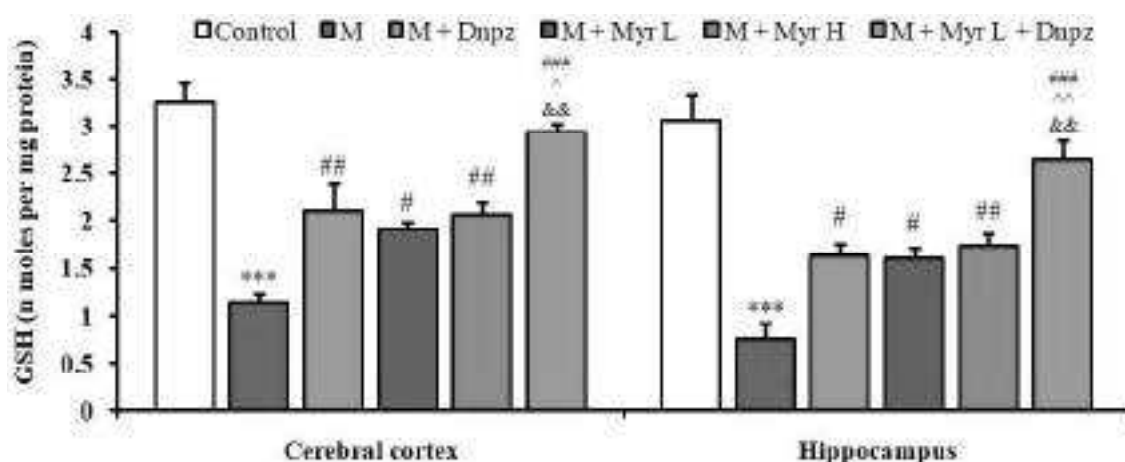


Figure 5.21: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on GSH level in the cerebral cortex and hippocampus of (AlCl₃ + D - galactose) administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.05 and ^^p<0.01 significant from Dnpz treated group; &&p<0.01 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; GSH = Glutathione.

Table 5.23: Effect of Myrcene on catalase levels of AD-induced mice

Treatment	Mice brain regions	Catalase (Units per mg protein)
Control	Cerebral cortex	0.975 ± 0.064
	Hippocampus	0.929 ± 0.101
M	Cerebral cortex	0.328 ± 0.052***
	Hippocampus	0.248 ± 0.021***
M + Dnpz	Cerebral cortex	0.645 ± 0.032##
	Hippocampus	0.560 ± 0.034##
M + Myr L	Cerebral cortex	0.613 ± 0.019#
	Hippocampus	0.554 ± 0.023##
M + Myr H	Cerebral cortex	0.685 ± 0.113##
	Hippocampus	0.622 ± 0.043###
M + Myr L + Dnpz	Cerebral cortex	0.9127 ± 0.0317###,^,&
	Hippocampus	0.896 ± 0.074###,^,&&

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.05 and ^^p<0.01 significant from Dnpz treated group; &p<0.05 and &&p<0.01 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

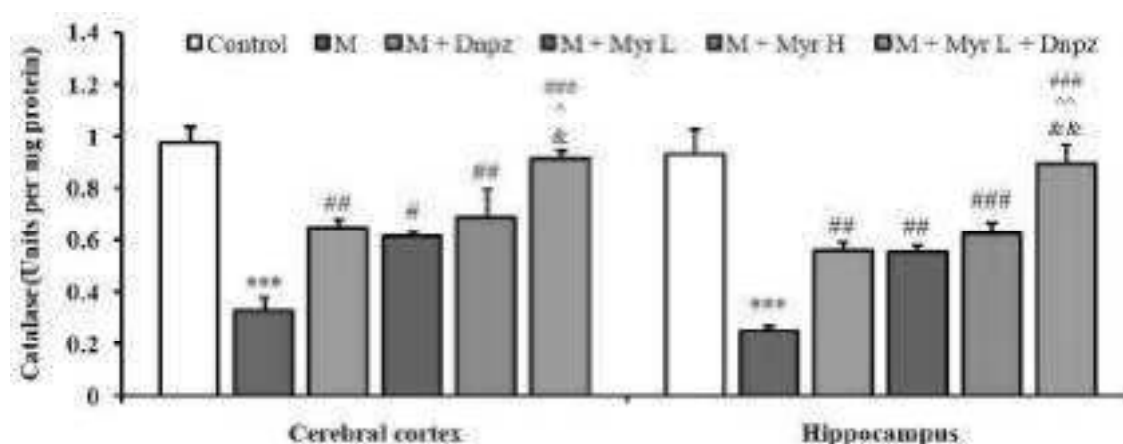


Figure 5.22: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on catalase level in the cerebral cortex and hippocampus of (AlCl₃ + D - galactose) administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.05 and ^^p<0.01 significant from Dnpz treated group; &p<0.05 and &&p<0.01 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

5.2.2.5. Myrcene alone and in combination with Donepezil increase the superoxide dismutase level in different brain regions of AlCl₃ and D-galactose induced AD mice

Superoxide dismutase level was calculated in cerebral cortex as well as hippocampus to assess treatments effect on antioxidant. The results of the one-way ANOVA showed the treatments had a significant impact on level of SOD in the cortex ($F_{(5, 30)} = 31.478$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 18.426$, $P < 0.001$) of the mice brain. In comparison to the control (vehicle-treated) group, the model (AlCl₃ + D-galactose treated) group had significantly attenuated SOD level in the cerebral cortex along with hippocampus. When, Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatments were given to AD mice significantly elevated SOD level in the cerebral cortex along with hippocampus than the model. In comparison to Dnpz and Myr L treated mice, concomitant treatment with Myr L + Dnpz considerably raised the SOD level in the cerebral cortex as well as hippocampus, as shown in **Table 5.24** and **Figure 5.23**.

5.2.2.6. Myrcene alone and in combination with Donepezil reduce the nitrite level in different brain regions of AlCl₃ and D-galactose induced AD mice

In one way ANOVA study, the level of nitrite in the cortex ($F_{(5, 30)} = 14.479$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 17.088$, $P < 0.001$) of the mice brain was considerably affected by the treatments. When compared to the control group, the model (AlCl₃ + D-galactose treated) showed a significantly raise nitrite amount in the cerebral cortex along with hippocampus. While, Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz in AD mice resulted in a significant decrease nitrite level in the cerebral cortex along with hippocampus than the model. In comparison to Dnpz and Myr L treated mice, concomitant treatment with Myr L + Dnpz considerably reduced nitrite amount in the cerebral cortex along with hippocampus, as shown in **Table 5.25** and **Figure 5.24**.

Table 5.24: Effect of Myrcene on SOD levels of AD-induced mice

Treatment	Mice brain regions	SOD (Units per mg protein)
Control	Cerebral cortex	2.184 ± 0.167
	Hippocampus	2.105 ± 0.196
M	Cerebral cortex	0.610 ± 0.055 ^{***}
	Hippocampus	0.406 ± 0.110 ^{***}
M + Dnpz	Cerebral cortex	1.382 ± 0.047 ^{###}
	Hippocampus	1.137 ± 0.122 [#]
M + Myr L	Cerebral cortex	1.149 ± 0.027 ^{##}
	Hippocampus	1.098 ± 0.077 [#]
M + Myr H	Cerebral cortex	1.355 ± 0.086 ^{###}
	Hippocampus	1.061 ± 0.099 [#]
M + Myr L + Dnpz	Cerebral cortex	1.949 ± 0.140 ^{###,^,&&&}
	Hippocampus	1.795 ± 0.190 ^{###,^,&}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; [&]p<0.05 and ^{&&&}p<0.001 significant from Myr L treated group. (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; SOD = Superoxide dismutase.

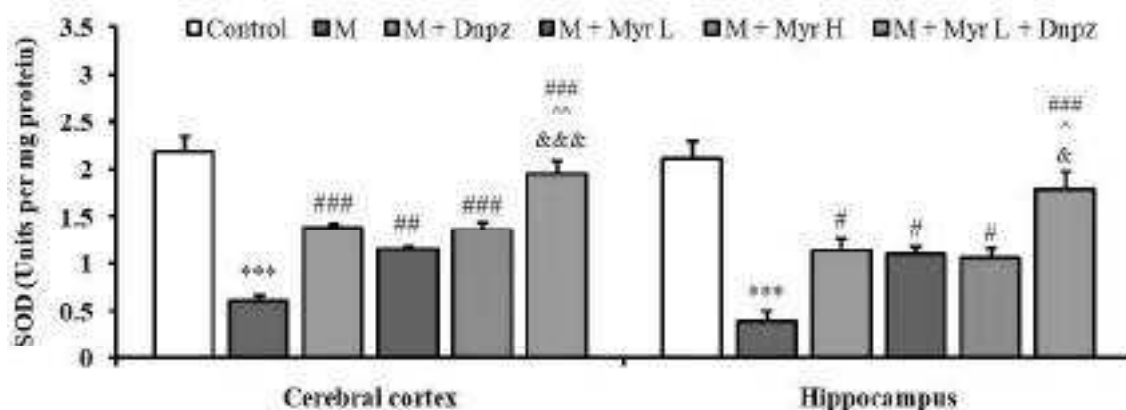


Figure 5.23: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on SOD level in the cerebral cortex and hippocampus of (AlCl₃ + D - galactose) administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; [&]p<0.05 and ^{&&&}p<0.001 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; SOD = Superoxide dismutase.

Table 5.25: Effect of Myrcene on Nitrite levels of AD-induced mice

Treatment	Mice brain regions	Nitrite (μM per mg protein)
Control	Cerebral cortex	0.961 ± 0.223
	Hippocampus	0.772 ± 0.205
M	Cerebral cortex	$4.215 \pm 0.385^{***}$
	Hippocampus	$4.014 \pm 0.180^{***}$
M + Dnpz	Cerebral cortex	$2.454 \pm 0.351^{##}$
	Hippocampus	$2.214 \pm 0.279^{##}$
M + Myr L	Cerebral cortex	$2.823 \pm 0.375^{\#}$
	Hippocampus	$2.671 \pm 0.392^{\#}$
M + Myr H	Cerebral cortex	$2.432 \pm 0.326^{##}$
	Hippocampus	$2.312 \pm 0.369^{##}$
M + Myr L + Dnpz	Cerebral cortex	$1.052 \pm 0.195^{###,\wedge,\&\&}$
	Hippocampus	$0.970 \pm 0.229^{###,\wedge,\&\&}$

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). *** $p < 0.001$ significant from the control group; # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ significant from M treated group; $\wedge$ $p < 0.05$ significant from Dnpz treated group; $\&\&$ $p < 0.01$ significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

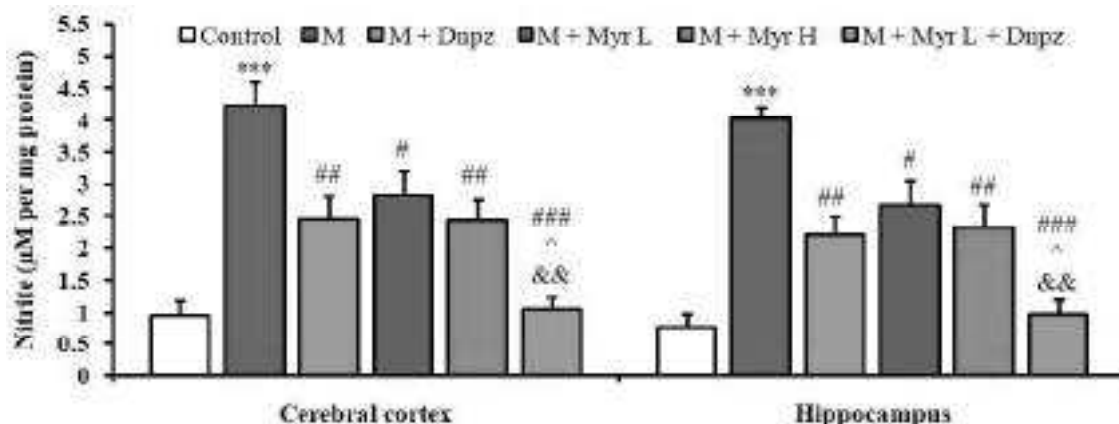


Figure 5.24: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on nitrite level in the cerebral cortex and hippocampus of (AlCl_3 + D - galactose) administered mice. Bars in the chart represent the mean $\pm$ S.E.M. of each group (n = 6). *** $p < 0.001$ significant from the control group; # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ significant from M treated group; $\wedge$ $p < 0.05$ significant from Dnpz treated group; $\&\&$ $p < 0.01$ significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

5.2.2.7. Myrcene alone and in combination with Donepezil increase the brain derived neurotrophic factor level in different brain regions of AlCl_3 and D-galactose induced AD mice

BDNF level in the cerebral cortex along with hippocampus was determined to assess the treatments impact on neurogenesis in AD mice. BDNF level was analyzed by ELISA. According to a one-way ANOVA study, the treatments had a significant impact on level of BDNF in the cortex ($F_{(5, 30)} = 22.137$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 21.194$, $P < 0.001$) of the mice brain. When compared to the control group, the model (AlCl_3 + D-galactose) group showed a considerably lower BDNF amount in the cerebral cortex along with hippocampus. Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatments were given to AD mice considerably enhance BDNF level in the cerebral cortex along with hippocampus than the model. In comparison to Dnpz and Myr L treated mice, concomitant treatment with Myr L + Dnpz considerably increased BDNF level in the cerebral cortex as well as hippocampus, as shown in **Table 5.26** and **Figure 5.25**.

5.2.2.8. Myrcene alone and in combination with Donepezil decrease the β -secretase level in different brain regions of AlCl_3 and D-galactose induced AD mice

β -secretase level alteration affects to amyloidogenic process of AD as a consequence of fluctuation of amyloid beta-peptide in the cerebral cortex along with hippocampus. β -secretase level in cerebral cortex along with hippocampus was examined to measure treatments effect on amyloidogenic process of AD. According to one way ANOVA study, the treatments showed a considerable impact on the level of β -secretase in the cortex ($F_{(5, 30)} = 43.719$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 41.356$, $P < 0.001$) of the mice brain. In comparison to the control group, the model (AlCl_3 + D-galactose) group was exposed to considerably higher β -secretase level in the cerebral cortex along with hippocampus. When, Dnpz (2 mg/kg), Myr L (10 mg/kg), Myr H (20 mg/kg), and Myr L + Dnpz treatments were given to AD mice significantly attenuate β -secretase level in the cerebral cortex along with hippocampus than the model. When compared to Dnpz and Myr L treated AD mice, concomitant treatment with Myr L + Dnpz considerably reduced β -secretase level in the cerebral cortex along with hippocampus, as shown in **Table 5.27** and **Figure 5.26**.

Table 5.26: Effect of Myrcene on BDNF levels of AD-induced mice

Treatment	Mice brain regions	BDNF (pg/mL)
Control	Cerebral cortex	559.833 ± 12.892
	Hippocampus	523.833 ± 16.924
M	Cerebral cortex	121.166 ± 31.834 ^{***}
	Hippocampus	110.166 ± 12.752 ^{***}
M + Dnpz	Cerebral cortex	346.833 ± 39.797 ^{###}
	Hippocampus	327.500 ± 29.722 ^{###}
M + Myr L	Cerebral cortex	270.166 ± 39.494 [#]
	Hippocampus	252.166 ± 49.226 [#]
M + Myr H	Cerebral cortex	323.333 ± 37.268 ^{##}
	Hippocampus	303.500 ± 37.281 ^{##}
M + Myr L + Dnpz	Cerebral cortex	502.500 ± 33.961 ^{###, ^, &&&}
	Hippocampus	474.500 ± 35.803 ^{###, ^, &&&}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{&&&}p<0.001 significant from Myr L treated group. (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; BDNF: brain-derived neurotrophic factor.

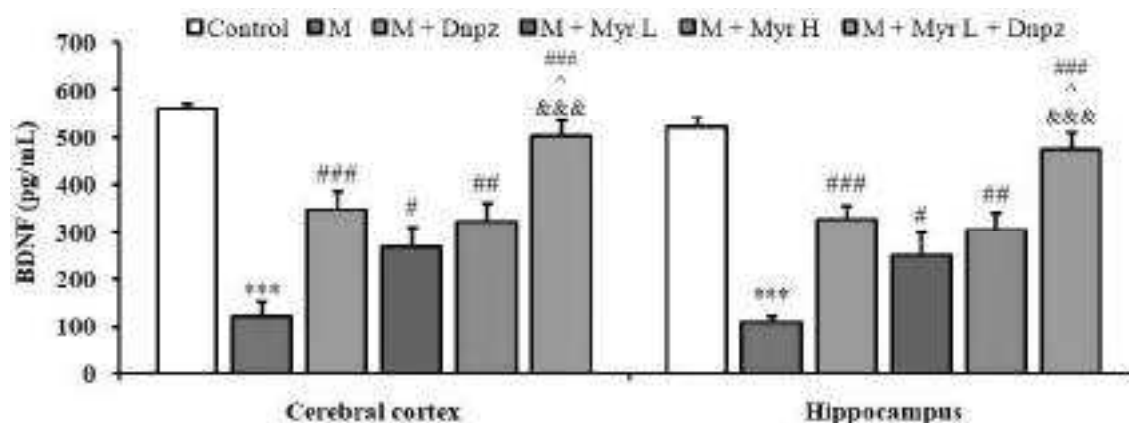


Figure 5.25: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on brain-derived neurotrophic factor level in the cerebral cortex and hippocampus of (AlCl₃ + D - galactose) administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01, and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{&&&}p<0.001 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

Table 5.27: Effect of Myrcene on β -secretase levels of AD-induced mice

Treatment	Mice brain regions	β -Secretase (U/L)
Control	Cerebral cortex	11.516 $\pm$ 0.839
	Hippocampus	9.450 $\pm$ 0.852
M	Cerebral cortex	41.366 $\pm$ 1.467 ^{***}
	Hippocampus	38.283 $\pm$ 1.726 ^{***}
M + Dnpz	Cerebral cortex	21.183 $\pm$ 2.543 ^{###}
	Hippocampus	18.416 $\pm$ 2.856 ^{###}
M + Myr L	Cerebral cortex	22.733 $\pm$ 1.179 ^{###}
	Hippocampus	20.850 $\pm$ 1.047 ^{###}
M + Myr H	Cerebral cortex	17.616 $\pm$ 1.181 ^{###}
	Hippocampus	16.050 $\pm$ 0.995 ^{###}
M + Myr L + Dnpz	Cerebral cortex	12.900 $\pm$ 1.445 ^{###, ^, &&}
	Hippocampus	10.916 $\pm$ 1.319 ^{###, ^, &&}

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{&&}p<0.01 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

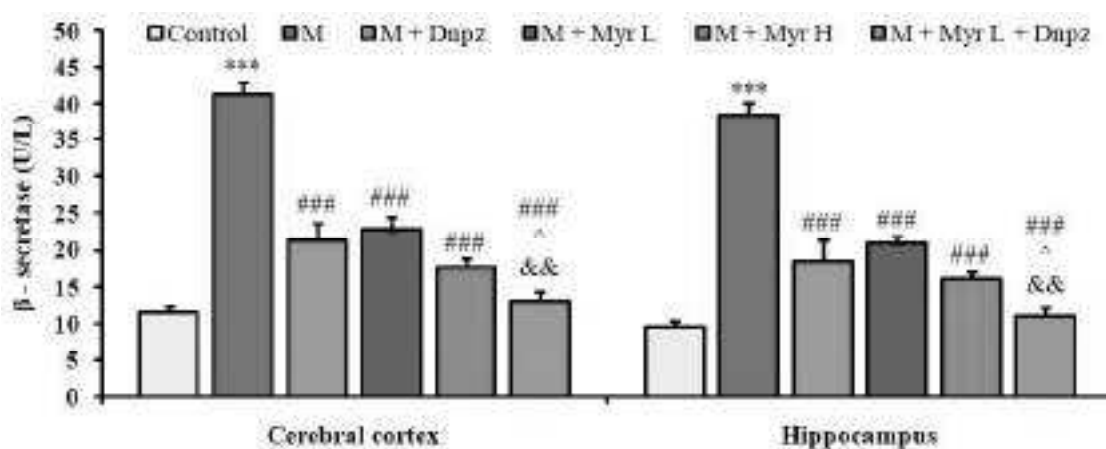


Figure 5.26: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on β - secretase level in the cerebral cortex and hippocampus of (AlCl_3 + D - galactose) administered mice. Bars in the chart represent the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{&&}p<0.01 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

5.2.2.9. Myrcene alone and in combination with Donepezil decrease the amyloid beta peptide₁₋₄₂ level in different brain regions of AlCl₃ and D-galactose induced AD mice

Amyloid beta peptide₁₋₄₂ level in the cerebral cortex along with hippocampus was analyzed by ELISA to assess the treatments impact on amyloidopathy of AD mice. According to one way ANOVA study, the treatments showed a significant impact on amyloid beta peptide₁₋₄₂ level in the cortex ($F_{(5, 30)} = 14.730$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 13.030$, $P < 0.001$) in the mice brain. When compared to control group, the model (AlCl₃ + D-galactose) group significantly raised the level of amyloid beta peptide₁₋₄₂ in the cerebral cortex as well as hippocampus. Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatments were given to AD mice significantly decrease amyloid beta peptide₁₋₄₂ level in the cerebral cortex along with hippocampus than the model. In comparison to Dnpz and Myr L treated mice, concomitant treatment with Myr L + Dnpz considerably reduced amyloid beta peptide₁₋₄₂ level in the cerebral cortex along with hippocampus, as shown in **Table 5.28** and **Figure 5.27**. Consequently, the treatment of drugs efficiently attenuated amyloid beta peptide₁₋₄₂ level which led to improvement in learning and memory in AD model mice.

5.2.2.10. Myrcene alone and in combination with Donepezil decrease the tumor necrosis factor- α level in different brain regions of AlCl₃ and D-galactose induced AD mice

TNF- α level was examined in cerebral cortex along with hippocampus to assess treatments effect on neuroinflammation. According to a one-way ANOVA study, the treatments showed a significant effect on level of TNF- α in the cortex ($F_{(5, 30)} = 59.546$, $P < 0.001$) as well as hippocampus ($F_{(5, 30)} = 39.100$, $P < 0.001$) in the mice brain. When compared to the control group, the model (AlCl₃ + D-galactose) group significantly enhanced TNF- α level in the cerebral cortex along with hippocampus. Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatments were given to AD mice considerably attenuate TNF- α level in the cerebral cortex along with hippocampus than the model. In comparison to Dnpz and Myr L treated mice, concomitant treatment with Myr L + Dnpz considerably decreased level of TNF- α in the cerebral cortex as well as hippocampus, as shown in **Table 5.29** and **Figure 5.28**. Therefore, the treatment of drugs efficiently decreased TNF- α level which lead to reduce neuroinflammation in AD-induced mice.

Table 5.28: Effect of Myrcene on amyloid-beta peptide₁₋₄₂ levels of AD-induced mice

Treatment	Mice brain regions	Amyloid beta peptide ₁₋₄₂ (ng/mL)
Control	Cerebral cortex	2.125 ± 0.591
	Hippocampus	1.810 ± 0.589
M	Cerebral cortex	12.351 ± 0.692 ^{***}
	Hippocampus	11.615 ± 1.003 ^{***}
M + Dnpz	Cerebral cortex	6.648 ± 1.718 ^{##}
	Hippocampus	6.333 ± 1.675 [#]
M + Myr L	Cerebral cortex	7.509 ± 0.567 [#]
	Hippocampus	6.592 ± 1.001 [#]
M + Myr H	Cerebral cortex	5.074 ± 1.185 ^{###}
	Hippocampus	4.023 ± 0.940 ^{###}
M + Myr L + Dnpz	Cerebral cortex	2.375 ± 0.600 ^{###,^,&}
	Hippocampus	1.930 ± 0.435 ^{###,^,&}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6).
^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; [&]p<0.05 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

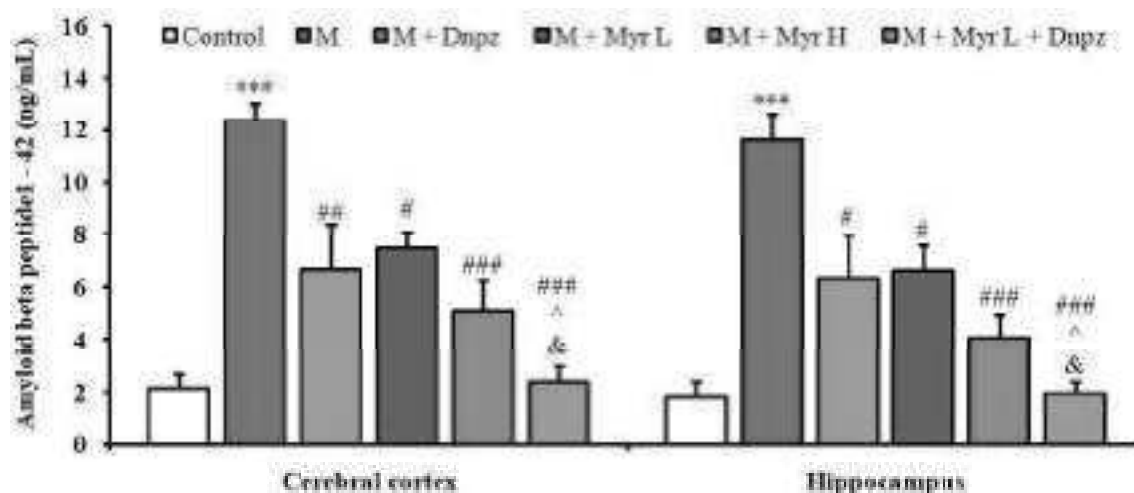


Figure 5.27: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on amyloid-beta peptide₁₋₄₂ level in the cerebral cortex and hippocampus of (AlCl₃ + D - galactose) administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01, and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; [&]p<0.05 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

Table 5.29: Effect of Myrcene on TNF- α levels of AD-induced mice

Treatment	Mice brain regions	TNF - α ($\mu\text{g/ml}$)
Control	Cerebral cortex	963.333 $\pm$ 70.019
	Hippocampus	884.166 $\pm$ 69.646
M	Cerebral cortex	3738.333 $\pm$ 141.370 ^{***}
	Hippocampus	3313.333 $\pm$ 209.230 ^{***}
M + Dnpz	Cerebral cortex	1817.500 $\pm$ 177.570 ^{###}
	Hippocampus	1688.333 $\pm$ 178.380 ^{###}
M + Myr L	Cerebral cortex	2630.000 $\pm$ 150.000 ^{###}
	Hippocampus	2325.833 $\pm$ 137.752 ^{###}
M + Myr H	Cerebral cortex	2280.000 $\pm$ 123.490 ^{###}
	Hippocampus	2000.833 $\pm$ 117.511 ^{###}
M + Myr L + Dnpz	Cerebral cortex	1146.666 $\pm$ 110.428 ^{###, ^, &&&}
	Hippocampus	955.000 $\pm$ 118.84 ^{###, ^, &&&}

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean $\pm$ S.E.M. of each group (n = 6).

^{***} $p < 0.001$ significant from the control group; ^{###} $p < 0.001$ significant from M treated group; [^] $p < 0.05$ significant from Dnpz treated group; ^{&&&} $p < 0.001$ significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; TNF- α : tumour necrosis factor - α .

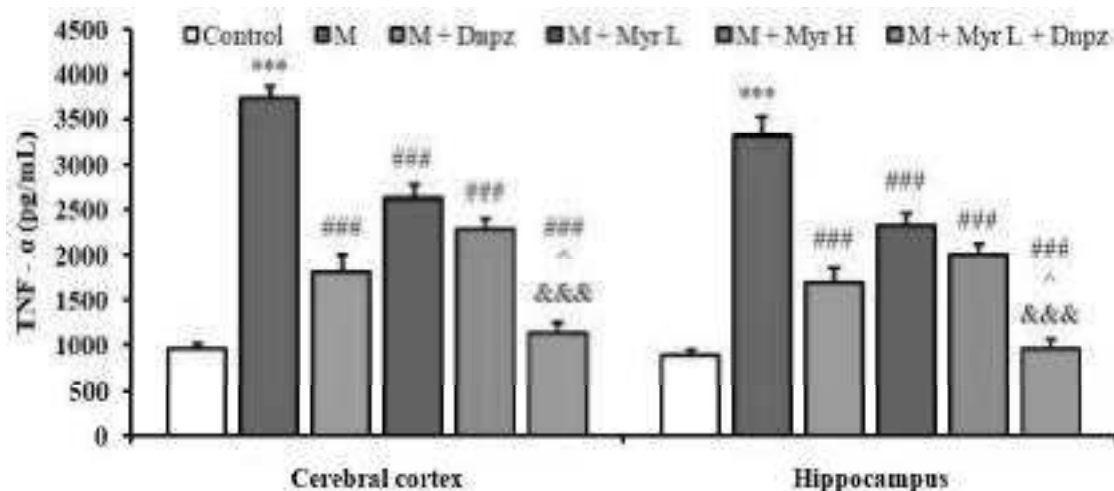


Figure 5.28: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on tumor necrosis factor - α level in the cerebral cortex and hippocampus of (AlCl_3 + D - galactose) administered mice. Bars in the chart represent the mean $\pm$ S.E.M. of each group (n = 6). ^{***} $p < 0.001$ significant from the control group; ^{###} $p < 0.001$ significant from M treated group; [^] $p < 0.05$ significant from Dnpz treated group; ^{&&&} $p < 0.001$ significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; TNF- α : tumor necrosis factor - α .

5.2.2.11. Myrcene alone and in combination with Donepezil decrease the interleukin-6 level in different brain regions of AlCl₃ and D-galactose induced AD mice

Level of interleukin - 6 was estimated in cerebral cortex along with hippocampus to assess treatments effect on neuroinflammation. According to one way ANOVA study, the treatments had a significant effect on IL-6 level in the cortex ($F_{(5, 30)} = 63.518$, $P < 0.001$) along with hippocampus ($F_{(5, 30)} = 35.521$, $P < 0.001$) in the mice brain. When compared to control, the model (AlCl₃ + D-galactose treated) showed a considerable increase level of IL-6 in the cerebral cortex along with hippocampus. Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatments were given to AD mice significantly reduce IL-6 level in the cerebral cortex along with hippocampus than the model. In comparison to Dnpz and Myr L treated AD mice, concomitant treatment with Myr L + Dnpz considerably attenuated level of IL-6 in the cerebral cortex along with hippocampus, as shown in **Table 5.30** and **Figure 5.29**. Thus, the treatment of drugs efficiently decreased IL-6 level which lead to attenuate neuroinflammation in AD-induced mice.

5.2.3. Histopathological analysis

The analysis of histopathology revealed no damage signs to the cerebral cortex along with hippocampus of control (vehicle-treated) mice. The model (AlCl₃ + D-galactose treated) group showed abnormal neuron cells, eosinophilic lesions, and hyperchromatic nuclei in cerebral cortex as well as hippocampus. Dnpz (2 mg/kg), Myr L (100 mg/kg), Myr H (200 mg/kg), and Myr L + Dnpz treatments were given to AD mice, showed the improvement in abnormal neuron cells, eosinophilic lesions, and hyperchromatic nuclei in cerebral cortex along with hippocampus than the model. The concomitant treatment with Myr L + Dnpz to AD mice showed the maximum protection in dispersedly arranged neurons, eosinophilic lesions and hyperchromatic nuclei in the cerebral cortex along with hippocampus than Dnpz and Myr L treated mice, as shown in **Figure 5.30**.

Table 5.30: Effect of Myrcene on IL-6 levels of AD-induced mice

Treatment	Mice brain regions	IL - 6 (pg/mL)
Control	Cerebral cortex	215.583 ± 28.884
	Hippocampus	198.500 ± 36.600
M	Cerebral cortex	1163.500 ± 23.013 ^{***}
	Hippocampus	1081.833 ± 32.925 ^{***}
M + Dnpz	Cerebral cortex	511.000 ± 29.467 ^{###}
	Hippocampus	471.833 ± 24.098 ^{###}
M + Myr L	Cerebral cortex	643.500 ± 56.741 ^{###}
	Hippocampus	598.500 ± 84.496 ^{###}
M + Myr H	Cerebral cortex	538.500 ± 54.799 ^{###}
	Hippocampus	493.500 ± 76.895 ^{###}
M + Myr L + Dnpz	Cerebral cortex	276.833 ± 46.655 ^{###, ^, &&&}
	Hippocampus	229.333 ± 35.371 ^{###, ^, &&&}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6).

^{***}p<0.001 significant from the control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{&&&}p<0.001 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; IL-6: interleukin – 6.

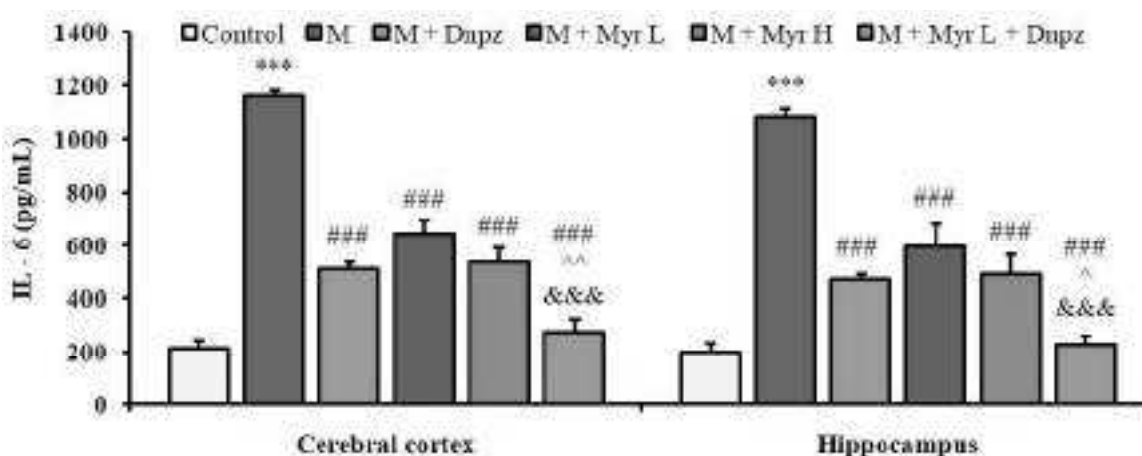


Figure 5.29: Effect of treatment with Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), Dnpz (2 mg/kg b. w.; i. p.) and (Myr L + Dnpz) on interleukin – 6 level in the cerebral cortex and hippocampus of (AlCl₃ + D - galactose) administered mice. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{&&&}p<0.001 significant from Myr L treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Myr L = Myrcene low dose; Myr H = Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight; IL-6: interleukin – 6.

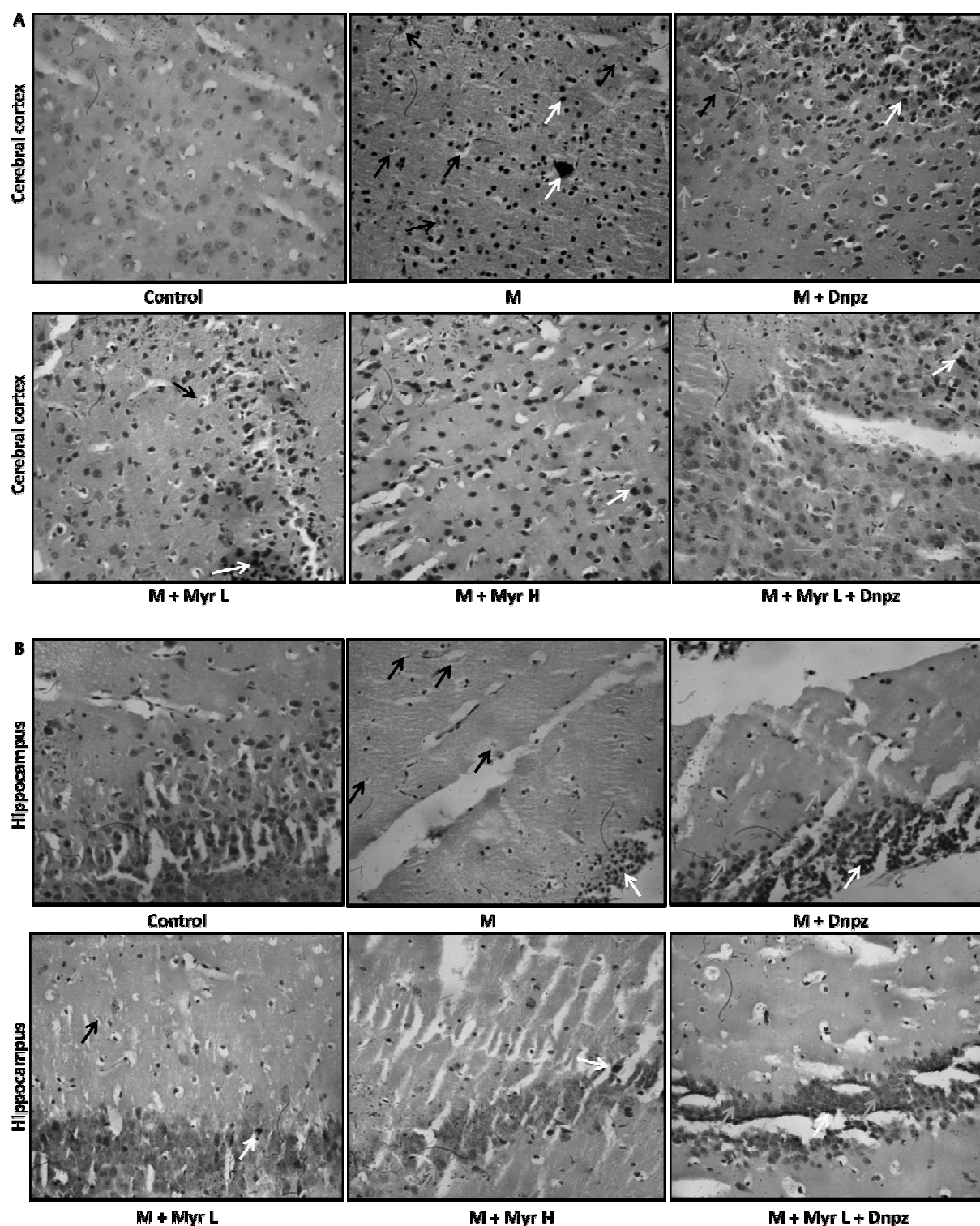


Figure 5.30: Analysis of histopathological data; (40X) of transverse section of the cerebral cortex (A) and hippocampus (B) using hematoxylin and eosin staining; Control (Normal saline), Model (AlCl_3 +D-galactose), Dnpz (2 mg/kg b. w.; i. p.), Myr L (100 mg/kg b. w.; i. p.), Myr H (200 mg/kg b. w.; i. p.), and co-administration (Myr L + Dnpz) of mice of all groups; Black, Yellow and Green arrows express the eosinophilic lesions, hyperchromatic nuclei, and normal neuron cells respectively. Where: M= Model (AlCl_3 + D-galactose); Dnpz= Donepezil; Myr L= Myrcene low dose; Myr H= Myrcene high dose; i. p. = Intraperitoneal route; b. w. = Body weight.

