FORMULATION, DEVELOPMENT AND EVALUATION OF FISETIN LOADED ORAL NANOEMULSION FOR THE TREATMENT OF ALZHEIMER'S DISEASE IN RATS

Thesis Submitted for the Award of the Degree of

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By

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DECLARATION

I hereby declare that the present dissertation entitled "**Formulation, development and evaluation of fisetin loaded oral nanoemulsion for the treatment of Alzheimer's disease in rats**" embodies the original research work carried out by me. It is further stated that no part of this dissertation has been submitted either in part or full for the award of any other degree of Lovely Professional University or any other University/Institution.

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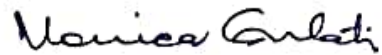Jalandhar-Delhi G.T. Road
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CERTIFICATE

This is to certify that the present dissertation entitled "**Formulation, development and evaluation of fisetin loaded oral nanoemulsion for the treatment of Alzheimer's disease in rats**" embodies the original research work carried out by **Sukriti Vishwas** under my supervision and guidance. It is further stated that no part of this dissertation has been submitted either in part or full for the award of any other degree of Lovely Professional University or any other University/Institution.

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It is not just an end; it is a new beginning-an emotional blend of fond memories and hopeful dreams. Closing this chapter stirs up a mix of emotions, and I am excited about what lies ahead. Here is to the emotions of the past and the heartfelt anticipation of the future. Cheers to the next emotional adventure.

Thankful I ever remain...   Sukriti Vishwas

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Abstract

Alzheimer's disease (AD) is a type of neurodegenerative disease (ND). Globally, more than 44 million people are affected with AD. Various pathophysiological factors are leading causes of AD including formation of accumulated Aβ, formation of neurofibrillary tangle, degeneration of cholinergic neurons, enhanced oxidative stress and neuroinflammation. The Food and Drug Administration (FDA) has approved few anti-Alzheimer's drugs for the treatment of AD. These drugs are AChE inhibitor (donepezil, galantamine and rivastigmine), glutamate N-methyl-D-aspartate (NMDA) receptor antagonist (memantine) and Aβ inhibitor (aducanumab and lecanemab). Numerous others treatment strategies have been also reported that synthetic, herbal and gene therapies shown significant response for the treatment of AD. Currently, herbal drugs and bioactives have shown excellent neuroprotective response for the treatment of AD such as curcumin, resveratrol, fisetin (FS), quercetin, proanthocyanidins, berberine and rosmarinic acid etc. They have shown very good antioxidant and anti-inflammatory effects are prevent neurons for degenerations. Among these FS has shown excellent response for the treatment of AD.

FS is a polyphenolic flavonoid that possesses various neuroprotective effects by attenuating the levels of AChE enzyme, accumulated Aβ, reduced oxidative stress, and neuroinflammation. It is inherently senolytic, reducing the impact of cellular senescence. However, FS suffers from a dissolution rate-limited oral bioavailability, poor blood brain barrier (BBB) permeability leading to decreased therapeutic efficacy. To address these challenges, an attempt has been made to formulate NE of FS using a Box-Behnken design (BBD) approach. NE offer numerous advantages such as enhance drug loading, easy to prepare, highly stable, less toxic, increased bioavailability and BBB permeability. To formulate NE, 10 mg of FS was added to mixture of 164 µL of Capmul MCM EP/NF (oil), 618 µL of Tween 80 (surfactant), 218 µL of Transcutol P (co-surfactant) and 4000 µL of distilled water (continuous phase). The optimized formulation resulted in a mean droplet size, drug loading, PDI, and zeta potential of 32 nm, 95.5%, 0.26, and -15.99 mV, respectively. TEM image revealed the spherical shape of FS-NE and average droplet size was observed 30 nm. Further, the optimized FS-NE were evaluated for their oral bioavailability, availability in the brain, and pharmacodynamic efficacy. Bioavailability and

availability of FS-NE in the brain were observed by pharmacokinetic studies. To evaluate pharmacodynamic studies behavioural, biochemical and histopathological studies were performed. Morris water maze (MWM) and open field test (OFT) were performed to evaluated cognitive function, motor function and anxiety level of the rats. Biochemical studies in cerebral cortex and hippocampus part of the brain reveals level of AChE, Aβ, oxidative stress and neuroinflammation (IL1β and TNFα). In the pharmacokinetic results, FS-NE showed an 842.93% enhancement in relative bioavailability compared to naïve FS. To evaluate pharmacodynamic effects, an aluminum chloride (AlCl$_3$)-induced AD rat model used. Cognitive functions were assessed using MWM test and OFT. The formulation showed dose dependent effects. Both low and high doses of FS-NE (groups VI and VII) showed significant improvements (P < 0.001) in cognitive functions in AD induced rats. The biochemical studies showed that FS-NE at both doses attenuated the levels of AChE enzyme, amyloid β (Aβ), oxidative stress, and neuroinflammation. Results of histopathology indicated that FS-NE attenuated the degeneration of neurons in the cerebral cortex and hippocampus parts of the brain.
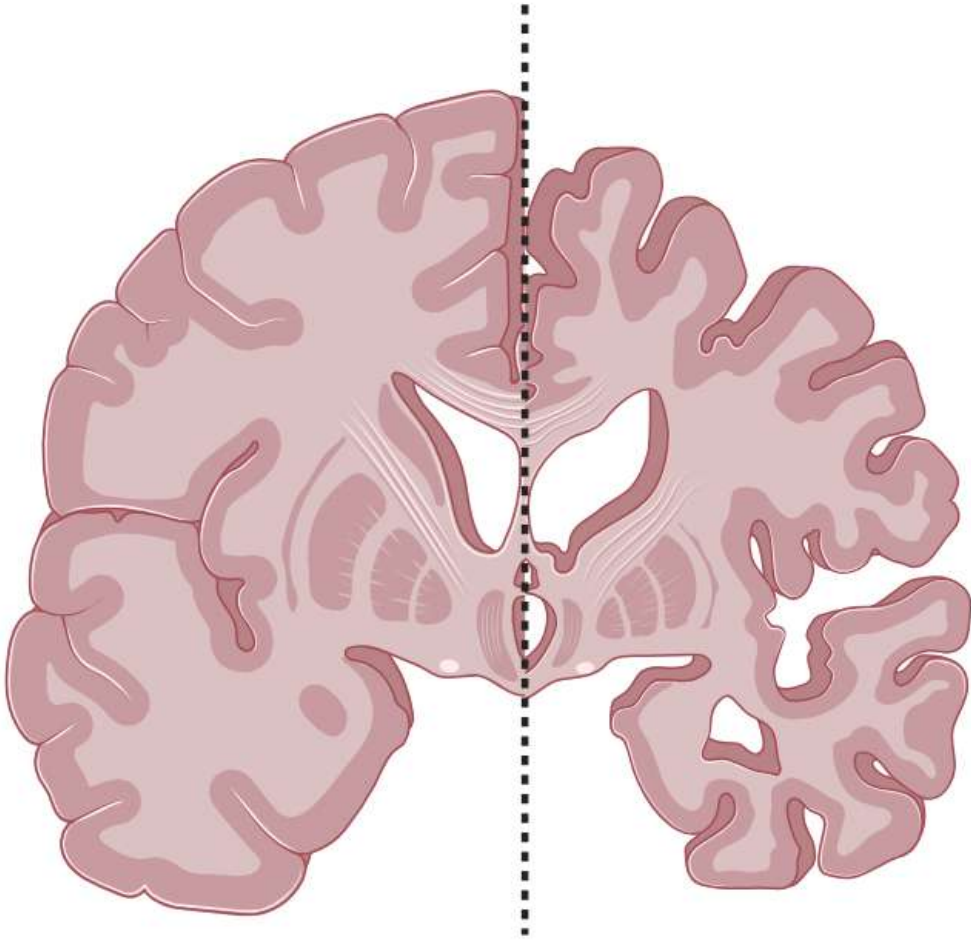
1. Introduction

1.0 Introduction

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease (ND). It manifests with cognitive, motor, and psychotic symptoms. Over the course of its development, AD impairs the ability to perform routine daily tasks [1]. As per the Alzheimer's Association of India, more than 44 million people are affected by AD till 2023 across the globe [2]. Numerous pathological symptoms of AD include the progression of accumulated amyloid beta (Aβ) protein, the formation of neurofibrillary tangles (NFT), the degeneration of cholinergic neurons, enhanced oxidative stress, and neuroinflammation [3]. Aging is one of the most significant factors of AD with individuals typically between the ages of 65 and 85 being most commonly affected.. However, it has been observed that 9% of people are affected with AD in early onset dementia (>65 years). Recent studies reported that people can lower the risk of AD by changing sedentary lifestyles, ensuring adequate sleep, doing daily physical activities, adopting a healthy eating regimen, controlling blood pressure, cholesterol, blood sugar levels, avoiding tobacco smoking, and alcohol consumption [3–6]

Till now very few treatments are available for AD such as donepezil, galantamine, rivastigmine, memantine, lecanemab and aducanumab [4]. These anti-AD drugs carry some serious limitations such as these drugs working through single mechanistic pathways and possess several side effects including Vomiting, nausea, muscle cramps, loss of appetite, increased frequency of bowel movements, headaches, constipation, confusion, and dizziness. These challenges make their oral delivery cumbersome. To overcome those challenges, new therapy is required that can overcome the side effects of drugs, enhance their solubility and dissolution rate, and function through multiple mechanistic pathways for the treatment of the disease [5].

Many plant-based bioactive drugs are reported for effective inhibition of Aβ, βChE, oxidative stress, and neuroinflammation markers (TNF α and IL1β) with fewer side effects and a broad therapeutic index. Among them, Fisetin (FS) exhibited numerous neuroprotective effects for treating AD. It is a polyphenolic flavonoid. Chemically, it is a 2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one [5,6]. It shows neuroprotective effects by attenuating the level of AChE enzymes, which decrease the degradation of cholinergic neurons and increase

the progression of acetylcholine in the cerebral cortex and hippocampus parts of the brain. FS has high potential against AD due to its attenuated progression of Aβ and neurofibrillation tangle (NFT). Furthermore, it also reduced oxidative stress, neuroinflammation, and cellular apoptosis [7].

Despite its potential, FS offers less aqueous solubility, bioavailability, and blood-brain barrier (BBB) permeability. In addressing the issue of low aqueous solubility, nanoformulations have demonstrated a significant potential. These systems contribute to an improved oral bioavailability of the drug. Currently, various nanoformulations have been developed, including NE, liposomes, solid lipid nanoparticles, transferosomes, nanostructured lipid carriers, inorganic nanoparticle, metallic nanoparticles, self-nanoemulsifying drug delivery systems etc [8]. Among these nanoformulations, NE have shown excellent advantages for the brain delivery of the drugs. It enhances the aqueous solubility, dissolution, and disintegration of the hydrophilic drugs, resulting in enhanced oral bioavailability and BBB permeability [9].

To evaluate anti-Alzheimer's effects various models have been developed such as scopolamine indued amnesia, streptozotocin induced AD, A$\beta_{1\text{-}42}$ induced AD, diazepam induced cognitive dysfunction, methionine induced AD aluminium chloride (AlCl$_3$) induced AD, etc [10]. Among these models, AlCl$_3$ induced AD in rats one of the well reported models for the treatment of AD. Aluminium is a heavy metal that causes neurotoxicity and alters the normal pathophysiology of various NDs, especially AD. It impairs cognitive functions by degenerating cholinergic neurons in the cerebral cortex and hippocampus regions of the brain [11]. Aluminium enhances oxidative stress and causes neuroinflammation by activating numerous inflammatory mediators. It also promotes the formation of Aβ oligomers and the accumulation of Aβ plaques. According to previous reports, the AlCl$_3$ model is one of the suitable screening methods for evaluating the anti-Alzheimer effects of neuroprotective drugs and bioactives [12–15]. In the present work, we have formulated, optimized and characterized FS-NE and evaluated their anti-Alzheimer's activities in pharmacokinetic and pharmacodynamic studies.

Chapter 2
Review of literature

2.0. Literature review

2.1. Literature review on AD

AD is a chronic condition characterized by the degeneration of cholinergic neurons in various regions of the brain, including the cerebral cortex and hippocampus [16]. The symptoms associated with AD are categorized by cognitive dysfunction, psychotic abnormalities and problems in speaking, walking, and performing daily activities. Many pathogenic factors are responsible for the disease progression, such as accumulation of Aβ, formation of NFT, enhanced oxidative stress, neuroinflammation, mitochondrial dysfunction, endoplasmic reticulum stress, degeneration of cholinergic neurons, and excitotoxicity [5,7,17].