5.3. Combination of Bromelain and Myrcene

5.3.1. Behavioral analysis

5.3.1.1. Bromelain and Myrcene improve the spatial reference memory and spatial working memory in AlCl_3 and D - galactose induced AD mice in the MWM test

SRM data analysis by two-way ANOVA revealed significant differences between treatments ($F_{(6, 105)} = 41.344$, $P < 0.001$) and test days ($F_{(2, 105)} = 4.956$, $P = 0.009$) in the MWM task. Treatments and days had no significant interaction ($F_{(12, 105)} = 0.0408$, $P = 1$) **Table 5.31a and Figure 5.31a**. On training days 81, 82, and 83, data analysis revealed that the model (AlCl_3 + D - galactose treated) group significantly ($P < 0.001$) increased escape latency compared to the control group, indicating greater cognitive dysfunction and a decline in mice's reference memory. When caused AD mice were given Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz, there was a substantial ($P < 0.001$) reduction in escape latency compared to the model. In comparison to Dnpz, Brm, Myr, Brm + Myr treated animals, combined therapy with Brm + Myr + Dnpz significantly ($P < 0.01$) reduced escape latency in AD caused mice, indicating decreased cognitive impairment and improved reference memory. However, there was no significant ($P > 0.05$) change among day 81, 82, and 83.

Table 5.31 a: Effect of Bromelain and Myrcene on spatial reference memory of AD induced mice in MWM

Day	Control	M	M + Dnpz	M + Brm	M + Myr	M + Brm + Myr	M + Brm + Myr + Dnpz
Day-81	14.208 ± 4.343	67.041 ± 3.515 ^{***}	38.125 ± 1.700 ^{##}	45.000 ± 3.985 [#]	42.416 ± 5.549 [#]	42.125 ± 8.490 ^{##}	14.500 ± 2.090 ^{###, ^, \$\$\$, &&, @@}
Day-82	9.500 ± 1.200	64.375 ± 5.791 ^{***}	32.291 ± 1.901 ^{###}	39.041 ± 3.764 ^{##}	40.250 ± 6.033 [#]	37.041 ± 9.024 ^{##}	9.833 ± 1.198 ^{###, ^, \$\$\$, &&, @@}
Day-83	7.250 ± 0.642	59.125 ± 7.938 ^{***}	29.041 ± 1.619 ^{###}	34.250 ± 2.317 [#]	35.250 ± 5.224 [#]	32.458 ± 7.726 ^{##}	7.500 ± 0.444 ^{###, ^, \$\$\$, &&, @}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&}p<0.01, ^{&&&}p<0.001 significant as compared to Myr treated group and [@]p<0.01 significant from Brm + Myr treated group, (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

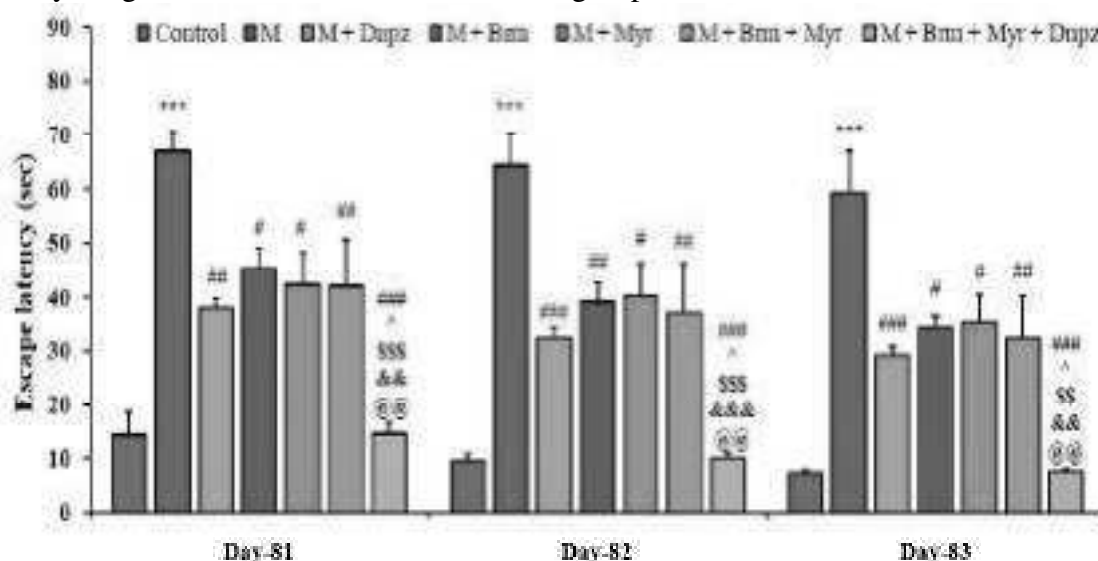


Figure 5.31a: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on spatial reference memory of (AlCl₃ + D - galactose) administered mice during training days 81, 82 and 83. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&}p<0.01, ^{&&&}p<0.001 significant as compared to Myr treated group and [@]p<0.01 significant from Brm + Myr treated group, (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

On day 84, a probing trial task was performed to assess memory retention. According to a one-way ANOVA investigation, significant differences between treatments were found as time passed in the target quadrant ($F_{(6, 35)} = 51.756$, $P < 0.001$) **Table 5.31b and Figure 5.31 b**. When compared to control, the model was found to considerably ($P < 0.001$) minimize the time spent in the target quadrant. Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz, treatments to generated AD mice significantly ($P < 0.001$) increased time spent in the target quadrant compared to the model. Concomitant treatment with Brm + Myr + Dnpz of AD-induced mice was observed to significantly ($P < 0.001$) enhance the time spent in the target quadrant compared to Dnpz, Brm, Myr, Brm + Myr treated mice, indicating improved memory in mice.

On days 87, 88, 89, and 90 of the SWM task, mice were given an acquisition and retrieval trial to assess their spatial working memory. The acquisition and retrieval trial data were analyzed using two-way ANOVA, which revealed significant differences in escape latency among the treatments. Two-way ANOVA analysis revealed significant differences between treatments ($F_{(6, 140)} = 43.453$, $P < 0.001$) and test days ($F_{(3, 140)} = 3.019$, $P = 0.032$) in the acquisition trial. There was no statistically significant interaction between treatments and days ($F_{(18, 140)} = 0.0224$, $P = 1.00$). **Table 5.31 c and Figure 5.31 c**. The model was observed to increase escape latency significantly ($P < 0.001$) when compared to the control, indicating increased cognitive dysfunction and decreased working memory in mice. When caused AD mice were given. Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz, the escape latency was significantly reduced ($P < 0.001$) compared to the model. In comparison to Dnpz, Brm, Myr, Brm + Myr treated animals, concurrent therapy with Brm + Myr + Dnpz was observed to significantly ($P < 0.05$) reduce escape latency in AD caused mice, indicating reduced cognitive impairment and increased working memory.

Table 5.31 b: Effect of Bromelain and Myrcene on time spent in the target quadrant (Probe trial) of AD induced mice in MWM

Day	Control	M	M + Dnpz	M + Brm	M + Myr	M + Brm + Myr	M + Brm + Myr + Dnpz
Day - 84	49.125 ± 2.739	6.458 ± 0.748 ^{***}	23.583 ± 1.452 ^{##}	16.625 ± 1.936 [#]	17.166 ± 2.917 [#]	28.041 ± 2.194 ^{###}	43.458 ± 2.055 ^{###,^^^,\$}

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p< 0.05. and ^{###}p<0.001 significant from M treated group; ^{^^^}p<0.001 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&&}p<0.001 significant from Myr treated group and ^{@@@}p<0.001 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

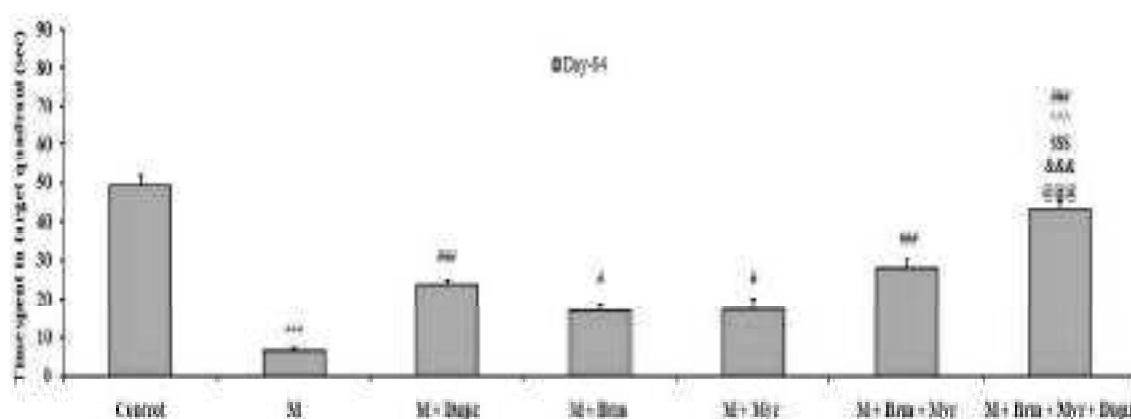


Figure 5.31 b: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on time spent in the target quadrant (Probe trial) of (AlCl_3 + D - galactose) administered mice in MWM test. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p< 0.05. and ^{###}p<0.001 significant from M treated group; ^{^^^}p<0.001 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&&}p<0.001 significant from Myr treated group and ^{@@@}p<0.001 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

Table 5.31 c: Effect of Bromelain and Myrcene on the spatial working memory (acquisition trial) of AD induced mice in MWM

Day	Control	M	M + Dnpz	M + Brm	M + Myr	M + Brm + Myr	M + Brm + Myr + Dnpz
Day-87	13.541 ± 2.447	81.583 ± 2.887 ^{***}	44.625 ± 3.848 ^{##}	51.458 ± 8.033 [#]	49.083 ± 8.560 [#]	46.125 ± 10.885 ^{##}	14.083 ± 3.245 ^{###, ^, \$, &, @}
Day-88	11.041 ± 2.805	76.166 ± 5.581 ^{***}	41.291 ± 3.586 ^{##}	46.541 ± 9.373 [#]	46.250 ± 11.938 [#]	41.500 ± 10.686 ^{##}	11.625 ± 1.713 ^{###, ^, \$, &, @}
Day-89	9.500 ± 3.032	75.500 ± 4.096 ^{***}	39.291 ± 2.979 ^{##}	44.833 ± 1.181 [#]	44.041 ± 12.352 [#]	40.041 ± 11.187 ^{##}	9.708 ± 2.562 ^{###, ^, \$, &, @}
Day-90	5.708 ± 0.350	67.916 ± 5.389 ^{***}	34.833 ± 3.279 [#]	38.583 ± 5.306 [#]	36.875 ± 8.244 [#]	35.041 ± 5.670 [#]	5.916 ± 0.546 ^{###, ^, \$, &, @}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01, and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$}p<0.01 significant from Brm treated group; [&]p<0.05 and [&]p<0.01 significant from Myr treated group and [@]p<0.05 significant from Brm + Myr treated group, (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

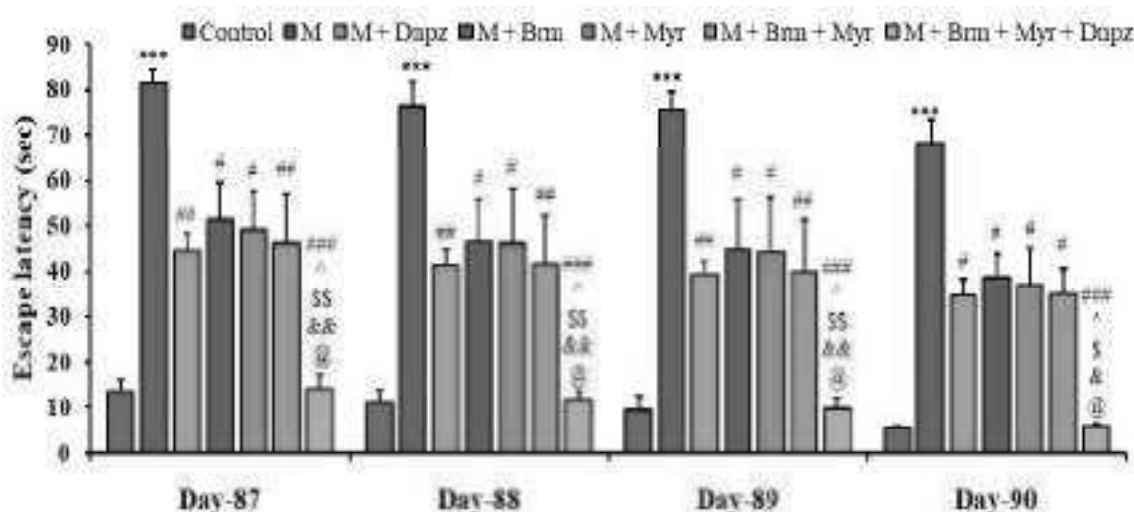


Figure 5.31 c: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on the spatial working memory of (AlCl₃ + D - galactose) administered mice at days 87, 88, 89 and 90, acquisition trial. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from the control group; [#]p<0.05, ^{##}p<0.01, and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$}p<0.01 significant from Brm treated group; [&]p<0.05 and [&]p<0.01 significant from Myr treated group and [@]p<0.05 significant from Brm + Myr treated group, (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

Similarly, two-way ANOVA analysis revealed significant differences between treatments ($F_{(6, 140)} = 52.681$, $P < 0.001$) and test days ($F_{(3, 140)} = 2.680$, $P = 0.049$) in the retrieval trial. There were no significant differences between treatments and days ($F_{(18, 140)} = 0.0502$, $P = 1.000$) **Table 5.31 d** and **Figure 5.31 d**. In the model and treatments, there was a substantial ($P < 0.001$) increase and decrease in escape delay, respectively. In the acquisition and retrieval experiment, however, no significant differences were seen between days 87, 88, 89, and 90.

5.3.1.2. Bromelain and Myrcene improve the learning memory of $AlCl_3$ and D - galactose induced AD mice in the EPM test

Two way ANOVA analysis were found significant variations among treatments ($F_{(6, 105)} = 33.81$, $P < 0.001$) as well as test days ($F_{(2, 105)} = 98.181$, $P < 0.001$). A significant interaction between treatments and days were obtained ($F_{(12, 105)} = 5.611$, $P < 0.001$) **Table 5.31e** and **Figure 5. 31e**. Thus, it was observed that on day 81; in any treatment, transfer latency did not differ significantly. On days 82 and 90; the Model ($AlCl_3$ + D - galactose treated) group was found to significantly ($P < 0.001$) increase transfer latency than the control (vehicle-treated) group. The treatments with Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz, to induce AD mice were found significantly ($P < 0.001$) attenuation in transfer latency as compared to the model. The concomitant treatment with Brm + Myr + Dnpz to AD induced mice was observed to significantly ($P < 0.001$) decrease transfer latency as compared to Dnpz, and Brm + Myr treated mice, demonstrating improvement in learning along with the memory of mice. However, significant ($P < 0.05$) differences in transfer latency were observed on days 82 and 90 as compared to day 81.

Table 5.31 d: Effect of Bromelain and Myrcene on the spatial working memory (retrieval trial) of AD induced mice in MWM

Day	Control	M	M + Dnpz	M + Brm	M + Myr	M + Brm + Myr	M + Brm + Myr + Dnpz
Day-87	12.875 ± 3.579	80.916 ± 1.227***	43.791 ± 1.951###	48.833 ± 6.218##	47.791 ± 7.674##	45.750 ± 9.079##	14.250 ± 3.314###, ^, \$, &, @
Day-88	10.458 ± 1.975	79.333 ± 2.413***	41.875 ± 5.939###	45.666 ± 9.744##	44.583 ± 5.173##	41.958 ± 11.433###	12.958 ± 4.707###, ^, \$, &, @
Day-89	7.416 ± 0.554	73.500 ± 5.569***	39.541 ± 4.596##	42.958 ± 7.818#	44.625 ± 11.595#	39.250 ± 9.472##	7.708 ± 1.153###, ^, \$\$\$, &, @
Day-90	6.291 ± 0.705	67.041 ± 6.446***	36.125 ± 5.312##	39.750 ± 7.167#	39.791 ± 6.628#	36.500 ± 7.210#	6.458 ± 0.986###, ^, \$, &, @

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01, and ###p<0.001 significant from M treated group; ^p<0.05 and ^^p<0.01 significant from Dnpz treated group; \$p<0.01 significant from Brm treated group; &p<0.01 and &&p<0.001 significant from Myr treated group and @p<0.05 and @@p<0.01 significant from Brm + Myr treated group, (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

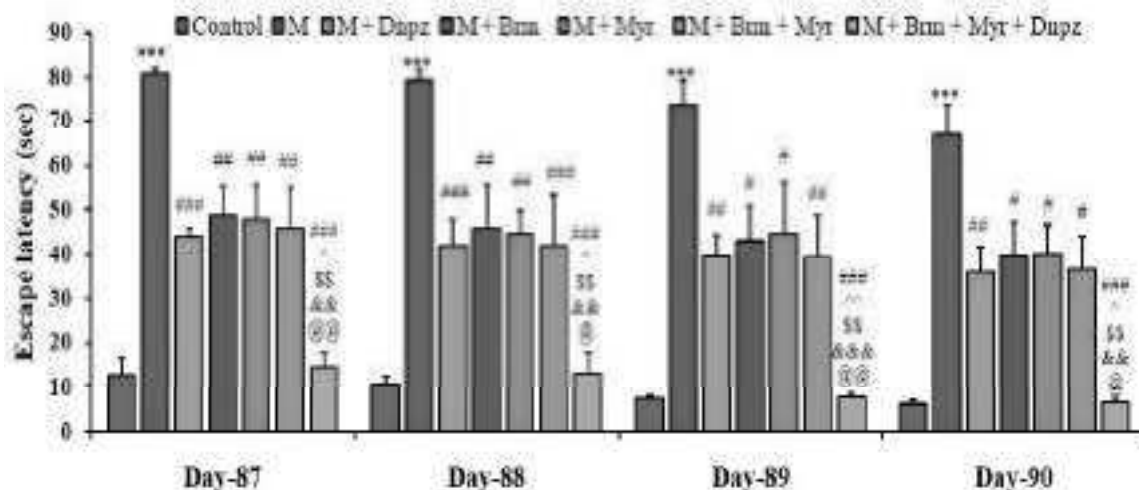


Figure 5.31 d: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on the spatial working memory of (AlCl₃ + D - galactose) administered mice at days 87, 88, 89 and 90, retrieval trial. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01, and ###p<0.001 significant from M treated group; ^p<0.05 and ^^p<0.01 significant from Dnpz treated group; \$p<0.01 significant from Brm treated group; &p<0.01 and &&p<0.001 significant from Myr treated group and @p<0.05 and @@p<0.01 significant from Brm + Myr treated group, (two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

Table 5.31 e: Effect of Bromelain and Myrcene on the transfer latency of AD induced mice in EPM

Day	Control	M	M + Dnpz	M + Brm	M + Myr	M + Brm + Myr	M + Brm + Myr + Dnpz
Day-81	45.500 ± 3.956	51.666 ± 3.062	46.500 ± 3.712	48.500 ± 3.452	49.166 ± 3.646	47.333 ± 4.971	45.833 ± 4.110
Day-82	10.333 ± 1.475 ^a	52.166 ± 2.937 ^{***}	29.500 ± 1.056 ^{###,a}	37.666 ± 2.333 ^{#,a}	36.333 ± 2.043 ^{#,a}	32.000 ± 4.234 ^{###,a}	11.500 ± 2.473 ^{###,^^,\$ \$\$,&&&,@ @ @, a}
Day-90	5.666 ± 0.954 ^a	47.333 ± 4.835 ^{***}	23.000 ± 2.236 ^{###,a}	31.166 ± 2.650 ^{##,a}	33.666 ± 2.996 ^{#,a}	25.500 ± 5.542 ^{###,a}	5.833 ± 1.492 ^{###,^^,\$ \$\$,&&&,@ @ @, a}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01, and ###p<0.001 significant from M treated group, ^p<0.01 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&&p<0.001 significant from Myr treated group, @@@p<0.001 significant from Brm + Myr treated group, ^a represent significant from day 8, (Two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

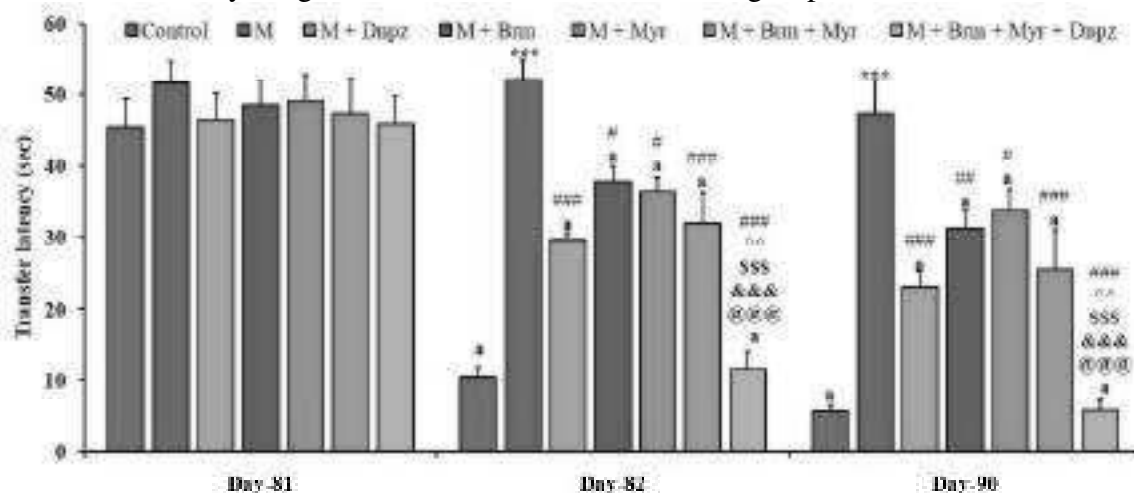


Figure 5.31 e: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) treatment on the transfer latency of (AlCl₃ + D - galactose) administered mice in the elevated plus-maze test. Bars in the chart represent the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from the control group; #p<0.05, ##p<0.01, and ###p<0.001 significant from M treated group, ^p<0.01 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&&p<0.001 significant from Myr treated group. @@@p<0.001 significant from Brm + Myr treated group, ^a represent significant from day 81, (Two-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil;

Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; b. w. = Body weight; n = Number of animal in each group.

5.3.1.3. Bromelain alone and in combination with Donepezil show no locomotor activity alteration of AlCl₃ and D - galactose induced AD mice

Locomotor activity was performed on days 0, 80, and 90 to assess the effect of treatments on spontaneous locomotion of mice of all groups. Data analysis by one-way ANOVA showed that the model (AlCl₃ + D-galactose) group was not found significantly than the control (vehicle-treated) group. The treatments with Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz to induced AD mice were not observed significantly as compared to the model. The concomitant treatment with Brm + Myr + Dnpz to AD-induced mice was not found significantly as compared to Dnpz and Brm + Myr treated mice, signifying no alteration in locomotor activity of mice throughout the study as shown in **Table 5.32**.

Table 5.32: Effect of Bromelain and Myrcene on locomotor activity of AD-induced mice

Groups	Day 0		Day 80		Day 90	
	Before dose	After dose	Before dose	After dose	Before dose	After dose
Control	388.33 ± 31.43	367.67 ± 22.16	361.17 ± 17.43	355.17 ± 13.95	376.33 ± 10.41	364.50 ± 8.86
M	355.5 ± 21.92	328.17 ± 18.11	348.33 ± 10.22	342.67 ± 8.41	362.00 ± 6.60	349.67 ± 10.73
M + Dnpz	379.17 ± 20.93	351.00 ± 15.46	359.00 ± 8.33	355.00 ± 7.02	358.00 ± 10.55	340.33 ± 4.48
M + Brm	361.5 ± 15.74	367.33 ± 14.52	334.33 ± 15.06	333.17 ± 14.03	366.17 ± 25.04	329.50 ± 22.44
M + Myr	332.17 ± 22.67	332.83 ± 19.40	311.50 ± 16.60	310.00 ± 15.49	329.50 ± 14.71	328.67 ± 14.41
M + Brm + Myr	335.00 ± 13.50	328.50 ± 14.08	342.17 ± 15.81	339.33 ± 14.93	321.50 ± 7.48	321.83 ± 7.55
M + Brm + Myr + Dnpz	340.67 ± 17.12	336.17 ± 14.58	324.33 ± 18.07	325.00 ± 16.93	329.50 ± 20.70	329.33 ± 17.69

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6).

5.3.2. Biochemical analysis

5.3.2.1. Bromelain and Myrcene inhibit the acetylcholinesterase activity in different brain regions of AlCl₃ and D - galactose induced mice

AChE activity in both the cerebral cortex and hippocampus was measured at the end of the trial to see how medications affected the cholinergic system's function. The treatments had a significant influence on AChE enzyme activity in both the cerebral cortex ($F_{(6, 35)} = 29.203$, $p < 0.001$) as well as hippocampus ($F_{(6, 35)} = 22.802$, $p < 0.001$), according to a one-way ANOVA analysis. As compared to the control, the model (AlCl₃ + D - galactose) showed a substantial rise in AChE levels in both the cerebral cortex and hippocampus. When induced AD mice were given Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz, AChE levels in the cerebral cortex and hippocampus were shown to be significantly lower than in the model. The concomitant treatment with Brm + Myr + Dnpz to exposed AD mice was found to significantly decline AChE level in both the cerebral cortex and hippocampus as compared to Dnpz, Brm, Myr, Brm + Myr treated mice groups as shown in **Table 5.33 and Figure 5.32**.

5.3.2.2. Bromelain and Myrcene decrease the MDA level in different brain regions of AlCl₃ and D - galactose induced mice

Lipid peroxidation measured MDA level in the cerebral cortex and hippocampus to consider the effect of medication on antioxidants. One-way ANOVA analysis showed a significant effect of the treatments on MDA level in the cerebral cortex ($F_{(6, 35)} = 15.053$, $p < 0.001$) along with the hippocampus ($F_{(6, 35)} = 11.575$, $p < 0.001$). Model (AlCl₃ + D - galactose) was observed to significantly increase MDA level in both the cerebral cortex and hippocampus as compared to control. The treatments with Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz to induced AD mice were evaluated to significantly reduce MDA level in both the cerebral cortex and hippocampus as compared to the model. The concomitant treatment with Brm + Myr + Dnpz to AD-induced mice was obtained significantly decrease MDA level in both the cerebral cortex and hippocampus as compared to Dnpz, Brm, Myr, Brm + Myr treated mice as shown in **Table 5.34 and Figure 5.33**.

Table 5.33: Effect of Bromelain and Myrcene on **acetylcholinesterase** levels of AD-induced mice

Treatment	Mice brain regions	AChE (n moles per mg protein)
Control	Cerebral cortex	2.093 ± 0.284
	Hippocampus	2.026 ± 0.592
M	Cerebral cortex	8.372 ± 0.465***
	Hippocampus	8.154 ± 0.666***
M + Dnpz	Cerebral cortex	5.161 ± 0.378###
	Hippocampus	5.001 ± 0.368###
M + Brm	Cerebral cortex	6.234 ± 0.393##
	Hippocampus	5.751 ± 0.337#
M + Myr	Cerebral cortex	6.174 ± 0.573##
	Hippocampus	5.949 ± 0.353#
M + Brm + Myr	Cerebral cortex	4.532 ± 0.357###
	Hippocampus	4.213 ± 0.473###
M + Brm + Myr + Dnpz	Cerebral cortex	2.706 ± 0.272###, ^, \$\$\$, &&&, @
	Hippocampus	2.099 ± 0.284###, ^, \$\$\$, &&&, @

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.01 significant from Dnpz treated group. \$\$\$p<0.001 significant from Brm treated group; &&&p<0.001 significant from Myr treated group, and @p<0.05 significant from Brm + Myr treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃+ D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; AChE= Acetylcholinesterase.

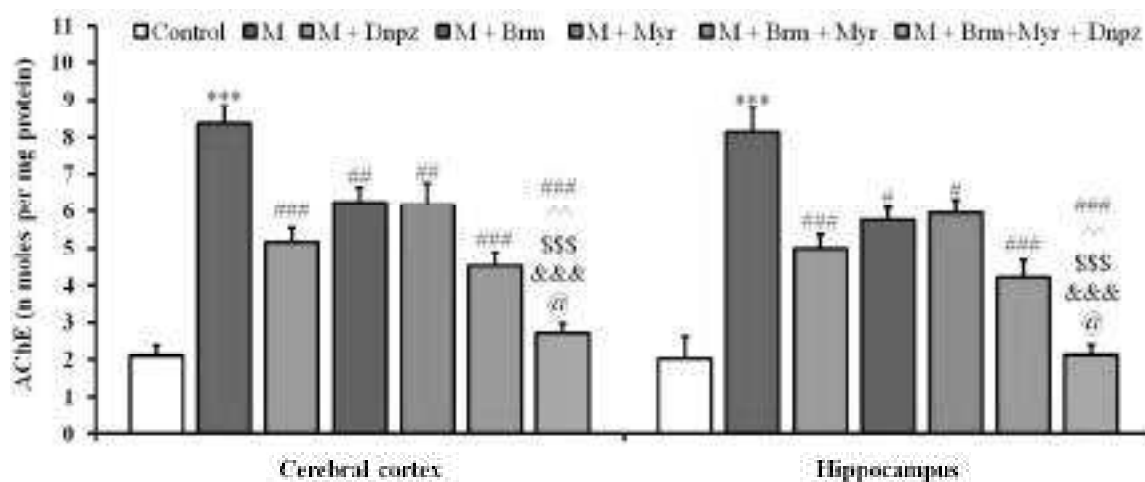


Figure 5.32: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on AChE levels in the cerebral cortex and the hippocampus of (AlCl₃ + D - galactose) given mice. The mean ± S.E.M. of each group (n = 6) is represented by bars in the graph. ***p<0.001 significant from control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.01 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&&p<0.001 significant from Myr treated group; and @p<0.05 significant from Brm + Myr treated group, (one way ANOVA followed by Tukey test). Where: M = Model (AlCl₃+ D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; AChE= Acetylcholinesterase.

Table 5.34: Effect of Bromelain and Myrcene on MDA levels of AD-induced mice

Treatment	Mice brain regions	MDA (n moles per mg protein)
Control	Cerebral cortex	0.843 ± 0.079
	Hippocampus	0.753 ± 0.127
M	Cerebral cortex	4.251 ± 0.389 ^{***}
	Hippocampus	3.743 ± 0.300 ^{***}
M + Dnpz	Cerebral cortex	2.404 ± 0.262 ^{##}
	Hippocampus	2.285 ± 0.483 [#]
M + Brm	Cerebral cortex	2.651 ± 0.314 [#]
	Hippocampus	2.269 ± 0.330 [#]
M + Myr	Cerebral cortex	2.792 ± 0.494 [#]
	Hippocampus	2.314 ± 0.289 [#]
M + Brm + Myr	Cerebral cortex	2.303 ± 0.195 ^{###}
	Hippocampus	2.184 ± 0.314 [#]
M + Brm + Myr + Dnpz	Cerebral cortex	0.874 ± 0.199 ^{###, ^, \$, &, &, @}
	Hippocampus	0.800 ± 0.070 ^{###, ^, \$, &, @}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$}p<0.01 significant from Brm treated group; [&]p<0.05 and [&]p<0.01 significant from Myr treated group; and [@]p<0.05 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; MDA= Malondialdehyde.

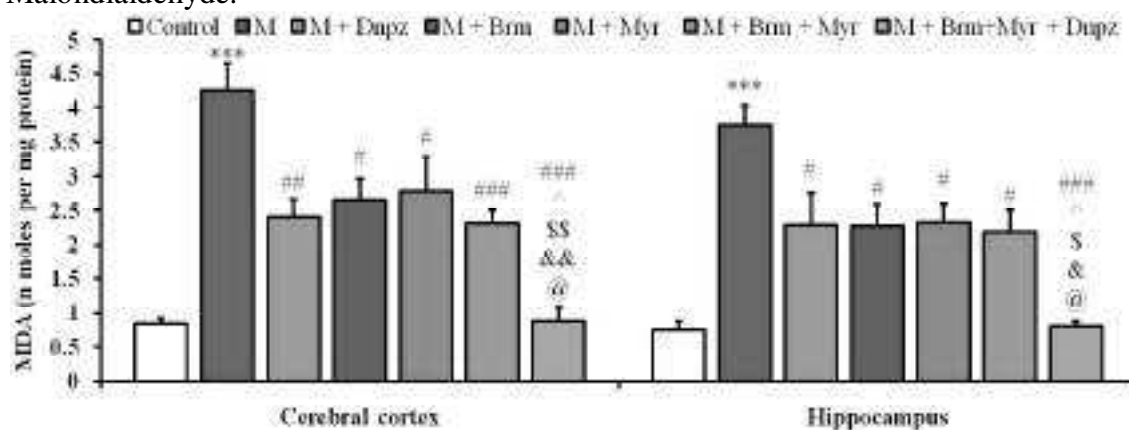


Figure 5.33: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on MDA levels in the cerebral cortex and the hippocampus of (AlCl₃ + D - galactose) administered mice. The mean ± S.E.M. of each group (n = 6) is indicated by bars in the graph. ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$}p<0.01 significant from Brm treated group; [&]p<0.05 and [&]p<0.01 significant from Myr treated group; and [@]p<0.05 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; MDA= Malondialdehyde.

5.3.2.3. Bromelain and Myrcene increase the reduced glutathione level in different brain regions of AlCl_3 and D - galactose induced mice

GSH amount in the cerebral cortex and hippocampus was measured to see how treatments affected antioxidant levels. The treatments had a significant influence on GSH levels in the cerebral cortex ($F_{(6, 35)} = 16.868$, $p < 0.001$) and hippocampus ($F_{(6, 35)} = 17.505$, $p < 0.001$), according to one-way ANOVA analysis. Model (AlCl_3 + D - galactose) was observed to significantly attenuate GSH level in both the cerebral cortex and hippocampus as compared to control. The treatments with Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz to induced AD mice were obtained to significantly enhance GSH level in both the cerebral cortex and hippocampus as compared to the model. The concomitant treatment with Brm + Myr + Dnpz to AD-induced mice was observed to significantly enhance GSH level in both the cerebral cortex and hippocampus as compared to Dnpz, Brm, Myr, Brm + Myr treated mice as shown in **Table 5.35** and **Figure 5.34**.

5.3.2.4. Bromelain and Myrcene increase the catalase level in different brain regions of AlCl_3 and D - galactose induced mice

To calculate the effect of treatments on antioxidants, the amount of CAT was measured in the cerebral cortex and hippocampus. The treatments had a significant influence on the level of CAT in the cerebral cortex ($F_{(6, 35)} = 26.371$, $p < 0.001$) and hippocampus ($F_{(6, 35)} = 20.070$, $p < 0.001$), according to a one-way ANOVA analysis. Model (AlCl_3 + D - galactose) was obtained significantly attenuate CAT level in both the cerebral cortex and hippocampus as compared to control. The treatments with Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz to induced AD mice were observed to significantly enhance CAT levels in both the cerebral cortex and hippocampus as compared to the model. The concomitant treatment with Brm + Myr + Dnpz to AD-induced mice was found to significantly augment CAT level in both the cerebral cortex and hippocampus as compared to Dnpz, Brm, Myr, Brm + Myr treated mice as shown in **Table 5.36** and **Figure 5.35**.

Table 5.35: Effect of Bromelain and Myrcene on GSH levels of AD-induced mice

Treatment	Mice brain regions	GSH (n moles per mg protein)
Control	Cerebral cortex	3.255 ± 0.210
	Hippocampus	3.056 ± 0.274
M	Cerebral cortex	1.130 ± 0.099 ^{***}
	Hippocampus	0.750 ± 0.166 ^{***}
M + Dnpz	Cerebral cortex	2.101 ± 0.288 ^{##}
	Hippocampus	1.629 ± 0.126 [#]
M + Brm	Cerebral cortex	1.950 ± 0.113 [#]
	Hippocampus	1.641 ± 0.179 [#]
M + Myr	Cerebral cortex	1.912 ± 0.070 [#]
	Hippocampus	1.601 ± 0.118 [#]
M + Brm + Myr	Cerebral cortex	2.002 ± 0.191 [#]
	Hippocampus	1.905 ± 0.234 ^{##}
M + Brm + Myr + Dnpz	Cerebral cortex	3.020 ± 0.157 ^{###,^,\$\$,&&,@}
	Hippocampus	2.806 ± 0.166 ^{###,^,\$\$,&&,@}

Note: Control (Normal saline), Model (AlCl₃ +D - galactose),Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$\$}p<0.01 significant from Brm treated group; ^{&&}p<0.01 and ^{&&&}p<0.001 significant from Myr treated group; [@]p<0.05 and ^{@@}p<0.01 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃+ D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; GSH= Glutathione.

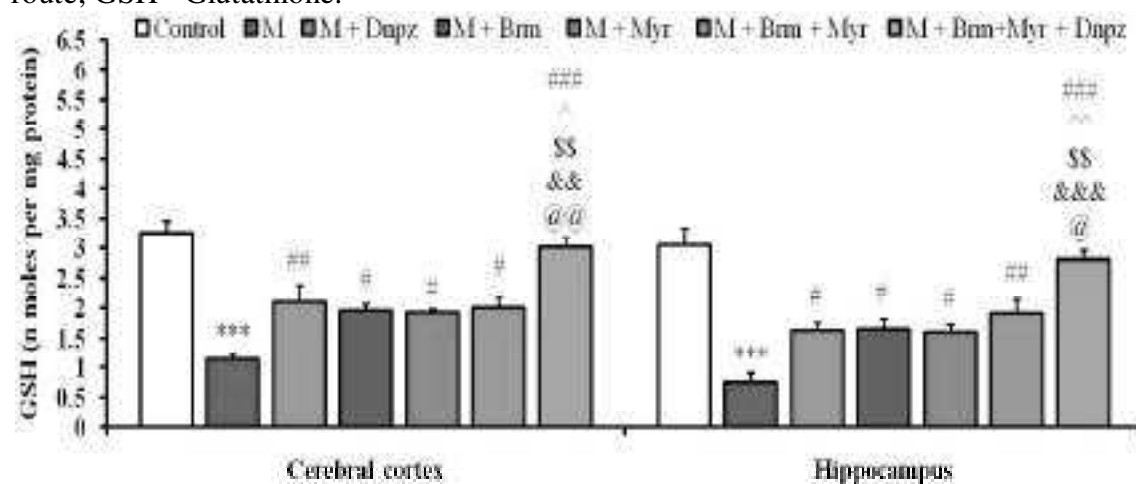


Figure 5.34: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on GSH levels in the cerebral cortex and the hippocampus of (AlCl₃ +D - galactose) induced mice. The mean ± S.E.M. of each group (n = 6) is represented by bars in the graph. ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$\$}p<0.01 significant from Brm treated group; ^{&&}p<0.01 and ^{&&&}p<0.001 significant from Myr treated group; [@]p<0.05 and ^{@@}p<0.01 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃+ D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; GSH= Glutathione.

Table 5.36: Effect of Bromelain and Myrcene on catalase levels of AD-induced mice

Treatment	Mice brain regions	CAT (Units per mg protein)
Control	Cerebral cortex	0.975 ± 0.064
	Hippocampus	0.929 ± 0.101
M	Cerebral cortex	0.328 ± 0.052***
	Hippocampus	0.248 ± 0.021***
M + Dnpz	Cerebral cortex	0.645 ± 0.032###
	Hippocampus	0.560 ± 0.034##
M + Brm	Cerebral cortex	0.612 ± 0.031###
	Hippocampus	0.542 ± 0.020##
M + Myr	Cerebral cortex	0.613 ± 0.019###
	Hippocampus	0.554 ± 0.023##
M + Brm + Myr	Cerebral cortex	0.715 ± 0.032###
	Hippocampus	0.601 ± 0.0319###
M + Brm + Myr + Dnpz	Cerebral cortex	0.945 ± 0.052###,^^,\$\$\$,&&&,@
	Hippocampus	0.907 ± 0.073###,^^,\$\$\$,&&&,@

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ***p<0.001 significant from control group; ##p<0.01 and ###p<0.001 significant from M treated group; ^^p<0.001 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&&p<0.001 significant from Myr treated group; @@p<0.01 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

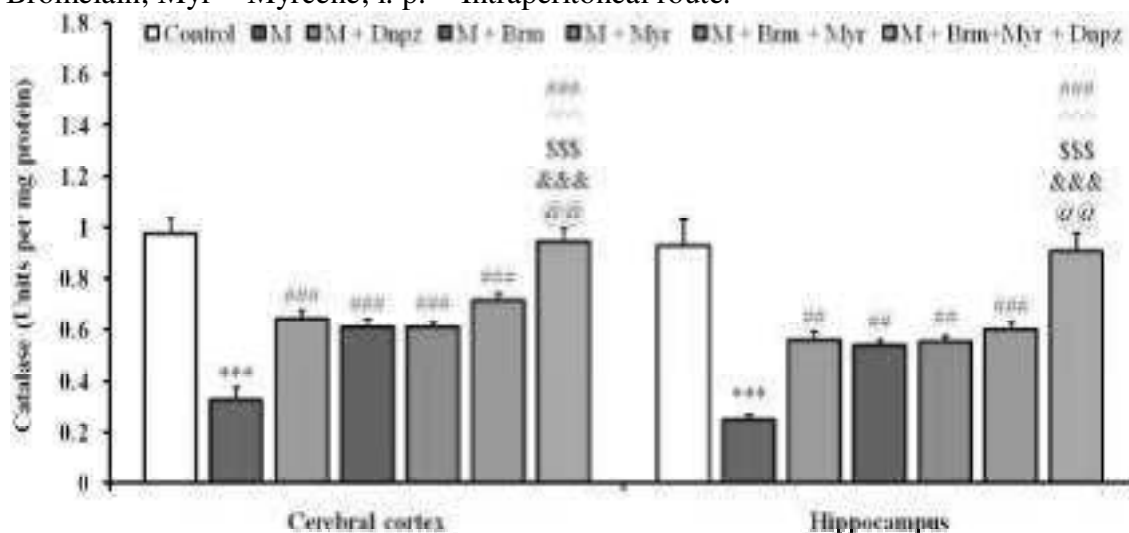


Figure 5.35: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on CAT levels in the cerebral cortex, as well as the hippocampus of (AlCl_3 + D - galactose) administered mice. The mean $\pm$ S.E.M. of each group (n = 6) is indicated by bars in the graph. ***p<0.001 significant from control group; ##p<0.01 and ###p<0.001 significant from M treated group; ^^p<0.001 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&&p<0.001 significant from Myr treated group; @@p<0.01 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

5.3.2.5. Bromelain and Myrcene increase the superoxide dismutase level in different brain regions of AlCl_3 and D - galactose induced mice

Superoxide dismutase level was calculated in the cerebral cortex and hippocampus to assess treatments effect on antioxidants. The results of the one-way ANOVA showed the treatments had a significant impact on the level of SOD in the cerebral cortex ($F_{(6, 35)} = 21.308$, $p < 0.001$) and hippocampus ($F_{(6, 35)} = 15.140$, $p < 0.001$) of the mice brain. In comparison to the control group, the model (AlCl_3 + D - galactose) group had significantly attenuated SOD levels in the cerebral cortex and hippocampus. When, Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz treatments were given to AD mice significantly elevate SOD level in the cerebral cortex and hippocampus than the model. In comparison to Dnpz, Brm, Myr, Brm + Myr treated mice, concomitant treatment with Brm + Myr + Dnpz significantly raised the SOD level in the cerebral cortex and hippocampus, as shown in **Table 5.37** and **Figure 5.36**.

5.3.2.6. Bromelain and Myrcene reduce the nitrite level in different brain regions of AlCl_3 and D - galactose induced mice

In a one-way ANOVA study, the level of nitrite in the cerebral cortex ($F_{(6, 35)} = 15.330$, $p < 0.001$) and hippocampus ($F_{(6, 35)} = 14.901$, $p < 0.001$) of the mice brain was significantly affected by the treatments. The model (AlCl_3 + D - galactose) demonstrated a significantly higher nitrite level in the cerebral cortex and hippocampus when compared to the control group. While, Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz in AD mice resulted in a significant decrease in nitrite level in the cerebral cortex and hippocampus than the model. In comparison to Dnpz, Brm, Myr, Brm + Myr mice, concomitant treatment with Brm + Myr + Dnpz significantly reduced nitrite amount in the cerebral cortex and hippocampus, as shown in **Table 5.38** and **Figure 5.37**.

Table 5.37: Effect of Bromelain and Myrcene on SOD levels of AD-induced mice

Treatment	Mice brain regions	SOD (Units per mg protein)
Control	Cerebral cortex	2.184 ± 0.167
	Hippocampus	2.105 ± 0.196
M	Cerebral cortex	0.610 ± 0.055 ^{***}
	Hippocampus	0.406 ± 0.110 ^{***}
M + Dnpz	Cerebral cortex	1.382 ± 0.047 ^{###}
	Hippocampus	1.137 ± 0.122 [#]
M + Brm	Cerebral cortex	1.163 ± 0.077 [#]
	Hippocampus	1.102 ± 0.135 [#]
M + Myr	Cerebral cortex	1.149 ± 0.027 [#]
	Hippocampus	1.098 ± 0.077 [#]
M + Brm + Myr	Cerebral cortex	1.5106 ± 0.210 ^{###}
	Hippocampus	1.187 ± 0.144 [#]
M + Brm + Myr + Dnpz	Cerebral cortex	2.090 ± 0.128 ^{###, ^, \$\$\$, &&&, @}
	Hippocampus	2.014 ± 0.222 ^{###, ^, \$\$, &&, @}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05 and ^{###}p<0.001 significant from M treated group; [^]p<0.01 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&}p<0.01 and ^{&&&}p<0.001 significant from Myr treated group; [@]p<0.05 and ^{@@}p<0.01 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; SOD= Superoxide dismutase.

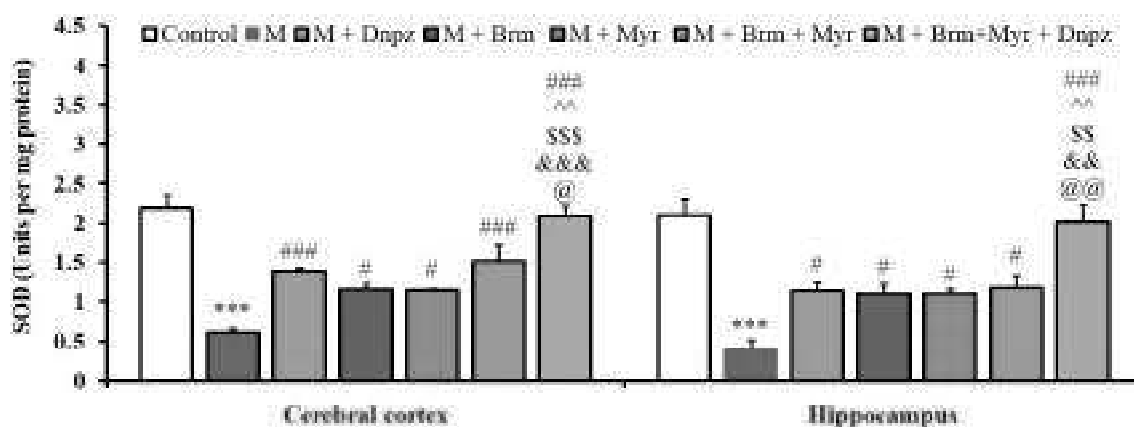


Figure 5.36: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on SOD levels in the cerebral cortex and the hippocampus of (AlCl₃ + D - galactose) induced mice. The mean ± S.E.M. of each group (n = 6) is represented by bars in the graph. ^{***}p<0.001 significant from control group; [#]p<0.05 and ^{###}p<0.001 significant from M treated group; [^]p<0.01 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&}p<0.01 and ^{&&&}p<0.001 significant from Myr treated group; [@]p<0.05 and ^{@@}p<0.01 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; SOD= Superoxide dismutase.

Table 5.38: Effect of Bromelain and Myrcene on nitrite levels of AD-induced mice

Treatment	Mice brain regions	Nitrite (μM per mg protein)
Control	Cerebral cortex	0.961 ± 0.223
	Hippocampus	0.772 ± 0.205
M	Cerebral cortex	$4.215 \pm 0.385^{***}$
	Hippocampus	$4.014 \pm 0.180^{***}$
M + Dnpz	Cerebral cortex	$2.454 \pm 0.351^{##}$
	Hippocampus	$2.214 \pm 0.279^{##}$
M + Brm	Cerebral cortex	$2.918 \pm 0.207^{\#}$
	Hippocampus	$2.702 \pm 0.273^{\#}$
M + Myr	Cerebral cortex	$2.823 \pm 0.375^{\#}$
	Hippocampus	$2.671 \pm 0.392^{\#}$
M + Brm + Myr	Cerebral cortex	$2.291 \pm 0.238^{###}$
	Hippocampus	$2.156 \pm 0.442^{###}$
M + Brm + Myr + Dnpz	Cerebral cortex	$0.993 \pm 0.176^{###,\wedge,\$ \$ \$,\&\&,@}$
	Hippocampus	$0.827 \pm 0.155^{###,\wedge,\$ \$ \$,\&\&,@}$

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ***p<0.001 significant from control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.05 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&p<0.01 significant from Myr treated group; @p<0.05 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

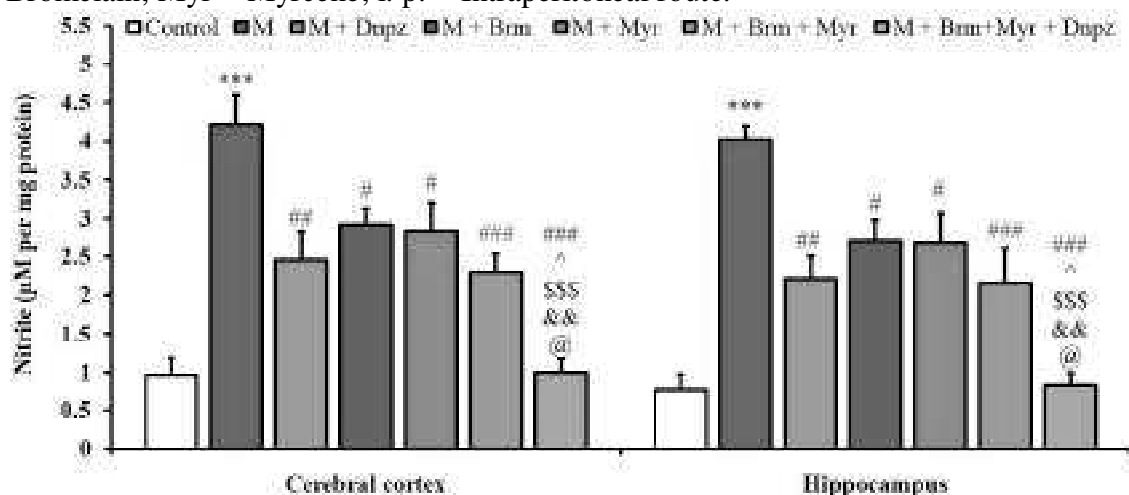


Figure 5.37: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on nitrite levels in the cerebral cortex and the hippocampus of (AlCl_3 + D - galactose) administered mice. The mean $\pm$ S.E.M. of each group (n = 6) is indicated by bars in the graph. ***p<0.001 significant from control group; #p<0.05, ##p<0.01 and ###p<0.001 significant from M treated group; ^p<0.05 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&p<0.01 significant from Myr treated group; @p<0.05 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

5.3.2.7. Bromelain and Myrcene increase the brain-derived neurotrophic factor level in different brain regions of AlCl₃ and D - galactose induced mice

BDNF was analyzed by ELISA test. The level of BDNF in the cerebral cortex and hippocampus was determined to assess the treatment's impact on neurogenesis in AD mice. According to a one-way ANOVA study, the treatments had a significant impact on the level of BDNF in the cerebral cortex ($F_{(6, 35)} = 23.464, p < 0.001$) and hippocampus ($F_{(6, 35)} = 21.399, p < 0.001$) of the mice brain. The model (AlCl₃ + D - galactose) group had significantly reduced levels of BDNF in the cerebral cortex and hippocampus when compared to the control group. While, Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz treatments were given to AD mice significantly enhance the level of BDNF in the cerebral cortex and hippocampus than the model. In comparison to Dnpz, Brm, Myr, Brm + Myr treated mice, concomitant treatment with Brm + Myr + Dnpz significantly augmented the level of BDNF in the cerebral cortex and hippocampus, as shown in **Table 5.39** and **Figure 5.38**.

5.3.2.8. Bromelain and Myrcene decrease the β -secretase level in different brain regions of AlCl₃ and D - galactose induced mice

β -secretase level alteration affects the amyloidogenic process of AD as a consequence of the fluctuation of amyloid beta-peptide in the cerebral cortex and hippocampus. β -secretase levels in the cerebral cortex and hippocampus were examined to measure treatment's effect on the amyloidogenic process of AD. According to one way ANOVA study, the treatments showed a significant impact on the level of β -secretase in the cerebral cortex ($F_{(6, 35)} = 44.985, p < 0.001$) and hippocampus ($F_{(6, 35)} = 29.682, p < 0.001$) of the mice brain. In comparison to the control group, the model (AlCl₃ + D-galactose) group was exposed significantly higher β -secretase levels in the cerebral cortex and hippocampus. When, Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz treatments were given to AD mice significantly attenuate β -secretase level in the cerebral cortex and hippocampus than the model. When compared to Dnpz, Brm, Myr, Brm + Myr treated AD mice, concomitant treatment with Brm + Myr + Dnpz significantly reduced β -secretase level in the cerebral cortex and hippocampus, as shown in **Table 5.40** and **Figure 5.39**.

Table 5.39: Effect of Bromelain and Myrcene on BDNF levels of AD-induced mice

Treatment	Mice brain regions	BDNF (pg/mL)
Control	Cerebral cortex	559.833 ± 12.892
	Hippocampus	523.833 ± 16.924
M	Cerebral cortex	121.166 ± 31.834***
	Hippocampus	110.166 ± 12.752***
M + Dnpz	Cerebral cortex	346.833 ± 39.797###
	Hippocampus	327.500 ± 29.722###
M + Brm	Cerebral cortex	273.500 ± 37.501 [#]
	Hippocampus	250.000 ± 49.890 [#]
M + Myr	Cerebral cortex	270.166 ± 39.494 [#]
	Hippocampus	252.166 ± 49.226 [#]
M + Brm + Myr	Cerebral cortex	385.000 ± 19.464###
	Hippocampus	344.666 ± 17.182###
M + Brm + Myr L + Dnpz	Cerebral cortex	525.500 ± 29.625####,^,\$\$\$,&&&,@
	Hippocampus	486.833 ± 13.179####,^,\$\$\$,&&&,@

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ***p<0.001 significant from control group; [#]p<0.05 and ###p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&&p<0.001 significant from Myr treated group; @p<0.05 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; BDNF = Brain Derived Neurotrophic Factor.

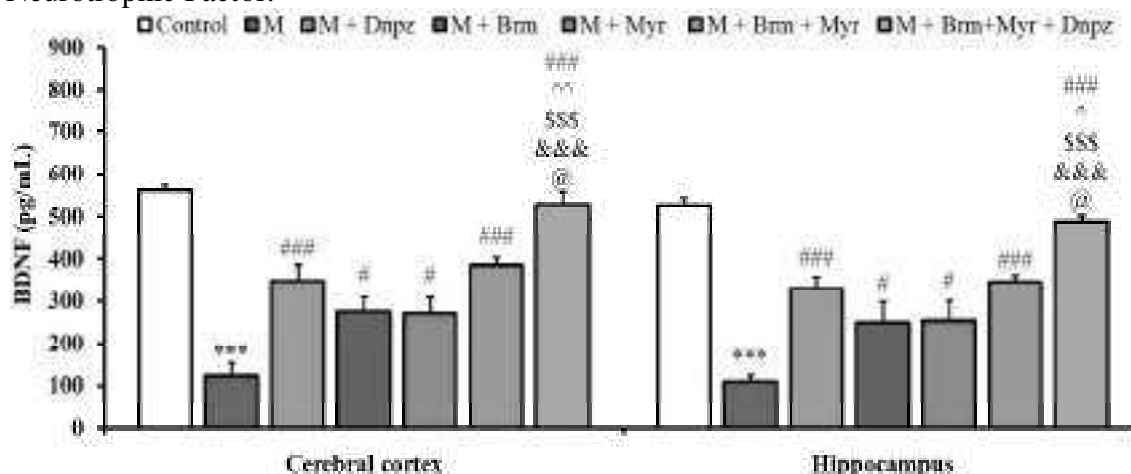


Figure 5.38: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on BDNF levels in the cerebral cortex and the hippocampus of (AlCl₃ + D - galactose) administered mice. The mean ± S.E.M. of each group (n = 6) is indicated by bars in the graph. ***p<0.001 significant from control group; [#]p<0.05 and ###p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; \$\$\$p<0.001 significant from Brm treated group; &&&p<0.001 significant from Myr treated group; @p<0.05 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; BDNF = Brain Derived Neurotrophic Factor.

Table 5.40: Effect of Bromelain and Myrcene on β -secretase levels of AD-induced mice

Treatment	Mice brain regions	β -Secretase (U/L)
Control	Cerebral cortex	11.516 $\pm$ 0.839
	Hippocampus	9.450 $\pm$ 0.852
M	Cerebral cortex	41.366 $\pm$ 1.467 ^{***}
	Hippocampus	38.283 $\pm$ 1.726 ^{***}
M + Dnpz	Cerebral cortex	21.183 $\pm$ 2.543 ^{###}
	Hippocampus	18.416 $\pm$ 2.856 ^{###}
M + Brm	Cerebral cortex	25.550 $\pm$ 0.933 ^{###}
	Hippocampus	18.983 $\pm$ 1.008 ^{###}
M + Myr	Cerebral cortex	22.733 $\pm$ 1.179 ^{###}
	Hippocampus	20.850 $\pm$ 1.047 ^{###}
M + Brm + Myr	Cerebral cortex	19.000 $\pm$ 1.204 ^{###}
	Hippocampus	18.033 $\pm$ 2.620 ^{###}
M + Brm + Myr + Dnpz	Cerebral cortex	11.850 $\pm$ 0.978 ^{###, ^, \$\$\$, &&&, @}
	Hippocampus	9.700 $\pm$ 0.882 ^{###, ^, \$, &&, @}

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&}p<0.01 and ^{&&&}p<0.001 significant from Myr treated group; [@]p<0.05 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

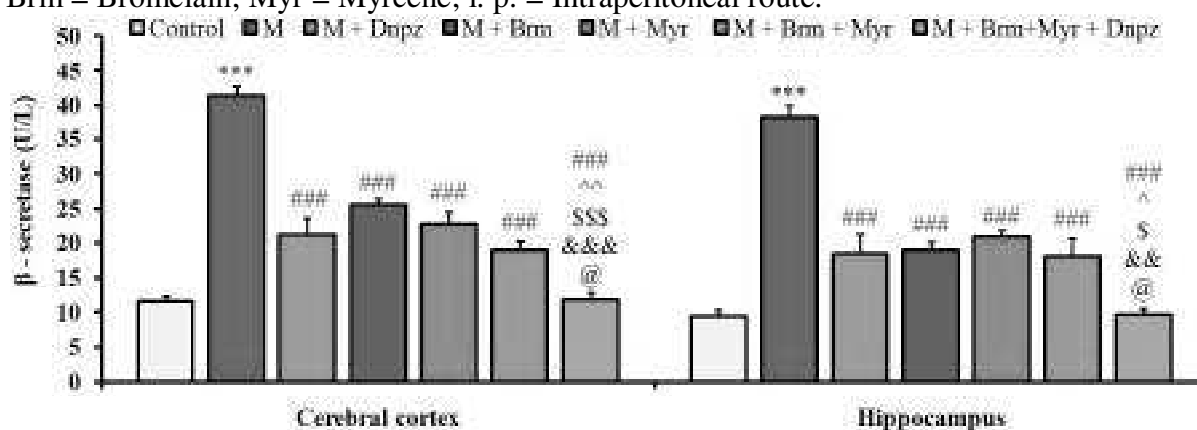


Figure 5.39: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on β -secretase levels in the cerebral cortex and the hippocampus of (AlCl_3 + D - galactose) administered mice. The mean $\pm$ S.E.M. of each group (n = 6) is represented by bars in the graph. ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 and ^{^^}p<0.01 significant from Dnpz treated group; ^{\$}p<0.05 and ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&}p<0.01 and ^{&&&}p<0.001 significant from Myr treated group; [@]p<0.05 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

5.3.2.9. Bromelain and Myrcene decrease the amyloid-beta peptide₁₋₄₂ level in different brain regions of AlCl₃ and D - galactose induced mice

Amyloid-beta peptide₁₋₄₂ level in the cerebral cortex and hippocampus was analyzed by ELISA to assess the treatment's impact on amyloidopathy of AD mice. According to one way ANOVA study, the treatments showed a significant impact on the amyloid-beta peptide₁₋₄₂ level in the cerebral cortex ($F_{(6, 35)} = 16.434, p < 0.001$) and hippocampus ($F_{(6, 35)} = 12.892, p < 0.001$) in the mice brain. When compared to the control group; the model (AlCl₃ + D-galactose) group significantly raised the level of amyloid-beta peptide₁₋₄₂ in the cerebral cortex and hippocampus. While, Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz treatments were given to AD mice significantly decrease amyloid-beta peptide₁₋₄₂ level in the cerebral cortex and hippocampus than the model. In comparison to Dnpz, Brm, Myr, Brm + Myr treated mice, concomitant treatment with Brm + Myr + Dnpz significantly reduced amyloid beta peptide₁₋₄₂ level in the cerebral cortex and hippocampus, as shown in **Table 5.41** and **Figure 5.40**.

5.3.2.10. Bromelain and Myrcene decrease the tumor necrosis factor- α level in different brain regions of AlCl₃ and D - galactose induced mice

The level of tumor necrosis factor- α was examined in the cerebral cortex and hippocampus to assess medication effect on neuroinflammation. According to a one-way ANOVA study, the treatments showed a significant effect on the level of TNF- α in the cerebral cortex ($F_{(6, 35)} = 55.884, p < 0.001$) and hippocampus ($F_{(6, 35)} = 33.197, p < 0.001$) in the mice brain. The model (AlCl₃ + D-galactose) group significantly increased TNF- α levels in both the cerebral cortex and hippocampus when compared to the control group. While, Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz treatments were given to AD mice significantly attenuate the TNF- α level in the cerebral cortex and hippocampus than the model. In comparison to Dnpz, Brm, Myr, Brm + Myr treated mice, concomitant treatment with Brm + Myr + Dnpz significantly decreased the level of TNF- α in the cerebral cortex and hippocampus, as shown in **Table 5.42** and **Figure 5.41**.

Table 5.41: Effect of Bromelain and Myrcene on amyloid-beta peptide₁₋₄₂ levels of AD-induced mice

Treatment	Mice brain regions	Amyloid beta peptide ₁₋₄₂ (ng/mL)
Control	Cerebral cortex	2.125 ± 0.591
	Hippocampus	1.810 ± 0.589
M	Cerebral cortex	12.351 ± 0.692 ^{***}
	Hippocampus	11.615 ± 1.003 ^{***}
M + Dnpz	Cerebral cortex	6.648 ± 1.718 ^{###}
	Hippocampus	6.333 ± 1.675 ^{##}
M + Brm	Cerebral cortex	7.796 ± 0.350 [#]
	Hippocampus	7.004 ± 0.710 [#]
M + Myr	Cerebral cortex	7.509 ± 0.567 ^{##}
	Hippocampus	6.592 ± 1.001 ^{##}
M + Brm + Myr	Cerebral cortex	6.240 ± 0.969 ^{###}
	Hippocampus	5.583 ± 0.406 ^{###}
M + Brm + Myr + Dnpz	Cerebral cortex	2.143 ± 0.405 ^{###, ^, \$\$\$, &, @}
	Hippocampus	1.875 ± 0.526 ^{###, ^, \$\$, &}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm treated group; [&]p<0.05 and ^{&&}p<0.01 significant from Myr treated group; [@]p<0.05 significant from Brm + Myr treated group. (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

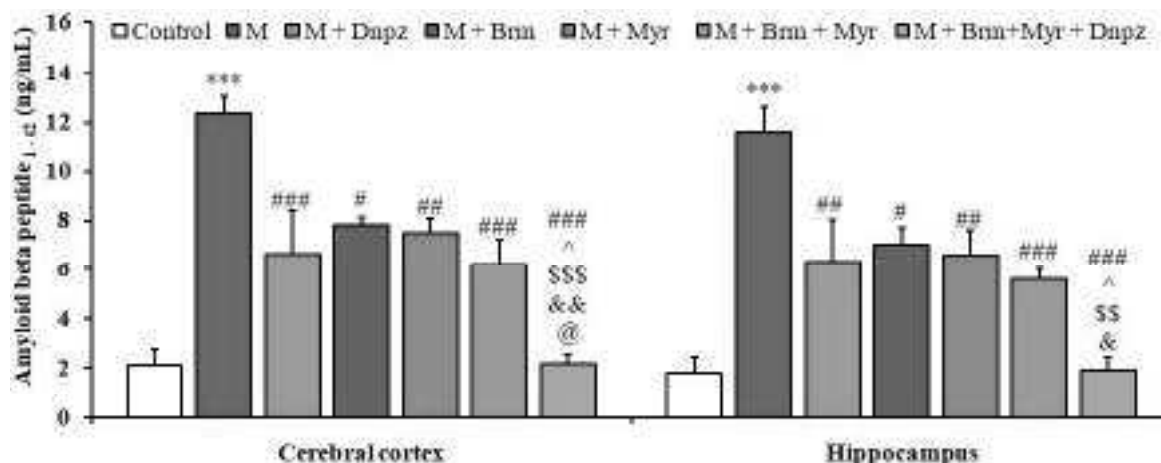


Figure 5.40: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on amyloid beta peptide₁₋₄₂ levels in the cerebral cortex and the hippocampus of (AlCl₃ + D - galactose) administered mice. The mean ± S.E.M. of each group (n = 6) is represented by bars in the graph. ^{***}p<0.001 significant from control group; [#]p<0.05, ^{##}p<0.01 and ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$\$}p<0.01 and ^{\$\$\$}p<0.001 significant from Brm treated group; [&]p<0.05 and ^{&&}p<0.01 significant from Myr treated group; [@]p<0.05 significant from Brm + Myr treated group. (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

Table 5.42: Effect of Bromelain and Myrcene on TNF- α level of AD-induced mice

Treatment	Mice brain regions	TNF - α ($\mu\text{g/ml}$)
Control	Cerebral cortex	963.333 $\pm$ 70.019
	Hippocampus	884.166 $\pm$ 69.646
M	Cerebral cortex	3738.333 $\pm$ 141.370 ^{***}
	Hippocampus	3313.333 $\pm$ 209.230 ^{***}
M + Dnpz	Cerebral cortex	1817.500 $\pm$ 177.570 ^{###}
	Hippocampus	1688.333 $\pm$ 178.380 ^{###}
M + Brm	Cerebral cortex	2534.166 $\pm$ 154.301 ^{###}
	Hippocampus	2180.000 $\pm$ 104.880 ^{###}
M + Myr	Cerebral cortex	2630.000 $\pm$ 150.000 ^{###}
	Hippocampus	2325.833 $\pm$ 137.752 ^{###}
M + Brm + Myr	Cerebral cortex	2071.666 $\pm$ 87.955 ^{###}
	Hippocampus	1855.000 $\pm$ 148.042 ^{###}
M + Brm + Myr + Dnpz	Cerebral cortex	988.333 $\pm$ 99.093 ^{###, ^, \$\$\$, &&&, @@@}
	Hippocampus	905.000 $\pm$ 139.940 ^{###, ^, \$\$\$, &&&, @@@}

Note: Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean $\pm$ S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.01 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&&}p<0.001 significant from Myr treated group; ^{@@@}p<0.001 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; TNF - α = Tumor Necrosis Factor - α .

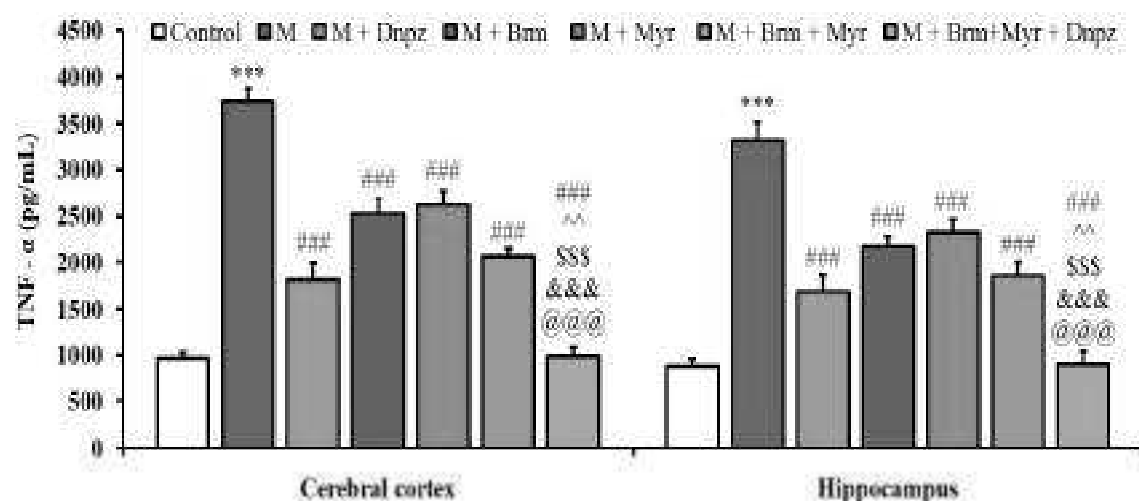


Figure 5.41: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on TNF- α levels in the cerebral cortex and the hippocampus of (AlCl_3 + D - galactose) administered mice. The mean $\pm$ S.E.M. of each group (n = 6) is indicated by bars in the graph. ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.01 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&&}p<0.001 significant from Myr treated group; ^{@@@}p<0.001 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; TNF - α = Tumor Necrosis Factor - α .

5.3.2.11. Bromelain and Myrcene decrease the interleukin-6 level in different brain regions of AlCl₃ and D - galactose induced mice

The interleukin – 6 levels were estimated in the cerebral cortex and hippocampus to assess treatment's effect on neuroinflammation. According to one way ANOVA study, the treatments had a significant effect on IL-6 level in the cerebral cortex ($F_{(6, 35)} = 42.819$, $p < 0.001$) and hippocampus ($F_{(6, 35)} = 26.947$, $p < 0.001$) in the mice brain. When compared to control, the model (AlCl₃ + D - galactose) showed a significant increase level of IL-6 in the cerebral cortex and hippocampus. While, Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz treatments were given to AD mice significantly reduce IL-6 level in the cerebral cortex and hippocampus than the model. In comparison to Dnpz, Brm, Myr, Brm + Myr treated AD mice; concomitant treatment with Brm + Myr + Dnpz significantly attenuated the level of IL-6 in the cerebral cortex and hippocampus, as shown in **Table 5.43** and **Figure 5.42**.

5.3.3. Histopathological analysis

The cerebral cortex and hippocampus of control mice revealed no signs of injury, according to the histological investigation. In the cerebral cortex and hippocampus, the model (AlCl₃ + D – galactose) revealed aberrant neuron cells, eosinophilic lesions, as well as hyperchromatic nuclei. When developed AD mice were given Dnpz (2 mg/kg), Brm (10 mg/kg), Myr (100 mg/kg), Brm + Myr, and Brm + Myr + Dnpz, normal neuron cells, eosinophilic lesions, along with hyperchromatic nuclei in the cerebral cortex and hippocampus improved compared to the model. In comparison to Dnpz, Brm, Myr, and Brm + Myr treated mice, concomitant treatment with Brm + Myr + Dnpz to AD-induced mice demonstrated the most protection in radially distributed neurons, eosinophilic lesions, as well as hyperchromatic nuclei in the cerebral cortex and hippocampus **Figure 5.43**.

Table 5.43: Effect of Bromelain and Myrcene on IL - 6 level of AD-induced mice

Treatment	Mice brain regions	IL - 6 (pg/mL)
Control	Cerebral cortex	215.583 ± 28.884
	Hippocampus	198.500 ± 36.600
M	Cerebral cortex	1163.500 ± 23.013 ^{***}
	Hippocampus	1081.833 ± 32.925 ^{***}
M + Dnpz	Cerebral cortex	511.000 ± 29.467 ^{###}
	Hippocampus	471.833 ± 24.098 ^{###}
M + Brm	Cerebral cortex	711.000 ± 63.888 ^{###}
	Hippocampus	628.500 ± 64.688 ^{###}
M + Myr	Cerebral cortex	643.500 ± 56.741 ^{###}
	Hippocampus	598.500 ± 84.496 ^{###}
M + Brm + Myr	Cerebral cortex	562.666 ± 78.194 ^{###}
	Hippocampus	511.833 ± 89.334 ^{###}
M + Brm + Myr + Dnpz	Cerebral cortex	272.666 ± 23.118 ^{###, ^, \$\$\$, &&&, @@}
	Hippocampus	216.833 ± 25.736 ^{###, ^, \$\$\$, &&&, @}

Note: Control (Normal saline), Model (AlCl₃ + D - galactose), Dnpz (2 mg/kg b. w.; i. p.), Brm (10 mg/kg b. w.; i. p.), Myr (100 mg/kg b. w.; i. p.), co-administration (Brm + Myr) and (Brm + Myr + Dnpz) treatment. Value expressed as the mean ± S.E.M. of each group (n = 6). ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&&}p<0.001 significant from Myr treated group; [@]p<0.05 and ^{@@}p<0.01 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; IL - 6 = Interleukin - 6.