2.1.1. Global prevalence and economic burden

Globally, more than 44 million people have been reported with dementia till now [18]. In the USA about 5.8 million people above 65 years of age have been affected by AD. The total expenditure on healthcare, long-term care, and hospice services for individuals aged 65 and older with dementia amounted to approximately $305 billion in 2023 [19]. Additionally, projections indicate that this figure may rise to 13.8 million by the year 2050.[19]. About 4.72 million Australians have been diagnosed with dementia till the end of 2023 and this number is predicted to rise to 5.9 million by 2028 and 10.76 million by 2058 [20]. In Japan, about 3.5 million people suffered from AD till 2016 and it is expected to reach up to 4.9 million by 2026 [21]. In China, the prevalence rate of dementia was 5.60% till 2019 [22]. As per statistics, in India, around 4 million people are suffering from AD [18].

2.1.2. Pathophysiology

The Pathophysiology of AD is not fully clear but the development of AD is attributed to neuronal changes in molecular biochemical parameters. Oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, and inflammation in neuronal cells are leading causes of AD. Accumulation of Aβ and formation of NFT are observed in AD. Various cytokines such as interleukins (IL)1β, IL-6, IL-8, tumor necrosis factor (TNF)-α, and nuclear factor kappa B (NF-kB) contribute to the development of neuroinflammation, which further disrupts the normal transcription and translation processes of neuronal cells [23,24]. Free

radicals such as hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical cause abnormal mitochondrial DNA damage and negatively affect the electron transport chain (ETC) [25]. Abnormal mitochondrial function limits the function of whole cells, including endoplasmic reticulum stress, which is responsible for protein synthesis. Abnormal mitochondria also lead to attenuated protein synthesis. These pathogenic factors contribute to the degeneration of neuronal cells [26]. The term "neuroinflammation" refers to an inflammatory reaction that occurs within the central nervous system (CNS). The formation of cytokines, chemokines, reactive oxygen species (ROS), reactive nitrogen species (RNS) and secondary messengers mediates this inflammation. These mediators are provided by resident CNS glia (microglia and astrocytes), endothelial cells, and immune cells originating from the peripheral circulation [27]. These neuroinflammatory reactions have immune, neurological, metabolic, and psychological effects. Inflammation can leads to several problem in the brain such as immune cell recruitment, edema, tissue damage, and potentially cell apoptosis or death [28]. Numerous pro-inflammatory cytokines IL-1, IL-6, and TNFα, chemokines [Chemokine (C-C motif) ligand (CCL)2, CCL5, and chemokine (C-X-C motif) ligand 1 (CXCL1)], and ROS contribute to neuroinflammatory responses. These mediators are synthesised by activated resident CNS cells, such as microglia and astrocytes [28]. Nuclear factor kappa light chain enhancer of activated NFκB signalling pathway activated by TNFα and IL-6, IL-8 and IL-1β in microphase and leads to neuroinflammation and cell apoptosis [29]. Oxidative stress is an abnormal production of free radical (oxidants) generated by ROS. ROS include singlet oxygen (O_2), superoxide (O_2.), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH·). They originate from a range of sources such as, aerobic systems, oxidative metabolism, nicotinamide adenine dinucleotide (NADPH) oxidase activity, and environmental stress such as, ultraviolet (UV) radiation, and contaminants affected neurons [30]. Pathophysiology of AD and their pathogenetic biomarkers are presented in **Figure 1**.
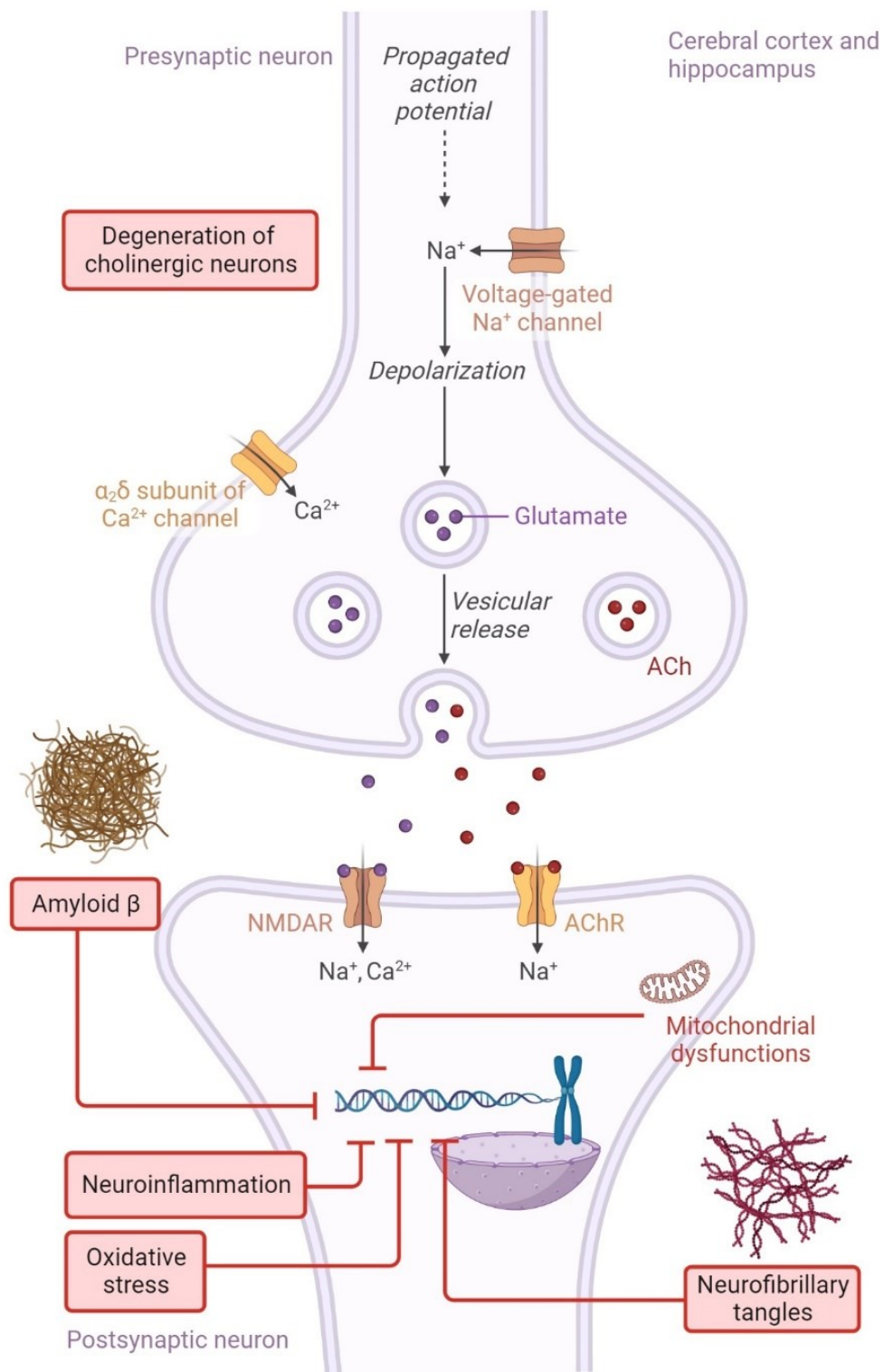
Figure 1: Pathophysiology of AD

2.1.3. Therapeutic approaches

2.1.3.1. Anti-AChE drugs

Several studies have reported that degeneration of cholinergic neurons leads to cognitive dysfunction. Acetylcholine is a neurotransmitter which is responsible for maintaining cognitive function. Degeneration of acetylcholine neurons leads to cognitive dysfunction and behavioural abnormalities. Acetylcholine is metabolised by the acetylcholinesterase enzyme, which is present in the synapse. It can degrade acetylcholine into acetic acid and choline. Acetylcholine metabolism reduces the level of acetylcholine in synapses and postsynaptic neurons [31]. Galantamine, donepezil, and rivastigmine are among the cholinomimetic drugs approved by the FDA for the treatment of AD [16]. It inhibits AChE and enhances the levels of acetylcholine in post-synaptic neurons. Donepezil is used for all stages of AD, whereas galantamine and rivastigmine are used for mild and moderate AD. These drugs produce common side effects such as hepatotoxicity, nausea, abdominal pain, diarrhoea, loss of appetite, and vomiting [32]. Recent studies suggests that AChE inhibitors attenuated accumulation of Aβ proteins which is one of the potential biomarkers of AD [33].

2.1.3.2. NMDA antagonists

The development of AD is influenced by glutamate-mediated neurotoxicity. Since glutamate is the most prevalent excitatory neurotransmitter in the mammalian central nervous system (CNS), it has a critical role in most of the CNS functions [34]. Glutamate binds with NMDA receptor. NMDA receptors are ligand-gated cationic channels. According to the glutamatergic hypothesis, excessive NMDA receptor activation can lead to an abnormal rise in intracellular calcium, which is responsible for neuronal cell death. Furthermore, degeneration of neuronal cells declines cognitive functions. Memantine is a NMDA receptor antagonist that has a quick on/off kinetics with a moderate affinity. It prevents excessive calcium influx brought by overstimulation of NMDA receptor. It reduces excitotoxicity, cell apoptosis and enhances cognitive functions [35].

2.1.3.3. Anti-Aβ therapeutics

Aβ is a fragment of the protein called APP. The exact function of APP is not defined yet. The APP is cleaved by other proteins into portions that remain within and outside of cells. In certain cases, Aβ is produced as a fragment during the diverse cleavage processes of APP. Aβ exhibits greater chemical adhesiveness compared to other fragments resulting from APP fragmentation. It progressively aggregates to form small amyloid plaques, a hallmark of the brain affected by AD. The fragments first form tiny clusters known as oligomers, then chains of clusters known as fibrils, and finally "mats" of fibrils known as beta-sheets.

These beta sheets, in later stages forms clusters and accumulate in the brain. The amyloid hypothesis states that these phases of Aβ accumulation impede cell-to-cell communication and activate immune cells. These immune cells (cytokines) initiate an inflammatory response which leads to neuronal cell apoptosis.

Aducanumab (ABC) is the most recent drug approved by the FDA for the treatment of AD. It is available in transparent to opalescent and colourless to yellow solution form. It comes in single-dose vials and is administered via intravenous infusion. ABC is human immunoglobulin gamma 1 (IgG1) monoclonal antibody. which can bind and reduce both soluble and insoluble accumulated Aβ as well as plaque formation. ABC is used for the treatment of mild AD as well as dementia [36]. ABC binds to a linear epitope, which is released by Aβ amino acids 3 to 7. After binding with a linear epitope, it suppresses the aggregation of Aβ monomers, oligomers, and polymers. ABC also inhibits phosphorylation of tau proteins and NFT[37]. ABC exhibits some common side effects like headache, diarrhoea, hypersensitivity, immunogenicity, confusion, delirium, altered mental status, and disorientation. **Figure 2** depicts the mechanism of action of various anti-Alzheimer's drugs. FDA approved anti-Alzheimer's drugs are tabulated in **Table 1**.
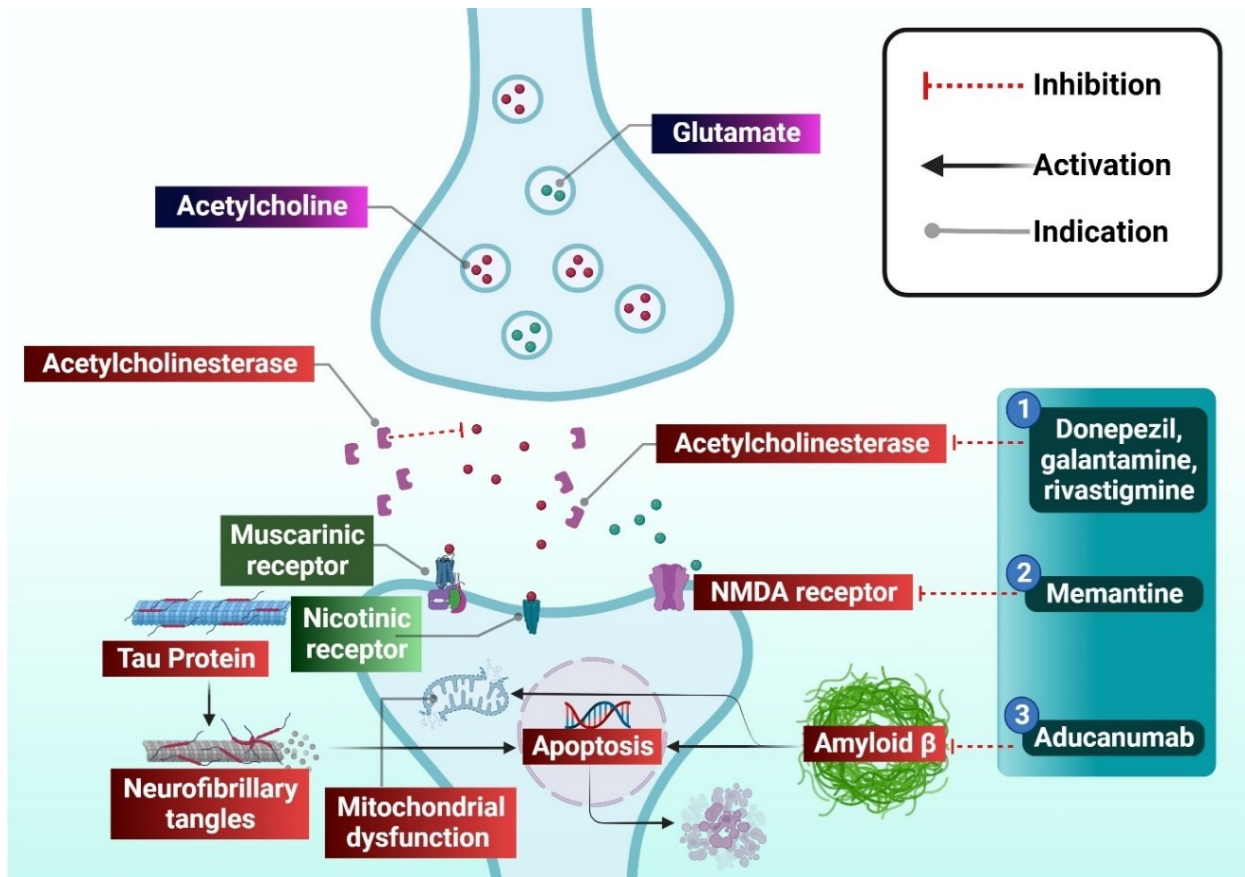
Figure 2: Mechanism of action of galantamine, donepezil, rivastigmine, memantine and aducanumab