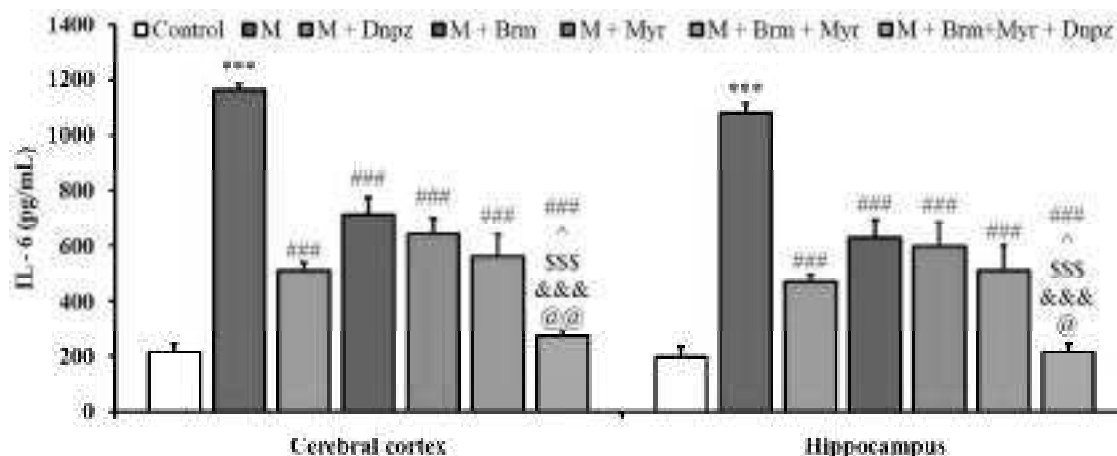


Figure 5.42: Effects of Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), Dnpz (2 mg/kg, i. p.), (Brm + Myr), and (Brm + Myr + Dnpz) on IL - 6 levels in the cerebral cortex and the hippocampus of (AlCl₃ + D - galactose) administered mice. The mean ± S.E.M. of each group (n = 6) is represented by bars in the graph. ^{***}p<0.001 significant from control group; ^{###}p<0.001 significant from M treated group; [^]p<0.05 significant from Dnpz treated group; ^{\$\$\$}p<0.001 significant from Brm treated group; ^{&&&}p<0.001 significant from Myr treated group; [@]p<0.05 and ^{@@}p<0.01 significant from Brm + Myr treated group, (one-way ANOVA followed by Tukey test). Where: M = Model (AlCl₃ + D -

galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route; IL – 6 = Interleukin - 6.

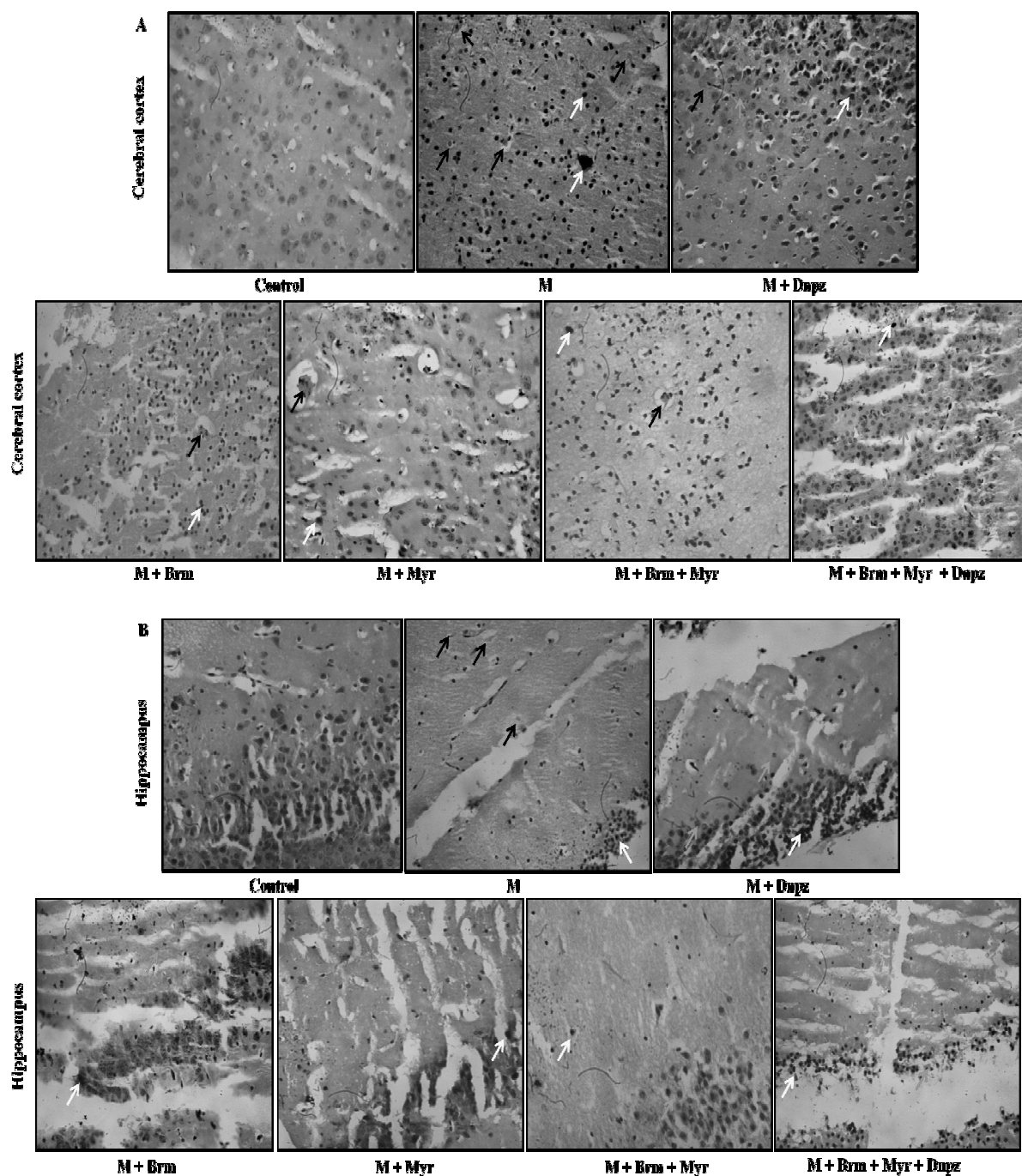


Figure 5.43: Histopathological analysis (40X) of transverse section of the cerebral cortex (A) and the hippocampus (B) using hematoxylin and eosin staining; Control (Normal saline), Model (AlCl_3 + D - galactose), Dnpz (2 mg/kg, i. p.), Brm (10 mg/kg, i. p.), Myr (100 mg/kg, i. p.), (Brm + Myr), and co-administration (Brm + Myr + Dnpz) of mice of all groups; Black, Yellow and Green arrows express the eosinophilic lesions, hyperchromatic nuclei and normal neuron cells respectively. Where: M = Model (AlCl_3 + D - galactose); Dnpz = Donepezil; Brm = Bromelain; Myr = Myrcene; i. p. = Intraperitoneal route.

CHAPTER – 6

DISCUSSION

6. Discussion

In the current study, we explored the potential impact of Bromelain, Myrcene, and Brm L + Myr L against the oxidative stress markers (MDA, GSH, CAT, SOD, and NO), cholinergic markers (AChE), inflammatory markers (IL-6 and TNF- α), neurotrophic factor (BDNF), and amyloidogenic markers (β -secretase and A β_{1-42}) in AlCl₃ and D - galactose induced AD mice. This study also investigated the efficacious effect of Brm L, Brm H, Myr L, Myr H, Brm L + Myr L, Brm L + Dnpz, Myr L + Dnpz, and Brm L + Myr L + Dnpz in AlCl₃ and D - galactose induced AD mice.

The AlCl₃ and D - galactose mouse model is considered appropriate for investigating age-related changes in the brain such as neuronal damage and memory deficits due to cholinergic dysfunction, alteration of amyloidogenic markers, fluctuation of neurotrophic factor, disturbance in oxidative markers and inflammatory markers of AD mice (Luo et al., 2009; Wei et al., 2017; Abulfadl et al., 2018; Chiroma et al., 2018; Haider et al., 2020). In this study, AlCl₃ and D - galactose induced mice were observed significant learning and memory deficit indicating a suitable choice of AD model for the present study. AlCl₃ (5 mg/kg/day; p.o.), and D - galactose (60 mg/kg/day; i.p.), doses were selected to co-administer for 90 days (Peng et al., 2015) that induced Alzheimer's disease in mice by the disturbing level of enzymes as well as altering the pathological markers of AD such as β -secretase and A β_{1-42} in mice brain as reported by the previous researcher (Sun et al., 2009).

Bromelain doses (10, 20 mg/kg, b.w.; i. p.) were selected based on previous research studies (Lotz-Winter, 1990; Baez R et al., 2007). LD₅₀ dose is considered as 36.7 mg/kg, i.p. in mice (Lotz-Winter, 1990). The earlier *in vivo* studies (Chaudhary et al., 2018) provided evidence that Bromelain improved oxidative degeneration and cholinergic deficits in dichlorvos induced mice brains. Bromelain was reported to enhance the permeability of the blood-brain barrier (BBB) to nutrients (Laurer et al., 2001). The previous *In vitro* study provided evidence that Bromelain was investigated to degrade A β_{1-42} monomers and soluble aggregate in cerebrospinal fluid (CSF) of AD patients (Sancesario et al., 2018). Therefore, based on *In vitro* study, the first-time Bromelain *In vivo* study was conducted to evaluate the impact on A β_{1-42} peptide aggregation, alteration of β -secretase, reduced BDNF, and increased AChE enzyme using a combination of AlCl₃ and D - galactose induced AD mice. Consequently, the present study of Bromelain was performed to explore the potential effect on neuroprotection, restoring learning and memory of AlCl₃ and D - galactose induced AD mice.

Myrcene dose (100, 200 mg/kg, b.w.; i.p.) were selected based on previous research studies (**Osman et al., 2014; Burcu et al., 2016**). Myr LD₅₀ dose is considered as 2.25 g/kg, i.p. in mice (**Zeiger 1997**). Myr improved the oxidative damage and histopathological neuronal damage in mice brain tissue induced by global cerebral ischemia/ reperfusion in C57BL/J6 mice (**Osman et al., 2014**). Consequently, the current study of Myr was performed to explore the potential effect on neuroprotection, restoring learning and memory of AlCl₃ and D-galactose induced AD model of mice.

A combination of Bromelain low dose and Myrcene low dose was tested to explore the potential effect on neuroprotection, restoring learning and memory of AlCl₃ and D-galactose induced AD model of mice.

However, Donepezil is marketed as a standard drug for the symptomatic treatment of AD and acts by inhibiting the AChE enzyme in the brain (**Birks and Harvey, 2018; Hampel et al., 2018**). Donepezil (2 mg/kg, b.w.; i.p.) dose was selected as per the previously reported studies (**Yu et al., 2015; Wang et al., 2019**). Thus, Bromelain was found similar to effective as compared with standard drug and the combination of Bromelain low dose and Donepezil was observed significant effect as compared with Bromelain low dose and standard drug-treated mice.

Spatial learning in addition to memory in mice was investigated by MWM assessment (**Morris, 1984; Wei et al., 2017**). In this study, we demonstrated that the AlCl₃ and D - galactose treated mice showed a cognitive impairment as a result of the increased latency time to reach the secret platform. SRM was increased with treatments on training days 81, 82, and 83 by reducing escape latencies to enter the secret platform. Reduced escape latencies are an indicator of improved spatial memory (**Morris, 1984**). On day 84, a probe trial was performed without the use of a secret platform to decide if the memory trace is reserved as well as consolidated for navigation (**Morris, 1984**). Bromelain's low and high dose considerably enhanced the time passed in the target quadrant throughout probe trials, suggesting preservation of spatial memory of an earlier target quadrant platform. As aforementioned parameters, Donepezil and a combination of Bromelain low dose and Donepezil significantly improved memory efficiency (**Zhi et al., 2008**). On days 87, 88, 89, and 90, SRM was performed. The animal had to look for a forum in a new role in the SWM's first acquisition trial. However, in the second retrieval trial, which took place 75 min after the first trial, the platform was returned to its earlier location (**Davoodi et al., 2009**). Brm L, Brm H, Myr L, Myr H, and Brm L + MyrLtreatments considerably reduced the escape latency time in acquisition as well as

retrieval trial indicating improvement in SRM. Therefore, a significantly decreased escape latency time in the acquisition, as well as retrieval trials, was observed in the Donepezil group, and in the combination of Brm L + Dnpz, Myr L + Dnpz, and Brm L + Myr L + Dnpz. The improved memory by treatments can be recommended by possessing nootropic activity.

In the elevated plus-maze (EPM) test, mice naturally expend more time in enclosed arms while less time in open arms. Transfer latency defines the time engaged by mice to reach from open arms to enclosed arms. It was suggested that decreased transfer latency time can be related to learning and memory. In the development of learning along with memory, neurons in the central cholinergic system possess an imperative action (Itoh et al., 1990; Everitt and Robbins, 1997). AlCl₃ and D - galactose treated mice have significantly increased the transfer latency in the EPM test indicating learning and memory deficits. Treatments with Brm L, Brm H, Myr L, Myr H, and Brm L + Myr L significantly decreased the transfer latency time in AlCl₃ and D - galactose treated mice. Dnpz, Brm L + Dnpz, Myr L + Dnpz, and Brm L + Myr L + Dnpz were also significantly reduced transfer latency time indicating the improvement in learning and memory of AlCl₃ and D - galactose induced AD mice.

The acetylcholine (ACh) hydrolysis in presence of AChE terminates cholinergic transmission. The AChE enzyme is a marker for the loss of cholinergic neurons and maintains normal function of the nervous system because AChE breaks down ACh in the synaptic cleft (Dunnett et al., 1991; Ballard et al., 2005). ACh is an essential neurotransmitter released in the synaptic cleft for proper neuronal transmission to assess proper cognitive function, learning, and memory. An increased level of AChE enzyme degrades the large amount of ACh and leads to reduce ACh level in the brain. Therefore, decreased level of ACh is regarded as the cause of cognitive dysfunction, learning and memory deficits in AD (Mattson, 2004, Mikkilineni et al., 2012). AlCl₃ and D - galactose induced mice were reported to increase the level of AChE enzyme in the animal brain (Ravi et al., 2000; Zatta et al., 2002; Xian et al., 2011). In the present study, we found that enhanced AChE enzyme level in the cerebral cortex along with the hippocampus of AlCl₃ and D - galactose treated mice, but Brm L, Brm H, Myr L, Myr H, and Brm L + Myr L have significantly inhibited AChE enzyme in the cerebral cortex along with the hippocampus of AlCl₃ and D - galactose induced AD mice. However, Donepezil is reported as a potent AChE inhibitor (Birks and Harvey, 2018; Hampel et al., 2018) that significantly attenuated AChE amount in the cerebral cortex along with the

hippocampus of AlCl_3 and D - galactose induced mice. The combination of Brm L + Dnpz, Myr L + Dnpz, and Brm L + Myr L + Dnpz were also markedly inhibited AChE activity of AlCl_3 and D - galactose treated mice. Therefore, our finding suggested that Bromelain, Myrcene, Brm L + Myr L, and combination with Dnpz respectively can be used to inhibit the AChE enzyme and restore ACh level in the brain, accompanied by improving memory and spatial learning abilities of mice.

Previous studies have reported that oxidative stress is an imbalance between reactive oxygen species (ROS), and antioxidants (**Birnboim, 1986**). Oxidative stress is projected as an essential factor in the pathogenesis of AD and contributes to A β aggregation and neurofibrillary tangles (NFTs) in the brain (**Aliev, 2011**). “Excessive production of ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^*\text{OH}$) can lead to break down of biomacromolecules in the body as result the oxidative damage. Thus, the endogenous antioxidants such as SOD, CAT, and GSH-Px are activated to protect cells in the body against oxidative destruction by ROS” (**Zhang et al., 2008**). “SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide” (**Hayyan et al., 2016**). CAT reacts with peroxide to catalyze the formation of oxygen and water (**Chelikani et al., 2004**). GSH-Px was reported that reduces hydrogen peroxide to small sulfur-containing proteins such as glutathione (**Lubos et al., 2011**). MDA is an end product of polyunsaturated peroxidation in cells. Overproduction of MDA is caused by increased ROS (**Ayala, et al., 2014**). Similarly, NO is a ROS synthesized in presence of nitric oxide synthase (NOS). However, NOS interacts with superoxide to give highly reactive peroxynitrite (**Pacher et al., 2007; Johnson et al., 2017**). NOS is strongly regulated by neuronal NOS and generates NO as a signaling molecule to influence the synaptic function and cerebral blood flow (**Calabrese et al., 2000**). In this study, we found that AlCl_3 and D - galactose treated mice were observed to reduce SOD level, decrease CAT level, decrease GSH level, enhance MDA level and increase NO level in the cerebral cortex and the hippocampus. However, Brm L, Brm H, Myr L, Myr H, Brm L + Myr L, and Dnpz treatments have significantly improved an imbalance between ROS and antioxidants in the cerebral cortex along with the hippocampus of AlCl_3 and D - galactose induced mice. Similarly, the combination of Brm L + Dnpz, Myr L + Dnpz, and Brm L + Myr L + Dnpz also markedly improved the oxidative destruction indicating improvement in learning and memory function of AlCl_3 and D - galactose induced mice.

BDNF is a small protein encoded by the BDNF gene, neurotrophic factor, and found in both brain and the periphery (**Binder and scharfman, 2004; Maisonpierre et al., 1991**). BDNF plays an essential role in neurogenesis such as proliferation, differentiation, maturation, and synapse plasticity (**Schinder and Poo, 2000**). BDNF is active in the cerebral cortex, the hippocampus along the basal forebrain for learning and memory (**Yamada and Nabeshima, 2003; Bekinschtein et al., 2008**). “BDNF is released from neural tissues as a consequence of neural cell death signaling and various stimuli under normal and pathological conditions. Activation of tropomyosin related kinase B (TrkB) by BDNF, potentiate signal transaction pathway via tyrosine kinase for leading neurogenesis and repair neural plasticity”(Yamada and Nabeshima, 2003; Sossin and Barker, 2007). The decreased level of BDNF was explored in brain tissues of AD patients (**Mattson, 2008**). While some researchers reported that BDNF is an important contributor to the etiology of neurodegenerative diseases (**Fumagalli et al., 2006; Schindowski et al., 2008**). It has been found that $AlCl_3$ and D - galactose induced mice showed a decrease in BDNF amount in the brain (**Abulfadl et al., 2018; Auti and Kulkarni, 2019**). In this study, we investigated that a lower BDNF amount in the cerebral cortex, as well as the hippocampus, was observed in $AlCl_3$ and D - galactose treated mice. But, Brm L, Brm H, Myr L, Myr H, Brm L + Myr L, and Dnpz treatments were significantly elevated BDNF amount in the cerebral cortex along with the hippocampus of $AlCl_3$ and D - galactose treated mice. Similarly, the combination of Brm L + Dnpz, Myr L + Dnpz, and Brm L + Myr L + Dnpz were markedly enhanced BDNF amount in the cerebral cortex along with the hippocampus of $AlCl_3$ and D - galactose induced mice. Consequently, our finding was suggested that Bromelain, Myrcene, Brm L + Myr L, and combination with Dnpz respectively can protect neurons, improve cognitive function, learning and memory by elevating BDNF levels in the brain.

Previous studies reported that amyloid precursor protein (APP) metabolism increases in the non-amyloidogenic pathway, while decreases in the amyloidogenic process as the α -secretase and β -secretase participate in the APP substrate (**Vassar et al., 1999, Skovronsky et al., 2000**). β -secretase (BACE1) enzyme is important for the formation of β -amyloid ($A\beta$). BACE1 is the initiating enzyme in $A\beta$ generation that is considered a prime drug target for lower cerebral $A\beta$ levels in the prevention of AD (**Lewczuk and Wiltfang, 2008**). The highest activity levels were considered in neural tissue and neural cell lines. BACE1 predominates in neurons with reduced BACE1 levels in astrocytes (**Seubert et al., 1993; Zhao et al., 1996**). However, increased BACE1

protein expression was seen in the brain of sporadic AD patients (Kao et al., 2003; Hebert et al., 2008). BACE1 is an essential drug target enzyme for the treatment of AD. BACE1 mRNA and protein levels were found greater in the cerebral cortex along with the hippocampus of AlCl_3 and D - galactose induced mice. BACE1 activity and protein levels were up-regulated in cerebrospinal fluid and brain samples of AD patients (Kao et al., 2003; Fukumoto et al., 2004; Lewczuk and Wiltfang, 2008). In this study, we found that Brm L, Brm H, Myr L, Myr H, Brm L + Myr L, and Dnpz treatments decreased the BACE1 levels in the cerebral cortex along with the hippocampus of AlCl_3 and D- galactose induced mice. Similarly, the combination of Brm L + Dnpz, Myr L + Dnpz, Brm L + Myr L + Dnpz was remarkably attenuated BACE1 levels in the cerebral cortex along with the hippocampus of AlCl_3 and D - galactose induced mice.

$\text{A}\beta$ peptide is formed following sequential cleavage of APP by β -secretase and γ -secretase in the amyloidogenic process (Vassar, 2004). $\text{A}\beta$ has a key role in mitochondrial dysfunction, mitochondrial deficits such as oxidation, and energy deficiency with mitochondrial depolarization were observed in $\text{A}\beta$ treated cells and animal models (Gao and Tang, 2006; Dragicevic et al. 2011; Sarkar et al., 2014). The APP deficiency influenced synapse generation and transmission in cultured hippocampal neurons (Priller et al., 2006). Previous evidence suggested that $\text{A}\beta$ and NFTs are hypothesized major markers in AD and $\text{A}\beta_{1-42}$ aggregation triggers a complex pathological cause of AD (Huang et al., 2016). The combination of AlCl_3 and D - galactose treated mice showed a markedly enhance level of $\text{A}\beta_{1-42}$ accumulation in mice brains (Sun et al., 2009; Luo et al., 2009). In this study, we investigated that Brm L, Brm H, Myr L, Myr H, and Brm L + Myr L treatments were observed to decrease $\text{A}\beta_{1-42}$ peptide monomer aggregations in the cerebral cortex along with the hippocampus of AlCl_3 and D - galactose AD model of mice. However, Donepezil was found to attenuate $\text{A}\beta_{1-42}$ accumulations in the cerebral cortex along with the hippocampus of mice (Ye et al., 2015). Similarly, the combination of Brm L + Dnpz, Myr L + Dnpz, Brm L + Myr L - Dnpz remarkably reduced the $\text{A}\beta_{1-42}$ accumulations in the cerebral cortex along with the hippocampus of AlCl_3 and D - galactose induced mice. Subsequently, our finding was recommended that Bromelain, Myrcene, Brm L + Myr L, and combination with Dnpz respectively can be considered for pathological treatment of AD by inhibiting BACE1 enzyme and decreasing $\text{A}\beta_{1-42}$ aggregations in the brain.

Proinflammatory cytokines such as TNF - α as well as IL - 6 (Heneka and O-Banion, 2007) were secreted by the active form of microglial cells and astrocytes.

Various studies reported that neuroinflammation plays a critical role in the pathogenesis of AD (**Ravichandran et al., 2018**). In this study, we obtained that AlCl_3 and D - galactose treated mice were elevated TNF- α as well as IL-6 levels in the cerebral cortex and the hippocampus (**Xing et al., 2018; Justin-Thenmozhi et al., 2018, Ibrahim et al., 2020**). However, Brm L, Brm H, Myr L, Myr H, Brm L + Myr L, and Dnpz treatments considerably decreased the TNF- α along with IL-6 levels in the cerebral cortex along with the hippocampus of AlCl_3 and D - galactose treated mice. Similarly, the combination of Brm L + Dnpz, Myr L + Dnpz, and Brm L + Myr L + Dnpz markedly reduced the amount of TNF- α along with IL-6 in the cerebral cortex along with the hippocampus of AlCl_3 and D - galactose induced mice.

Previously reported literature strengthens the present study results in which the cells were darkly stained in an AD-like model of rats, indicating oxidative stress (**Miyata et al., 2010**). A study has reported dense chromatic nuclei and shrunken neuronal cells (**Naidu et al., 2013**). However, AlCl_3 and D - galactose have been reported in neuronal disarrangement and acidophilic degeneration (**Li et al., 2010**). D - galactose administration showed unorganized nerve fiber with irregular neurons in the hippocampal region (**Kenawy et al., 2017**). The rats were reported with pericellular edema in the cerebral cortex and cell distortion in hippocampal slides (**Coban et al., 2014**). Thus, the histopathological study was performed to investigate morphological changes in the cerebral cortex along with the hippocampus of mice brains. Our present study found that long-term exposure to AlCl_3 and D - galactose in mice showed neuronal disarrangement, eosinophilic lesion, or hyperchromatic nuclei in the cerebral cortex along with the hippocampus. However, treatment with Dnpz, Brm L, Brm H, Myr L, Myr H, Brm + Myr, Brm L + Dnpz, Myr L + Dnpz, and Brm + Myr + Dnpz were prevented the AlCl_3 and D - galactose induced histopathological alterations.

CHAPTER – 7
SUMMARY AND CONCLUSION

7. Summary and conclusion

7.1 Summary

Neurodegeneration is the process of neurons losing their form, function, or dying in the central nervous system. The most common neurodegenerative diseases are Alzheimer's, Parkinson's, Huntington's, and Amyotrophic lateral sclerosis. Among many, aging remains one of the major risk factor of neurodegenerative disorders. Aging is associated with mitochondrial DNA mutation and oxidative stress. Dementia is a widespread word that describes a decline in cognitive function, including memory loss, language impairment, and disorientation, as well as a degree of mental ability in the patient that interferes with daily life. Alzheimer's disease is the most common kind of dementia. Alzheimer's disease accounts for around 80% of all dementia cases, which is higher than vascular dementia, Lewy body dementia, and frontotemporal dementia.

Alzheimer's disease (AD) belongs to neurodegenerative disorder which is characterized by severe progressive loss of neurons in the central nervous system. AD is the basic type of dementia among geriatrics that slowly decreases memory and alters the thought process, reduces the understanding of the simple tasks of the daily routine, and with passing time, it becomes more problematic. Neuronal destruction occurs in AD with less viable neuron cells. Its progressive nature ultimately leads to shrinkage of the brain. The exact pathology of AD is still unknown but, it involves the accumulation of the Amyloid- β ($A\beta$) and neurofibrillary tangles (NFTs) in the hippocampus and cortex region of the brain that both $A\beta$ and NFTs lead to degeneration and alteration of function of the neurons. The peptide monomer $A\beta_{1-42}$ or $A\beta_{40-42}$ is formed by proteolytic cleavage of amyloid precursor protein in the presence of the enzyme γ -secretase. Mutations in the amyloid precursor protein, presenilin-1, and presenilin-2 genes cause familial AD. The level of acetylcholine in the brain diminishes with AD, according to the cholinergic theory.

The risk factors for Alzheimer's disease include neuroinflammation and brain damage. Potentiation of free radical-induced oxidative stress increases with age, inhibiting endogenous antioxidant enzymes and, as a result, influencing the pathogenesis of Alzheimer's disease through extracellular A and intracellular NFT deposition. $TNF-\alpha$ and interleukin-6 production are increased when amyloid-beta aggregates. By reducing oxidation and mitochondrial dysfunction, a high-antioxidant diet lowers the risk of Alzheimer's disease. Neuronal death and degeneration of neural connections in the cerebral cortex region of the brain occur with Alzheimer's disease, resulting in significant

brain mass loss. The development of Alzheimer's disease is linked to mitochondrial malfunction, which leads to amyloidosis. This resulted in cell cycle re-entry and phosphorylation of tau.

For the treatment of Alzheimer's disease, cholinesterase inhibitors and memantine are available. Despite their designs to detect extremely minor or minimum clinical effects from the experimental medications, drug development efforts aimed at new targets, such as the amyloid cascade, have failed thus far. However, the medicines provide only symptomatic relief and are not able to nurse the underline pathological cause.

The treatment with Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil, prevent AlCl_3 and D-galactose-induced spatial learning and memory deficits, as well as cognitive impairment, in the cerebral cortex and the hippocampus, by enhancing cholinergic activity and synaptic plasticity, as well as attenuating oxidative damage, neuroinflammation, and $\text{A}\beta_{1-42}$ aggregations. Thus, **Figure 7.1 and 7.2.** show potential hypothesis, mechanism of action of Bromelain, Myrcene alone and in combination with Donepezil in AlCl_3 and D - galactose induced mice respectively.

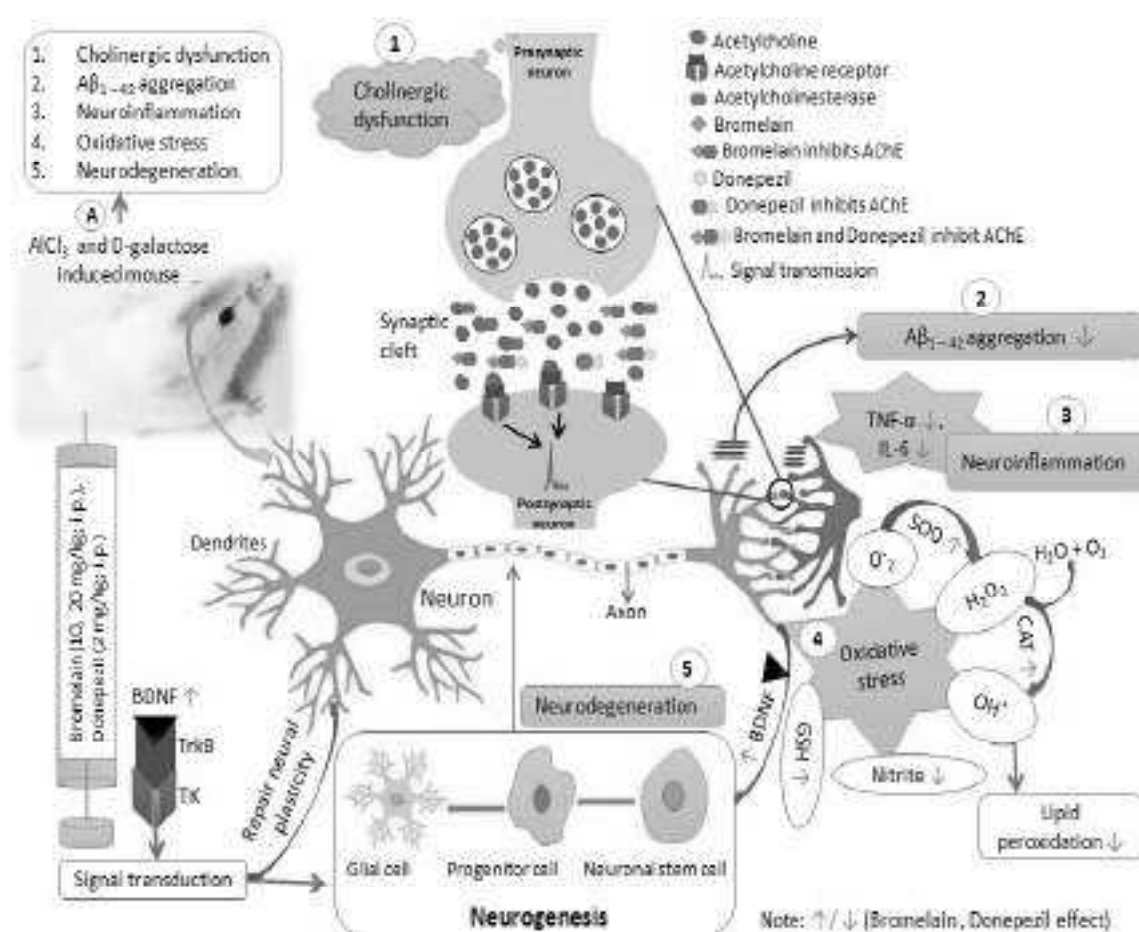


Figure 7.1: Schematic diagram indicates the possible mechanism of Bromelain alone and in combination with Donepezil. **A.** the $AlCl_3$ and D - galactose induced alteration of cholinergic dysfunction, oxidative stress, amyloid-beta peptide₁₋₄₂ aggregations, neuroinflammation, and brain-derived neurotrophic factor in mice brain. Thus, Bromelain alone and in combination with Donepezil could **1.** stimulate the cholinergic system by inhibiting AChE and release acetylcholine in the synaptic cleft for proper signal transmission that acts to increase memory, **2.** attenuate $A\beta_{1-42}$ aggregation by inhibiting β -secretase, **3.** reduce neuroinflammation to protect neurons, **4.** attenuate oxidative stress by enhancing GSH, SOD, and CAT levels and reducing nitrite and lipid peroxidation, and **5.** increase BDNF levels which release from neuronal tissues, BDNF activates tropomyosin related kinase B (TrkB) initiate signal transduction pathway via tyrosine kinase (TK) activity to lead neurogenesis in addition to repair neural plasticity.

7.2. Conclusion

This study concludes that Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil, prevent AlCl_3 and D - galactose-induced spatial learning and memory deficits, as well as cognitive impairment, in the cerebral cortex and the hippocampus, by enhancing cholinergic activity and synaptic plasticity, as well as attenuating oxidative damage, neuroinflammation, and $\text{A}\beta_{1-42}$ aggregations.

✓ In behavioral study

- Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil were significantly ($p < 0.05$) observed to improve the spatial reference memory and spatial working memory in mice by decreasing the escape latency time and increasing the time spent in the target quadrant of Morris water maze. This signifies cognitive function improvement in mice brain against the model.
- Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil were significantly ($p < 0.05$) observed transfer latency time in the elevated plus-maze that signifies learning memory improvement in mice against the model.
- Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil observed no significant ($p > 0.05$) effect on locomotor activity of the mice which signifies that Bromelain alone and with Donepezil do not effect the motor function in mice.

✓ In biochemical study

- Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil were significantly ($p < 0.05$) observed to decrease the acetylcholinesterase level in mice brain that signify the cholinergic function improvement by increasing acetylcholine level in mice brain against the model.
- Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil were significantly ($p < 0.05$) observed to decrease the lipid peroxidation, and nitrite levels and increase the superoxide dismutase, catalase, and glutathione level in mice brain that signify the decrease the oxidative stress in mice against the model.
- Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil were significantly ($p < 0.05$) observed to increase the brain-derived neurotrophic factor that signifies the neuron growth in mice brain against the model.
- Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil were significantly ($p < 0.05$) observed to decrease IL-6 and TNF- α level in mice brain signifying reduce neuroinflammation in the brain against the model.

- Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil were significantly ($p < 0.05$) observed to decrease the β -secretase and amyloid-beta peptide₁₋₄₂ level in mice brain as compared to the model.

✓ **In histopathological study**

- Histopathology of mice brain of Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil treatments showed the improvement in eosinophilic lesions and neuron cells as compared to the model.

Consequently, Bromelain, Myrcene alone and in combination with Donepezil show potential for neuroprotection. Moreover, the co-administration of (Bromelain + Myrcene) and (Bromelain + Myrcene + Donepezil) may be a beneficial neuroprotective drug combination in the treatment of Alzheimer's disease. Henceforth, Bromelain, Myrcene, Bromelain + Myrcene, and in combination with Donepezil can be a useful neuroprotective agent for the treatment of Alzheimer's disease.

Despite the fact that Bromelain, Myrcene, and the combination of Bromelain and Myrcene have neuroprotective and anti-Alzheimer's effects, there are some inherent challenges also associated with its molecular based mechanism and the blood-brain barrier studies. To overcome these obstacles, more research is needed before its clinical use. Furthermore, the pharmaceutical formulation could aid in improving the efficacy of the chosen biomolecules. These studies if done in the future on these molecules can prove to be an important breakthrough in connecting its developmental path from pre-clinical to clinical use.

Bibliography:

- Armstrong, R.A., 2013. Review article What causes alzheimer's disease? *Folia Neuropathol.* 3, 169–188. <https://doi.org/10.5114/fn.2013.37702>.
- Abdulrahman, A.A., 2015. Antimicrobial Effects of Crude Bromelain Extracted from Pineapple Fruit (*Ananas comosus*). *Adv. Biochem.* 3, 1. <https://doi.org/10.11648/j.ab.20150301.11>.
- Abulfadl, Y.S., El-Maraghy, N.N., Ahmed, A.A.E., Nofal, S., Badary, O.A., 2018. Protective effects of thymoquinone on D-galactose and aluminum chloride induced neurotoxicity in rats: biochemical, histological and behavioral changes. *Neurol. Res.* 40, 324–333. <https://doi.org/10.1080/01616412.2018.1441776>.
- Agarwal, S., Chaudhary, B., Bist, R., 2016. Bacoside A and Bromelain relieve dichlorvos induced changes in oxidative responses in mice serum. *Chem. Biol. Interact.* 254, 173–178. <https://doi.org/10.1016/j.cbi.2016.05.017>.
- Aisen, P.S., 2002. The potential of anti-inflammatory drugs for the treatment of Alzheimer's disease. *The Lancet Neurol.* 1, 279–284. [https://doi.org/10.1016/s1474-4422\(02\)00133-3](https://doi.org/10.1016/s1474-4422(02)00133-3).
- Alagiakrishnan, K., Wiens, C.A., 2004. An approach to drug induced delirium in the elderly. *Postgrad. Med. J.* 80, 388–393. <https://doi.org/10.1136/pgmj.2003.017236>.
- Aliev, G., 2011. Oxidative stress induced-metabolic imbalance, mitochondrial failure, and cellular hypoperfusion as primary pathogenetic factors for the development of Alzheimer disease which can be used as an alternate and successful drug treatment strategy: past, present and future. *CNS Neurol. Disord. Drug Targets*, 10, 147–8. <https://doi.org/10.2174/187152711794480492>.
- Allen, S.J., Watson, J.J., Dawbarn, D., 2011. The Neurotrophins and Their Role in Alzheimer's disease. *Curr. Neuropharmacol.* 9, 559–573. <https://doi.org/10.2174/157015911798376190>.
- Nóbrega de Almeida, R., Agra, M. de F., Negromonte Souto Maior, F., De Sousa, D.P., 2011. Essential Oils and Their Constituents: Anticonvulsant Activity. *Molecules*, 16, 2726–2742. <https://doi.org/10.3390/molecules16032726>.
- Alzheimer's Association, 2015. Alzheimer's disease facts and figures. *Alzheimers Dement.* 11, 332–84. [https://doi.org/10.1016/j.jalz.\(2015\).02.003](https://doi.org/10.1016/j.jalz.(2015).02.003).
- Alzheimer's Association, 2014. AD Facts and Figures. *Alzheimer's & Dementia.* 10, 1–80.

- Amjad, S., Umesalma, S., 2015. Protective effect of *Centella asiatica* against aluminium-induced neurotoxicity in cerebral cortex, striatum, hypothalamus and the hippocampus of rat brain- histopathological, and biochemical approach, *J. Mol. Biomark. Diagn.* 6-1. <https://doi.org/10.4172/2155-9929.1000212>.
- Apostolova, L.G., Risacher, S.L., Duran, T., Stage, E.C., Goukasian, N., West, J.D., Do, T.M., Grotts J., Wilhalme, H., Nh0, K., Pkilips, M., Elashoff, D.Saykin, A.J., 2018. Associations of the Top 20 Alzheimer Disease Risk Variants With Brain Amyloidosis. *JAMA Neurol*, 75, 328. <https://doi.org/10.1001/jamaneurol.2017.4198>.
- Aprahamian, I., Stella, F., Forlenza, O.V., 2013. New treatment strategies for AD: is there a hope? *Indian J. Med. Res.* 138, 449–460.
- Ataide, J.A., de Carvalho, N.M., Rebelo, M. de A., Chaud, M.V., Grotto, D., Gerenutti, M., Rai, M., Mazzola P.G., Jozala, A.F., 2017. Bacterial Nanocellulose Loaded with Bromelain: Assessment of Antimicrobial, Antioxidant and Physical-Chemical Properties. *Sci.Rep.* 7, 1. <https://doi.org/10.1038/s41598-017-18271-4>.
- Auti, S.T., Kulkarni, Y.A., 2019. Neuroprotective Effect of Cardamom Oil Against Aluminum Induced Neurotoxicity in Rats. *Front. Neurol.* 10, 399. <https://doi.org/10.3389/fneur.2019.00399>.
- Ayala, A., Munoz, M. F., Arguelles, S., 2014. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid. Med. Cell. Longev.* 2014, 1–31. <https://doi.org/10.1155/2014/360438>.
- Ayton, S., Lei, P., Bush, A.I., 2013. Metallostasis in Alzheimer's disease. *Free Radic. Biol. Med.* 62, 76 – 89. [https://doi.org/10.1016/j.freeradbiomed.\(2012\).10.558](https://doi.org/10.1016/j.freeradbiomed.(2012).10.558).
- Baez, R., Lopes, M.T.P., Salas, C.E., Hernández, M., 2007. In vivo antitumoral activity of stem pineapple (*Ananas comosus*) Bromelain. *Planta. Med.* 73, 1377–1383. <https://doi.org/10.1055/s-2007-990221>.
- Ballard, C.G., Greig, N.H., Guillozet-Bongaarts, A.L., Enz, A., Darvesh, S., 2005. Cholinesterases: roles in the brain during health and disease. *Curr. Alzheimer Res.* 2, 307–18. <https://doi.org/10.2174/1567205054367838>.
- Banks, W.A., Niehoff, M.L., Drago, D., Zatta, P., 2006. Aluminum complexing enhances amyloid beta protein penetration of blood-brain barrier. *Brain Res.* 1116, 215–221.
- Baydas, G., Kutlu, S., Naziroglu, M., Canpolat, S., Sandal, S., Ozcan, M., 2003. Inhibitory effects of melatonin on neural lipid peroxidation induced by intracerebroventricularly administered homocysteine. *J. Pineal Res.* 34, 36–39.

- Baykalir Burcu, G., Osman, C., Ash, C., Namik, O.M., Nese, B.T., 2016. The protective cardiac effects of beta-Myrcene after global cerebral ischemia/reperfusion in C57BL/J6 mouse. *Acta. Cirúrgica. Bras.* 31, 2016–457. [https://doi.org/10.1590/S0102-8650\(2016\)00700000005](https://doi.org/10.1590/S0102-8650(2016)00700000005).
- Baynes, W.J., 2001. The role of AGEs in aging: causation or correlation. *Expt. Geront.* 36, 1527–1537. [https://doi.org/10.1016/s0531-5565\(01\)00138-3](https://doi.org/10.1016/s0531-5565(01)00138-3).
- Béez, R., Lopes, M.T.P., Salas, C.E., Hernández, M., 2007. In vivo antitumoral activity of stem pineapple (*Ananascomosus*) Bromelain. *Planta. Med.* 73, 1377–1383. [https://doi.org/10.1055/s-\(2007\)-990221](https://doi.org/10.1055/s-(2007)-990221).
- Bekinschtein, P., Cammarota, M., Kathe, C., Slipczuk, L., Rossato, J. I., Goldin, A., Izquierdo, I., Medina, J.H., 2008. BDNF is essential to promote persistence of long-term memory storage. *Proc. Natl. Acad. Sci.* 105, 2711–2716. <https://doi.org/10.1073/pnas.0711863105>.
- Berríos-Torres, S.I., Umscheid, C.A., Bratzler, D.W., Leas, B., Stone, E.C., Kelz, R. R., 2017. Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection. *JAMA Surgery.* 152, 784.
- Bhattacharya, A., Ghosal, S., Bhattacharya, S.K., 2001. Anti-oxidant effect of Withaniasomniferaglycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J. Ethnopharmacol.* 74, 1–6. [https://doi.org/10.1016/S0378-8741\(00\)00309-3](https://doi.org/10.1016/S0378-8741(00)00309-3).
- Bhattacharyya, B.K., 2008. Bromelain: an overview. *Nat. Prod. Radiance.* 7, 359–363. <http://nopr.niscair.res.in/handle/123456789/5694>.
- Binder, D.K., Scharfman, H.E., 2004. Brain-derived neurotrophic factor; Mini Review. *Growth Fact.* 22, 123–131. <https://doi.org/10.1080/08977190410001723308>.
- Bird, T.D., 2009. Genetic Aspects of Alzheimer's disease. *Health Care (Don. Mills)* 10: 231–239. <https://doi.org/10.1097/GIM.0b013e31816b64dc>.
- Birks, J., 2008. Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database of Systematic Reviews.* <https://doi.org/10.1002/14651858.CD005593>.
- Birks, J., 2012. Cholinesterase inhibitors for Alzheimer's disease: Review. *Cochrane Libr.* 5, 1–51.
- Birks, J.S., Harvey, R.J., 2018. Donepezil for dementia due to Alzheimer's disease. *Cochrane Database of Syst. Rev.* 0, 1–338. <https://doi.org/10.1002/14651858.CD001190.pub3>.

- Birnboim, H.C., 1986. DNA strand breaks in human leukocytes induced by superoxide anion, hydrogen peroxide and tumor promoters are repaired slowly compared to breaks induced by ionizing radiation. *Carcinogenesis* 7, 1511–1517. Doi:10.1093/carcin/7.9.1511.
- Blennow, K., Zetterberg, H., 2018. Biomarkers for Alzheimer disease - current status and prospects for the future. *J. Intern. Med* 0–2. <https://doi.org/10.1111/joim.12816>.
- Blom, K., Koek, H.L., Graaf, Y., Van, Der, Zwartbol, M.H.T., Wisse, L.E.M., 2018. Hippocampal sulcal cavities: prevalence, risk factors and association with cognitive performance. The SMART-Medea study and PREDICT-MR study. *Brain Imaging and Behavior*. <https://doi.org/10.1007/s11682-018-9916-y>.
- Bloom, G.S., 2014. Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. *JAMA Neurol.* 71, 505–508. <https://doi.org/10.1001/jamaneurol.2013.5847>.
- Boccardi, V., Baroni, M., Mangialasche, F., Mecocci, P., 2016. Vitamin E family: Role in the pathogenesis and treatment of Alzheimer's disease. *Alzheimer's Dement. Transl. Res. Clin. Interv.* 2, 182–191. [https://doi.org/10.1016/j.trci.\(2016\).08.002](https://doi.org/10.1016/j.trci.(2016).08.002).
- Boller, F., Forbes, M.M., 1998. History of dementia and dementia in history: An overview. *J. Neurol. Sci.* 158, 125–133. [https://doi.org/10.1016/S0022-510X\(98\)00128-2](https://doi.org/10.1016/S0022-510X(98)00128-2).
- Bonamin, F., Moraes, T.M., Dos Santos, R.C., Kushima, H., Faria, F.M., Silva, M.A., Junior, I.V., Nogueira, L., Bauab, T.M., Souza Brito, A.R., Rocha, L.R.M., Hiruma-Lima, C.A., 2014. The effect of a minor constituent of essential oil from *Citrus aurantium*: The role of beta-Myrcene in preventing peptic ulcer disease. *Chem. Biol. Interact.* 5, 11-9. <https://doi.org/10.1016/j.cbi.2014.01.009>.
- Bromley-Brits, K., Deng, Y., Song, W., 2011. Morris Water Maze Test for Learning and Memory Deficits in Alzheimer's Disease Model Mice. *J. Vis. Exp.* 2–6. <https://doi.org/10.3791/2920>.
- Brookmeyer, R., Johnson, E., Ziegler-Graham, K., Arrighi, H.M., 2007. Forecasting the global burden of Alzheimer's disease. *Alzheimer's & Dementia*, 3, 186–191. <https://doi.org/10.1016/j.jalz.2007.04.381>.
- Brunden, K.R., Ballatore, C., Crowe, A., I, A.B.S., Lee, V.M., Trojanowski, J.Q., 2011. NIH Public Access 223, 304–310. [https://doi.org/10.1016/j.expneurol.\(2009\).08.031](https://doi.org/10.1016/j.expneurol.(2009).08.031).

- Burcu, G.B., Osman, C., Asli, C., Namik, O.M., Nese, B.T., 2016. The protective cardiac effects of β -Myrcene after global cerebral ischemia-reperfusion in C57BL/6 mouse. *Acta. Cir. Bras.* 31, 456-62. <https://doi.org/10.1590/S0102-8650201600700000005>.
- Burns, A., Iliffe, S., 2009. Alzheimer's disease. *B.M.J.* 338, b158–b158. <https://doi.org/10.1136/bmj.b158>.
- Burton, G. W., Joyce, A., Ingold, K.U., 1983. Is vitamin E, the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch. Biochem. Biophys.* 221, 281–290.
- Calabrese, V., Bates, T. E., Stella, A.M.G., 2000. NO Synthase and NO-dependent signal pathways in brain aging and neurodegenerative disorders: The role of oxidant/antioxidant balance. *Neurochem. Res.* 25, 1315–1341. <https://doi.org/10.1023/A:1007604414773>.
- Calhoun, M.E., Wiederhold, K.H., Abramowski, D., Phinney, A.L., Probst, A., Sturchler-Pierrat, C., Jucker, M., 1998. Neuron loss in APP transgenic mice. *Nature*, 39, 755–756. <https://doi.org/10.1038/27351>.
- Campbell, A., Becaria, A., Lahiri, D.K., Sharman, K., Bondy, S.C., 2004. Chronic exposure to aluminium in drinking water increases inflammatory parameters selectively in the brain. *J. Neurosci. Res.* 75, 565–72. <https://doi.org/10.1002/jnr.10877>.
- Cesta, M.F., Hard, G.C., Boyce, J.T., Ryan, M.J., Chan, P.C., Sills, R.C., 2013. Complex histopathologic response in rat kidney to oral β -Myrcene: unusual dose-related nephrosis and low-dose alpha2uglobulin nephropathy. *Toxicol. Pathol.* 41, 1068-77. <https://doi.org/10.1177/0192623313482057>.
- Chaudhary, B., Agarwal, S., Bist, R., 2018. Invulnerability of Bromelain against oxidative degeneration and cholinergic deficits imposed by dichlorvos in mice brains. *Frontiers in Biology* 13, 56-62. <https://doi.org/10.1007/s11515-018-1479-1>.
- Chelikani, P., Fita, I., Loewen, P.C., 2004. Diversity of structures and properties among catalases. *Cell. Mol. Life Sci.* 61, 192–208. <https://doi.org/10.1007/s00018-003-3206-5>.
- Chen, W.W., Zhang, X., Huang, W.J., 2016. Role of neuroinflammation in neurodegenerative diseases. *Mol. Med. Rep.* 13, 3391–3396. [https://doi.org/10.3892/mmr.\(2016\).4948](https://doi.org/10.3892/mmr.(2016).4948).
- Chen, K.H., Reese, E.A., Kim, H.W., Rapoport, S.I., Rao, J.S., 2011. Disturbed neurotransmitter transporter expression in Alzheimer's disease brain. *Journal of Alzheimer's disease* 26, 755-766.

- Chiroma, S.M., Mohd Moklas, M.A., Mat Taib, C.N., Baharuldin, M.T.H., Amon, Z., 2018. D -galactose and aluminium chloride induced rat model with cognitive impairments. *Biomedicine & Pharmacotherapy* 103, 1602–1608. <https://doi.org/10.1016/j.biopha.2018.04.152>.
- Ciftci, O., Ozdemir, I., Tanyildizi, S., Yildiz, S., Oguzturk, H., 2011. Antioxidative effects of curcumin, β -Myrcene and 1,8-cineole against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced oxidative stress in rats liver. *Toxicol. Ind. Health*, 27, 447–453. <https://doi.org/10.1177/0748233710388452>.
- Ciftci, O., Oztanir, M.N., Cetin, A., 2011. Neuroprotective Effects of β -Myrcene Following Global Cerebral Ischemia/Reperfusion-Mediated Oxidative and Neuronal Damage in a C57BL/J6 Mouse. *Neurochem. Res.* 39, 1717–1723. <https://doi.org/10.1007/s11064-014-1365-4>.
- Çoban, J., Doğan-Ekici, I., Aydın, A. F., Betül-Kalaz, E., Doğru-Abbasoğlu, S., Uysal, M., 2014. Blueberry treatment decreased D-galactose-induced oxidative stress and brain damage in rats. *Metabolic Brain Disease* 30, 793–802. <https://doi.org/10.1007/s11011-014-9643-z>.
- Cohen, A., Goldman, J., 1964. Bromelains therapy in rheumatoid arthritis. *P.A. Med. J.* 67, 27–30. <https://doi.org/10.1093/ptj/44.7.571>.
- Coyle, J., 1982. The cholinergic system in Alzheimer's disease Lord Adrian and the nature of neural conduction. *Trends in Neurosciences*, 5, 261-262. [https://doi.org/10.1016/0166-2236\(82\)90165-5](https://doi.org/10.1016/0166-2236(82)90165-5).
- Cui, X., Zuo, P., Zhang, Q., Li, X., Hu, Y., Long, J., Packer, L., Liu, J., 2006. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: protective effects of R-alpha-lipoic acid. *J. Neurosci. Res.* 84, 647–654.
- Cummings, J., Lefèvre, G., Small, G., Appel-Dingemanse, S., 2007. Pharmacokinetic rationale for the rivastigmine patch. *Neurol.* 69, S10-S13. <https://doi.org/10.1212/01.wnl.0000281846.40390.50>.
- Damage, O., 2015. HHS Public Access, 34, 670–678. <https://doi.org/10.1007/s11064-008-9808-4>.
- Darusman, H.S., Gjedde, A., Sajuthi, D., Schapiro, S.J., Kalliokoski, O., Kristianingrum, Y.P., Handaryani, E., Hau, J., 2014. Amyloid Beta1-42 and the Phosphorylated Tau Threonine 231 in Brains of Aged Cynomolgus Monkeys (*Macaca fascicularis*). *Front. Aging Neurosci.* 6. <https://doi.org/10.3389/fnagi.2014.00313>.

- Das. S., Ghosal, M., Pal, S., 2012. Dementia: Indian scenario. *Neurol. India*, 60, 618. <https://doi.org/10.4103/0028-3886.105197>.
- Dave, S., Kaur, N.J., Nanduri, R., Dkhar, H.K., Kumar, A., Gupta, P., 2012. Inhibition of adipogenesis and induction of apoptosis and lipolysis by stem Bromelain in 3T3-L1 adipocytes. *PLoS One* 7. <https://doi.org/10.1371/journal.pone.0030831>.
- Davies, P., Maloney, A.J.F., 1976. Selective Loss of Central Cholinergic Neurons in AD. *The Lancet*. 308, 1403.
- Davoodi, F.G., Motamedi, F., Naghdi, N., Akbari, E., 2009. Effect of reversible inactivation of the reuniens nucleus on spatial learning, memory in rats using morriswater maze task. *Behav. Brain Res.* 198, 130–135. <https://doi.org/10.1016/j.bbr.2008.10.037>.
- De Sousa, D.P., Gonçalves, J.C.R., Quintans-Júnior, L., Cruz, J.S., Araújo, D.A.M., De Almeida, R.N., 2006. Study of anticonvulsant effect of citronellol, a monoterpene alcohol, in rodents. *Neurosci. Letters*, 401, 231–235. <https://doi.org/10.1016/j.neulet.2006.03.030>.
- DeKosky, S.T., 2001. Epidemiology and pathophysiology of Alzheimer's disease. *Clinical Cornerstone*, 3, 15–26. [https://doi.org/10.1016/s1098-3597\(01\)90045-6](https://doi.org/10.1016/s1098-3597(01)90045-6).
- Deng, Y., Li, B., Liu, F., Iqbal, K., Grundke-Iqbal, I., Brandt, R., Gong, C.X., 2007. Regulation between O-GlcNAcylation and phosphorylation of neurofilament-M and their dysregulation in Alzheimer's disease. *FASEB J.* 22, 138–145. <https://doi.org/10.1096/fj.07-8309com>.
- Diamond, B.J., Bailey, M.R., 2013. Ginkgo biloba, Indications, Mechanisms, and Safety. *Psychiatr. Clin. North Am.* 36, 73–83. [https://doi.org/10.1016/j.psc.\(2012\).12.006](https://doi.org/10.1016/j.psc.(2012).12.006).
- Dodart, J.C., Bales, K.R., Gannon, K.S., Greene, S.J., DeMattos, R.B., Mathis, C., DeLong, C.A., Wu, S., Wu, X., Holtzman, D.M., Paul, S.M., 2002. Immunization reverses memory deficits without reducing brain A β burden in Alzheimer's disease model. *Nat. Neurosci.* 5, 452–457. <https://doi.org/10.1038/nn842>.
- Doody, R.S., Gavrilova, S.I., Sano, M., Thomas, R.G., Aisen, P.S., Bachurin, S.O., 2008. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate AD: a randomised, double-blind, placebo-controlled study. *The Lancet*. 372, 207–215.
- Dragicevic, N., Smith, A., Lin, X., Yuan, F., Copes, N., Delic, V., Tan, J., Cao, C., Shytle, R.D., Bradshaw, P.C., 2011. Green tea epigallocatechin-3-gallate (EGCG) and

- other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. *J. Alzheimers Dis.* 26, 507-521. <https://doi.org/10.3233/JAD-2011-101629>.
- Dunnett, S., Everitt, B., Robbins, T., 1991. The basal forebrain-cortical cholinergic system: interpreting the functional consequences of excitotoxic lesions. *Trends in Neurosciences* 14, 494–501. [https://doi.org/10.1016/0166-2236\(91\)90061-x](https://doi.org/10.1016/0166-2236(91)90061-x).
- Ellman, G.L., Courtney, D.K., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7, 88–95. [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9).
- Eric, R., Secor, Jr., William, F., Carson, I.V., Cloutier, M.M., Guernsey, L.A., Schramm, C.M., Wu, CA., Thrall, R.S., 2005. Bromelain exerts anti-inflammatory effects in an ovalbumin-induced murine model of allergic airway disease. *Cellular Immunol.* 237, 68–75. <https://doi.org/10.1016/j.cellimm.2005.10.002>.
- Everitt, B.J., Robbins, T.W., 1997. Central cholinergic systems and cognition. *Annual Review of Psychology* 48, 649–684. <https://doi.org/10.1146/annurev.psych.48.1.649>.
- Farina, M., Lara, F.S., Brandao, R., Jacques, R., Rocha, J.B.T., 2002. Effects of aluminumsulfate on erythropoiesis in rats. *Toxicology Letters* 132, 131–139. [https://doi.org/10.1016/S0378-4274\(02\)00077-2](https://doi.org/10.1016/S0378-4274(02)00077-2).
- Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y., Jorm, A., Mathers, C., Menezes, P.R., Rimmer, E., Sczufca, M., 2005. Global prevalence of dementia: a Delphi consensus study. *The Lancet*, 366, 2112–2117. [https://doi.org/10.1016/s0140-6736\(05\)67889-0](https://doi.org/10.1016/s0140-6736(05)67889-0).
- Filipcik, P., Zilka, N., Bugos, O., Kucerak, J., Koson, P., Novak, P., Novak, M., 2012. First transgenic rat model developing progressive cortical neurofibrillary tangles. *Neurobiol. Aging*, 33, 1448–1456. <https://doi.org/10.1016/j.neurobiolaging.2010.10.015>.
- Flint-Garcia, S.A., Thornsberry, J.M., Buckler, E.S., 2003. Structure of Linkage Disequilibrium in Plants. *Annu. Rev. Plant. Biol.* 54, 357–374.
- Flood, J.F.E., Morley, J., 1997. Learning and memory in the SAMP8 mouse. *Neurosci. Biobehav. Rev.* 22, 1–20. [https://doi.org/10.1016/S0149-7634\(96\)00063-2](https://doi.org/10.1016/S0149-7634(96)00063-2).
- Forman, M.S., 2005. Transgenic Mouse Model of Tau Pathology in Astrocytes Leading to Nervous System Degeneration. *J. Neurosci.* 25, 3539–3550. [https://doi.org/10.1523/JNEUROSCI.0081-05.\(2005\)](https://doi.org/10.1523/JNEUROSCI.0081-05.(2005)).

- Förstl, H., Sattel, H., Bahro, M., 1993. Alzheimer's disease: Clinical features. *Int. Rev. Psychiatry*, 5, 327–349. <https://doi.org/10.3109/09540269309037797>.
- Fukumoto, H., Rosene, D. L., Moss, M.B., Raju, S., Hyman, B. T., Irizarry, M.C., 2004. β -Secretase Activity Increases with Aging in Human, Monkey, and Mouse Brain. *The American Journal of Pathology* 164, 719–725. [https://doi.org/10.1016/s0002-9440\(10\)63159-8](https://doi.org/10.1016/s0002-9440(10)63159-8).
- Fumagalli, F., Racagni, G., Riva, M.A., 2006. The expanding role of BDNF: a therapeutic target for Alzheimer's disease? *Pharmacogenomics J.* 6, 8–15. <https://doi.org/10.1038/sj.tpj.6500337>.
- Galimberti, D., Scarpini, E., 2017. Pioglitazone for the treatment of AD. *Expert Opin. Investig. Drugs*. 26, 97–101.
- Games, D., Adams, D., Alessandrini, R., Barbour, R., Borthellette, P., Blackwell, C., Car, T., Clemens, J., Donaldson, T., Gillespie, F., Guido, T., Hagopian, S., Johnson-wood, K., Khan, K., Lee, M., Leibowitz, P., Lieberburg, I., Little, S., Masliah, E., McConlogue, L., Montoya-Zavala, M., Mucke, L., Paganini, L., Penniman, E., Power, M., Shenk, D., Seubert, P., Snyder, B., Soriano, F., Tan, H., Vitale, J., Wadsworth, S., Wolozin, B., Zhao, J., 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F β -amyloid precursor protein. *Nat.* 373, 523–527. <https://doi.org/10.1038/373523a0>.
- Gao, X., Tang, X.C., 2006. Hyperzinc A attenuates mitochondrial dysfunction in β -amyloid-treated PC12 cells by reducing oxygen free radicals accumulation and improving mitochondrial energy metabolism. *J. Neurosci. Res.* 83, 1048–1057. <https://doi.org/10.1002/jnr.20791>.
- German, D.C., 2007. Mouse Models of Alzheimer's disease. *The Brain Research Bulletin*, 88, 259–273. [https://doi.org/10.1016/j.brainresbull.\(2011\).11.017](https://doi.org/10.1016/j.brainresbull.(2011).11.017).
- Ghara, T.K., Roy, K., 2017. Alzheimer's disease - An Analytical View, 6, 31–35. <https://doi.org/10.9790/1959-0604043135>.
- Ghribi, O., Dewitt, D.A., Forbes, M.S., Herman, M.M., Savory, J., 2001. Co-involvement of mitochondria and endoplasmic reticulum in regulation of apoptosis: changes in cytochrome-c, Bcl-2 and Bax in the hippocampus of aluminium treated rabbits. *Brain Res.* 8, 66–73. [https://doi.org/10.1016/s0006-8993\(01\)02406-4](https://doi.org/10.1016/s0006-8993(01)02406-4).
- Gibson, Q.S., G.E., 2011. Oxidative Stress and Transcriptional Regulation in Alzheimer's disease. *Alzheimer Dis. Assoc. Disord.* 21, 276–291. <https://doi.org/10.1097/WAD.0b013e31815721c3>.

- Gitler, A.D., Dhillon, P., Shorter, J., 2017. Neurodegenerative disease: models, mechanisms, and a new hope. *Dis. Model Mech.* 10, 499–502. <https://doi.org/10.1242/dmm.030205>.
- Giunta, B., Fernandez, F., Nikolic, W.V., Obregon, D., Rrapo, E., Town, T., Tan, J., 2008. Inflammaging as a prodrome to Alzheimer's disease. *J. Neuroinflammation*, 5, 1–15. <https://doi.org/10.1186/1742-2094-5-51>.
- Götz, J., Chen, F., Van Dorpe, J., Nitsch, R.M., 2001. Formation of neurofibrillary tangles in P301L tau transgenic mice induced by A β 42 fibrils. *Sci.* 293, 1491–1495. <https://doi.org/10.1126/science.1062097>.
- Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal. Biochem.* 126, 131–138. [https://doi.org/10.1016/0003-2697\(82\)90118-X](https://doi.org/10.1016/0003-2697(82)90118-X).
- Greenamyre, J.T., Young, A.B., 1989. Excitatory amino acids and Alzheimer's disease. *Neurobiology of aging* 10, 593–602.
- Greig, N.H., Sambamurti, K., Yu, Q., Brossi, A., Bruinsma, G.B., Lahiri, D. K., 2005. An overview of phenserine tartrate, a novel acetylcholinesterase inhibitor for the treatment of AD. *Curr. Alzheimer Res.* 2, 281–290.
- Guerreiro, R., Bras, J., 2015. The age factor in Alzheimer's disease. *Genome Med.* 7, 1–3. <https://doi.org/10.1186/s13073-015-0232-5>.
- Gulya, K., Rakonczay, Z., Kasa, P., 1990. Cholinotoxic effects of aluminium in rat brain. *J. Neurochem.* 54, 1020–6. <https://doi.org/10.1111/j.1471-4159.1990.tb02352.x>.
- Habashi, S., Sabouni, F., Moghimi, A., 2012. Reduction of NO production in LPS-stimulated primary rat microglial cells by Bromelain. *Profdoc. Um. Ac. Ir.* 3, 57–65. <https://doi.org/10.22067/jcmr.v3i2.14613>.
- Haider, S., Liaquat, L., Ahmad, S., Batool, Z., Siddiqui, R. A., Tabassum, S., Shahzad, S., Rafiq, S., Naz, N., 2020. Naringenin protects A β 13/D-galactose induced neurotoxicity in a rat model of AD via attenuation of acetylcholinesterase levels and inhibition of oxidative stress. *Plos One* 15, e0227631. <https://doi.org/10.1371/journal.pone.0227631>.
- Hakk, H., Larsen, G.KW.E., 2002. Tissue disposition , excretion and metabolism in the male Sprague $\pm$ Dawley rat. *Xenobiotica.* 32, 369–382.
- Hale, L.P., 2004. Proteolytic activity and immunogenicity of oral Bromelain within the gastrointestinal tract of mice. *Int. Immunopharmacol.* 4, 255–264. <https://doi.org/10.1016/j.intimp.2003.12.010>.

- Hampel, H., 2012. Current insights into the pathophysiology of Alzheimer's disease: selecting targets for early therapeutic intervention. *International Psychogeriatrics* 24, S10–S17. <https://doi.org/10.1017/S1041610212000579>.
- Hampel, H., Mesulam, M.M., Cuello, A.C., Farlow, M.R., Giacobini, E., Grossberg, G.T., Khachaturian, A.S., Vergallo, A., Cavedo, E., Synder, P.J., Khachaturian, Z.S., 2018. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* 141, 1917–1933. <https://doi.org/10.1093/brain/awy132>.
- Hardy, J., Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Sci.* 297, 353–356. <https://doi.org/10.1126/science.1072994>.
- Harrington, M.J., Razghandi, K., Ditsch, F., Guiducci, L., Rueggeberg, M., Dunlop, J.W.C., 2011. Origami-like unfolding of hydro-actuated ice plant seed capsules. *Nat. Commun.* 2, 337.
- Hayyan, M., Hashim, M.A., AlNashef, I.M., 2016. Superoxide Ion: Generation and Chemical Implications. *Chem. Rev.* 116, 3029–3085. <https://doi.org/10.1021/acs.chemrev.5b00407>.
- Hebert, S.S., Horre, K., Nicolai, L., Papadopoulou, A.S., Mandemakers, W., Silahatoglu, A.N., Kauppinen, S., Delacourte, A., De Strooper, B., 2008. Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/-secretase expression. *Proceedings of the National Academy of Sciences USA* 105, 6415–6420. <https://doi.org/10.1073/pnas.0710263105>.
- Heinonen, T.; Gaus, W. Cross matching observations on toxicological and clinical data for the assessment of tolerability and safety of Ginkgo biloba leaf extract. *Toxicology*. 2015, 327, 95–115.
- Heneka, M.T., O'Banion, M.K., 2007. Inflammatory processes in Alzheimer's disease. *J. Neuroimmunol.* 184, 69–91. <https://doi.org/10.1016/j.jneuroim.2006.11.017>.
- Herculano, B., Tamura, M., Ohba, A., Shimatani, M., Kutsuna, N., Hisatsune, T., 2013. β -Alanyl-L-histidine rescues cognitive deficits caused by feeding a high fat diet in a transgenic mouse model of Alzheimer's disease. *J. Alzheimer's Dis.* 33, 983–997. [https://doi.org/10.3233/JAD-\(2012\)-121324](https://doi.org/10.3233/JAD-(2012)-121324).
- Hewlings, S., Kalman, D., 2017. Curcumin: A Review of Its' Effects on Human Health. *Foods*, 6, 92. <https://doi.org/10.3390/foods6100092>.

- Hollman, P.C.H., Van Trijp, J.M.P., Buysman, M.N.C.P., Martijn, M.S., Mengelers, M.J.B., De Vries, J.H.M., 1997. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Letters*. 418, 152–156.
- Howard, A.C., McNeil, A.K., McNeil, P.L., 2011. Promotion of plasma membrane repair by vitamines. *Nat. Commun.* 2, 1–8.
- Hozumi, I., Hasegawa, T., Honda, A., Ozawa, K., Hayashi, Y., Hashimoto, K., Inuzuka, T. (2011). Patterns of levels of biological metals in CSF differ among neurodegenerative diseases. *J. Neurological Sci.* 303, 95–99. <https://doi.org/10.1016/j.jns.2011.01.003>.
- Hsieh, H., Wua, W., Hu, M., 2009. Soy isoflavones attenuate oxidative stress and improve parameters related to aging and Alzheimer's disease in C57BL/6J mice treated with D-galactose. *Food Chem. Toxicol.* 47, 625–632. <https://doi.org/10.1016/j.fct.2008.12.026>.
- Hua, X., Lei, M., Ding, J., Han, Q., Hu, G., Xio, M., 2008. Pathological and biochemical alterations of astrocytes in ovariectomized rats injected with D-galactose: a potential contribution to Alzheimer's disease processes. *Exp. Neurol.* 210, 709–718.
- Huang, W.J., Zhang, X.I., Chen, W.W., 2016. Role of oxidative stress in Alzheimer's disease. *Biomed Rep.* 4, 519–22. <https://doi.org/10.3892/br.2016.630>.
- Humanson, G.L., 1962. Animal tissue technique. San Francisco: Freeman W.H., 3–126. <https://www.worldcat.org/title/animal-tissue-techniques/oclc/558154>.
- Hwang, E., Ngo, H.T.T., Park, B., Seo, S.A., Yang, J.E., Yi, T.H., 2017. Myrcene, An Aromatic Volatile Compound, Ameliorates Human Skin Extrinsic Aging via Regulation of MMPs Production. *Am. J. Chin. Med.* 45, 1–12. <https://doi.org/10.1142/S0192415X17500604>.
- Ibrahim, W.W., Ismail, H.M., Khattab, M. M., Abdelkader, N.F., 2020. Cognitive enhancing effect of diapocynin in D-galactose-ovariectomy-induced Alzheimer's-like disease in rats: Role of ERK, GSK-3 β and JNK signaling. *Toxicology and Applied Pharmacology*, 115028. <https://doi.org/10.1016/j.taap.2020.115028>.
- Islam, I., 2018. Protective effect of chloroform extract of *Stereospermum chelonoides* bark against amyloid beta 42 induced cell death in SH-SY5Y cells and against inflammation in Swiss albino mice. *J. Basic Clin. Physiol. Pharmacol.* [https://doi.org/10.1515/jbcpp-\(2017\)-0123](https://doi.org/10.1515/jbcpp-(2017)-0123).
- Islam, A.U.S., Hellman, B., Nyberg, F., Amir, N., Jayaraj, R.L., Petroianu, G., Adem, A., 2020. Myrcene attenuates renal inflammation and oxidative stress in the

- adrenalectomized rat model. *Molecules*, 25, 4492.
<https://doi.org/10.3390/molecules25194492>.
- Israel, M.A., Yuan, S.H., Bardy, C., Reyna, S.M., Mu, Y., Herrera, C., Hefferan, M.P., Gorp, S.V., Nazor, K.L., Boscolo, F.S., Carson, C.T., Laurent, L.C., Marsala, M., Gage, F.H., Remes, A.M., Koo, E.H., Goldstein, L.S.B., 2012. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nat.* 482, 216–220. <https://doi.org/10.1038/nature10821>.
- Itoh, J., Nabeshima, T., Kameyama, T., 1990. Utility of an elevated plus maze for the evaluation of memory in mice: Effects of nootropics, scopolamine and electroconvulsive shock. *Psychopharmacology* 101, 27–33.
<https://doi.org/10.1007/BF02253713>.
- Jaen, M.W., Shirley, K.L., Small, G.W., 2002. Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. *Clin. Pharmacokinet.* 41, 719–739.
- Johnson, L.C., DeVan, A.E., Justice, J.N., Seals, D.R., 2017. Nitrate and Nitrite in Aging and Age-Related Disease. *Nitrite and Nitrate in Human Health and Disease* 0, 259–277. https://doi.org/10.1007/978-3-319-46189-2_18.
- Jollow, D.J., Mitchell, J.R., Zampaglione, N., Gillette, J.R., 1974. Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3, 4-bromobenzenoxide as the hepatotoxic intermediate. *Pharmacology* 11, 151–169.
<https://doi.org/10.1159/000136485>.
- Jones, L., Lambert, J.C., Wang, L.S., Choi, S.H., Harold, D., Vedernikov, A., Bellenguez, C., 2015. Convergent genetic and expression data implicate immunity in Alzheimer's disease. *Alzheimer's & Dementia*, 11, 658–671. <https://doi.org/10.1016/j.jalz.2014.05.1757>.
- Jones, R.W., 2002. Drug treatment of Alzheimer's disease. *Rev. Clin. Gerontol.* 12, 165–173.
- Jones, R.W., 2010. Dimebon disappointment. *Alzheimers. Res. Ther.* 2, 3–5.
- Jones, R.W., 2002. Drug treatment of AD. *Rev. Clin. Gerontol.* 12, 165–173 9.
- Justin-Thenmozhi, A., Dhivya Bharathi, M., Kiruthika, R., Manivasagam, T., Borah, A., Essa, M.M., 2018. Attenuation of aluminum chloride induced neuroinflammation and caspase activation through the AKT/GSK-3 β pathway by hesperidin in Wistar Rats. *Neurotox. Res.* 34, 463–476. <https://doi.org/10.1007/s12640-018-9904-4>.