Table 1: Drug approved by FDA for the treatment of AD

Class of the drug	Chemical name (brand name)	Drug delivery and dose	Molecular mechanism of the drug	Therapeutical uses	Side effects
Parasympathomimetic drugs	Donepezil (Aricept®, Donep®, Namzaric®, Alzil®, Donetaz®, Lapezil®, Dnp®, Adlarity®, Dopezil® and Cognidep®)	Oral (5, 10 and 23 mg) and oral disintegrates tablet (5 and 10 mg)	Donepezil inhibits the AChE enzyme and enhances the level of acetylcholine in synapse	It treats the cognitive dysfunction and improve the behavioural effects in AD. Donepezil used to treat moderate and severe stage of disease.	Common side effects: Nausea, loss of appetite and vomiting Serious side effects: Muscle cramps and diarrhoea
	Galantamine (Razadyne®, Galamer®)	Oral (4, 8 and 12 mg), extended-release capsule (8, 16 and 24 mg) and oral solution (4 mg/mL)	Galantamine is reversible AChE enzyme inhibitor and increase the function of muscarinic and nicotinic receptor which increase cholinergic neurotransmission.	Galantamine is used to treat mild and moderate stage of AD. It reduced cognitive dysfunction in AD.	Similar as donepezil
	Rivastigmine (Exelon®, Rivamer®, Exelon Tts®, Rivaplast®, Ritas, Veloxan®, Rivasmine®, Vastmin®, Rivastina®)	Capsule (1.5, 3, 4.5 and 6 mg), transdermal patch (4.6, 6.5 and 13.3 mg/24 hr)	Rivastigmine enhanced cognitive function in Alzheimer patients.	Rivastigmine is used for the treatment of mild and moderate stage of AD.	Similar as donepezil

NMDA glutamate antagonist	Memantine (Namenda®, Admenta®, Nemdaa®, Larentine®, Ebixa®, Dmentin®, Almantin®, Demenzil®, Lemix®, Alzitin®, Cormatin®)	Oral (5 and 10 mg), extended-release capsule (7, 14, 21 and 28mg) and oral solution (2 mg/mL)	Memantine is NMDA glutamate receptor antagonist. It reduces excitotoxicity induced by enhanced level of intracellular glutamate.	Memantine used to treat cognitive dysfunction and improve the behavioural effects in AD. It used to treat moderate and severe stage of disease.	Common side effects: similar as donepezil It can also show headache, constipation, confusion and dizziness.
Combination of parasympathomimetic drugs and NMDA glutamate antagonist	Memantine + Donepezil (Namzaric®, Donamen®, Alzil-M®, Larentin D®, Donecept M®, Donamind M®, Congmentin®, Dopa plus®, Memancad D®)	Capsule ER (7, 14, 21 and 28 mg/10 mg)	Combination and Donepezil work in multiple pathways such as inhibit AChE enzyme and produce antagonistic effects in NMDA receptor	It used in the treatment of moderate and severe stage of disease.	Similar as donepezil
Aβ inhibitor	Aducanumab (Aduhelm®) and lecanemab (Leqembi®)	Aducanumab: Injectable (100 mg/mL, 3 mL single-dose vial) Lecanemab: Injectable (200 mg/2mL and 500 mg/5mL single-dose vials)	Aducanumab is a monoclonal antibody, specifically an immunoglobulin gamma 1 (IgG1), designed to target aggregated soluble and insoluble forms of Aβ and soluble forms of Aβ monomers.	Aducanumab and lecanemab are used for the treatment of mild, moderate and severe stage of AD.	Common side effects as donepezil Bleeding, headache or falls

2.1.3.4. Antioxidants and anti-inflammatory

Oxidative stress and neuroinflammation play key role in progression of AD. Enhanced level of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to increased production of cytokinins such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL8, IL-1β, Nuclear factor kappa B (NFκB) [38,39]. These inflammatory mediators also promote cellular apoptosis. In the Alzheimer's brain, several inflammatory indicators, such as higher levels of cytokines and their receptors have been identified. These cytokines are responsible for various brain dysfunctions such as the production of neurotoxic free radicals, pro-inflammatory cytokines, and neurotoxic prostaglandins. Furthermore, pro-inflammatory cytokines may be involved in activating β-secretase and increasing Aβ formation, triggering a chain reaction of neurotoxic events in the brain [39]. Several preclinical studies have been conducted where the results revealed that various neuroprotective drugs and bioactives attenuated oxidative stress and neuroinflammation. Some of the important studies are mentioned below. In one of the studies, Yang and co-workers reported the neuroprotective effects of FS in mice. Lead is a heavy metal and it can cause various neurotoxic effects in the brain such as cognitive dysfunction, enhanced oxidative stress and neuroinflammation. The open maze test was conducted to check the behavioural studies. The study revealed that FS enhanced the latency (second) and reduced the number of errors in open maze test. This further revealed the improvement of mice cognitive function. FS shown antioxidant and anti-inflammatory effects by downregulating the levels of ROS and cytokines such as NFκB p65, TNF-α and IL-6 [40]. Another, study reported by Zhang et al., where FS has shown antioxidant effect in traumatic brain injury (TBI) mice. TBI is a highly intricate condition that can lead to both primary and secondary brain damage. Such injuries disrupt calcium homeostasis, induce oxidative stress, and trigger neuroinflammation in the brain.

FS has shown antioxidant and anti-inflammatory effects. It has shown neuroprotective effects by reducing primary and secondary brain injury [41].

Another study reported the antioxidant and anti-amyloid effects of quercetin (Qu) in mice wherein the oxidative stress was induced by hydrogen peroxide (H_2O_2) and H_2O_2 enhanced the levels of ROS, neuronal toxicity, mitochondrial dysfunctions. Moreover (Qu) attenuated the

toxic effects of H_2O_2 suppress oxidative stress and mitochondrial dysfunction. It has also inhibited the accumulation of Aβ in the hippocampus parts of the brain which exhibit its anti-Alzheimer's effects [42]. Chen *et al.,* have evaluated antioxidant and antiapoptotic effects of Qu in rat glioma C6 cells. Qu attenuated the levels of ROS and significantly inhibited oxidative stress in rat glioma C6 cells [43].

Furthermore, lycopene, a carotiniod aliphatic found in red coloured fruits and vegetables including tomatoes, pomegranates, and watermelons has strong antioxidant, anti-inflammatory, anti-amyloidogenic, and anti-apoptotic characteristics [44]. As a result, it has protective properties against neurodegenerative illnesses including AD and Parkinson's disease (PD) [45]. According to Liu et al., lycopene can reduce the neuroinflammation brought on by Aβ and suppress NF-κB signalling in the brain's choroid plexus during the early stages of AD. This study documented the occurrence of Aβ-induced neuroinflammation in rats. Behavioral traits were assessed using the water maze test. The in vivo examination of Aβ in conjunction with lycopene demonstrated neuroprotective effects. The anti-inflammatory and antioxidant attributes of lycopene have been elucidated in the conducted biochemical research. [46].

Prakash et al., explored the role of lycopene in restoration of BDNF and mitochondrial levels in AD which is induced by β-amyloid. They observed that the animals exposed to -A1-42 had changed brain-derived neurotrophic factor (BDNF) levels compared to the controls. In rats treated with Aβ_{1-42}, chronic treatment of lycopene improved memory retention, attenuated mitochondrial-oxidative damage, decreased neuro-inflammation, and restored BDNF levels. These investigations showed that lycopene inhibits amyloidogenesis and protects against β -A1-42-induced cognitive impairment [47]. Huang et al. documented that lycopene safeguarded neurons from oxidative damage caused by butyl hydroperoxide. The researchers administered lycopene as a pretreatment to neural stem cells (NSCs) [48].

Qu is another phytoconstituent that has neuroprotective activity against AD. The neuroprotective action of Qu is primarily attributable to the suppression of polyglutamine aggregation, AChE, amyloid-fibrillogenesis, 6-hydroxydopamine, 3-nitropropionic acid, and elevated ApoE level [49]. Mehta et al. claim that Qu, at a dosage of 30 mg/kg, has been shown to lessen chronic unexpected stress (CUS), including anxiety and depression, and to enhance

cognitive function. Along with the maze and open field, the behavior of the mice was also investigated to examine CUS. Nitric oxide, pro-inflammatory cytokines (IL-6, TNF-a, Interleukin 1 beta, and cyclooxygenase-2) and oxidative stress indicators (thiobarbituric acid reactive compounds and nitric oxide) are said to be increased by CUS. In the hippocampus, this further results in the apoptosis of neuronal cells. Qu substantially decreased the levels of all these inflammatory markers, which avoided neuronal cell injury [50]. Qu's effects on cadmium-induced neuronal damage in the frontal cortex of Sprague Dawley (SD) rats were documented by Unsal et al [50]. Qu demonstrated anti-inflammatory and antioxidant properties, which led to neuroprotective benefits. Several biochemical experiments, including catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), and histopathological analyses, were carried out to evaluate these activities. The findings demonstrated that Qu has antioxidant properties by lowering the levels of SOD, SPX, and CAT in the rats' frontal brain and raising the levels of MDA. Qu also inhibited neuronal cell degeneration in the frontal cortex region of the brain, according to histopathological examinations [50].

Similarly, Curcumin is an another important phytoconstituent which has the significant effect against AD. In respect to Aβ in AD, Ferrari and colleagues addressed the chemical connections between curcuminoids and metal chelating agents. They looked at substituted curcuminoids' metal-complexing potential as novel possible AD therapeutics. Curcumin exhibited metal chelating, anti-inflammatory, and antioxidant effects in addition to preventing the aggregation of mutant proteins such Aβ- and α-synuclein and huntingtin [51].

Traditional Chinese Medicine uses the powerful antioxidant baicalein, which is derived from Scutellaria baicalensis Georgi (Lamiaceae) (TMC). Additionally, baicalein has been shown to prevent Aβ-induced toxicity in PC12 cells, promote A-oligomerization and fibrillation, and cause preformed amyloid fibrils to disaggregate [52]. Baicalein has also been found to prevent Aβ-induced toxicity in PC12 cells, cause Aβ-oligomerization, fibrillation, and disaggregate preformed A amyloid fibrils [53].

The myricetin polyphenolic molecule, also known as hydroxyquercetin, has neuroprotective properties against Aβ-induced neuronal cell damage. Myricetin's hydrophobic properties and

low molecular weight facilitate BBB bridging, creating a good therapeutic environment. According to Shimmyo et al., myricetin, has a dual function that excludes its impact on protein expression by reducing BACE1 activity (IC_{50} =2.8 μM) and activating the α-secretase in the cell-free enzyme activity. It has been determined that the impact of myricetin on neuronal cells is less pronounced than originally anticipated [54].