- Jyotshna Srivastava, N., Singh, B., Chanda, D., Shanker, K., 2015. Chemical composition and acetylcholinesterase inhibitory activity of *Artemisia maderaspatana* essential oil. *Pharm. Biol.* 53, 1677–1683. [https://doi.org/10.3109/13880209.\(2014\).1001405](https://doi.org/10.3109/13880209.(2014).1001405).
- Kalaria, R.N., Maestre, G.E., Arizaga, R., Friedland, R.P., Galasko, D., Hall, K., Luchsinger, J.A., Ogunniyi, A., Parry, E.K., Potocnik, F., Prince, M., Stewart, R., Wimo, A., Zhang, Z.X., Antuono, P., 2008. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. *The Lancet Neurol.* 7, 812–826. [https://doi.org/10.1016/s1474-4422\(08\)70169-8](https://doi.org/10.1016/s1474-4422(08)70169-8).
- Kalra, N., Bhui, K.B., Roy, P., Srivastava, S., George, J., Prasad, S., Shukla, Y., 2008. Regulation of p53, nuclear factor κ B, and cyclooxygenase-2 expression by Bromelain through targeting mitogen-activated protein kinase pathway in mouse skin. *Toxicol. Appl. Pharm.* 226, 30–37. <https://doi.org/10.1016/j.taap.2007.08.012>.
- Kao, S.C., Krichevsky, A.M., Kosik, K.S., Tsai, L.H., 2003. BACE1 suppression by RNA interference in primary cortical neurons. *Journal of Biological Chemistry*, 279, 1942–1949. <https://doi.org/10.1074/jbc.M309219200>.
- Kaplan, L.A., Pesce, A.J., 1996. *Clinical Chemistry*, third ed. Mosby, St. Louis. p. 954. <https://www.elsevier.com/books/clinical-chemistry/kaplan/978-0-323-03658-0>.
- Kawahara, M., 2003. Aluminum induced conformational changes of β -Amyloid protein and the pathogenesis of Alzheimer's disease. *Journal of Health Science* 49, 341–347. <https://doi.org/10.1248/jhs.49.341>.
- Kawahara, M., 2005. Effects of aluminium on the nervous system and its possible link with neurodegenerative diseases. *J. Alzheimer Dis.* 8, 171–182. <https://doi.org/10.3233/jad-2005-8210>.
- Kenawy, S., Hegazy, R., Hassan, A., El-Shenawy, S., Gomaa, N., Zaki, H., Attia, A., 2017. Involvement of insulin resistance in D-galactose-induced age-related dementia in rats: Protective role of metformin and saxagliptin. *Plos One* 12, e0183565. <https://doi.org/10.1371/journal.pone.0183565>.
- Khurana, N., Gajbhiye, A., 2013. Ameliorative effect of *Sidacordifolia* in rotenone induced oxidative stress model of Parkinson's disease. *Neurotoxicol.* 39, 57–64. [https://doi.org/10.1016/j.neuro.\(2013\).08.005](https://doi.org/10.1016/j.neuro.(2013).08.005).
- Khurana, N., Ishar, M.P.S., Gajbhiye, A., Goel, R.K., 2011. PASS assisted prediction and pharmacological evaluation of novel nicotinic analogs for nootropic activity in mice. *Eur. J. Pharmacol.* 662, 22–30. <https://doi.org/10.1016/j.ejphar.2011.04.048>.

- Kono, Y., 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch. Biochem. Biophys.* 186, 189–195. [https://doi.org/10.1016/0003-9861\(78\)90479-4](https://doi.org/10.1016/0003-9861(78)90479-4).
- Kulkarni, K., Kasture, S., Mengi, S., 2010. Efficacy study of prunus amygdalus (almond) nuts in scopolamine-induced amnesia in rats. *Indian Journal of Pharmacology* 42, 168. <https://doi.org/10.4103/0253-7613.66841>.
- Lacor, P.N., Buniel, M.C., Furlow, P.W., Sanz Clemente, A., Velasco, P. T., Wood, M., Viola, K.L., Klein, W.L., 2007. Aβ Oligomer-Induced Aberrations in Synapse Composition, Shape, and Density Provide a Molecular Basis for Loss of Connectivity in Alzheimer's disease. *Journal of Neuroscience* 27, 796–807. <https://doi.org/10.1523/JNEUROSCI.3501-06.2007>.
- Lauer, D., Reichenbach, A., Birkenmeier, G., 2001. α2-Macroglobulin mediated degradation of amyloid β1-42: A mechanism to enhance amyloid β catabolism. *Exp. Neurol.* 167, 385–392. <https://doi.org/10.1006/exnr.2000.7569>.
- Laurijssens, B., Aujard, F., Rahman, A., 2013. Animal models of Alzheimer's disease and drug development. *Drug Discov. Today Technol.* 10, 1–9. [https://doi.org/10.1016/j.ddtec.\(2012\).04.001](https://doi.org/10.1016/j.ddtec.(2012).04.001).
- Lei, M., Hua, X., Xiao, M., Ding, J., Han, Q., Hu, G., 2008. Impairments of astrocytes are involved in the d-galactose-induced brain aging. *Biochem. Biophys. Res. Commun.* 369, 1082–1087. <https://doi.org/10.1016/j.bbrc.2008.02.151>.
- Lewczuk, P., Wiltfang, J., 2008. Neurochemical dementia diagnostics: State of the art and research perspectives. *Proteomics*, 8, 1292–1301. <https://doi.org/10.1002/pmic.200700703>.
- Li, X., Bao, X., Wang, R., 2016. Experimental models of Alzheimer's disease for deciphering the pathogenesis and therapeutic screening. *Int. J. Mol. Med.* 37, 271–283. [https://doi.org/10.3892/ijmm.\(2015\).2428](https://doi.org/10.3892/ijmm.(2015).2428).
- Li, F., Gong, Q.H., Wu, Q., Lu, Y.F., Shi, J.S., 2010. Icariin isolated from *Epimedium brevicornum* Maxim attenuates learning and memory deficits induced by d-galactose in rats. *Pharmacol. Biochem. Behav.* 96, 301–305. <https://doi.org/10.1016/j.pbb.2010.05.021>.
- Li, G.L., Farooque, M., Holtz, A., Olsson, Y., 1995. Changes of beta-amyloid precursor protein after compression trauma to the spinal cord: an experimental study in the rat using immunohistochemistry. *J. Neurotrauma.* 12, 269–277.

- Li, H., Song, J., Zhang, J., Wang, T., Yan, Y., Tao, Z., Li, S., Zhang, H., Kang, T., Yang, J., 2016. Ginseng Protein Reverses Amyloid Beta Peptide and H₂O₂ Cytotoxicity in Neurons, and Ameliorates Cognitive Impairment in AD Rats Induced by a Combination of D-Galactose and AlCl₃. *Phytotherapy Research* 3, 284–295. <https://doi.org/10.1002/ptr.5747>.
- Limon, A., Reyes-Ruiz, J.M., Miledi, R., 2012. Loss of functional GABA (A) receptors in the Alzheimer diseased brain. *Proceedings of the National Academy of Sciences of the United States of America* 109, 10071-10076.
- Lin, M.T., Beal, M.F., 2006. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nat.* 443, 787–795. <https://doi.org/10.1038/nature05292>.
- Lotz-Winter, H., 1990. On the Pharmacology of Bromelain: An Update with Special Regard to Animal Studies on Dose-Dependent Effects. *Planta. Medica.* 56, 249–253. <https://doi.org/10.1055/s-2006-960949>.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275. <https://www.jbc.org/content/193/1/265.full.pdf>.
- Lu, J., Zheng, Y.L., Luo, L., Wu, D.M., Sun, D.X., Feng, Y.J., 2006. Quercetin reverses D- galactose induced neurotoxicity in mouse brain. *Behav. Brain Res.* 171, 251–260. <https://doi.org/10.1016/j.bbr.2006.03.043>.
- Lubos, E., Loscalzo, J., Handy, D.E., 2011. Glutathione Peroxidase-1 in Health and Disease: From Molecular Mechanisms to Therapeutic Opportunities. *Antioxid. Redox Signal.* 15, 1957–1997. <https://doi.org/10.1089/ars.2010.3586>.
- Luck, H., 1965. Catalase. *Methods of Enzymatic Analysis*, 885-894. <http://dx.doi.org/10.1016/B978-0-12-395630-9.50158-4>.
- Lukiw, W.J., LeBlanc, H.J., Carver, L.A., McLachlan, D.R., Bazan, N.G., 1998. Run on gene transcription in human neocortical nuclei. Inhibition by nanomolar aluminium and implications for neurodegenerative diseases. *J. Mol. Neurosci.* 11, 67–78. <https://doi.org/10.1385/JMN:11:1:67>.
- Luo, Y., Niu, F., Sun, Z., Cao, W., Zhang, X., Guan, D., Lv, Z., Zhang, B., Xu, Y., 2009. Altered expression of A beta metabolism-associated molecules from D-galactose/AlCl₃ induced mouse brain. *Mechanisms of Ageing and Development* 130, 248-252. <https://doi.org/10.1016/j.mad.2008.12.005>.
- Maisonpierre, P.C., Le Beau, M.M., Espinosa, R., Ip, N.Y., Belluscio, L., De la Monte, S.M., Squinto, S., Furth, M.E., Yancopoulos, G.D., 1991. Human and rat brain-derived

- neurotrophic factor and neurotrophin-3: Gene structures, distributions, and chromosomal localizations. *Genomics* 10, 558–568. [https://doi.org/10.1016/0888-7543\(91\)90436-i](https://doi.org/10.1016/0888-7543(91)90436-i).
- Malekzadeh, S., Edalatmanesh, M. A., Mehrabani, D., Shariati, M. 2017. Drugs Induced Alzheimer's Disease in Animal Model. *Galen Medical J.* 6, 185-196. <https://doi.org/10.31661/gmj.v6i3.820>.
- Malhotra, S., Sawhney, G., Pandhi, P., 2012. The Therapeutic Potential of Melatonin: A Review of the Science. *Med. Gen. Med.* 6, 1–10.
- Martorana, A., Esposito, Z., Koch, G., 2010. Beyond the cholinergic hypothesis: Do current drugs work in alzheimer's disease? *CNS Neurosci. Ther.* 16, 235–245. <https://doi.org/10.1111/j.1755-5949.2010.00175.x>.
- Masters, C.L., Bateman, R., Blennow, K., Rowe, C.C., Sperling, R.A., Cummings, J.L., 2015. Alzheimer's disease. *Nat. Rev. Dis. Prim.* 1, 1–18. [https://doi.org/10.1038/nrdp.\(2015\).56](https://doi.org/10.1038/nrdp.(2015).56).
- Mattson, M.P., 2004. Pathways towards and away from Alzheimer's disease. *Nature*, 430, 631-639. <https://doi.org/10.1038/nature02621>.
- Mattson, M.P., 2008. Glutamate and neurotrophic factors in neuronal plasticity and disease. *Ann. N.Y. Acad. Sci.* 1144: 97–112. <https://doi.org/10.1196/annals.1418.005>.
- Mauri, P.L., Iemoli, L., Gardana, C., Riso, P., Simonetti, P., Porrini, M., 1999. Liquid chromatography/electrospray ionization mass spectrometric characterization of flavonol glycosides in tomato extracts and human plasma. *Rapid Commun. Mass Spectrom.* 13, 924–931.
- McCartney, M., Margaret, M.C., 2015. The “breakthrough” drug that's not been shown to help in AD. *BMJ (Clinical Research Ed.)*. 351, h4064.
- McLachlan, D.R., Kruck, T.P., Lukiw, W.J., Krishnan, S.S., 1991. Would decreased aluminium ingestion reduce the incidence of Alzheimer's disease? *C.M.A.J.* 145, 793–804. <https://pubmed.ncbi.nlm.nih.gov/1822096/>.
- McShane, R., Areosa Sastre, A., Minakaran, N., 2006. Memantine for dementia. *Cochrane Database Syst. Rev.* 1.
- Mikkilineni, S., 2012. The anticholinesterase phenserine and its enantiomer posiphen as 5' untranslated-region-directed translation blockers of the Parkinson's alpha synuclein expression. *Parkinsons Dis* 5–8. [https://doi.org/10.1155/\(2012\)/142372](https://doi.org/10.1155/(2012)/142372).

- Miles, L.A., Crespi, G.A.N., Doughty, L., Parker, M.W., 2013. Bapineuzumab captures the N-terminus of the Alzheimer's disease amyloid-beta peptide in a helical conformation. *Sci. Rep.* 3, 1–6. <https://doi.org/10.1038/srep01302>.
- Minicozzi, V., Chiaraluce, R., Consalvi, V., Giordano, C., Narcisi, C., Punzi, P., Rossi, G.C., Morante, S., 2014. Computational and experimental studies on β -sheet breakers targeting A β 1-40 fibrils. *J. Biol. Chem.* 289, 11242–11252. <https://doi.org/10.1074/jbc.M113.537472>.
- Mitchell, M.J., King, M.R., 2014. NIH Public Access, 1–23. <https://doi.org/10.1088/1367-2630/15/1/015008>.
- Miyata, T., Takizawa, S., Van Ypersele de Strihou, C., 2010. Hypoxia. 1. Intracellular sensors for oxygen and oxidative stress: novel therapeutic targets. *Am. J. Physiol. Cell Physiol.* 300, 226–31. <https://doi.org/10.1152/ajpcell.00430.2010>.
- Momtazi-borojeni, A.A., Sadeghi-aliabadi, H., Rabbani, M., 2017. Cognitive enhancing of pineapple extract and juice in scopolamine- induced amnesia in mice. *Res. Pharm. Sci.* 12, 257–264. <https://doi.org/10.4103/1735-5362.207198>.
- Moreira, P.I., 2018. Sweet Mitochondria: A Shortcut to Alzheimer's Disease. *J Alzheimer's Dis.* 62, 1391–1401. <https://doi.org/10.3233/JAD-170931>.
- Morris, R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods* 11, 47–60. [https://doi.org/10.1016/0165-0270\(84\)90007-4](https://doi.org/10.1016/0165-0270(84)90007-4).
- Morris, R.G.M., Garrud, P., Rawlins, J.N.P., O-Keefe, J., 1982. Place navigation impaired in rats with hippocampal lesions. *Nature* 297, 681–683. <https://www.nature.com/articles/297681a0>.
- Mount, C., Downton, C., 2006. Alzheimer disease: Progress or profit? *Nat. Med.* 12, 780–784. <https://doi.org/10.1038/nm0706-780>.
- Munoz, D.G., Feldman, H., 2000. Causes of Alzheimer's disease. *Cmaj.* 162, 65–72. <https://doi.org/10.1108/09526860010336984>.
- MW, J., KL, S., Jann, M.W., Shirley, K.L., Small, G.W., 2002. Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. *Clin Pharmacokinet* 41: 719–739. [https://doi.org/10.2165/00003088-\(2002\)41100-00003](https://doi.org/10.2165/00003088-(2002)41100-00003).
- Naidu, R.N., Shankar, B., Dsouza, U., 2013. Effect of long term administration of aluminium chloride on oxidativestress and acetylcholinesterase activity in rat brains. *Int. J. Pharm. Bio. Sci.* 3, 616–22. <https://doi.org/10.1371/journal.pone.0227631>.

- Oakley, H., Cole, S. L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M., Disterhoft, J., Eldik, L.V., Berry, R., Vassar, R., 2006. Intraneuronal beta-Amyloid Aggregates, Neurodegeneration, and Neuron Loss in Transgenic Mice with Five Familial Alzheimer's Disease Mutations: Potential Factors in Amyloid Plaque Formation. *J. Neurosci.* 26, 10129–10140. <https://doi.org/10.1523/jneurosci.1202-06.2006>.
- Oberley, L.W., 1998. Inhibition of tumor cell growth by overexpression of manganese-containing superoxide dismutase. *Age (Omaha)*, 21, 95–97. <https://doi.org/10.1007/s11357-998-0014-8>.
- Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M. P., Golde, T.E., Kaye, R., Metherate, R., Mattson, M.P., Akbari, Y., LaFerla, F.M., 2003. Triple-Transgenic Model of Alzheimer's Disease with Plaques and Tangles. *Neuron*, 39, 409–421. [https://doi.org/10.1016/s0896-6273\(03\)00434-3](https://doi.org/10.1016/s0896-6273(03)00434-3).
- Ojha, R., Sahu, A.N., Muruganandam, A.V., Singh, G.K., Krishnamurthy, S., 2010. Asparagus racemosus enhances memory and protects against amnesia in rodent models. *Brain and Cognition* 74, 1–9. <https://doi.org/10.1016/j.bandc.2010.05.009>.
- Olson, R.E., Albright, C.F., 2008. Recent progress in the medicinal chemistry of gamma-secretase inhibitors. *Curr. Top Med. Chem.* 8, 17–33.
- Osman, C., Ilknur, O., Sadettin, T., Sedat, Y., Hakan, O., 2011. Antioxidative effects of curcumin, b-Myrcene and 1,8-cineole against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced oxidative stress in rats liver. *Toxicol. Ind. Health.* 27, 447–453. <https://doi.org/10.1177/0748233710388452>.
- Osman, C., M. N.O., Asli, C., 2014. Neuroprotective effects of b-Myrcene following ~~global cerebral ischemia/reperfusion~~-mediated oxidative and neuronal damage in a C57BL/J6 mouse. *Neurochem. Res.* 39, 1717–1723. <https://doi.org/10.1007/s11064-014-1365-4>.
- Pacher, P., Beckman, J. S., Liaudet, L., 2007. Nitric Oxide and Peroxynitrite in Health and Disease. *Physiological Reviews* 87, 315–424. <https://doi.org/10.1152/physrev.00029.2006>.
- Peng, X.M., Gao, L., Huo, S.X., Liu, X.M., Yan, M., 2015. The Mechanism of Memory Enhancement of Acteoside (Verbascoside) in the Senescent Mouse Model Induced by a Combination of d-galactose and AlCl₃. *Phytother. Res.* 29, 1137–1144. <https://doi.org/10.1002/ptr.5358>.

- Penttilä, M., Partanen, J.V., Soininen, H., Riekkinen, P.J., 1985. Quantitative analysis of occipital EEG in different stages of Alzheimer's disease. *Electroencephalogr. Clin. Neurophysiol.* 60, 1–6. [https://doi.org/10.1016/0013-4694\(85\)90942-3](https://doi.org/10.1016/0013-4694(85)90942-3).
- Pereira, E., Barros, L., Ferreira, I.C.F R., 2013. Chemical characterization of Ginkgo biloba L. and antioxidant properties of its extracts and dietary supplements. *Ind. Crops Prod.* 51, 244–248.
- Perl, D.P., 2010. Neuropathology of Alzheimer's disease. *Mt Sinai J. Med.* 77, 32–42. [https://doi.org/10.1002/msj.\(2015\)7](https://doi.org/10.1002/msj.(2015)7).
- Perry, E.E., Perry, R.H., 1982. The cholinergic system in AD. *Trends Neurosci.* 5, 261–262.
- Prakash, A., Kalra, J., Mani, V., Ramasamy, K., Majeed, A.A., 2015. Pharmacological approaches for Alzheimer's disease: neurotransmitter as drug targets. *Expert Review of Neurotherapeutics* 15, 53–71.
- Priller, C., Bauer, T., Mitteregger, G., Krebs, B., Kretschmar, H.A., Herms, J., 2006. Synapse Formation and Function Is Modulated by the Amyloid Precursor Protein. *J. Neurosci.* 26, 7212–7221. <https://doi.org/10.1523/JNEUROSCI.1450-06.2006>.
- Prince, M., Comas-Herrera, A., Knapp, M., Guerchet, M., Karagiannidou, M., 2016. World Alzheimer Report: Improving healthcare for people living with dementia. Coverage, Quality and costs now and in the future. *AD Intern.* 1–140.
- Qian, Z. M., Ke, Y., 2014. Huperzine A: Is it an effective disease-modifying drug for AD? *Front Aging Neurosci.* 6, 1–6.
- Querfurth, H.W., Laferla, F.M., 2010. Alzheimer's Disease. *New England J. Med.* 362, 329–344. <https://doi.org/10.1056/nejmra0909142>.
- Rafii, M.S., Walsh, S., Little, J.T., Behan, K., Reynolds, B., Ward, C., 2011. A phase II trial of huperzine A in mild to moderate Alzheimer disease. *Neurology.* 76, 1389–1394.
- Rafii, M.S., Aisen, P.S., 2015. Advances in Alzheimer's Disease Drug Development. *B.M.C. Med.* 13. <https://doi.org/10.1186/s12916-015-0297-4>.
- Rahman, K., 2007. Studies on free radicals, antioxidants, and co-factors. *Clin. Interv. Aging.* 2, 219–236. <https://pubmed.ncbi.nlm.nih.gov/18044138/>.
- Ramkumar, S., Raghunath, A., Raghunath, S., 2016. Statin therapy: Review of safety and potential side effects. *Acta. Cardio.l Sin.* 32, 631–639. [https://doi.org/10.6515/ACS\(2016\)0611A](https://doi.org/10.6515/ACS(2016)0611A).

- Ravi, S.K., Ramesh, B.N., Mundugaru, R., Vincent., B., 2018. Multiple pharmacological activities of *Caesalpinia crista* against aluminium-induced neurodegeneration in rats: Relevance for Alzheimer's disease. *Environ. Toxicol. Pharmacol.* 58, 202–211. [https://doi.org/10.1016/j.etap.\(2018\).01.008](https://doi.org/10.1016/j.etap.(2018).01.008).
- Ravi, S.M., Prabhu, B.M., Raju, T.R., Bindu, P.N., 2000. Long-term effects of postnatal aluminium exposure on acetylcholinesterase activity and biogenic amine neurotransmitters in rat brain. *Indian J. Physiol. Pharmacol.* 44, 473–488. <https://pubmed.ncbi.nlm.nih.gov/11214504/>.
- Ravichandran, S., Michelucci, A., Del Sol, A., 2018. Integrative computational network analysis reveals site-Specific mediators of inflammation in Alzheimer's disease. *Front. Physiol.* 9, 1-15. <https://doi.org/10.3389/fphys.2018.00154>.
- Ribé, E. M., Pérez, M., Puig, B., Gich, I., Lim, F., Cuadrado, M., Sesma, T., Catena, S., Sanchez, B., Nieto, M., Gomez-ramos, P., Moran, M.A., Cabodevilla, F., Samaranch, L., Ortiz, L., Perez, A., Ferrer, I., Avila, J., Gómez-Isla, T., 2005. Accelerated amyloid deposition, neurofibrillary degeneration and neuronal loss in double mutant APP/tau transgenic mice. *Neurobiol. Diseases.* 20, 814–822. <https://doi.org/10.1016/j.nbd.2005.05.027>.
- Richards, J. G., Higgins, G. A., Ouagazzal, A.-M., Ozmen, L., Kew, J. N. C., Bohrmann, B., Malherbe, P., Brockhaus, M., Loetscher, H., Czech, C., Huber, G., Bluethmann, H., Jacobsen, H., Kemp, J.A., 2003. PS2APP Transgenic Mice, Coexpressing hPS2mut and hAPPswe, Show Age-Related Cognitive Deficits Associated with Discrete Brain Amyloid Deposition and Inflammation. *J. Neurosci.* 23, 8989–9003. <https://doi.org/10.1523/jneurosci.23-26-08989.2003>.
- Risacher, S.L., Saykin, A.J., 2013. Neuroimaging and other biomarkers for Alzheimer's disease: the changing landscape of early detection. *Annu. Rev. Clin. Psychol.* 9, 621-648. <https://doi.org/10.1146/annurev-clinpsy-050212-185535>.
- Rizvi, S., Raza, S.T., Ahmed, F., Ahmad, A., Abbas, S., Mahdi, F., 2014. The Role of Vitamin E in Human Health and Some Diseases. *Sultan Qaboos Univ. Med. J.* 14, 157–165.
- Roskams, A.J., Connor, J.R., 1990. Aluminium access to the brain: a role for transferrin and its receptor. *Proceedings of the National Academy of Sciences* 87, 9024–9027. <https://doi.org/10.1073/pnas.87.22.9024>.
- Rufino, A.T., Ribeiro, M., Sousa, C., Judas, F., Salgueiro, L., Cavaleiro, C., Mendes, A.F., 2015. Evaluation of the anti-inflammatory, anti-catabolic and pro-anabolic

- effects of E-caryophyllene, Myrcene and limonene in a cell model of osteoarthritis. *Eur. J. Pharmacol.* 750, 141–150. [https://doi.org/10.1016/j.ejphar.\(2015\).01.018](https://doi.org/10.1016/j.ejphar.(2015).01.018).
- Russell, R.W., 2012. Previously Published Works UC Irvine, Oceanography. [https://doi.org/10.1016/j.cognition.\(2008\).05.007](https://doi.org/10.1016/j.cognition.(2008).05.007).
- Salloway, S., Sperling, R., Fox, N.C., Blennow, K., Klunk, W., Raskind, M., 2014. Two Phase III Trials of Bapineuzumab in Mild-to-Moderate AD. *N Engl. J. Med.* 370, 322–333.
- Sancesario, G.M., Nuccetelli, M., Cerri, A., Zegeer, J., Severini, C., Ciotti, M.T., Pieri, M., Martorana, A., Caltagirone, C., Nistico, R., Bernardini, S., 2018. Bromelain degrades A β 1-42 monomers and soluble aggregates: An In Vitro study in cerebrospinal fluid of Alzheimer's disease patients. *Current Alzheimer Research* 15, 628-636.<https://doi.org/10.2174/1567205015666180123124851>.
- Sarkar, P., Zaja, I., Bienengraeber, M., Rarick, K.R., Terashvili, M., Canfield, S., Falck, J.R., Harder, D.R., 2014. Epoxyeicosatrienoic acids pretreatment improves amyloid beta induced mitochondrial dysfunction in cultured rat hippocampal astrocytes. *Am. J. Physiol. Heart Circ. Physiol.* 306, 475-484. <https://doi.org/10.1152/ajpheart.00001.2013>.
- Schinder, A.F., Poo, M., 2000. The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci.* 23, 639–45. [https://doi.org/10.1016/s0166-2236\(00\)01672-6](https://doi.org/10.1016/s0166-2236(00)01672-6).
- Schindowski, K., Belarbi, K., Buee, L., 2008. Neurotrophic factors in Alzheimer's disease: role of axonal transport. *Genes Brain Behav.* 1, 43–56. <https://doi.org/10.1111/j.1601-183X.2007.00378.x>.
- Schneider, L., 2013. Alzheimer disease pharmacologic treatment and treatment research. *Continuum Lifelong Learning in Neurology.* 19, 339–357.
- Schu, M.C., Sherva, R., Farrer, L.A., Green, R.C., 2012. The genetics of alzheimer's disease. *Alzheimer's Dis. Mod. Concept Biol. Diagnosis Ther.* 28, 15–29. <https://doi.org/10.1159/000335376>.
- Seubert, P., Oltersdorf, T., Lee, M.G., Barbour, R., Blomquist, C., Davis, D.L., Bryant, K., Fritz, L.C., Galasko, D., Thal, L.J., Lieberburg, I., Schenk, D.B., 1993. Secretion of b-amyloid precursor protein cleaved at the amino-terminus of the b-amyloid peptide. *Nature* 361, 260-263.<https://doi.org/10.1038/361260a0>.
- Shati, A.A., Elsaid, F.G., Hafez, E.E., 2011. Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of Crocus

- sativus L. extraction and honey syrup. *Neuroscience* 175, 66–74. <https://doi.org/10.1016/j.neuroscience.2010.11.043>.
- Shoshan-Barmatz, V., Nahon-Crystal, E., Shteinifer-Kuzmine, A., Gupta, R., 2018. VDAC1, Mitochondrial Dysfunction, and Alzheimer's disease. *Pharmacol. Res.* [https://doi.org/10.1016/j.phrs.\(2018\).03.010](https://doi.org/10.1016/j.phrs.(2018).03.010).
- Shoshan-Barmatz, V., Nahon-Crystal, E., Shteinifer-Kuzmine, A., Gupta, R., 2018. VDAC1, Mitochondrial Dysfunction, and AD. *Pharmacol. Res.* 1- 45.
- Skovronsky, D.M., Moore, D.B., Milla, M.E., Doms, R.W., Lee, V.M., 2000. Protein kinase C-dependent alpha-secretase competes with betasecretase for cleavage of amyloid-beta precursor protein in the trans-golginetwork. *J. Biol. Chem.* 275, 2568-2575. <https://doi.org/10.1074/jbc.275.4.2568>.
- Song, X., Bao, M., Li, D., Li, Y.M., 1999. Advanced glycation in D - galactose induced mouse aging model. *Mech. Ageing Dev.* 108, 239–251. [https://doi.org/10.1016/s0047-6374\(99\)00022-6](https://doi.org/10.1016/s0047-6374(99)00022-6).
- Sossin, W.S., Barker, P.A., 2007. Something old, something new: BDNF-induced neuron survival requires TRPC channel function. *Nat. Neurosci.* 10, 537–8. <https://doi.org/10.1038/nn0507-537>.
- Sun, Z.Z., Chen, Z.B., Jiang, H., Li, L.L., Li, E.G., Xu, Y., 2009. Alteration of A β metabolism-related molecules in predementia induced by AlCl₃ and d-galactose. *AGE* 31, 277–284. <https://doi.org/10.1007/s11357-009-9099-y>.
- Tabgled Bank Studios, 2016. A Journalist ' S Guide To Alzheimer ' s disease and drug development. 1- 20.
- Tagami, S., Attwater, J., Holliger, P., 2017. Simple peptides derived from the ribosomal core potentiate RNA polymerase ribozyme function. *Nat. Chem.* 9, 325–332.
- Taylor, M.J., McNicholas, C., Nicolay, C., Darzi, A., Bell, D., Reed, J.E., 2014. Systematic review of the application of the plan-do-study-act method to improve quality in healthcare. *B.M.J. Qual Saf.* 23, 290–298.
- Thenmozhi, A.J., Raja, T.R.W., Janakiraman, U., Manivasagam, T., 2015. Neuroprotective Effect of Hesperidin on Aluminium Chloride Induced Alzheimer's Disease in Wistar Rats. *Neurochem. Res.* 40, 767–776. <https://doi.org/10.1007/s11064-015-1525-1>.
- Theuns, J., Van Broeckhoven, C., 2000. Transcriptional regulation of Alzheimer's disease genes: implications for susceptibility. *Hum. Mol. Genet.* 9, 2383–2394. <https://doi.org/10.1093/hmg/9.16.2383>.

- Tian, J., Ishibashi, K., Ishibashi, K., Ishibashi, K., Reiser, K., grebe, R., Biswal, S., gehlbach, P., Handa, J.T., 2005. Advanced glycation end product-induced aging of the retinal pigment epithelium and choroid: a comprehensive transcriptional response. *Proc. Natl. Acad. Sci. U. S. A.* 102, 11846–11851.
- Tougu, V., Karafin, A., Zovo, K., Chung, R.S., Howells, C., West, A.K., Palumaa, P., 2009. Zn(II)- and Cu(II)-induced non-fibrillar aggregates of amyloid- β (1-42) peptide are transformed to amyloid fibrils, both spontaneously and under the influence of metal chelators. *J. Neurochem.* 110, 1784–1795. [https://doi.org/10.1111/j.1471-4159.\(2009\).06269.x](https://doi.org/10.1111/j.1471-4159.(2009).06269.x).
- Uddin, M.S., Amran, M.S., 2018 *Handbook of research on critical examinations of neurodegenerative disorders*, 1st ed.; USA: IGI Global, 00.
- Ulbricht, C., Chao, W., Costa, D., Rusie-Seamon, E., Weissner, W., Woods, J., 2008. Clinical Evidence of Herb-Drug Interactions: A Systematic Review by the Natural Standard Research Collaboration. *Curr. Drug Metab.* 9, 1063–1120.
- Upton, N., Chuang, T.T., Hunter, A.J., Virley, D.J., 2008. 5-HT₆ Receptor Antagonists as Novel Cognitive Enhancing Agents for AD. *Neurotherapeutics.* 5, 458–469.
- Uyeda, S., Sharmin, T., Satho, T., Irie, K., Watanabe, M., Hosokawa, M., Hiramatsu, Y., Koga, T., Nakashima, Y., Kashige, N., Toda, A., Mishima, K., Miake, F., 2016. Enhancement and regulation effect of Myrcene on antibody response in immunization with ovalbumin and Ag85B in mice. *Asian Pac. J. Allergy Immunol.* 34, 314–323. <https://doi.org/10.12932/AP0734>.
- Vassar, R., 2004. **BACE1: The β -Secretase Enzyme in Alzheimer's Disease.** *J. Mol. Neurosci.* 23, 105–114. <https://doi.org/10.1385/JMN:23:1-2:105>.
- Vassar, R., Bennett, B.D., Babu-Khan, S., Kahn, S., Mendiaz, E.A., Denis, P., Teplow, D.B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M.A., Biere, A.L., Curran, E., Burgess, T., Louis, J.C., Collins, F., Treanor, J., Rogers, G., Citron, M., 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Sci.* 286, 735–741. <https://doi.org/10.1126/science.286.5440.735>.
- Verma, N., Meena, N.K., Majumdar, I., Paul, J., 2017. Role of Bromelain as Herbal Anti-Inflammatory Compound Using In Vitro and In Vivo Model of Colitis. *iMedPub Journals* 1–8.
- Vetrivel, K.S., Thinakaran, G., 2006. Amyloidogenic processing of β -amyloid precursor protein in intracellular compartments. *Neurology.* 66, S69–S73.

- Vlassara, H., Bucala, R., Striker, L., 1994. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. *Lab. Invest.* 70, 138–151. <https://pubmed.ncbi.nlm.nih.gov/8139257/>.
- Wang, R., Yan, H., Tang, X.C., 2006. Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine. *Acta. Pharmacol. Sin.* 27, 1–26. <https://doi.org/10.1111/j.1745-7254.2006.00255.x>.
- Wang, Y., Xia, J., Shen, M., Zhou, Y., Wu, Z., Shi, Y., Xu, J., Hou, I., Zhang, R., Qui, Z., Xie, Q., Chen, H., Zhang, Y., Wang, H., 2019. Effects of BIS-MEP on reversing amyloid plaque deposition and spatial learning and memory impairments in a mouse model of β -amyloid peptide- and ibotenic acid-induced Alzheimer's disease. *Front. Aging Neurosci.* 11, 1-11. <https://doi.org/10.3389/fnagi.2019.00003>.
- Wei, H., Li, L., Song, Q., Ai, h., Chu, J., Li, W., 2005. Behavioral study of the D -galactose induced aging model in C57BL/6J mice. *Behav. Brain Res.* 157, 245–251. <https://doi.org/10.1016/j.bbr.2004.07.003>.
- Wei, Y., Liu, D., Zheng, Y., Li, H., Hao, C., Ouyang, W., 2017. Protective effects of kinetin against aluminum chloride and D -galactose induced cognitive impairment and oxidative damage in the mouse. *Brain Research Bulletin*, 134, 262–272. <https://doi.org/10.1016/j.brainresbull.2017.08.014>.
- Weinreb, O., Amit, T., Bar-Am, O., Youdim, B.H., Ladostigil, M., 2012. A Novel Multimodal Neuroprotective Drug with Cholinesterase and Brain-Selective Monoamine Oxidase Inhibitory Activities for Alzheimers Disease Treatment. *Curr. Drug Targets.* 13, 483–494.
- Weinstock, M., Bejar, C., Wang, R.H., Poltyrev, T., Gross, A., Finberg, J.P., 2000. TV3326, a novel neuroprotective drug with cholinesterase and monoamine oxidase inhibitory activities for the treatment of AD. *J Neural Transm. Suppl.* 60, 157–169.
- Wenk, G.L., 2003. Neuropathologic changes in Alzheimer's disease. *The Journal of Clinical Psychiatry* 64, 7–10. <https://pubmed.ncbi.nlm.nih.gov/12934968/>.
- Whitehouse, P., Price, D., Struble, R., Clark, A., Coyle, J., Delon, M., 1982. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Sci.* 215, 1237–1239. <https://doi.org/10.1126/science.7058341>.
- W.H.O., 2002. Active Ageing: A Policy Framework. *Aging Male* 5: 1–37. <https://doi.org/10.1080/713604647>.
- Wills, E.D., 1966. Mechanism of lipid peroxide formation in animals. *Biochem. J.* 99, 667–676. <https://doi.org/10.1042/bj0990667>.

- Winblad, B., Grossberg, G., Frolich, L., Farlow, M., Zechner, S., Nagel, J., 2007. IDEAL: a 6-month, double-blind, placebo-controlled study of the first skin patch for Alzheimer disease. *Neurology*. 69, S14 - 22.
- Woodhouse, A., Dickson, T.C., Vickers, J.C., 2007. Vaccination strategies for AD: A new hope? *Drugs & Aging*. 24, 107–119.
- World Health Organization, 2012. Dementia: a public health priority. *Dementia*. 112.
- World Health Organization, 2016. World Health Statistics Monitoring Health For theSDGs. WHO. 2016, 1, 121.
- Xian, Y.F., Lin, Z.X., Zhao, M., Mao, Q.Q., Ip, S.P., Che, C.T., 2011. Uncariarhynchophylla ameliorates cognitive deficits induced by Dgalactose in mice. *Planta. Med.* 77, 1977–1983. <https://doi.org/10.1055/s-0031-1280125>.
- Xing, Z., He, Z., Wang, S., Yan, Y., Zhu, H., Gao, Y., Zhao, Y., Zhang, L., 2018. Ameliorative effects and possible molecular mechanisms of action of fibrauretin from *Fibraurearecisa* Pierre on d-galactose/ AlCl_3 -mediated Alzheimer's disease. *RSC Advances* 8, 31646–31657. <https://doi.org/10.1039/C8RA05356A>.
- Xiong, J., Wang, C., Chen, H., Hu, Y., Tian, L., Pan, J., Geng, M., 2014. Abeta-induced microglial cell activation is inhibited by baicalin through the JAK2/STAT3 signaling pathway. *Int. J. Neurosci.* 124, 609–620.
- Yadav, J., 2018. Genetic and Environmental Risk Factors of Alzheimer's Disease. *J. Aging Sci.* 06, 1–3. <https://doi.org/10.4172/2329-8847.1000187>.
- Yamada, K., Nabeshima, T., 2003. Brain-Derived Neurotrophic Factor/TrkB Signaling in Memory Processes. *Journal of Pharmacological Sciences* 91, 267–270. [https://doi.org/10.1254/S1347-8613\(19\)32708-2](https://doi.org/10.1254/S1347-8613(19)32708-2).
- Yang, Y., Mufson, E.J., Herrup, K., 2003. Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease. *J. Neurosci.* 23, 2557–63. <https://doi.org/10.1523/JNEUROSCI.23-07-02557.2003>.
- Yap, S.P., Yuen, K.H., Wong, J.W., 2001. Pharmacokinetics and bioavailability of α -, γ - and δ -tocotrienols under different food status. *J. Pharm. Pharmacol.* 53, 67–71.
- Ye, C.Y., Lei, Y., Tang, X.C., Zhang, H.Y., 2015. Donepezil attenuates $\text{A}\beta$ -associated mitochondrial dysfunction and reduces mitochondrial $\text{A}\beta$ accumulation in vivo and in vitro. *Neuropharma.* 95, 29–36. <https://doi.org/10.1016/j.neuropharm.2015.02.020>.
- Yi, C., Li, R., Wolbeck, J., Xu, X., Nilsson, M., Aires, L., 2010. Climate control of terrestrial carbon exchange across biomes and continents. *Environ. Res. Lett.* 5, 10pp.

- Yu, T.S., Kim, A., Kernie, S.G., 2015. Donepezil rescues spatial learning and memory deficits following traumatic brain injury independent of its effects on neurogenesis. *PloS One* 10, e0118793. <https://doi.org/10.1371/journal.pone.0118793>.
- Zatta, P., Ibn Lkhayat Idrissi, M., Zambenedetti, P., Kilyen, M., Kiss, T., 2002. In vivo and In vitro effects of aluminium on the activity of mouse brain acetylcholinesterase. *Brain Res. Bull.* 59, 41–5. [https://doi.org/10.1016/s0361-9230\(02\)00836-5](https://doi.org/10.1016/s0361-9230(02)00836-5).
- Zeiger, E., 1997. β -Myrcene, review of toxicological literature. National Institute of Environmental Health Sciences, Integrat. Lab. Syst. 01-97.
- Zhang, J., Li, P., Wang, Y., Liu, J., Zhang, Z., Cheng, W., Wang, Y., 2013. Ameliorative Effects of a Combination of Baicalin, Jasminoidin and Cholic Acid on Ibotenic Acid-Induced Dementia Model in Rats. *PLoS One*, 8, 1–12. <https://doi.org/10.1371/journal.pone.0056658>.
- Zhang, H.Y., Zheng, C.Y., Yan, H., Wang, Z.F., Tang, L.L., Gao, X., 2008. Potential therapeutic targets of huperzine A for AD and vascular dementia. *Chem.-Biol. Interact.* 175, 396–402.
- Zhang, X., Zhang, A., Jiang, B., Bao, Y., Wang, J., An, L., 2008. Further pharmacological evidence of the neuroprotective effect of catalpol from *Rehmanniaglutinosa*. *Phytomedicine* 15, 484–490. <https://doi.org/10.1016/j.phymed.2008.01.001>.
- Zhao, J., Paganini, L., Mucke, L., Gordon, M., Refolo, L., Carman, M., Sinha, S., Oltersdorf, T., Lieberburg, I., McConlogue, L., 1996. β -Secretase processing of the β -amyloid precursor protein in transgenic mice is efficient in neurons but inefficient in astrocytes. *J. Biol. Chem.* 271, 31407–31411. <https://doi.org/10.1074/jbc.271.49.31407>.
- Zhi, H., Yun, L., Min, Z., Fan, Z., Lian, G., 2008. Piracetam improves cognitive deficits caused by chronic cerebral hypoperfusion in rats. *Cellular and Molecular Neurobiology* 28, 613–627. <https://doi.org/10.1007/s105>.
- Zilka, N., Filipcik, P., Koson, P., Fialova, L., Skrabana, R., Zilkova, M., Rolkova, G., Kontsekkova, E., Novak, M., 2006. Truncated tau from sporadic Alzheimer's disease suffices to drive neurofibrillary degeneration in vivo. *FEBS Lett.* 580, 3582–3588. <https://doi.org/10.1016/j.fe>.
- Zusso, M., Barbierato, M., Facci, L., Skaper, S.D., Giusti, P., 2018. Neuroepigenetics and Alzheimer's disease: An update. *J Alzheimer's Dis.* 64, 671–688. <https://doi.org/10.3233/JAD-180259>.



Provisional Academic Transcript

Student Name : Rakesh Kumar

Programme : PSD0-NN2 :: Doctor of Philosophy
(Pharmacology)

Prov. Regd. No. : 11719625

Father's Name : Mr. Charan Singh

Mother's Name : Ms. Mohshri Devi

Mode : Regular

Date of initial registration : August, 2017

Term : I		TGPA : 8.44	Equivalent percentage : 84.40 %	
S.No.	Course	Credit	Grade	
1	GEN703 :: RESEARCH METHODOLOGY	4.00	A+	
2	GEN730 :: DATA ANALYSIS AND REPORTING	2.00	A	
3	PHR782 :: MODERN BIOANALYTICAL TECHNIQUES	3.00	A	

This is a provisional transcript and is valid till a regular transcript is issued

Student yet not completed the programme.

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Associate Dean

Division of Examination

Lovely Professional University

Phagwara (Punjab)

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LPU/CRDP/EC/210918/001

Dated: Tuesday, March 12, 2019

Rakesh Kumar
Registration Number: 11719625
Program Name: Ph.D. - Pharmacology (Full Time)

Subject: Letter of Candidacy for Ph.D.

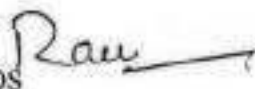
Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on Friday, September 21, 2018 by accepting your research proposal entitled: "Pharmacological Evaluation of Selected Phytoconstituents for Alzheimer's disease using Animal Model" under the supervision of Dr. Navneet Khurana.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your programme, please contact Centre of Research Degree Programmes.


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Registration Number -954/PO/ReRcBiBu/S/06/CPCSEA

CERTIFICATE

This is to certify that the project titled "*Pharmacological Evaluation of Selected Phytoconstituents for Alzheimer's disease using Animal Model*" [Thesis title: *Pharmacological Evaluation of Phytoconstituents (Bromelain and Myrcene) for Alzheimer's disease using Animal Model*] has been approved by the IAEC.

Name of Principal Investigator: Dr. Navneet Khurana

IAEC approval number: LPU/IAEC/2019/51

Date of Approval: 19th April 2019

Animals approved: Swiss albino female mice 66 in no. and SD Rats either sex, 12 in no

Remarks if any: - NA


Dr. Monica Gulati

Biological Scientist,
Chairperson IAEC


Dr. Navneet Khurana

Scientist from different discipline


Dr. Bimlesh Kumar

Scientist In-Charge of Animal House,
Member Secretary IAEC

Annexure IV



Annexure V

DIVISION OF STUDENT WELFARE

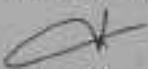
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Certificate No. 160026

Certificate of Merit

This is to certify that Mr./Ms. Rakesh Kumar S/O, D/O, W/O Mr. Charan Singh
student of LIT (Pharmacy), Department of Pharmaceutical Sciences
Registration No. 11719625 pursuing Ph.D. (Pharmacology) [Full Time]
stood Second in the event Graduation Project Expo 2019
held from 26-04-2019 to 27-04-2019 organized by Division of Student welfare
Lovely Professional University (Punjab).

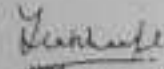
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Division of Student Welfare

Annexure VI



Annexure VII

 LOVELY PROFESSIONAL UNIVERSITY <i>Transforming Education Transforming India</i> Jalandhar-Delhi G.T. Road, Phagwara (Punjab)		Certificate No. 140648
Certificate of Recognition		
Presented to Mr. / Ms. <u>Rakesh Kumar</u> S/o, D/o, W/o <u>Mr. Charan Singh</u>		
student of <u>LJT (Pharmacy), Department of Pharmaceutical Sciences, Lovely Professional University</u> Registration No. <u>11719625</u>		
pursuing <u>Ph.D. (Pharmacology) [Full Time]</u> in recognition of his / her		
contribution as Student Volunteer of <u>Medical Science (including Physiology) Conference</u>		
Committee in organizing the event 106th Indian Science Congress on theme "Future India : Science & Technology"		
held from 3rd to 7th January, 2019 organized by Lovely Professional University, Punjab.		
Date of Issue : 12-04-2019 Place : Phagwara (Punjab)		
 Prepared By (Administrative Officer-Records)	 Member Local Organizing Committee	 Local Secretary 106th Indian Science Congress



Annexure IX



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Pharmacological evaluation of bromelain in mouse model of Alzheimer's disease

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ARTICLE INFO

Edited by Chan-Jang Chou

Keywords

Bromelain
Donepezil
Aluminum trichloride
D - galactose
Oxidative stress
Alzheimer's disease

ABSTRACT

The current study elucidates pharmacological evaluation of bromelain as a bioactive compound obtain from pineapple stem belongs to family Bromeliaceae in $AlCl_3$ and D - galactose induced mice. In mice, co-administration of $AlCl_3$ at dose 5 mg/kg b.w., via the oral route, and D - galactose at dose 60 mg/kg b.w., via intraperitoneal route for 90 days resulted in cognitive impairment, spatial learning, and memory deficits, as well as neurotoxicity. However, 30 consecutive days, treatments via an intraperitoneal route with bromelain low dose (Brm L) at dose 10 mg/kg b.w., bromelain high dose (Brm H) at dose 20 mg/kg b.w., donepezil (Dnpt) at dose 2 mg/kg b.w., and Brm L + Dnpt at doses 10, 2 mg/kg b.w. were considerably reversed the effect of $AlCl_3$ and D - galactose induced AD mice. Consequences of behavioral parameters (Morris water maze, elevated plus maze and locomotor), biochemical estimation (MDA, GSH, SOD, CAT, Nitrite and AChE), and ELISA tests (mouse BACE, $A\beta_{1-42}$, TNF- α , IL-6, and BDNF) confirmed significant ($p < 0.05$) neuroprotective effect of treatments in $AlCl_3$ and D - galactose induced mice. Additionally, hematoxylin and eosin staining of the cerebral cortex and the hippocampus exposed eosinophilic lesions and hyperchromatic nuclei in AD mice, but these neurodegenerative effects were eliminated by Brm L, Brm H, Dnpt, and Brm L + Dnpt treatments. Thus, bromelain alone and in combination with donepezil prevent $AlCl_3$ and D - galactose induced spatial learning and memory deficits, as well as cognitive impairment, by increasing cholinergic activity and synaptic plasticity, as well as reducing oxidative damage, neuroinflammation, $A\beta_{1-42}$ aggregations, and histopathological damage, according to our findings. The present study consequences indicate that bromelain alone and in combination with donepezil appears to have neuroprotective properties. Henceforward, this may be a promising treatment option for Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is an insidious and progressive neurodegenerative disease (Wenk, 2003; Jorna, 2009). AD is characterized by three major hallmarks such as synapse loss (Priller et al., 2006; Lacer et al., 2007), amyloid-beta ($A\beta$) accumulation extracellularly, and neurofibrillary tangles (NFTs) intracellularly (Blascher and Saitkin, 2015; Bloom, 2014). $A\beta_{1-42}$ or $A\beta_{40-42}$ peptide monomer is derived from proteolytic cleavage of amyloid precursor protein in presence of the enzyme β -secretase along with γ -secretase (DeGosky, 2001; Hampel, 2012). AD is an age-related inflammatory disease (Candore et al., 2006). Potentiate of free radical initiated oxidative stress enhances with age, attenuates endogenous anti-oxidant enzymes (Rahman et al., 2007), and influences the pathophysiology of AD as a result, deposition of

extracellular $A\beta$ and intracellular NFTs (Huang et al., 2016). The aggregation of $A\beta$ has been shown to activate microglia and increase the development of tumor necrosis factor- α (TNF- α) as well as interleukin-6 (IL-6) (Xiong et al., 2014).

Aluminum (Al) is a neurotoxic agent (Shari et al., 2011) as well as the third most basic rich element on the earth (Farina et al., 2002). The regular consumption of Al from various items used in our daily life like food additives, deodorants, and a few antacid medicines enhanced the Al concentration in the brain. Al enters the brain by binding with high-affinity receptors for transferrin (TfR) in the blood-brain barrier (BBB) (Rokkum et al., 1990). In the brain, Al affects fast and slow axonal transport and induces inflammatory responses (Amjad et al., 2015; Campbell et al., 2004). Long-term potentiation is inhibited by Al, resulting in synaptic and structural abnormalities. The result was

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Ameliorative effect of myrcene in mouse model of Alzheimer's disease

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ARTICLE INFO

Keywords:

Aluminium trichloride
D - galactose
Myrcene
Donepezil
Alzheimer's disease
Oxidative stress

ABSTRACT

Myrcene (Myr) has been reported to show neuroprotective effects in cerebral ischemia. In this research work, we investigated the Myr effect on neurobehavioral, and neuropathological alteration in mice induced by Aluminium trichloride ($AlCl_3$) and D - galactose. The administration of $AlCl_3$ (5 mg/kg, p. o.), and D - galactose (60 mg/kg, i. p.) for 90 days in mice resulted in spatial learning and memory deficits, cognitive decline, as well as neurotoxicity. The treatments with Myr low dose (100 mg/kg), Myr high dose (200 mg/kg), donepezil (2 mg/kg), and Myr low dose + donepezil (100 + 2 mg/kg) were administered via intraperitoneal route for 30 days significantly reversed the neurobehavioral, and neuropathological effects of $AlCl_3$ and D - galactose in mice. The results of behavioural tests such as Morris water maze, elevated plus maze, and locomotor; biochemical analysis such as malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), nitrite, and acetylcholinesterase (AChE); and ELISA tests such as mouse β - secretase (BACE), amyloid-beta peptide₁₋₄₂ ($A\beta_{1-42}$), tumor necrosis factor - α (TNF- α), interleukin - 6 (IL-6), and brain-derived neurotrophic factor (BDNF) demonstrated a significant ($p < 0.05$) neuroprotective effect of the Myr and donepezil co-treatments. In addition, hematoxylin and eosin staining of the cerebral cortex and hippocampus revealed eosinophilic lesions and hyperchromatic nuclei in Alzheimer's disease mice, but treatments with Myr low dose, Myr high dose, donepezil, and Myr low dose + donepezil reversed these neurodegenerative effects. Myr showed these activities by enhancing synaptic plasticity and cholinergic activity, as well as reducing oxidative damage, neuroinflammation, $A\beta_{1-42}$ aggregations, and histopathological damage. Myr alone and in combination with donepezil may serve as a potential candidate for the treatment of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is the most economically exhausting in society (Cavallaro and Battore, 1997). AD is an age-related neuronal degenerative disease characterized by the occurrence of three major pathological hallmarks such as loss of synapses, extracellular accumulation of amyloid-beta ($A\beta$) peptide, and intracellular neurofibrillary tangles (NFTs) (Cardenas et al., 2012). However, $A\beta_{1-42}$ can add as oligomers into the lipid layer and commence lipid peroxidation. 40–42 amino acid peptides are derived from proteolytic cleavage of amyloid precursor protein by action of β -secretases and γ -secretases (Hampel, 2012). The aggregate of $A\beta$ has been reported to activate microglia and enhanced the proinflammatory cytokines such as interleukin -1 β , interleukin-6 as well as tumor necrosis factor- α production (Xiong et al., 2014). Neurotransmitters such as acetylcholine, serotonin, and glutamate deficits occur at the early stage of AD while γ -aminobutyric acid (GABA) show to be distressed at a later stage of AD (Limón et al., 2012;

Prakash et al., 2015). Moreover, in AD pathology, fluctuations of excitatory and inhibitory neurotransmitters were reported whereas these neurotransmitters play an important role in learning, memory, and cognition (Greenamyre and Young, 1999; Chen et al., 2011). Therefore, Donepezil is marketed as a standard drug for the symptomatic treatment of AD and acts by inhibiting the AChE enzyme in the brain (Birks and Harvey, 2018; Hampel et al., 2018).

Co-administration of $AlCl_3$ and D - galactose has been reported for studying AD mechanism and screening molecules for various years (Wei et al., 2005). However, long-time co-administration of $AlCl_3$ and D - galactose has been found to cause AD-like pathological alteration in mice. AD pathological changes were mediated through several mechanisms such as oxidative stress (Cui et al., 2006); Advanced glycation end-product formation (Tian et al., 2005); gene transcriptional changes (Li et al., 1995); accumulation of $A\beta$ peptides (Banks et al., 2006); biochemical in addition to pathological changes of astrocytes (Hua et al., 2006). Consequently, the co-administration of $AlCl_3$ and D - galactose

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Potential of Prediction of Activity Spectra of Substances Software to Justify 3Rs Ethics for *In Vivo* Anti-Alzheimer's Studies of Phytochemicals

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Gopal Lal Khatik¹, Navneet Duggal¹, Meenu Mehta¹, Saurabh Satija¹,
Neha Sharma¹, Navneet Khurana¹

¹Department of Pharmacology, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India. ²Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA

Abstract

Context: Alzheimer's disease (AD) is a progressive neurodegenerative disorder having important concern in today's society. The prediction of activity spectra of substances (PASS) software allows prediction of the pharmacological activity on behalf of canonical simplified molecular-input line-entry system (SMILES) of the substance. PASS predicted the activity of substances in two manners, i.e. probable activity (Pa) and probable inactivity. **Objective:** In this study, we elaborated the benefit of PASS to define 3Rs ethics (replacement, reduction, and refinement) in animal study for anti-Alzheimer's activities of phytochemicals. **Materials and Methods:** Pa values of different phytochemicals important for AD predicted using PASS. For the prediction of activity in the different targets of AD, canonical SMILES of phytochemicals was obtained from PubChem website. **Results and Discussion:** Based on these Pa values predicted for different target sites of AD, screening of pharmacologically important phytochemicals was performed so that those phytochemicals can be further explored for *in vivo* pharmacological activity against AD to justify the 3Rs ethics. **Conclusion:** PASS online software provides an informative data to support the reported activities of the phytochemicals.

Key words: 3Rs ethics, Alzheimer's disease, phytochemicals, prediction of activity spectra of substances software

INTRODUCTION

More than 100 years ago, the Alzheimer's disease (AD) was first identified, but 70 years passed before, it was recognized as the most common cause of dementia and a "major killer." Although research has revealed a great deal about AD, much is yet to be discovered about the precise biologic changes that cause this disease and why it progresses more quickly in some than in others, and how the disease can be prevented, slowed, or stopped. Researchers believe that early detection will be key to prevent, slowing and stopping AD. Under the proposed criteria, the disease begins before symptoms such as memory loss appear, while earlier criteria require memory loss and a decline in thinking abilities for an AD diagnosis to be made. Due to the proposed criteria of the scientific evaluation are

ongoing, in this report, AD refers to the disease as defined by the earlier criteria.^[1]

AD is a neurodegenerative disorder that generally appears in late adulthood. It is associated with a progressive and rather irreversible decline in memory various other cognitive capabilities. In AD, there is neuronal destruction and deterioration of neural connections in the cerebral cortex region of the brain along with a substantial loss of brain mass. In some rare cases, it appears in people in their 40s and 50s, but otherwise, it is a disease of old age. Based on

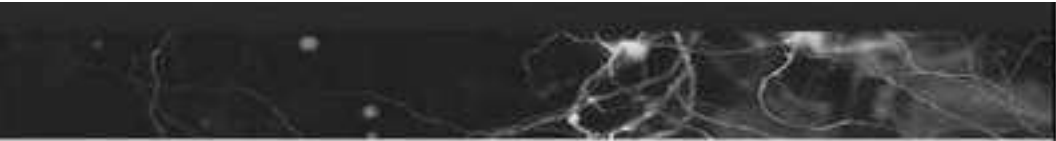
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Received: 21-02-2018

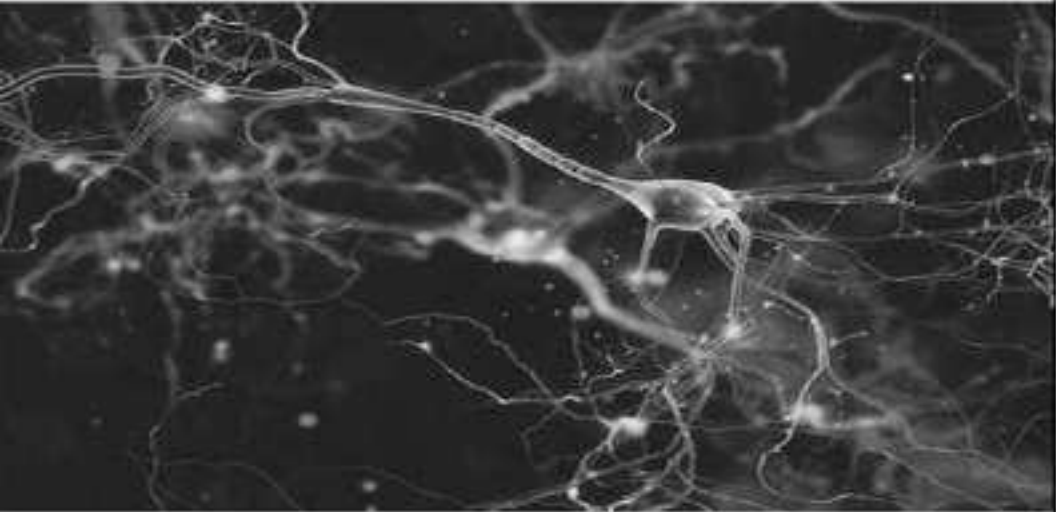
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CHAPTER 5

Pharmacological Management of Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder, which is characterized by severe progressive loss of neurons in the central nervous system. AD is the basic type of dementia among the geriatrics that slowly decreases memory and alters the thought process, reduces understanding the simple tasks of the daily routine, and with passing time, it becomes more problematic. A neuronal destruction occurs in the AD with less number of viable neuron cells. As it is progressive in nature, it ultimately leads to

5.1 INTRODUCTION

The word dementia, obtained from the Latin word "de" and "mente" means "out (de) of one's mind (mente)." Dementia is a common term which is characterized by deterioration of cognitive function, involving loss of memory, impairment of language and disorientation as well as a degree of mental ability in the patient to interfere daily life (Boller and Forbes, 1998). Alzheimer's disease (AD) is the general type of dementia. AD accounts about 80% cases that are more than other all type of dementia such



Improved neuroprotective activity of Fisetin through SNEDDS in ameliorating the behavioral alterations produced in rotenone-induced Parkinson's model

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Abstract

Fisetin is a polyphenolic flavonoid reported to have antioxidant, anti-inflammatory, and anti-cancer activities. However, it loses its importance as an effective phytochemical due to its poor water solubility and lower bioavailability. In the present study, the self-emulsifying drug delivery system (SNEDDS) of fisetin was developed in order to improve its pharmacological activity. The developed SNEDDS of fisetin was evaluated for improving the rotenone-induced behavioral changes in the rats, and its efficacy was compared with native fisetin. It was noticed that fisetin loaded in the SNEDDS formulation significantly ($p < 0.001$) ameliorated the rotenone-induced alteration in the body weight, grip strength, beam walk, postural instability, etc., in rats when compared to the effect of native fisetin. Native fisetin significantly ($p < 0.05$) ameliorated the effect of rotenone on the level of dopamine only at a higher dose. Whereas, SNEDDS of fisetin produced a significant ($p < 0.05$) effect at both dose levels when compared with the diseased group as well as also produced a significant ($p < 0.05$) effect when compared with the native fisetin group. The results of histopathological examination revealed about the neuroprotective effect of SNEDDS loaded with fisetin as observed through the prevention of neuronal damage. From this study, it was concluded that SNEDDS improved the anti-Parkinsonian activity of fisetin by improving the behavioral alteration produced by rotenone due to enhancement in its solubility and bioavailability.

Keywords Fisetin · SNEDDS · Parkinson's disease · Rotenone · Dopamine

Introduction

Neurodegenerative diseases (NDDs) are a group of uncontrollable diseases which affect the health of the person. The NDDs include Alzheimer's disease, Parkinson's disease,

Huntington's disease, spinocerebellar ataxia, amyotrophic lateral sclerosis, and spinal and bulbar muscular atrophy (Huang and Adachi 2016). Parkinson's disease (PD) is the second most common neurodegenerative disease that affects approximately 6 million people worldwide (Gopalakrishna and Alexander 2015). PD was described by a British physician James Parkinson in 1817 in a published paper, "the shaking palsy." In his paper, he described the symptoms of the disease, which was later on known by his name (Bala and Gupta 2013). PD is related to the loss of the dopaminergic neurons in the substantia nigra pars compacta of the brain; hence, the level of dopamine (DA) gets reduced in the brain. This loss of dopamine reduces the control over the voluntary muscles and causes the appearance of motor symptoms. The symptoms include bradykinesia, resting tremor, rigidity, postural instability, non-motor symptoms also appeared as sleep disturbance and dementia (Blesa et al. 2015). Aggregation of alpha-synuclein protein has also been observed in

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Fail-safe nano-formulation of prodrug of sulfapyridine: Preparation and evaluation for treatment of rheumatoid arthritis



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ABSTRACT

Aim of the present study was to give a second life to the long abandoned drug, sulfapyridine (SP) for its anti-arthritis potential by design of nano-vesicular delivery system. For this, intra-articular delivery of liposomal formulation was tried. As the prepared formulation exhibited rapid drug leakage, an arthritis responsive prodrug of SP showing labile covalent synovial enzymes was synthesized to exploit the overexpression of arthritis specific enzymes. Prodrug (SP-90) exhibited the retention in synovium as compared to the drug pro-drug, because of its low synovial permeability (SP-90) and its high plasma clearance. This delivery of its high permeability to synovium (SP-90) and its high plasma clearance (SP-90) and higher retention in synovium (SP-90) compared to the SP liposomes. In arthritis rats, prodrug liposomes were found to reverse the symptoms of inflammation including the levels of biochemical markers. Liposomes of pro-drug as drug, therefore, offer a revolutionary approach in the treatment of rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune inflammatory joint disease, characterized by chronic synovitis and hyperplastic synovial tissue, ultimately leading to destruction of cartilage, joint and bone tissue [1]. Currently used drugs include nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying antirheumatic drugs (DMARDs) and biologics [2]. DMARDs are preferred over other agents as they modify the clinical course of disease and apart from providing symptomatic relief, also arrest the progression of disease [3].

Sulfapyridine (SP), an active moiety of sulfadiazine for the management of RA, is the third oldest drug still in use in treatment of RA [4,5]. The mechanism of anti-inflammatory action of SP has not been clearly understood, but it is reported to inhibit folate dependent enzymes leading to an impaired function of lymphocytes. SP exhibits direct immunosuppressive effects on T and B cells, thus suppressing the levels of interleukins (ILs) and tumor necrosis factor- α (TNF- α) [6]. The recommended oral dose of SP is 2 g per day [7]. The high dose of SP is associated with side effects such as nausea/vomiting, rashes, dizziness,

anxiety, abdominal pain and high creatinine level [7].

Local administration of drugs offers numerous physiological and practical advantages over systemic medications i.e. increased local bioavailability, lower systemic exposure, less non-target effects and lower treatment cost [8]. The potential of direct intra-articular (IA) administration of therapeutic agents in the treatment of joint diseases such as RA, osteoarthritis and for the management of joint pain has been extensively explored [9].

However, even IA route proves to fail occasionally due to the rapid efflux of the drugs from synovial cavity after injection [10]. Rapid clearance of drugs following IA injection is attributed to highly vascularized synovial membrane that offers little resistance to diffusion of even large molecules [11]. The rate of clearance of drugs from synovial cavity largely depends on the size of the molecule and its solubility. Low molecular weight (MW) compounds (MW < 10,000 Da) are removed for less than 1 h in joints after IA administration while molecules with MW > 10,000 Da exhibit resistance more in the order of hours [12]. Half-lives of methotrexate, piroxicam, salicylate, ibuprofen, indomethacin and chlorzoxazone have been reported to be 0.59–2.9, 1.1–2.4, 1.9, 2.8 and 2.2 h respectively after local injection [12–14].

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Anti-ulcerogenic effect of methanolic extract of *Elaeagnus conferta* Roxb. seeds in Wistar rats

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ABSTRACT

Ethnopharmacological relevance: *Elaeagnus conferta* seeds (Elaeagnaceae) is a subtropical shrub commonly native to India, Vietnam, Malaysia and Bangladesh where various reports are used for treatment of diabetes, gastric ulcers, joint, skin, heart stress and other many ailments. Though the therapeutic use about seeds is not proper for their utilization as fruits are stringy and costly and for commercial use, they have been widely utilized for medicinal and industrial purposes. The present study is aimed to study the medicinal properties of the seeds of *Elaeagnus conferta* seeds. Based on the fact that seeds of closely resembling species i.e. *Elaeagnus thymoides* has demonstrated significant anti-gastro-therapeutic properties, the probability of the seeds of *E. conferta* possessing similar activity seemed quite significant.

Aims of the study: Phytochemical analysis, in-vitro assessment of ulcerogenic and gastro-protective activity in rat stomach by using methanolic extract of the seeds of *E. conferta*.

Materials and methods: Standard phytochemicals were isolated by column chromatography. These were identified by a variety of techniques including infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) and mass spectrometry. Methanolic extract (MEC) of the seeds was prepared by cold extraction and its anti-ulcerogenic potential was evaluated using indomethacin (50 mg/kg) and water immersion stress models in rats. The animals were treated with different doses of MEC (400 and 800 mg/kg) and the therapeutic effect was compared to the standard H_2 inhibitor, ranitidine (50 mg/kg). The anti-ulcerogenic effect of MEC were investigated against gastric juice acidity, gastric mucus, gastric acid, gastric lipase. The activity of the seeds against gastric acid (GAT), gastric mucus (GCM), gastric acid (GSA) and gastric lipase (GSL) and per-*in vivo* demonstration of ulcerogenic activity (ulcer index) and tumor necrosis factor alpha (TNF- α) were carried out to find out the possible mechanisms of action. Gastric histopathological changes were also studied.

Results: Chromatography studies and further confirmation by a variety of techniques revealed the presence of four different compounds in MEC i.e. oleic acid (1), stearic acid (2), arachidic acid (3) and myristic acid (4). MEC exhibited anti-ulcerogenic effect in all models other than water immersion stress model. It has a suppression of 20% indomethacin-induced (IC₅₀ 1.46 mg/ml), 100% indomethacin (IC₅₀ 0.1107 mg/ml) and hypergastric acid secretion (IC₅₀ 0.0048 mg/ml), 100% (IC₅₀ 0.0077 mg/ml) and 100% (IC₅₀ 0.0013 mg/ml) ulcer inhibition, reduction in total and free gastric acid, mucus in gastric juice. pH were observed in MEC treated rats as compared to disease control animals. Histopathological findings confirmed decreased cell infiltration, less epithelial cell damage and hyperplasia of gastric mucosa in dose dependent manner.

Conclusions: The anti-ulcerogenic effect of MEC may be attributed to its ability to scavenge free radicals and anti-inflammatory property via suppression of TNF- α and IL-6. These effects are comparable to standard drugs such as ranitidine and omeprazole.

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Development and validation of RP-HPLC method for estimation of fisetin in rat plasma

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ABSTRACT

Fisetin is an important phyto-flavonoid that possesses very good anti-oxidant, anti-Parkinson's and anti-cancer activities. A bioanalytical method was developed and validated for the estimation of fisetin in the rat's plasma using reverse phase ultra-fast liquid chromatography combined with a C-18 reverse phase column. Quercetin was used as an internal standard. The mobile phase was composed of acetonitrile and orthophosphoric acid (0.2% v/v) in the ratio of 30:70 v/v. Flow rate was set at 1 mL/min and chromatogram of both compounds was detected at a wavelength of 362 nm. Protein precipitation method was used to extract drug from plasma samples. The retention times for fisetin and quercetin were found at 8.3 min and 16.9 min, respectively. The developed method was found to be linear in the range of 25–125 ng/mL with regression coefficient (r^2) of 0.9996. The method was validated as per the ICH Q2 (R1) guidelines. The percentage recovery was in the range of 95–105%, which indicated that method was accurate. The percentage relative standard deviation was found to be less than 2% which indicates that method was precise. Limit of detection (LOD) and limit of quantification (LOQ) were found to be 3.18 ng/mL and 9.66 ng/mL, respectively. The developed method was found to be robust as there was no any significant change in response with change in flow rate and composition of mobile phase. Obtained results indicated that the developed method has passed all the validation test parameters and can be applied successfully for estimation of fisetin in the rat plasma.

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1. Introduction

Flavonoids are the naturally occurring plant molecules. Fisetin (2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one or 3,3',4',7-tetrahydroxyflavone) is one of the polyphenolic flavonol (Fig. 1). It is a pale-yellow color compound having molecular weight of 286.26 g/mol. The melting point of fisetin is 330 °C. It is naturally synthesized in various fruits (strawberries, apples, persimmons, mangoes, grapes and kiwis), vegetables (tomatoes, onions and cucumbers) and also acts as a coloring agent. It is also synthesized in various trees and shrubs belonging to Anacardiaceae family members, for instance Japanese fruit wax tree (*Rhus succedanea*), honey locust (*Gleditsia triacanthos*), parrot tree (*Butea fronds*), smoke tree (*Cotinus coggygria*), lac tree (*Rhus verniciiflua* Stokes), *rhus cotinus* (*Cotinus coggygria*) and quebracho

colorado (*Schinopsis balansae*); Fabaceae family, such as *senegalia greggii* (*Acacia greggii*), *senegalia berlandieri* (*Acacia berlandieri*); Pinopyta species, such as yellow cypress (*Callitropsis nootkatensis*) (Mehta et al., 2018). Fisetin reported to have various pharmacological activities like anti-inflammatory (Goh et al., 2012; Gelderblom et al., 2012), anti-epileptic (Das et al., 2017), nephroprotective (Sahu et al., 2014), ulcerative colitis (Sahu et al., 2016), diabetic neuropathy (Sandireddy et al., 2016), hepatoprotective (Koneru et al., 2016), neurotropic activity (Maher, 2006) and anti-depressant (Zhen et al., 2012). Various scientists reported different methods for the estimation of fisetin concentration in plasma samples. Kadari et al. (2017) administered fisetin orally as a polymeric nanoparticle to rats and then estimated the concentration of fisetin in blood using an LC-MS/MS gradient method combined with ammonium formate (10 mm, pH 3.5) and acetonitrile as the mobile phase at flow rate of 0.4 mL/min (Kadari et al., 2017). Similarly, Jo et al. (2016) evaluated the concentration of fisetin and geraldol in the mouse plasma using a LC-MS/MS gradient method of 0.1% formic acid (0.1%) in acetonitrile and formic acid (0.1%) in water at a flow rate of 0.25 mL/min (Jo et al., 2016). It is important to note that development of an analytical method using a LC-MS or LC-MS/MS is an

Abbreviations: UFLC, Ultra-fast liquid chromatography; LOD, Limit of detection; LOQ, Limit of quantification; ICH, International conference on harmonization; LQC, Lower quantified concentration; MQC, Medium quantified concentration; HQC, Higher quantified concentration; RSD, Relative standard deviation

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Enhanced oral bioavailability and neuroprotective effect of fisetin through its SNEDDS against rotenone-induced Parkinson's disease rat model

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ABSTRACT

The TS (TS) was reported to have various adverse effects on fish. Third, for the first time, we requested reliability and oral bioavailability, it is not in much use. For reliability and bioavailability plays an important role in the pharmacological studies. In this research work, we used to improve the oral bioavailability of TS. In this study, we developed self-nanoemulsifying drug delivery system (SNTNFS) of TS. In TS developed SNTNFS were compared the decrease in drug and the decrease in gastric emptying and gastric motility. First study of TS in rats because TS is used in rats. Higher China and area under the curve of drug decrease in rats only. In this study, but SNTNFS improved the oral bioavailability of TS and also increase of the gastric emptying time and gastric motility. Results of behavior parameters (locomotion, muscle coordination and cataplexis), locomotor, behavioral, and motor (RTNR, mRNR, GSR, SDN and GAT) and clinical (absolute and percentage, 30N-4, 4N-6 and 6N-7) confirmed that significantly improved ($p < 0.05$) in response in rats. Our results with SNTNFS as compared to TS as compared to TS. This study suggests that SNTNFS improved the oral bioavailability of TS and SNTNFS also improved the gastric emptying time and gastric motility. First study of TS in rats. It is suggested that potential use of SNTNFS in the treatment of TS and other.

1. Introduction

Parkinson's disease (PD) is most common age related and second most common chronic neurodegenerative disorder which affects the people worldwide. In PD, there is loss of dopaminergic neurons which occurs in the substantia nigra pars compacta of the mid brain, ultimately causing the reduction of dopamine level in the brain. There are various reasons for the development of the PD. Among these reasons, oxidative stress plays an important role in the development of pathological condition of PD leading occurrence of alpha-synuclein, an important pathological hall mark of PD [1]. Pathobiology of Parkinson's disease [20]. Medical Journal, India's Inspiration, 2016]. Rotenone, which is used as an inducing agent in the present study, is now potent member of rotenone family. It is a natural pesticide and classical mitochondrial complex I inhibitor. Rotenone was reported to be dopaminergic toxin. Many studies had been reported that administration of rotenone is able to produce the symptoms of PD. These symptoms include behavioral

alteration, relative degradation of the nigrostriatal dopaminergic system, production of reactive oxygen and nitric oxide species and elevation in the level of inflammatory cytokines (Garcia et al., 2012; Tinkler and Schenk, 2014).