An isoquinoline alkaloid known as berberine was discovered in the plant Coptis chinensis Franch (Ranunculaceae). Berberine has neuro-pharmacological effects that control APP processing and decrease Aβ protein levels. For four months, transgenic AD mice were given berberine orally at doses of 25 or 100 mg/kg per day, which shows a significant reduction in Aβ pathology and no effect on BACE1 protein levels [55].

Although the use of herbal medicines is expanding globally, their clinical applicability as medicines is constrained by their poor solubility, bioavailability, and pharmacological activity, as well as their physical and chemical instability and ease of degradation. Therefore, one potential technique for boosting their pharmacological action may be to create herbal medications with nanotechnology-based delivery systems. To assure their safety and efficacy in treating many types of illnesses, these nanotechnology-based delivery systems' development still has to be further examined, particularly with regard to their safety and toxicity profiles [56]. Some other herbal medicine and synthetic drugs and their anti-AD effects are listed in **Table 2** and **Table 3**. Antioxidant and anti-inflammatory effects of herbal drugs and their bioactive are presented in **figure 3.**
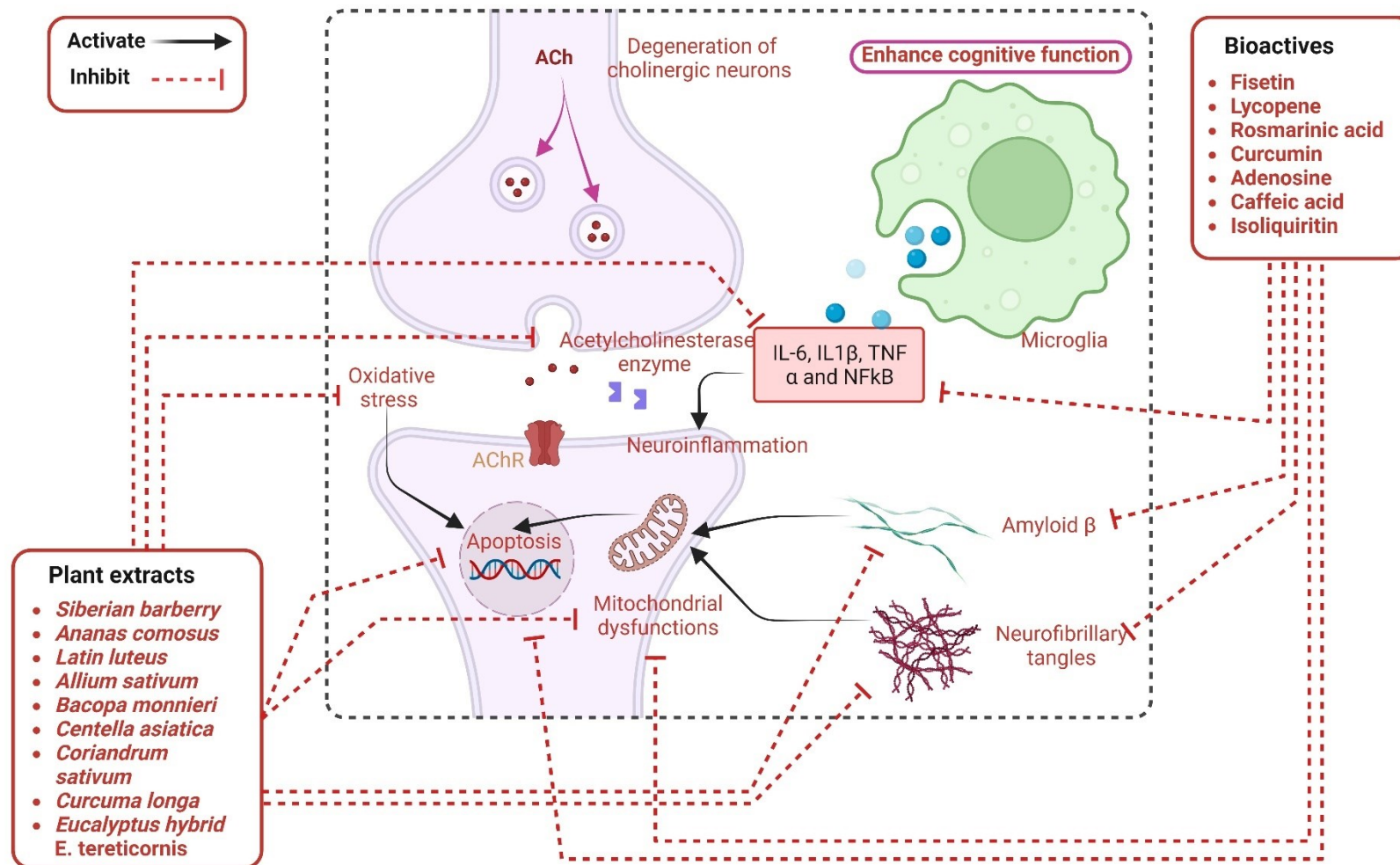
Figure 3: Antioxidant and anti-inflammatory effects of numerous plant extract and their bioactives

Table 2: Herbal medicine and its bioactive component against AD

Name of Herbal Medicine and bioactives	Bioactive Component	Animal Models	Pharmacological effects	Reference
Lycium barbarum	• Polysaccharides	Homocysteine-induced toxicity in rats	• Reverses Aβ and homocyteine induced apoptosis	[57]
Moringa olifera extract	• Qu 3-methyl ether	Homocysteine-Induced AD in rats	• Improved cognitive function • Reduced oxidative stress	[58]
Ginkgo biloba	• Qu • Kaempferol	Oxidative-stress-induced cytotoxicity in SHSY5Y neuroblastoma cells	• Improved cognitive function • Improved motor functions	[59]
Glycyrrhiza glabra	• Glycyrrhizin • Glycyrrhetic acid • Isoliquiritin	Diazepam induced amnesia in rats	• Produced antioxidant • Anti-inflammatory effects • Improve memory function	[60]
Curcuma longa	• Curcumin • Turmerone • Germacrone • Ar-curcumene	Aβ induced AD in Tg2576 mice brain	• Attenuated accumulation of Aβ • Inhibited Aβ	[61]
Salvia officinalis extract	• Cineole • Borneol • Fumaricacid	Human patients	• Inhibition of AChE • Use for mild and moderate stages of AD	[62,63]

	• Chlorogenic			
	• Acidthujone,			
	• Tannic acid,			
	• Carnosic acid			
	• Rosmarinic acid			
Rosmarinus officinalis	• Camphor	Lipoperoxidation induced AD in rats	• Inhibitor of lipoperoxidation	[64]
	• Caffeic acid			
	• Ursolic acid			
	• Betulinic acid			
Phyllanthus acidus L extract	• 4-hydroxybenzoic acid	Scopolamine-induced dementia and oxidative stress in rats	• Enhanced cognitive and learning function	[65]
	• Caffeic acid		• Produced antioxidant effects	
	• Adenosine			
	• Kaempferol		• Inhibited AChE enzymes	
	• Hypogallic acid			
Lepidium meyenii (Black maca)	• Macamides	Ovariectomy induced by AD in mice	• Enhanced cognitive functions	[66]
	• Macaene		• Inhibited neuroinflammations	
Siberian barberry	• Palmatine	Alzheimer's littermates mice	• Palmatine improve learning and cognitive function	[67]
			• Palmatine easily bypass through BBB	

Holothuria scabra extracts	--	Anti-amyloid effects in transgenic *C. elegans* model	• Inhibit Aβ • Reduced the paralysis and enhanced the chemotaxis behaviour	[68]
Indian Catechu methanolic extract	--	$AlCl_3$-induced AD in rats	• Inhibited AChE enzyme • Produced antioxidant effects	[69]
--	Rosmarinic acid	Inhibition of Aβ activities in endothelial cell	• Inhibition aggregation of Aβ	[70]
--	Ursolic acid	$Aβ_{1-42}$-induced AD in mice	• Enhanced hippocampal neurogenesis • Enhanced cognitive functions • Attenuated $Aβ_{1-42}$	[71]
--	Hesperidin methylchalcone	$Aβ_{1-42}$-induced AD in wister rats	• Attenuated $Aβ_{1-42}$ • Inhibited enzymes such as AChE and butyrylcholinesterase • Inhibited $Aβ_{1-42}$ neuroinflammations • Inhibited oxidative stress	[72]
--	FS	Galactose induced oxidative stress in mice	• Inhibited ROS • Inhibited neuroinflammations • Enhanced cognitive functions	[73]

	Lycopene	Lipopolysaccharide (LPS) induced AD in rats	• Inhibited LPS level • Reduced neuroinflammations • Reduced degeneration	[74]
--	Bromelain	AlCl₃-induced AD in rats	• Improved exploratory activity, cognitive function, anxiety, and depression in rats • Modulated thioredoxin binding protein	[75]
--	Lutein	Aβ induced AD in rats	• Inhibited Aβ • Inhibited oxidative stress	[76]

Table 3: Synthetic drugs against AD

Drugs	Animal Models	Pharmacological effects	Reference
Thiazolidin-4-one	Streptozotocin induced AD in rats	• Attenuated oxidative damages • Inhibited AChE enzyme • Inhibited tau protein	[77]
Interferon-beta	AD in rats	• Attenuated oxidative damages • Inhibit amyloid formation • Inhibit tau proteins • Anti-inflammatory effects • Antiapoptotic effects	[78]
Sitagliptin	3xTg-AD	• Enhanced learning and memory	[79]

	triple transgenic AD	• Enhanced regenerations of neuronal cells	
Acetylsalicylic acid	Human	• Low dose of acetylsalicylic acid for long term treats all stages of dementia	[80]
Fluoxetine	AlCl$_3$-induced AD in rats	• Activated Nrf2/HO-1 • Attenuated toll-like receptor 4/ NLR family pyrin domain containing 3 (TLR4/NLRP3) inflammasome signalling pathway	[81]
Amoxapine	Aβ induced AD in rats	• Attenuated accumulation Aβ multiple serotonin receptor 6 mediated targets, including β-arrestin2 and cyclin-dependent kinase 5 (CDK5)	[82]

2.1.3.5. Ongoing and completed clinical trials of AD

Currently, numerous clinical trials are underway for AD. However, only a small fraction has successfully progressed through all phases and received approval from the FDA. Different ongoing (Aug-2018 to Jan-2023) and completed clinical trials on AD are listed in **Table 4** and **Table 5.**

Table 4: Ongoing clinical trial for AD [83]

Intervention	Sample size	Phase	Design	Study start month	Estimated study completion date	NCT number
ABBV-916	195	2	R	Jan-2023	Dec-2024	NCT05291234
UPLIFT-AD	2000	NA	NR	Mar-23	Nov-24	NCT04520698
Wei Li Bai capsules	130	2	R	Jan-2023	Nov-2024	NCT05670912
MK-1942	408	2	R	Nov-2022	May 2026	NCT05602727

Active acupuncture, sham and Donepezil Hydrochloride	160	NA	R	June-2022	Dec-2025	NCT05078944
Caffeine and placebo	248	3	R	Mar-2021	Nov-2024	NCT04570085
Probiotics	40	NA	R	Dec-2021	June-2023	NCT05145881
Sargramostim	42	2	R	Jun-2022	Jul-2024	NCT04902703
Memantine and placebo	88	4	R	Jan-2019	Jun-2024	NCT03703856
LX1001	15	1,2	NR	Aug-2018	Jan-2023	NCT03634007

Abbreviation: R; randomized, NR; non randomized; NA not applicable

Table 5: Completed clinical trial for AD [83]

Intervention	Sample size	Phase	Design	Study start month	Completion date	NCT number
Donepezil HCL	171	4	R	Mar-2014	Jun-2016	NCT02097056
Donepezil HCl	97	4	NA	Dec-2007	Sep-2018	NCT00571064
Donepezil	199	4	NR	Sep-2014	Jau-2022	NCT00381381
Carvedilol	29	4	R	May-2011	Frb-2018	NCT01354444
Galantamine	99	4	NA	Jan-2010	Dec-2012	NCT01054976
ENA713	222	4	NA	Sep-2013	Feb-2017	NCT01948791
Rivastigmine	208	4	NR	Nov-2007	Apr-2012	NCT00561392
Memantine	60	4	R	May-2007	Aug-2014	NCT00476008
Memantine	12	4	NA	Oct-2007	Jan-2014	NCT00551161
Memantine, Placebo	265	4	R	May-2007	Dec-2009	NCT00469456
Memantine, Placebo	277	4	R	Mar-2009	Sep-2012	NCT00862940