Various phytochemicals having antioxidant and anti-inflammatory activities are tested previously in rodent models of IHD. These phytochemicals include curcuma (Fai et al., 2012), hesperidin (Hamisolemi et al., 2012), resveratrol (Ji et al., 2008), epigallocatechin gallate (Sevita et al., 2001), ficoidan (Moonaki et al., 2016), caffeine (Bernard, 2001) and hamamel (Chen et al., 2019). Thele (-5) is one of the polyphenolic flavonoid. It is present in various fruits and vegetables (Kumar et al., 2019b). It is reported to have various pharmacological activities like anti-inflammatory (Giri et al., 2012), anticonvulsant (Jha et al., 2017), antidepressant (Chen et al., 2012), hepatoprotective (Suman et al., 2016), nephroprotective (Sahu et al., 2014), antitumor (Sahu et al., 2016), neuroleptic (Scher, 2006). In our study Thele (-5) was reported to act as potent anti-fibrotic and also show potential to reduce

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Synthesis and evaluation of new 1,2,4-oxadiazole based *trans*-acrylic acid derivatives as potential PPAR- α /gamma dual agonist



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ABSTRACT

Diabetes is a ubiquitous metabolic disorder and life threatening disease. Peroxisome proliferator activated receptors (PPARs) belong to the class of nuclear receptors which are overexpressed in tissues to regulate lipid and glucose metabolism. PPAR α /gamma dual agonists tend to corroborate the functions of both Glucocorticoid hormones and thiazolidines and they hold substantial promise for ameliorating the type 2 diabetic symptoms and metabolic abnormalities of the type 2 diabetic individuals. New 1,2,4-oxadiazole based trans-acrylic acid derivatives compounds possessing glycidyl moiety as linker between a pharmacophore based on lipid cell receptors i.e. PPAR α /gamma dual agonists are studied. Antidiabetic Wistar rats for potential PPAR α /gamma dual γ agonists compounds 9a, 9b, 9c, 9d, 9e and 9f were identified comparable to PPAR α /gamma agonist Pioglitazone on the basis of their affinity scores and further their α effect values and in α 200 AICZ assays. The selected compounds showed better calculated lipophilicity (LogP) was found to be 0.02 to 0.19. Compounds 9a and 9b were found to be most potent on both PPAR α and gamma receptors with IC_{50} of $1.67 \pm 0.0015 \mu M$, $0.04 \pm 0.0015 \mu M$ and $1.781 \pm 0.004 \mu M$, $0.23 \mu M$ ± 0.001 respectively as α and γ agonists having IC_{50} of 2.38 ± 0.2 and 38.01 ± 0.15 for both receptors. The α agonist 9a and 9b reduce the plasma glucose level and total cholesterol level significantly in diabetic rats compared to pioglitazone at 5 mg/kg/day dose for 7 days of treatment. Trans- α substituted derivatives can be further developed as oral therapeutic agents for diabetic rats/rats as α PPAR α /gamma dual agonists.

1. Introduction

Among the lifestyle diseases, obesity and diabetes are prevailing in a remarkable rate across the globe. Worldwide, around 422 million adults are suffering with type 2 diabetes mellitus (T2M), according to the latest data from the World Health Organization (WHO) [1]. According to International Diabetes Federation says that among other countries in the world, until recently India had more diabetes compared to another country in the world and according to the World Health Organization (WHO), India had 69.2 million people living with diabetes in 2015 and are believed to increase approximately up to 98 million (approximately) by 2025 [2]. As per the study by the American Diabetes Association states that India would have the highest number of

diabetic people being diagnosed by 2030 [3]. Diabetes Mellitus is a very expensive disease and has profound long term complications and their associated cost [1,5]. World Health Organization also estimated that mortality related to heart disease and diabetes costs nearly \$210 billion each year and is expected to rise to \$335 billion in the coming 10 years [6]. This economic burden results in declining life expectancy and quality of life [7,8]. Peroxisome proliferator activated receptors (PPARs) are nuclear receptors classified as α , γ and δ . Among them, PPAR γ is considered a promising target for the treatment of diabetes mellitus because of its ability to improve insulin sensitivity and reduce the risk of cardiovascular complications particularly cardiovascular diseases

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Modified apple polysaccharide capped gold nanoparticles for oral delivery of insulin

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ABSTRACT

The study entailed formulation of gold nanoparticles (AuNPs) upon reduction of chloroauric acid by modified apple polysaccharide (MAP). AuNPs were conjugated with insulin (INS) for its oral delivery to treat type 1 diabetes mellitus (DM). The size of MAP conjugated AuNPs loaded with INS was 124 ± 8.55 nm with zeta potential -10.5 ± 0.54 mV. The animal study carried out in streptozotocin induced rat model revealed that AuNPs conjugated insulin (AuNPs-INS) in high dose caused 3.36 folds decrease in blood glucose level in 240 min, whereas, orally administered INS failed to decrease the blood glucose level. The 28-day study also revealed better improvement in body weight, lipid profile, urea, creatinine and liver parameters in AuNPs-INS (high dose) for which the observed value was close with respect to intraperitoneally administered insulin followed by medium dose of AuNPs-INS, low dose of AuNPs-INS, AuNPs alone and modified apple polysaccharide.

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1. Introduction

Type 1 diabetes mellitus (T1DM) is a severe autoimmune disease recognized by increased levels of blood glucose (hyperglycaemia), which is mainly due to the insulin (INS) insufficiency that arises as the outcome of the destruction of pancreatic islet β -cells [1,2]. It is the most common endocrine and metabolic condition arising in childhood. In around 70–90% of patients, the deprivation of β -cells is the outcome of T1DM-related autoimmunity (concomitant with the emergence of T1DM-associated autoantibodies); these individuals have autoimmune T1DM [3]. In a smaller section of patients, no immune reciprocation or autoantibodies are noticeable, and the cause of destruction of β -cells are unknown (idiopathic T1DM); this type usually has a strong genetic constituent. Unless otherwise specified, the word T1DM refers to autoimmune T1DM [4].

Oral hypoglycaemic agents/INS are the mainstay of the treatment of diabetes and are effective in controlling hyperglycaemia. However,

drawbacks of subcutaneous administration of INS are lipodystrophy, peripheral hyperinsulinemia and insulin neuropathy [5]. Moreover, multiple injections through this route may lead to local hypertrophy and infection and may also pose a challenge in case if the patients have trypanophobia resulting in non-compliance to this mode of therapy [6]. The main complications of INS therapy are hypoglycaemia which may result from delay in taking a meal, unusual physical exertion or a dose of INS that is too large for immediate needs [7]. Recent dosage of injectable INS required for maintaining blood glucose level, which comprises of subcutaneous injection of INS per day that can lead to psychological stress resulting in poor patient compliance [8].

Since ancient times, gold (Au) has been highly regarded as the 'elixir of life'. In 1925 gold complexes were being used in clinical trials to determine its efficacy to help alleviate rheumatoid arthritis [9]. Gold nanoparticles (AuNPs) were used by the Romans for the purpose of decorating and staining of glass. It is nearly 150 years ago when Michael Faraday noticed that the properties of colloidal gold solution were different from that of bulk metallic gold. Over the last half-century, different high yielding techniques for the synthesis of gold nanoparticles, including spherical and non-spherical shapes have been developed [10]. In general, all the methods for the synthesis of AuNPs include reduction of Au^{3+} ions to Au^{0+} ions with the help of some reducing agents which result in the nucleation of Au ions to nanoparticles (NPs).

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E-mail address: sachin.16030@lpu.co.in (S.K. Singh).

Validated Reverse Phase-High-Performance Liquid Chromatography Method for Estimation of Fisetin in Self-Nanoemulsifying Drug Delivery System

Rajan Kumar,¹ Rakesh Kumar,¹ Rubiya Khursheed,¹
Ankit Awasthi,¹ Arya Kadukkattil Ramanunny,¹ Jaskiran Kaur,¹
Navneet Khurana,¹ Sachin Kumar Singh,¹ Shelly Khurana,²
Narendra Kumar Pandey,¹ Bhupinder Kapoor,¹ and Neha Sharma¹

¹School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India.

²Department of Pharmacy, Government Polytechnic College, Amritsar, Punjab, India.

ABSTRACT

Fisetin (FS) is a polyphenolic phytoconstituent reported to have various pharmacological activities such as antioxidant, anti-parkinsonian, and antidepressant. An analytical method was developed and validated for the estimation of FS by ultrafast liquid chromatography using C-18 reverse phase column. Acetonitrile and orthophosphoric acid (0.2% v/v) in the ratio of 30:70 v/v was used as mobile phase. Flow rate was set at 1 mL/min. Chromatogram of FS was detected at wavelength of 362 nm. Retention time for FS was found to be 7.06 min. The developed method was found to be linear in the range of 2–10 µg/mL with regression coefficient of 0.9985. The method was validated as per the International Conference on Harmonization (ICH) Q2 (R1) guidelines. The percentage recovery was in the range of 95%–105%, which indicated the accuracy of the method. The percentage relative standard deviation (RSD) was found to be <2%, which indicates the precision of the method. Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.46 and 1.41 µg/mL, respectively. The developed method was found to be robust as there was no significant change in response with change in flow rate, ratio of mobile phase, and pH. The method was successfully applied for estimation of drug loading and drug release from self-nanoemulsifying drug delivery system (SNEDDS). The % drug loading of FS in prepared liquid SNEDDS formulation was found to be 101.95%. The results of dissolution studies indicated 67.78% FS release in water at the end of 60 min.

Keywords: fisetin, SNEDDS, HPLC, validation

INTRODUCTION

Flavonoids are the naturally occurring plant molecules. Fisetin (FS; 2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4H-chromen-4-one) is one of the polyphenolic flavonol (Fig. 1). It is a pale-yellow compound having molecular weight 286.24 g/mol. The melting point of FS is 330°C. It is naturally synthesized in various fruits (apples, mangoes, strawberries, kiwis, grapes, and persimmons) and vegetables (onions, tomatoes, and cucumbers). The reported pharmacological activities of FS are anti-inflammatory,^{1,2} antiepileptic,³ nephroprotective,⁴ antiulcer,⁵ antineuropathic,⁶ hepatoprotective,⁷ neurotrophic,⁸ and antidepressant.⁹ Besides having various pharmacological activities, FS also have some of the pharmaceutical challenges. FS have very low solubility and oral bioavailability.¹⁰ Therefore, researchers try to improve the solubility and bioavailability of FS by formulating nanoformulations such as liposomes,^{11,12} polymeric nanoparticles,¹³ and self-nanoemulsifying drug delivery system (SNEDDS).¹⁴ To evaluate these nanoformulations on various parameters such as drug loading, drug dissolution, and permeability, there is a need to develop a simple, sensitive, and reproducible method for its quantification.

There are some of the methods that have been reported for the quantification of FS. Bouzid *et al.* reported the presence of FS along with some other flavonoids in the extract of *Arbutus unedo* L. by analyzing the extract over high-performance liquid chromatography (HPLC) using methanol:water (50:50, v/v, pH 2.8) as mobile phase. But the information regarding method development and validation for FS was not available.¹⁵ Mignet *et al.* developed the liposomal formulation of FS and used HPLC technique to measure the percentage encapsulation of FS. To perform HPLC analysis they used methanol:2% glacial acetic acid (52:48, v/v) as mobile phase. But, in this study also details about method development and validation of FS were not reported.¹² Some bioanalytical methods are also reported for the evaluation of FS in the biological samples,^{16,17} but detailed information is not available in the literature regarding validation of these methods. Kadari *et al.* demonstrated application of LC-MS/MS for estimation of

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RESEARCH ARTICLE

Development and Validation of UV Spectroscopic Method for Estimation of Fisetin in Self Nanoemulsifying Drug Delivery System

Rajan Kumar¹, Rakesh Kumar¹, Rubiya Khursheed¹, Bhupinder Kapoor¹, Neha Sharma¹, Shelly Khurana², Navneet Khurana¹, Sachin Kumar Singh^{1*}, Manish Vyas¹

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²Department of Pharmacy, Govt. Polytechnic College, Amritsar, Punjab, India

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ABSTRACT:

Fisetin is a polyphenolic flavonoid which has been reported various pharmacological activities as a crude extract and in formulation form. This study was designed to develop and validate a simple and cost effective method for the estimation of fisetin in the liquid self nanoemulsifying drug delivery system (L-SNEDDS). Solutions having different concentration of fisetin were prepared in methanol to develop the calibration curve. The solutions were scanned at 362nm by using UV-spectrophotometer. Similarly, for validation of method recovery of drug was measured at three levels 80%, 100% and 120% of the mid concentration and data was recorded. Further, this method was used to measure the drug loading in L-SNEDDS of fisetin. The data was found to be linear in the range of 4 – 12µg/mL with regression coefficient of 0.999. The recovery of drug in the range of 95% to 105% indicated the accuracy of the method. The percentage relative standard deviation among all the responses was found to be less than 2% indicating the precision of the method. The LOD and LOQ was found to be 0.28 and 0.86µg/mL respectively. Drug loading in the L-SNEDDS was found to be 93.66%. A simple, accurate and cost-effective method for the estimation of the fisetin in pharmaceutical dosage forms was developed and validated using ultra violet spectroscopy.

KEYWORDS: Fisetin, Flavonoid, Spectroscopy, Phytochemical, SNEDDS.

INTRODUCTION:

Flavonoids are the polyphenolic compounds, present abundantly in various plants, and majorly exhibit activities like anti-oxidant, anti-inflammatory and anti-tumour. Fisetin (3,3',5',7-tetrahydroxyflavone) is one of the flavonoids belongs to the category flavanol. It is a pale-yellow powder having molecular weight 286.26 g/mol and melting point 330°C (fig. 1). It is soluble in methanol, acetone and acetic acid but very slightly soluble in water.

It is present in various fruits and vegetables and the highest concentration is reported in strawberries (160 µg/g) followed by apple (26.9µg/g) and persimmons (10.5µg/g)¹. It possesses pharmacological activities like antioxidant, anti-inflammatory, hypolipidemic, anti-adipocyte differentiation, inhibition of allergic airway inflammation^{2,3,4,5}, neurotropic activity⁶ and anti-depressant⁷. Despite having various pharmacological properties, there are some limitations like low aqueous solubility and oral bioavailability. As per literature, liquid self nano emulsifying drug delivery system (L-SNEDDS) are helpful to improve dissolution rate and bioavailability of poorly water-soluble drugs. SNEDDS are the homogenous mixture of oil, surfactant and co-surfactant in which drug is dissolved, which upon ingestion in the stomach produces oil in water micro or nano-emulsion. The nano size of droplets provide greater surface area for the absorption of the entrapped drug^{8,9}.

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Self-nanoemulsifying drug delivery system of fisetin: Formulation, optimization, characterization and cytotoxicity assessment

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ARTICLE INFO

Keywords:

Fisetin
Nanoemulsion
Formulation
Characterization
Cytotoxicity

ABSTRACT

Fisetin is a plant-derived flavonoid that possesses a diverse range of health and anti-cancer activities. However, due to its lipophilicity, it suffers from dissolution rate limitation, bioavailability, etc., making its therapeutic efficacy much lower. To overcome this issue, self-nanoemulsifying drug delivery system (SNEDDS) of Fisetin were formulated and characterized for droplet size, clarity, zeta potential, pH, stability, dissolution and permeability studies. Prepared SNEDDS (1.2 mL) were composed of cosolvent (0.1 mL), Laurethyl 900 (0.1 mL), Tween 80 (0.4 mL), Transcetyl 2 (0.5 mL) and Fisetin (5 mg). The formulation was optimized using Box-Behnken design. Droplet size and zeta potential of prepared SNEDDS was in the range 154 nm and -37 mV. Dissolution rate of Fisetin in pH 1.2, 4.5 and 6.8 SNEDDS formulation was 100%, 98.33% and 98.33% respectively compared to compressed Fisetin. Permeability studies revealed an increase in permeability (89.5%) for the loaded SNEDDS as compared to its compressed form (10.8%) at a fixed concentration (500 µg/mL). The in vitro cell line study revealed about 0.50 folds increase in permeation rate for Fisetin loaded in SNEDDS. The developed formulation was found to be stable with change in temperature, dilution and pH. The study entailed successful formulation of SNEDDS loaded with Fisetin.

1. Introduction

Fisetin (3,3',4',4'-tetrahydroxyflavone) is one of the flavonoids that belongs to the category of flavanol. The chemical structure of Fisetin is shown in Fig. 1. It is present in our fruits and vegetables and the highest concentration is reported in strawberries (160 µg/g) followed by apple (28.9 µg/g) and pimientos (19.5 µg/g) [1]. It possesses pharmacological activities like anti-oxidant, anti-inflammatory, hypolipidemic, anti-obesity, differentiation, inhibition of allergic airway inflammation [2–5], neurotrophic activity [6], anticancer [7,8] and anti-depressant [9]. Paul et al. (2012), reported neuroprotective effect of Fisetin on MPTP/APP¹ (methyl 4-phenyl 1, 2, 3, 6-tetrahydroxy-2-methyl-4-phenylpyridinium) induced neurotoxicity in PC12 cells to treat Parkinson's disease [10]. Despite having various pharmacological properties, Fisetin possesses low aqueous solubility and oral bioavailability [11,12]. There is no much literature available related to formulation strategy adopted to improve its solubility rate,

permeation and oral bioavailability of Fisetin.

Nanotechnology has a great impact on pharmaceutical science and numerous work is going on to realize its impact on other branches of the pharmaceutical sciences. The ultimate aim of using nanotechnology in pharmaceuticals is to improve the bioavailability, reducing the side effects of drug which ultimately improves their therapeutic potential. The various ways by which nanotechnology impacts is by using in pharmaceuticals are solubilizing the hydrophobic drugs, enhancing permeation of poorly soluble drugs, regulating the distribution, controlling drug release, target delivery and protecting drugs from degradation. Various types of nanocarriers like lipid based nanocarrier, inorganic nanocarrier, polymeric nanocarrier and drug nanoparticle are currently being used. The lipid based nanocarriers are further divided as lipid core micelles, liposomes, nanoemulsion, nanodispersion, oil in lipid nanoparticle and nanostructured lipid carriers [1,11–14]. Self-nanoemulsifying drug delivery system (SNEDDS) are thermodynamically stable systems that have been extensively explored to improve

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FISETIN: A PHYTOCHEMICAL WITH VARIOUS PHARMACOLOGICAL ACTIVITIES

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Abstract

Flavonoids are the plant secondary metabolites which work as growth hormone as well as defence mechanism for the plants. These are well known for their antioxidant properties and are part of our daily food. Fisetin is one of the polyphenolic flavonol, present in various fruits and vegetables. Fisetin is reported to have various pharmacological properties. Strawberries have the maximum concentration of fisetin. Despite having various pharmacological properties, low oral bioavailability and high lipophilicity meared its use. In this review we tried to collect the information regarding the various pharmacological properties and its developed formulations to improve its bioavailability.

Keywords: Fisetin, Flavonoids, Polyphenols, Antioxidant, Plant hormone

Abbreviations used: ALT: alanine aminotransferase, AST: aspartate aminotransferase, MPTP: 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, ROS: Reactive oxygen species, NF- κ B: Nuclear Factor kappa-light-chain-enhancer of activated B cells, TNF: Tumour necrosis factor, IL-6: Interleukin 6, GABA: Gamma-Aminobutyric acid, i.p: Intra-peritoneal, p.o: par oral, COX: Cyclooxygenase, iNOS: Inducible nitric oxide synthase, MAPK: Mitogen-activated protein kinases, SOD: Superoxide dismutase, CAT: Catalase.

Introduction

Flavonoids are the one of the important class of the chemicals which are available from the plants. Basically they are secondary metabolites of the plants and have polyphenolic basic structure. Flavonoids are abundantly found in various fruits, vegetables etc. They are also an integral part of various nutraceutical, pharmaceutical cosmetic and medicinal products because of their health promoting activity. Flavonoids are reported to help in the treatment of various diseases related to nervous and cardiovascular system. Flavonoids are extracted from the various plant parts depending on their concentration in different parts of the plant. In plants they are used as growth hormones and as defence mechanism (Panche *et al.*, 2016).

Fisetin is one of the flavonol bioactive molecules. It is present in various fruits and vegetables like: apple, strawberry, persimmon, grape, onion and cucumber etc. The concentration of fisetin in fruits and vegetables varies from 2 to 160 μ g/g (Figure 1). It is reported that strawberries have the maximum concentration of fisetin that is 160 μ g/g. For last few year fisetin becomes an interesting drug molecule for research because of its presence in human food and also because of its various pharmacological reported activities like: antioxidant, anti-diabetic etc. (Arai *et al.*, 2000)

Physicochemical Properties of fisetin:

Chemical name : 3, 3', 4', 7 – tetrahydroxyflavone

Molecular weight: 286.26 g/mol

Colour : Pale yellow

Melting point : 330 °C

Solubility : Soluble in methanol, acetone, and acetic acid, very slightly soluble in water

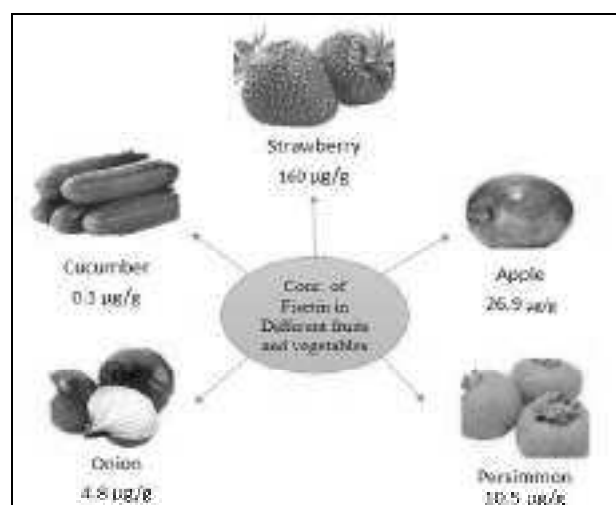


Fig. 1 : Fisetin concentration in different fruits and vegetable

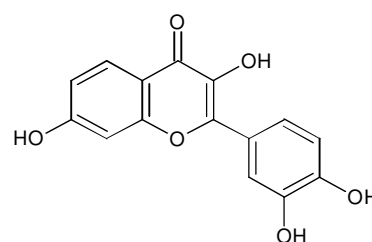


Fig. 2 : Structure of Fisetin

Antioxidant properties of fisetin

Fisetin was reported as a good antioxidant molecule as it is its inherent properties because of a flavonoid molecule. It acts as antioxidant by different activities like:

Annexure XXV

Certificate No.: 02548



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PHYTOCON-2018
November-April 14, 2018
LPU, Punjab, India



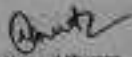
Certificate of Presentation

This is to certify that Prof./Dr./Mr./Ms. Rakesh Kumar
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as E-Poster Presentation / Oral Presentation in the **PHYTOCON 2018** - International Conference on "Commercialization of Medicinal Plant Products: Lab Techniques to Trade" held on April 14, 2018 organized by School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

Date of Issue: 14-04-2018
Place of Issue: Phagwara (India)



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Dr. Suresh Salje
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Prof. Monica Gulati
Chairman - LOC

Annexure XXVI



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presented Poster on Effects of Biomolecules on adaptation of β -secretase1
and β -amyloid in $AlCl_3$ and D-galactose induced mouse model of α in the ^{Alzheimer's disease}
International Conference on the theme "Emerging Techniques in Drug Discovery and
Drug Delivery: Current Challenges and Future Prospects", organized by Chandigarh
College of Pharmacy, Landran, Mohali, on 10th-11th February 2020.

Prof. (Dr.) M. Arackia Babu
Organising Secretary
Director-Principal, CCP

Prof. (Dr.) P. N. Hrisheekesha
Patron
Campus Director, CGC

Prof. (Dr.) Suresh
President, PCI
New Delhi

Annexure XXVII

Certificate No. 173869



Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. Rakesh Kumar
has Presented E-poster/Oral presentation on Recent Advancement in the
Management of Alzheimer's disease
presentation in International Conference of Pharmacy (ICP-2019) on the theme of "Pharmacy: Realigning the focus
on health" held on 13-14th September 2019 organized by School of Pharmaceutical Sciences, Lovely Professional
University, Punjab in a collaboration with Indian Pharmacy Graduates' Association (IPGA) Phagwara chapter.

Date of Issue: 14-09-2019
Place of Issue: Phagwara (India)


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Dr. Bindu Kumar
Organizing Secretary


Dr. Monica Gulati
Chairperson, Local Organizing Committee

Annexure XXVIII

DIVISION OF STUDENT WELFARE

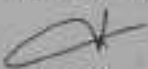
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student of LIT (Pharmacy)/Department of Pharmaceutical Sciences
Registration No. 11719625 pursuing Ph.D. (Pharmacology) [Full Time]
stood Second in the event Graduation Project Expo 2019
held from 26-04-2019 to 27-04-2019 organized by Division of Student welfare
Lovely Professional University (Punjab).

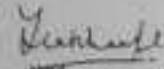
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Place of Issue: Phagwara (India)



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Organizing Secretary



Division of Student Welfare

Annexure XXIX



Annexure XXX

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This is to certify that Dr. / <i>Mr.</i> / <i>Ms.</i> <i>Rakesh Kumar</i> of <i>School of Pharmaceutical Sciences, Lovely Professional University</i> <i>Punjab</i> Has participated as resource person/delegate/presented a poster entitled <i>Potential effect of biomolecules in pathogenesis of Alzheimer's</i> <i>disease</i> In the M.P. Council of Science & Technology (MPCST) Bhopal sponsored National Seminar on "Recent Advancement in Pharmaceutical Research and Future Prospects" organized by Radharaman Institute of Pharmaceutical Sciences, Bhopal held on 22nd February 2019.		
 Chairman Shri R.R. Saxena	 Convener Dr. L.k. Omray	 Co-convener Dr. Kapil Malviya



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Paper Presentation Certificate

This is to certify that Prof./Dr./Shri/Smt. Rakesh Kumar
..... of Lovely Professional University
has presented a Paper (Oral/Poster) entitled 'In vivo and In vitro Pharmacological ----- Bromelain' in the Section of Medical Sciences
..... including Physiology during
the 106th Indian Science Congress held at Lovely Professional University,
Phagwara, Jalandhar from January 3 to 7, 2019.

His/Her Membership Number is L 36993

Date 05.01.2019



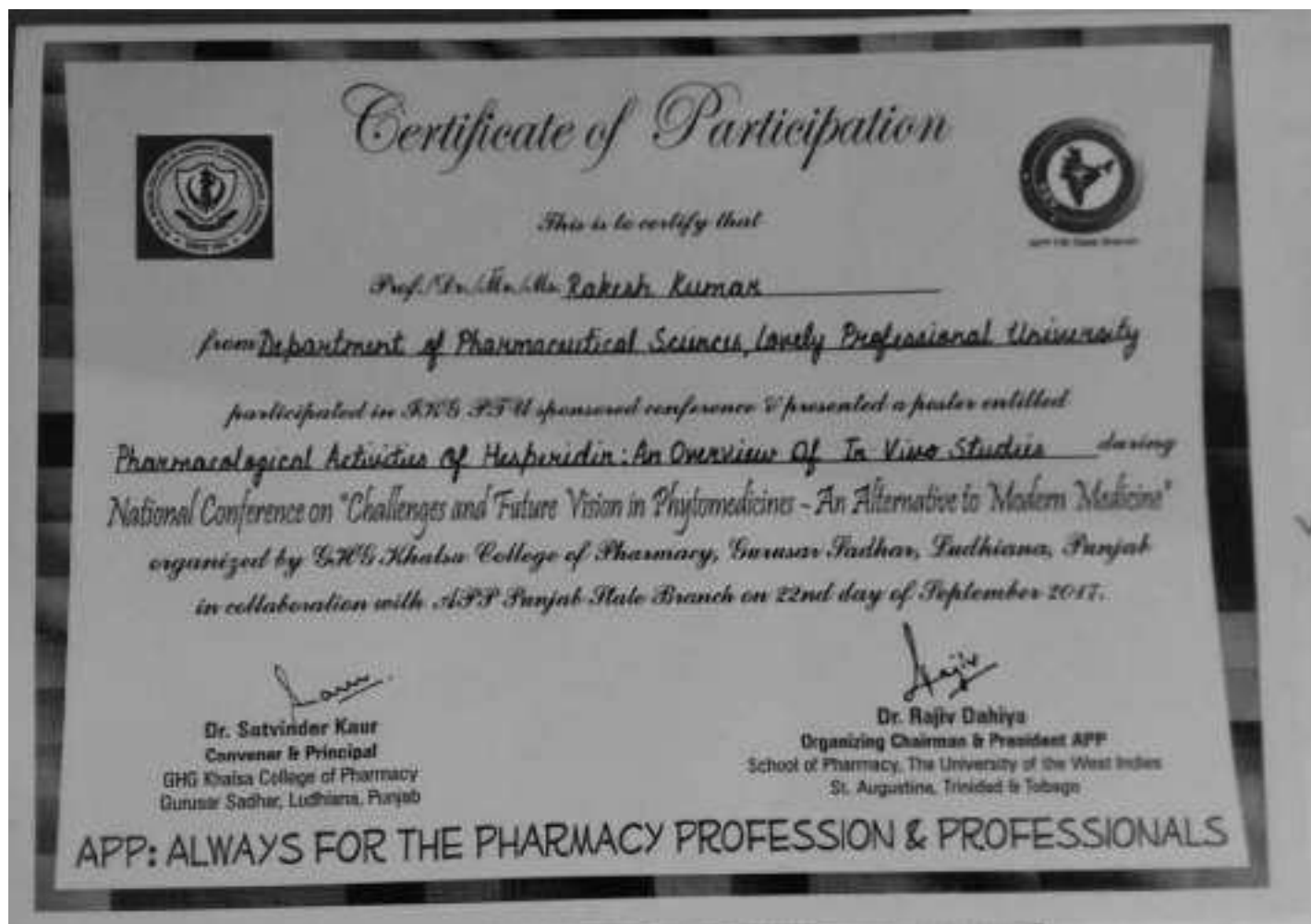
Office Seal

P. Dhara
Prof. Prakash C. Dhara
Sectional President
Medical Sciences (Including Physiology)
Indian Science Congress Association

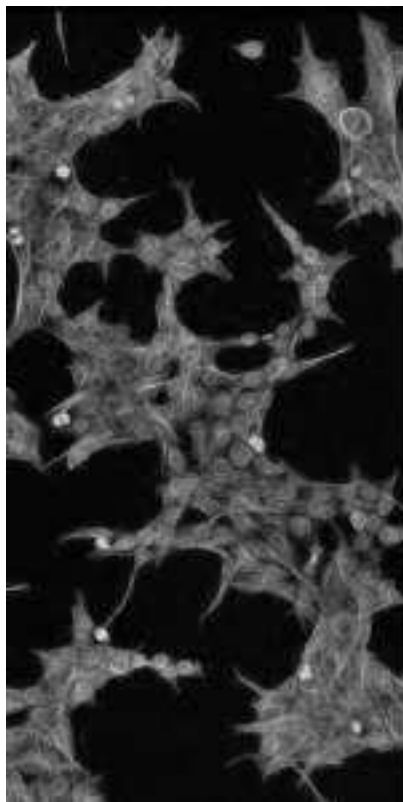
(Signature)
Sectional President

Annexure XXXII





Annexure XXXIV



**AMITY
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CERTIFICATE OF PARTICIPATION

THIS IS TO CERTIFY THAT

Rakesh Kumar

HAS PARTICIPATED IN THE WORKSHOP ENTITLED

“SERB-Scientific Social Responsibility–Research Facility
Training Workshop on Immunofluorescence Microscopy”

HELD ON 29th March 2022 AT

AMITY INSTITUTE OF PHYSIOLOGY AND ALLIED SCIENCES
AMITY UNIVERSITY UTTAR PRADESH, SECTOR 125, NOIDA, INDIA

Dr. Sudipta Saha
SERB-SRG Project PL AP(III)
Amity Institute of Physiology and
Allied Sciences

Prof. (Dr.) Subhrajit Bhowmik
Faculty Coordinator
Amity Institute of Physiology and
Allied Sciences

Prof. (Dr.) B.C. Das
Dean
Health and Allied Sciences
Amity University Uttar Pradesh

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HUMAN RESOURCE DEVELOPMENT CENTER

[Under the Aegis of Lovely Professional University, Jalandhar-Delhi G.T. Road, Phagwara (Punjab)]



Certificate No. 241698

Certificate of Participation

This is to certify that **Mr. Rakesh Kumar S/o Sh. Charan Singh** participated as Program Facilitator in **Training on The Care and Use of Laboratory Animals in Accordance with Ethical Guidelines** organized by Lovely Professional University w.e.f. **March 11, 2022 to March 12, 2022.**

Deputy Dean
Human Resource Development Center



Professor and Dean
Human Resource Development Center

Annexure XXXVI



Annexure XXXVII

FACULTY OF APPLIED MEDICAL SCIENCES

(Under the Aegis of Lovely Professional University, Jalandhar-Delhi G.T. Road, Phagwara (Punjab))

Certificate No: 235834

Certificate of Participation

This is to certify that Mr./Ms. Rakesh Kumar S/O, D/O, W/O Mr. Charan Singh
student of School of Pharmaceutical Sciences Registration No. 11719625
pursuing PhD participated in the National Pharmacovigilance Week-2021
on the theme of 'Pharmacovigilance: A step towards patient safety' held from 17-09-2021 to 23-09-2021
organized by Adverse Drug Reaction Monitoring Centre (AMC) and Medical Device Adverse Event Monitoring Centre (MDMC)
in association with School of Pharmaceutical Sciences at Lovely Professional University, Punjab.

Date of issue: 12-11-2021
Place of issue: Phagwara (India)



Prepared by
(Administrative Officer-Records)



Dr. Binod Kumar
Organizing Secretary



Prof. (Dr.) Monica Guleri
Executive Dean
LFAMS

Annexure XXXVIII

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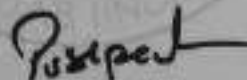
Certificate of Participation

This is to certify that Mr./Ms. Rakesh Kumar S/O,D/O,W/O Mr. Charan Singh
student of Lovely Faculty of Applied Medical Sciences
Registration No. 11719525 pursuing Ph.D. (Pharmacology)
participated in the National Workshop on Advanced Instrumentation
held on 20-04-2019 organized by Central Instrumentation Facility, Division of Research and Development
Lovely Professional University (Punjab).

Date of Issue : 13-05-2019
Place of Issue: Phagwara (India)



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Organizing Secretary
Head,
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Annexure XXXIX

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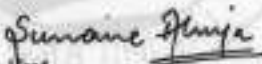
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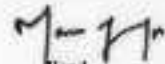
Certificate of Participation

This is to certify that **Mr. Rakesh Kumar S/o Sh. Charan Singh** participated in **Author Workshop on How to Write Manuscripts Grant Proposals** organized at Lovely Professional University on **October 12, 2018** and obtained **'B'** Grade.


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(Administrative Officer - Records)

Date of Issue: 27-10-2018
Place: Phagwara (Punjab)


Head
Human Resource Development Center


Head
Division of Human Resource

Annexure XXXX

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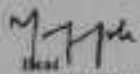
Certificate of Participation

This is to certify that **Mr. Rakesh Kumar S/o Sh. Charan Singh** participated in **Hands on Training to Improve Quality of Research Conducted on Experimental Animals** organized by Human Resource Development Center, Lovely Professional University from **March 23, 2018 to March 24, 2018** and obtained **'O' Grade**.


Presented by
(Administrative Officer - Records)

Date of Issue: 24.03.2018
Place: Phagwara (Punjab)


Head
Human Resource Development Center


Head
Division of Human Resource



CERTIFICATE OF PARTICIPATION

This is to certify that Prof. / Dr. / Mr. / Ms. Rakesh Kumar has participated as a **Delegate** / **Resource person** in Conference on "Design of Experiments" jointly organized by Qsutra & School of Pharmaceutical Sciences, Lovely Professional University, Punjab on 13th - 14th July 2018.

Monika Gulati
Prof. Monika Gulati
Sr. Dean - LFAMS, LPU

Mr. Madhu Madhavan
Mr. Madhu Madhavan
Founder & CEO - Qsutra

Dr. Saurabh Satija
Dr. Saurabh Satija
Organizing Secretary

Annexure XXXXII



Annexure XXXXIII

	Certificate This is to certify that	
Dr./Mr./Ms./Mrs. <u>RARESH KUMAR</u>		
Participated in		
Pre-conference workshop on Pharmacokinetics-Toxicokinetics		
under the aegis of		
Society of Toxicology (STOX)		
AS		
Speaker ☐ Demonstrator ☐ Delegate ☒		
at Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh on 17th November 2017		
Punjab Medical Council (PMC) has granted 4 credit hours for this program		
(Letter no. PMC/CME/2017/4395 dated 23/10/2017.)		
		
	<u>Dr. Sanjeev Mahajan</u> Registrar Punjab Medical Council (PMC)	<u>Prof. S.K. Garg</u> President PGIMER, Chandigarh
		<u>Prof. Bikash Medhi</u> Dept. of Pharmacology PGIMER, Chandigarh