Rivastigmine transdermal patch	228	4	NA	Frb-2008	Jul-2011	NCT00622713
Donepezil hydrochloride	14	4	NA	May-2007	Nov-2021	NCT00477659
Rivastigmine patch	142	4	R	Oct-2007	May-2011	NCT00549601
Methylphenidate and Placebo	60	4	R	Jul-2007	Nov-2015	NCT00495820
Cilostazol and Placebo	46	4	R	Aug-2011	May-2014	NCT01409564
ENA713	121	4	NA	Apr-2012	Apr-2018	NCT01585272
Neuraceq (florbetaben 18F)	218	4	NA	Feb-2018	Nov-2018	NCT02681172
Simvastatin and placebo	49	4	R	Jun-2010	Jun-2017	NCT01142336
Florbetapir (18F)	96	4	NA	Apr-2014	Jul-2015	NCT02107599
Risperidone	180	4	R	Jan-2007	Apr-2013	NCT00417482
Ramipril	14	4	R	Sep-2009	Aug-2020	NCT00980785

Abbreviation: R; Randomized, NR; Non randomized; NA not applicable

2.2. Literature review on FS

2.2.1. Pharmacological activities and molecular targets of FS for AD

FS exhibited numerous neuroprotective effects for the treatment of AD. It is a polyphenolic flavonoid. Chemically, it is a 2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one. The chemical structure of FS is presented in **figure 4**. FS has shown anti-AD effects by attenuating tau aggregation, fibril formation of Aβ, deoxyribonucleic acid (DNA) damage, oxidative stress [ROS, superoxide dismutases (SOD) 1], neuroinflammation (TNF-α, IL-6, IL-1β, nitric oxide, NF-κB), mitochondrial functions and cognitive function. A pictorial illustration of the neuroprotective effects of FS is presented in **Figure 5**. Several works have been conducted where FS showed neuroprotective effects against AD. Some of the studies showing the potential effects of naïve FS in AD are discussed below.
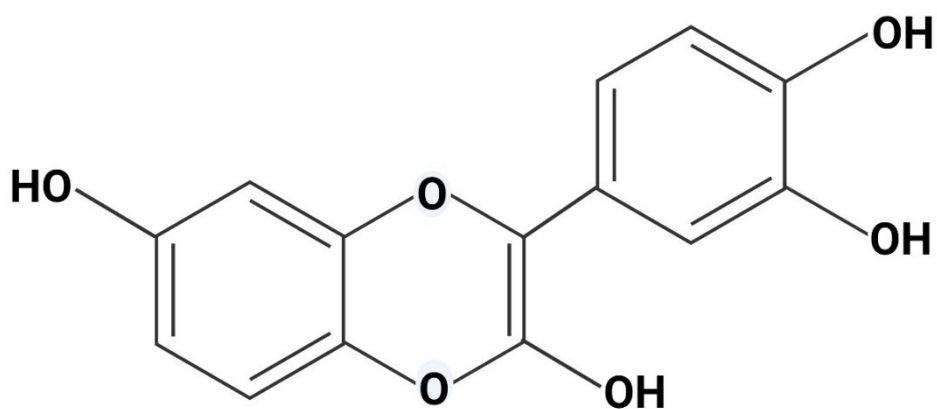
Figure 4: Chemical structure of FS

FS has showed neuroprotective effects against cognitive dysfunction, oxidative stress and neuroinflammation induced by lead in male institutional cancer research (ICR) mice. Pb is a heavy metal which causes neuronal cell death, cognitive dysfunction, inflammation, and oxidative stress. In order to evaluate its anti-AD potential in mice, behavioural and biochemical studies were conducted. In behavioural test, percentage latency and the numbers of errors were evaluated in open-field test in ICR mice. FS increased latency and reduced the number of errors in open-field tests. In the biochemical evaluation, FS has downregulated the levels of NFκB p65, TNF-α and IL-6 and reduced neuroinflammations [84]. In another study, Ahmad *et al.,* have reported anti-neuroinflammatory effects of FS against $A\beta_{1-42}$ induced cognitive and synaptic dysfunction in adult mice. The intraperitoneal (i.p.) administration of FS at dose of 20 mg/kg for two weeks improved memory efficiency in $A\beta_{1-42}$ treated mice by decreasing escape latency, increasing time spent in the goal quadrant, and increasing platform crossings throughout the in Morris water maze (MWM) test [85]. Cho *et al.,* (2013) have investigated neuroprotective and anti-inflammatory activities of FS in male ICR (Harlan Sprague–Dawley; 4 weeks old) mice. Various tests such as cell viability, 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, scopolamine-induced cognitive dysfunction, SOD and reduced glutathione (GSH) were evaluated. The results indicated that FS produced inhibitory effects in

MTT assay and upregulated the level of SOD and GSH. It also attenuated the effects of scopolamine, thereby improved cognitive function [86].

FS has been reported for its anti-inflammatory effects by inhibiting pro-inflammatory cytokines (IL-1, IL-6, and TNFα), chemokines (CCL2, CCL5, and CXCL1), NF-κB, toll-like receptor 1, etc. FS has been also reported to reduce inflammation-induced oxidative stress. Several studies have been conducted where FS has shown anti-inflammatory effects. Some of the important works carried out on anti-inflammatory potential of naïve FS are discussed below.

In a recent study, FS suppressed lead (Pb) induced apoptotic neurodegeneration, as shown by lower Bax and cleaved caspase-3 levels. FS has been reported to inhibit the activation of Toll-like receptor 4, myeloid differentiation factor 88, and βB, as well as pro-inflammatory factors such as IL-6 and TNF-α. It also increased phosphorylation of synaptosomal-related protein-25 (SNAP-25), postsynaptic density-95 (PSD-95), cyclic AMP response element binding protein (CREB), and calcium/calmodulin kinase II (CaMKII) in Pb-induced synaptic dysfunction [84]. In another study, FS has been reported to attenuate the synthesis of (lipopolysaccharide/gamma interferon) LPS/IFN-γ induced nitric oxide (NO) and peptidoglycan-induced inflammatory mediator IL-1β as well as decreased the expression of inducible nitric oxide synthase (iNOS) in microglial cell, migration of microglial cell and generation of ROS [87]. Additionally, another study reported FS to cause downregulation in the level of proinflammatory cytokines and suppressed NF-κB signalling pathway in intracerebral haemorrhage-induced injuries [88].
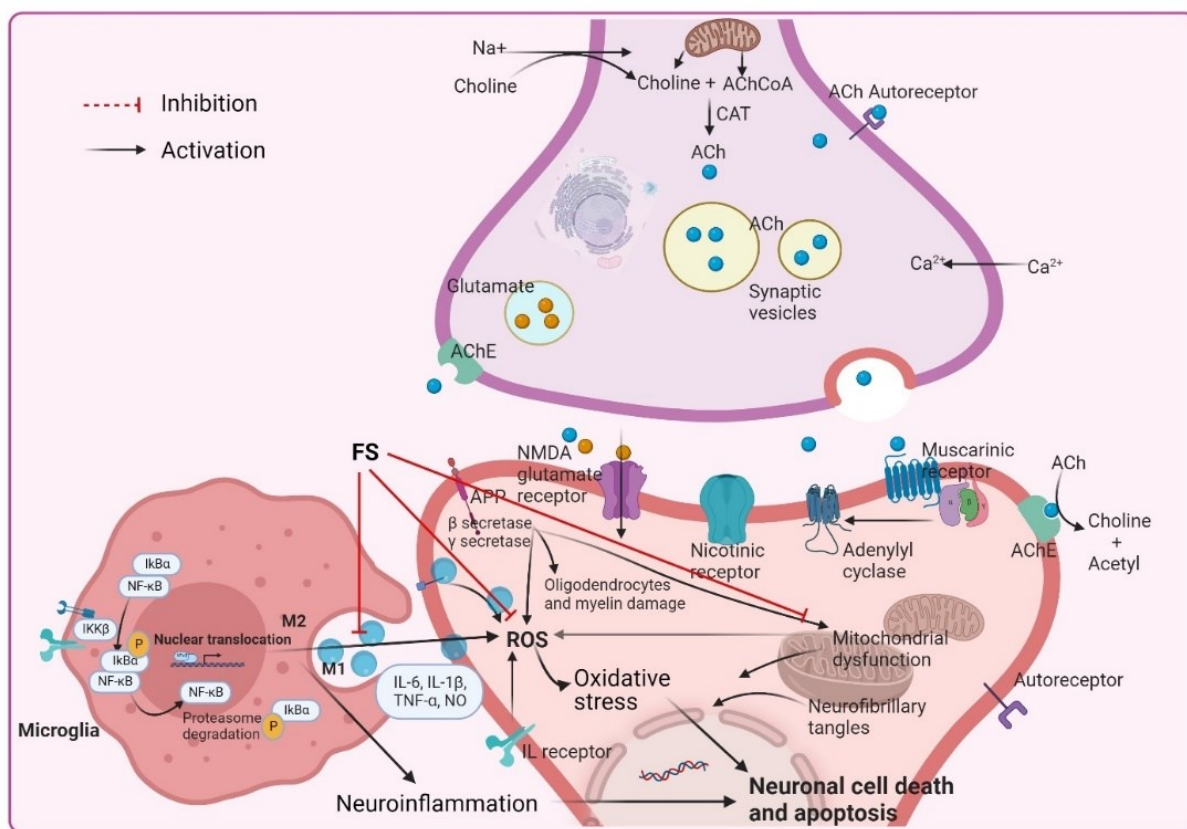
Figure 5: Molecular mechanism of FS against AD

2.3. *Advanced drug delivery system-based drugs for the treatment of AD*

Several studies have been reported where advanced drug delivery system-based nano-formulations are used to reduce symptoms of AD. In one of the studies, Ahmad *et al.,* found that Qu-loaded SNEDDS with the drug increased its bioavailability for the treatment of cerebral ischemia and degeneration of the neurons. The droplet size and zeta potential of Qu-loaded SNEDDS were found 94.63 ± 3.17 nm and -17.91 ± 1.02 mV respectively. The SNEDDS were developed for *in situ* formation of oil-in-water NE. [89]. Qu-loaded gold-palladium (AuPd) nanoparticles developed by Liu and coworkers against Aβ aggregation and mHtt genes for the treatment of AD and HD. Due to their lipidic composition and nanosize, Qu nanoparticles stimulated through autophagy and reduced Aβ aggregation in neuronal cells. These Qu nanoparticles also exhibited neuroprotective properties by attenuating the cytotoxicity generated by Aβ. The histopathological picture demonstrated that Qu loaded AuPd

nanoparticles exhibited no toxicity in the cortex, hippocampus, and thalamus regions of the mice brain as compared to the control group [90]. Some of the important studies are mentioned in **table 6**.

Table 6: Advanced drug delivery system based various drugs and bioactive substance for the treatment of AD

Sr. No.	Therapeutic moiety	NDDS	*In vivo* model	Method of preparation	Research highlights	References
1.	Rivastigmine	Nanoparticles	Wistar rats	Spontaneous emulsification	• Enhanced 1.96 folds of oral bioavailability compared with naïve rivastigmine • Reduced oxidative stress	[91]
2.	Pomegranate	Nanoparticles	$AlCl_3$ induced Wistar albino rats	Spontaneous emulsification	• Increased 3.25 folds of oral bioavailability compared with naïve drug • Attenuated thiobarbituric acid level from cerebral cortex • Shown antioxidant effects	[92]
3.	Curcumin + selenium	Nanoparticles	5XFAD mice	Emulsion solvent evaporation process	• Inhibited Aβ aggregation	[93]
4.	Nanoparticle-Chelator Conjugates	Nanoparticles	AD transgenic animal	-	• Inhibited Aβ aggregate formation • Protect human cortical neurons from Aβ oxidative toxicity	[94]
5.	Aβ Protein Antibody	Nanoparticles	AD transgenic	Hyeon method	• Target cerebrovascular amyloid formation	[95]

			mice (Tg2576)		• Enhanced passive immunization	
6.	Qu	Nanoparticles	Scopolamine induced amnesia	Spontaneous emulsification	• Enhanced memory function • Reduced oxidative stress	[96]
8.	Phosphatidic acid, or a modified Apolipoprotein E-derived peptide (mApoE) functionalized liposome	Liposomes	APP/PS1 Tg mice	Thin film hydration	• Sequester A β 42 in human biological fluids	[97]
9.	mApoE–PA	Liposomes	APP/PS1 Tg male mice	Thin film hydration	• Reduced brain Aβ aggregates • Reduced Aβ levels in mice brain	[98]
10	Qu	Lipid nanoparticles (SLN and NLC)	*In vitro* study in hCMEC/D3 cells	Hot homogenization technique	• Reduced fluorescence intensity of thioflavin T (ThT) in Aβ (1–42).	[99]
11.	Thymoquinone	nanoparticles	Mice	Single-emulsion solvent-evaporation technique	• Reduced SOD and oxidative stress • Reduced Aggregation of Aβ	[100]
12.	Selenium-chondroitin sulfate nanoparticles	Nanoparticles	SH-SY5Y cells	--	• Reduced aggregation of Aβ in SH-SY5Y cells • Reduced SPD, ROS, MDA, GSH-Px	[101]

13.	Erythropoietin	Solid Lipid Nanoparticle	Rats	Double emulsion solvent evaporation method	• Reduced aggregation of Aβ • Enhanced cognitive functions.	[102]
14.	Chiral penicillamine-capped selenium nanoparticles	Nanoparticles	Mice	--	• Inhibited aggregation of Aβ • Enhanced cognitive functions.	[103]
15.	Zinc	polymeric nanoparticles	APP23 mice	Double emulsion solvent evaporation method	• Inhibited aggregation of Aβ • Attenuated Pro-inflammation (IL-6, IL-8)	[104]
16.	Mesoporous silica	Gold nanoparticle	Mice	--	• Attenuated aggregation of Aβ • Reduced ROS	[105]
17.	Curcumin	magnetic nanoparticles	Mice	Antisolvent precipitation method	• Nanoparticles enhance penetration of the BBB	[106]
		Polylactide-co-glycolic-acid nanoparticles	PC12 cells	--	• Inhibited aggregation of Aβ • Inhibited formation of Aβ	[107]
18.	Sialic acid	Selenium nanoparticles	PC12 cells (neuroblastic cells and eosinophilic cells)	Homogeneous method	• Reduced aggregation of Aβ • Anti-apoptosis effects	[108]

19.	Resveratrol and Grape Extract	Solid lipid nanoparticles	HEK293 cells	High shear homogenization method	• Reduce aggregation of Aβ	[109]
20.	Chitosan	Polymeric nanoparticles	Rats	--	• Reduced oxidative stress • Enhanced cognitive function	[110]
21.	Memantine	NE	Rats	Homogenisation and ultrasonication methods	• Inhibited oxidative stress	[111]
22.	Magneto-plasmonic nanoparticles	Metallic nanoparticles	Rats	--	• Metallic nanoparticles work as a biosensor for the detection of tau protein	[112]
23	Tanshinone IIA	Chitosan nanoparticles	--	--	• ↑ accumulated Aβ • ↑ UNC-51, BEC-1, ATG-7, LGG-1 and ATG-18 • ↓ Oxidative stress	[113]
24.	Doxorubicin	β-cyclodextrin-poly(β-amino ester) nanoparticles	BME cells	--	• ↑ Permeability across the *in vitro* BBB models	[114]
25.	--	Gold nanoparticles	C57BL/6 inbred strain mice	--	• ↑ Permeation of BBB both *in vitro* and *in vivo* models.	[115]
26.	--	Gold nanoparticles	--	--	• Large AuNPs induce amorphous aggregates on the brain lipid bilayer. • ↓ Accumulated Aβ	[116]

31

| 27. | -- | Amine-modified gold nanoparticles, citrate-modified gold nanoparticles | -- | -- | • ↑ Positively charged gold nanoparticles attached to Aβ more tightly | [116] |
| 28 | Qu | -- | HBME cells | -- | • Enhanced permeability of the drug through paracellular drug delivery
• Attenuated level of Aβ | [117] |

2.3.1. NE

NE is colloidal dispersion systems containing two immiscible liquids that are stabilized with the addition of surface-active agents. Mostly, ionic or non-ionic surfactants are used in the NE which prevents flocculation due to electrostatic and steric stabilization. Furthermore, the nano droplets in the system are in continuous brownian motion, hence, it predominates over the gravitational force. This creates high resistance against droplet aggregation and therefore system maintains kinetic stability. However, the main instability issue reported in NE is Oswald ripening. The smaller globules grow into larger ones and finally separate out which leads to instability problems [118].

Typical, NE contain three components such as oil, water and emulsifier that are mixed in appropriate ratios to form simple NE or multiple NE. The simple NE are classified as oil in water (O/W) or water in oil (W/O). Multiple NE contain dispersed phase which in turn contain another internal droplet that use to be similar to the dispersion medium and it can be O/W/O or W/O/W. Hence, the composition of the system plays a significant role in determining the type of system [119]. Both the internal phase and external phase of NE contain multiple ingredients. Structure of O/W and W/O are presented in figure 6.

The oil phase represents the lipophilic portion of the NE and generally, this component is selected based on the solubility of the lipophilic active moiety. Edible oils, semisynthetic

triglycerides, synthetic oils, etc are frequently used in NE. Emulsifiers are amphiphilic molecules that are added to reduce the interfacial tension between the oily and aqueous phases of the dispersion. These agents stabilize the system through electrostatic repulsive forces and steric hindrance. Usually, surfactants are used as an emulsifier, but proteins, polysaccharides and lipids are also used as stabilizers. The added emulsifier will act at the interface of the two immiscible phases where the hydrophilic head interacts with the hydrophilic phase and the hydrophobic tail interacts with the oily phase. The selection of surfactants is important and they are preferably selected based on hydrophilic lipophilic balance (HLB) value. Surfactants with low HLB values ranging from 3 to 6 are selected for preparation of W/O type and higher HLB values ranging from 8 to 18 are chosen for O/W type. Mostly non-ionic surfactants such as sorbitan derivatives (Span 20, 40, 60, 80), polyoxyethylene sorbitan derivatives (Tween 20, 40, 60, 80), polyoxyethylene derivatives (Brij 30, 35, 50,56, 58) are chosen as emulsifiers for preparation of pharmaceutical NE.

2.3.1.1. Method of preparation of NE

For the preparation of thermodynamically stable NE, generally employed preparation methods include high energy method and low energy method. The high-energy method involves the use of mechanical devices which create disruptive forces resulting in the breakdown of the internal phase and later disperse it into a continuous phase. Some of the methods grouped under high energy techniques are stirring, ultrasonication (bath and probe), high-pressure homogenization, microfludization and membrane emulsification. The low-energy technique is advantageous over the high-energy technique. It ensures the least energy consumption and includes phase-inversion emulsification either by change in temperature or change in composition. Various techniques used to prepare NE are shown in **Table 7**.

The NE can load both hydrophilic and amphiphilic drugs; hence they can serve as a carrier to cross the BBB. Along with their high loading capacity, possible surface modification of the system can enable them to act specifically to the targeted site. The mechanism of enhancing oral bioavailability of drug using NE is similar to that of SNEDDS that is discussed in next section. The various advantages of NE is presented in Figure 7.
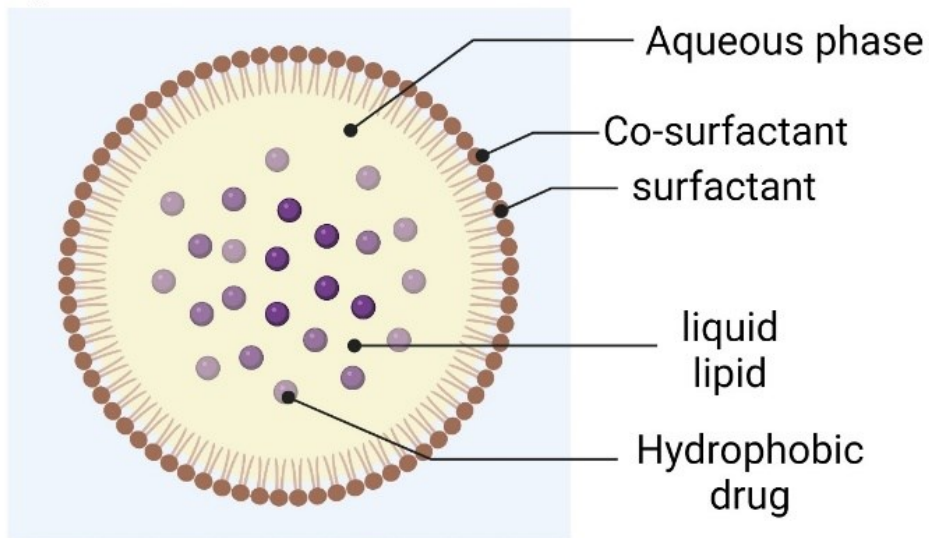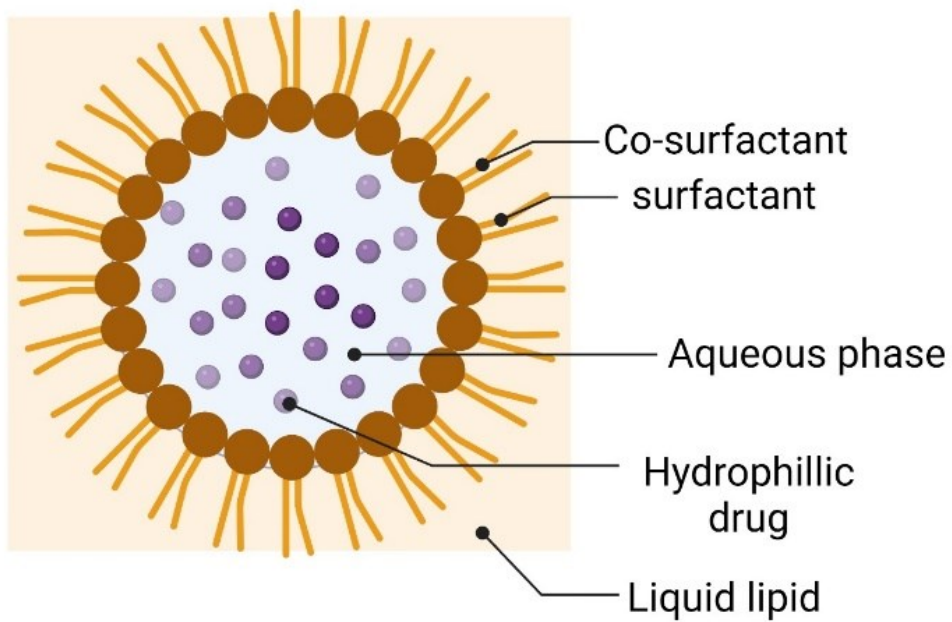
Figure 6: Structure of O/W and W/O nanoemulsion
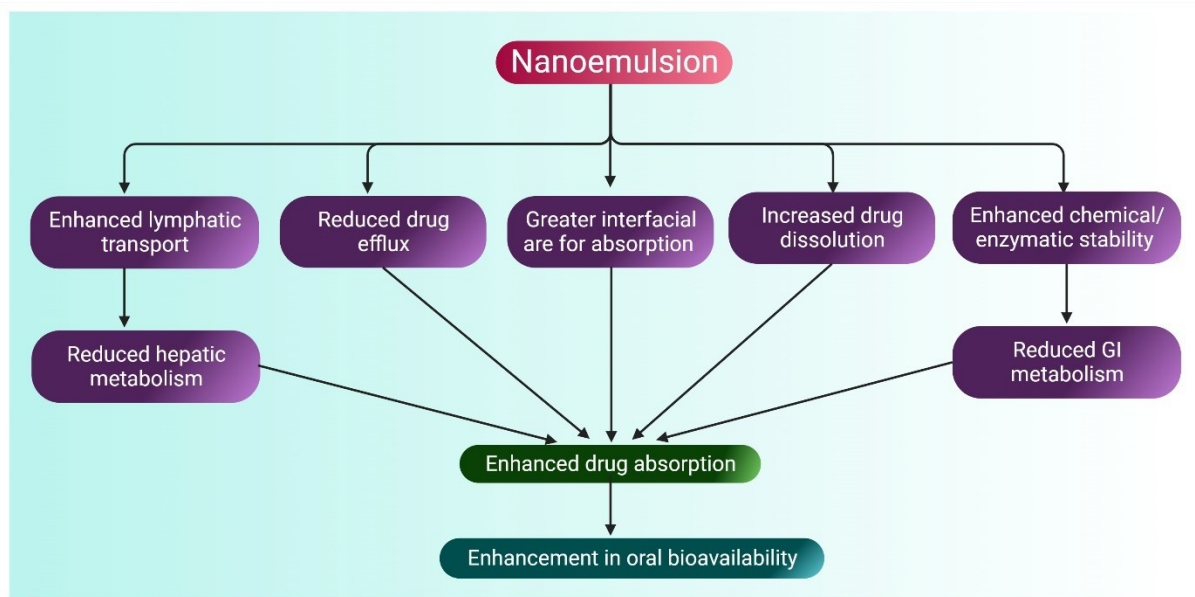
Figure 7: Advantages of NE

Table 7: Various techniques for preparing NE with their merits and limitations

S No.	Method of preparation of NE	Advantage	Disadvantage	Reference
1	Extreme emulsification	• Appropriate for a wide range of compositions beyond the dilute ϕ limit • Droplet size distribution tailored through both flow rate and compositional control	• More energy input required compared to low-flow methods • Apparatus can require significant up-front investment • Viscous heating can become appreciable at high-flow rates	[120]

2	Phase inversion composition	• Versatility of routes • Low-energy process • Not necessary to supply heat	• More limited range of compositions compared to high-flow methods • More prone to Ostwald ripening • Good mutual solubility of oil and water is needed • Limited to mostly nonionic surfactants	[121]
3	Phase inversion temperature	• Low-flow process	• Requires heat energy input • Limited stable T range • More prone to Ostwald ripening • Good mutual solubility of oil and water is needed	[122]
4	Micro/nanofluidic flow focusing	• Forms monodisperse droplets • Moderate flow	• Requires microfluidic apparatus • Limited range of flow rate ratios that produced NE-sized droplets • NE-sized droplets demonstrated for W/O only	[123]
5	Satellite Droplets	• Moderate flow	• Satellite droplet size and production are limited by • Viscosity ratio η_d/η_c • Radius before breakup • Interfacial tension • Producing a NE from satellite droplets	[124]

6	Membrane emulsification	• Low-pressure process • Forms monodisperse droplets if a membrane having a narrow pore size distribution is used	requires separation from the larger droplets • Coalescence of droplets near pores can occur wetting must be overcome and flow of outer liquid is needed to aid droplet detachment	[125]
7	Liquid-liquid nucleation (Ouzo effect)	• Low-energy process	• Continuous phase requires high mutual solubility • Need small solubility of oil in one continuous phase component	[126]

2.3.1.2. Characterization of NE

2.3.1.2.1. Appearance

The appearance of emulsion depends on the size of the droplets and varies from clear (smaller droplets) to opaque (larger droplets). When the size of the droplets is within the range of 10 to 100 nm, a clear and transparent nanoemulsion is obtained [118].

2.3.1.2.2. Droplet size and polydispersity index

The long-term stability of any nanocarriers is evaluated from their characteristic mean droplet size and droplet size distribution. These parameters are generally evaluated following the principle of light scattering technique. Diverse light scattering techniques have been employed for measuring globule size of nanoemulsion and are briefly discussed below with their respective methodologies, merits, and demerits [127–129].

2.3.1.2.3. Zeta potential

Zeta potential measures the surface charge of the droplets and helps in predicting the stability of NE. Electric repulsion due to electric charge existing between droplets prevents their agglomeration during storage. Thus, physically stable emulsions generally exhibit high zeta

potential values. ASX-NANEs were reported to possess good physical stability with a zeta potential of −31.2 ± 0.6 mV [130,131].

2.3.1.2.3. *Drug loading and entrapment efficiency*

Analytical techniques, such as ultraviolet-visible spectroscopy and high-performance liquid chromatography (HPLC), are used for the quantitative determination of the amount of drugs loaded in NE. Determination of entrapment efficiency requires separation techniques such as centrifugal filtration, solid-phase extraction, and centrifugation before its quantitative analysis. Entrapment efficiency constitutes a critical parameter that estimates drug-carrier affinity which, in turn, also determines the stability of the designed formulation [132].

There are various other techniques are used to characterized NA such as solid phase extraction, electrophoretic light scattering, TEM, cryo-TEM, laser diffraction techniques, atomic force microscopy, scanning electron microscopy, FTIR spectroscopy, etc. Those characterization techniques of NE and their significate are tabulated in **Table 8** and presented in figure 8.

Table 8: Characterization techniques of NE along with their significance.

S. No.	Characterization technique	Parameter characterized	Advantages	References
1.	Appearance	Droplet size	Fast, does not require any equipment	[133]
2.	Dynamic light scattering (DLS)/Photon correlation spectroscopy	Droplet size and PDI	Non-destructive, accurate, reliable, requires only a minute quantity of sample	[134]
3.	Static light scattering technique	Shape, size and structure of droplets	Non-destructive, possible to analyse partially hydrated/ gels/ solution samples	[135]
4.	Laser diffraction technique	Mean droplet diameter	Precise and fast	[136]
5.	Electrophoretic Light Scattering	Zeta potential	Non-destructive, precise, fast	[136]

6.	Centrifugal filtration	Drug loading and entrapment efficiency	Accurate and fast	[137]
7.	Solid-phase extraction	Drug loading and entrapment efficiency	Accurate, reproducible, automatable and fast	[138]
8.	Centrifugation	Drug loading and entrapment efficiency	Cost effective	[139]
9.	Optical microscopy	Morphology, structural imaging	Cost effective, easily available	[140]
10.	Transmission electron microscopy (TEM)	Morphology, structural imaging	Information about the internal morphological structure and size of the droplet	[134]
11.	Cryo-TEM	Morphology, structural imaging	Reveals internal structure also, no need of staining	[141]
12.	Atomic force microscopy	Morphology, structural imaging	Reveal droplet morphology and size of nano-emulsion	[142]
13.	Scanning electron microscopy	Used for visualizing Pickering emulsions	Reveal droplet morphology	[143]
14.	FTIR spectroscopy	Chemical compatibility between drug-excipients, vehicle/surfactant cross-linking, and drug loading	Gives information about the chemical interaction between the individual components, blank formulation, and drug-loaded formulation	[144]
15.	NMR spectroscopy	Provides extensive information regarding the chemical nature of the atomic nuclei	Gives information on morphological transition	[145]
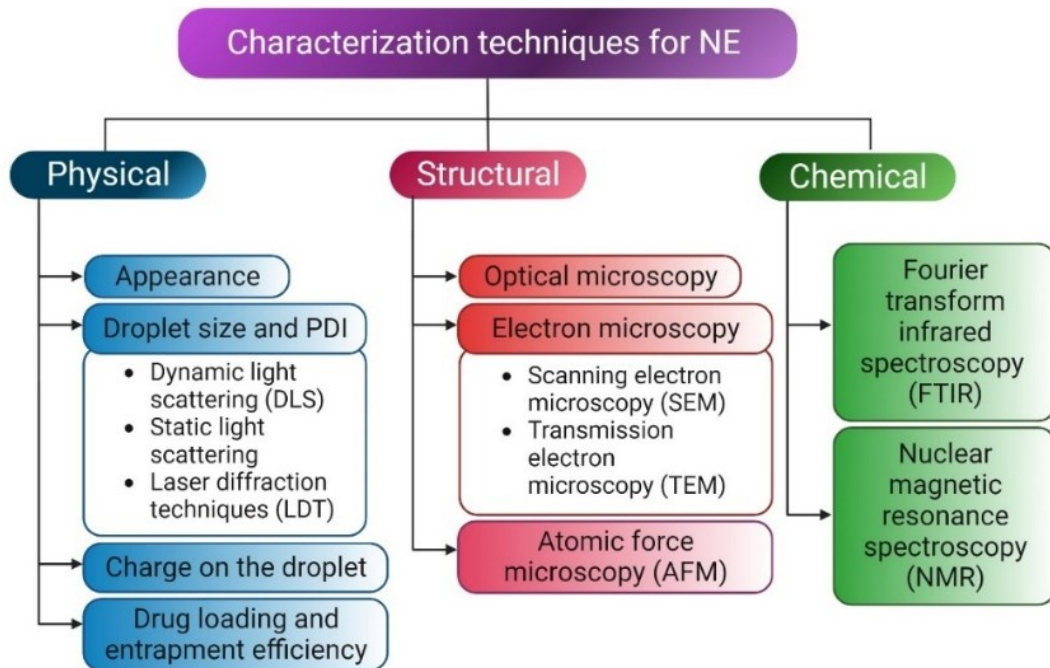
Figure 8: Characterization of NE

2.4. Animal models of AD

Various studies have been reported were post few decades researches observed behavioral symptoms of rodents (Rat and mice). Animal models played significant role in analysing treatment strategies of AD. To induced AD, numerous neurotoxins induced into rodents such as $AlCl_3$, streptozotocin, scopolamine, $A\beta$, okadaic (OKA) acid etc. These neurotoxins cause cognitive dysfunction, motor abnormalities and enhance symptoms of AD. Various animal models of AD are tabulated in Table 9.

Table 9: Various animal models used for inducing AD

Sr No.	Animal model	Species	Dosage	Biomarker	Reference
1.	$AlCl_3$	• Male Albina Wistar rats (Age 6-12 Months) •Male Sprague-Dawley (SD) rats (Age 6-16 weeks)	• $AlCl_3$ (4.2 mg/kg, intra peritoneal (i.p.)) 28 days studies • $AlCl_3$ (100 mg/kg, oral), 84 days study	• ↑ $A\beta$ • ↑ NFT • ↑ Neuroinflammation • ↑ Cognitive dysfunctions • ↑ ROS • ↑ AChE enzyme	[146–148]

2.	Aβ$_{1-42}$	• Male fisher rats of (Age 18-20 weeks) • Long-Evans hooded rats (Age 2-3months) • Male/female, aged Rhesus monkeys (Age 25-28 years) • Male/female aged Marmoset monkeys (Age 8-10 years) • Male/female vervet Monkeys (Age 19-23 years)	Acute/continuous infusion of Aβ (5μg/1μl) Continuous Infusion of (10/20 μg /3 days) into right ventricle	• ↑ Aβ • ↑ NFT • ↑ Neuroinflammation • ↑ Cognitive dysfunctions • ↑ ROS • ↑ AChE enzyme • Abnormal glutaminergic functions [149–152]
3.	Streptozotocin induced AD	• Male Albino Wistar rats (Age 03-04 months) • Either sex SD rats (Age 02-05 months) • Either sex Cynomolgus monkey (Age 05-08 years)	Administered 3mg/kg intracerebroventricularly every 48 hours in two doses for rodents, and delivering 2 mg/kg intracerebroventricularly divided into four doses for monkeys.	• ↑ Aβ plaques • ↑ NFT • ↑ Neuroinflammation • ↑ Cognitive dysfunctions • ↑ ROS • ↑ AChE enzyme [153–155]

			• ↑ Tauopathy	
			• ↑ Reactive gliosis	
4.	Scopolamine induced amnesia	• Male albino rats (Age 4-months) • Male/ female Swiss albino mice (Age 10-12 weeks)	Scopolamine 0.3, 0.5 1, 3 mg/kg (i.p.) (Duration of 28 days) and 72 µg/0.5 µl (i.c.v) (Duration of 21 days).	• ↑ Aβ • ↑ NFT • ↑ Neuroinflammation • ↑ Cognitive dysfunctions • ↑ ROS • ↑ AChE enzyme • ↑Cholinergic neurotransmitter decline	[156–158]
5.	Methionine	• Male Wistar albino rats or mice (aged 3 months)	1.7 g/kg per, orally (Duration of the study 32 days)	• ↑ Aβ • ↑ NFT • ↑ Neuroinflammation • ↑ Cognitive dysfunctions	[159,160]

				• ↑ ROS	
				• ↑ AChE enzyme	
				• ↑ endothelial dysfunction	
6.	Diazepam induced cognitive and motor impairment	• Male Swiss albino mice (Age 3 - 4 and 12 - 15 months)	Diazepam 1mg/kg., (i.p) (Duration of the study 28 days)	• ↑ Cognitive dysfunction • ↑ AChE enzyme • ↑ Astrogliosis • ↑ NFκB	[161,162]
7.	AF64A induced memory deficits	Male Wistar rats (Aged 7-8weeks) Male SD rats (Age 4-5 weeks)	AF64A 2, 6 and 8 nmol/2μl, i.v.c.	• ↑ AChE enzyme • ↑ Cholinergic neurotransmitter dysfunction	[163,164]
8.	Cholesterol and copper sulfate induced	New Zealand white rabbits (Age 3-4 months)	2% cholesterol + 0.12 ppm of copper ions, i.v.c	• ↑ Aβ • ↑ ROS • ↑ Reactive gliosis • ↑ Apoptosis	[165,166]

No.	Name	Model	Dose/Concentration	Effects	References
9.	Lysophosphatidic acid induced AD	•Mouse, neuroblastoma N2a cell line	25µM	• ↑ Aβ • ↑ NFT • ↑ Neuroinflammation • ↑ Mild cognitive impairment • ↑ ROS	[167,168]
10.	Okadaic (OKA) acid	Two to three-month-old male and female SD rats	Okadaic acid at the concentration of 70 ng/day icv, for the duration of 14 days	• • ↑ Aβ • ↑ NFT • ↑ Neuroinflammation • ↑ Cognitive dysfunctions • ↑ ROS • ↑ AChE enzyme	[169,170]

2.4.1. Limitations of various animal models used for the induction of AD

Developing models for the study of AD presents numerous challenges and limitations. It is crucial to acknowledge and consider these factors when interpreting the results. Models often simplify the intricate nature of AD, a condition characterized by multifactorial and progressive neurodegeneration. Consequently, certain elements of the disease may not be comprehensively reflected in these models. The replication of human pathophysiology by animal models is not perfect, and species differences pose limitations on the generalizability of findings from animal studies to human contexts [171]. Many models specifically concentrate on familial forms of AD linked to specific genetic mutations, overlooking the more prevalent sporadic forms that involve intricate interactions between genetic and environmental factors [172]. The precise temporal and spatial progression of Alzheimer's pathology in the human brain may be challenging for models to accurately capture. Additionally, when modelling the cognitive and behavioral aspects of AD in animals, the complexity and breadth of symptoms observed in humans may not be fully replicated [173]. The development of dependable biomarkers for AD in model systems might not entirely mirror the diverse spectrum of biomarkers observed in human patients. Furthermore, the establishment and upkeep of models for AD research can be demanding in terms of both time and financial resources. Among all the inducing models of AD, we have used $AlCl_3$ to induce AD due to its ability of marked impairment of working memory. Cognitive dysfunction and behavioral changes induced by Al have been documented in numerous studies, leading to a notable decline in working memory [174]. Treatment with $AlCl_3$ is associated with a significant rise in AChE activity. This elevated AChE activity is likely attributed to the genetic upregulation of AChE under oxidative stress, facilitated by an allosteric interaction between the cation and the peripheral anionic site of the enzyme. This interaction further amplifies AChE activity. Rats treated with $AlCl_3$ exhibit a substantial decrease in overall brain function compared to control rats. The hallmark pathological feature of AD is the deposition of Aβ plaques in the brain. Treatment with $AlCl_3$ is known to induce notable changes in the expression of proteins associated with Aβ metabolism, exacerbating Aβ deposition [175]. The accumulation of plaques and neurofibrillary tangles leads to neuronal dysfunction, synaptic destruction, and eventual impairment of neurotransmission. Collectively, these findings may offer an explanation for the observed neurochemical dysfunctions and

behavioral changes associated with the use of $AlCl_3$. Moreover, the mechanism underlying the harmful effects of co-administering $AlCl_3$ on the antioxidant defense system is notable. Elevated lipid peroxidation (LPO) contributes to neuronal damage and has the potential to trigger NDs such as AD. In conditions of oxidative stress, SOD plays a pivotal role by initially converting excess superoxide (O^{2-}) radicals into hydrogen peroxide radicals (H_2O_2) [176]. Subsequently, glutathione peroxidase (GPx) and CAT work to scavenge these H_2O_2 radicals, resulting in the production of water and molecular oxygen. The beneficial free radical scavenging properties of SOD are realized when followed by the scavenging activity of GPx and CAT. However, an excess of SOD coupled with decreased CAT and GPx activity is known to have a deleterious impact on tissues. MDA serves as a widely used biomarker for oxidative stress, formed as an end product of LPO induced by free radicals. Due to attenuated levels of cholinergic neurotransmitters, enhanced Aβ, oxidative stress, and neuroinflammation, $AlCl_3$ is considered one of the excellent models to induce AD. Aluminium is a neurotoxin that causes severe degeneration of neurons in the central nervous system (CNS). According to previous reports, histopathological images clearly show that $AlCl_3$ induces brain degeneration of neurons in rats [177].

Chapter 3
Rationale and plan of work

3.1. Rationale and plan of work

The available literature on AD and current therapies used to treat it indicated that the disease is progressing rapidly in aged people and currently available therapies are unable to completely cure the disease. This could be due to multiple pathways associated with AD's pathology, which cannot be inhibited by the use of a single drug as they have their unique mechanism. Some combination therapies have also been explored to overcome the issues associated with monotherapies. However, they also gained little success. Moreover, the synthetic drugs that are currently being used, cause some side effects such nausea, vomiting, decreased appetite, more frequent bowel movements, headaches, constipation, disorientation, and dizziness. Looking on these challenges, a safe and effective therapy is required that can work through inhibition of multiple pathways associated with AD. Plant based bioactives such as polyphenols have been found to be very effective to treat AD due to their action through inhibition of multiple pathways associated with AD and safety profile. Among them, FS has been reported to be very effective in treating AD through its oral administration due to its antioxidant, anti-inflammatory, senolytic and antiapoptotic activities. Despite this FS possesses dissolution rate limited oral bioavailability due to its lipophilic nature. Hence, its therapeutic efficacy to treat AD in its naïve form is limited. In addition to this the drugs administered through oral route have some other challenges such as first pass metabolism and poor blood brain permeability. Looking towards the challenges associated with oral delivery of naïve FS to treat AD, an attempt is made to deliver FS through NE. The advantage of NE includes ease of preparation using simple and single step procedures, higher drug loading and ease of oral administration. The NE upon oral administration reaches to GIT, where it remains in the form of micelles and does not allow the entrance of any GIT component within themselves. Thus, the drug loaded in it gets protected from the harsh environment of GIT (i.e., pH changes and enzymatic degradation). In addition to this, the absorption of NE takes place from lymphatic route; thus, the hepatic metabolism of drug gets bypassed. This further increases the oral bioavailability of FS loaded in NE. Upon intestinal as well as lymphatic absorption of the NE, it reaches to the brain through systemic circulation where it passes the BBB due to nanometer size of the droplet and elicits its action. The oral delivery of FS loaded NE to treat AD is reported for the first time. The hypothesis of present study reported in **Figure 9.**
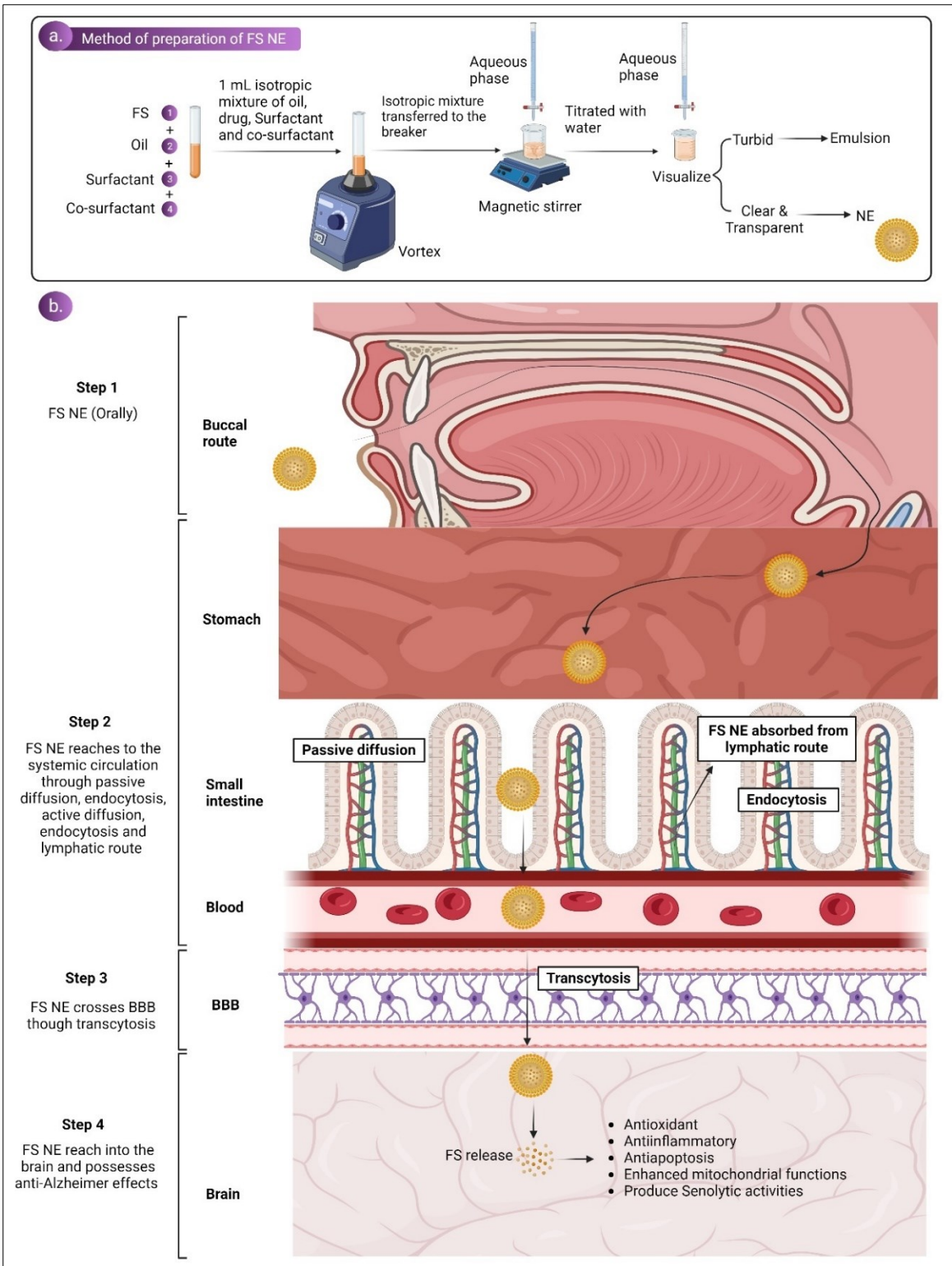
Figure 9: Hypothesis

3.2. Aim

The aim of this study is to develop an oral NE-loaded FS for effective treatment of AD.

3.3 Objectives

The objectives of the present study are to:

- Formulate and optimize FS loaded NE

- Characterize the optimized NE

- Assess/evaluate the stability of optimized NE

- *Evaluate the in vitro* and *in vivo* performance of NE

Chapter 4
Methodology

4.0. Materials and Methods

4.1. Materials

The list of various materials and equipment's used are summarized in **Table 10** and **Table 11** respectively.

Table 10. List of materials used in the study.

Chemicals	Manufacturers
Acetonitrile HPLC Grade	Ranbaxy laboratories Pvt. Ltd. India
$AlCl_3$	Ranbaxy laboratories Pvt. Ltd. India
Capmul MCM EP/NF	M/S Abitec Corp., Ohio
Cotton seed oil	Ranbaxy laboratories Pvt. Ltd. India
Ethanol	Ranbaxy laboratories Pvt. Ltd. India
Eucalyptus oil	Central Drug House (CDH) Pvt. Ltd., New Delhi, India
FS	Tokyo chemical industries, Japan
Formalin	Ranbaxy laboratories Pvt. Ltd. India
Formic acid	Ranbaxy laboratories Pvt. Ltd. India
Labrafil M I944CS	Gattefosse Pvt. Ltd., Mumbai, India
Labrasol	Gattefosse Pvt. Ltd., Mumbai, India
Lauroglycol FCC	Gattefosse Pvt. Ltd., Mumbai, India
Mustard oil	CDH Pvt. Ltd., New Delhi, India
Orthophosphoric acid	Lobachemie Pvt. Ltd., Mumbai, India
PEG 200	CDH Pvt. Ltd., New Delhi, India
Polyvinyl alcohol	CDH Pvt. Ltd., New Delhi, India
Transcutol P	Gattefosse Pvt. Ltd., Mumbai, India
Tween (80, 20)	CDH Pvt. Ltd., New Delhi, India

Table 11. List of equipment's used in the study

Equipment	Model/Manufacturer
Centrifuge	REMI RM-12C, Remi Elektrotechnic. Ltd, Vasai, India, Mumbai, India
Differential scanning calorimeter (DSC)	DSC 6000 PerkinElmer, USA
Disintegration apparatus	LabIndia, Analytical Instruments, Pvt. Ltd., Mumbai, India
Electronic weighing balance	CY360, Shimadzu Co. Ltd., Kyoto, Japan
HPLC	Analytical Ptv. Ltd.
Hot air oven	Cadmach Drying Oven, Cadmach Machinary Ltd., Ahmadabad, India
Homogenizer	Glass-Teflon potter homogenizer, Thomas Scientific, USA
Magnetic stirrer	Remi 5MLH, Vasai, Mumbai, India
Melting point apparatus	Popular, India
Optical microscope	KYOWA- Getner Instruments Pvt. Ltd., Japan
Particle size analyzer	Malvern Zetasizer, Nano ZS90, UK
pH meter	Phan, Lab India, Mumbai, India
Powder x-ray diffractometer	Bruker D8 Advance, USA
Scanning electron microscope	Jeol JSM-7610F Plus, Japan
Shaking water bath	LabFit, India
Stability chamber	Remi CHM 10S, Remi Sales & Engineering Ltd., Mumbai, India
Transmission electron microscope	JEM-2100 plus Electron microscope, Jeol, Japan
Ultrasonication bath	Loba Life, Lobachemie, Mumbai, India
UV spectrophotometer	UV-1800, Shimadzu Co. Ltd., Kyoto, Japan
Vortex mixer	REMI CM101, Delhi, India

0.45 μm syringe filter	Merck, Germany
0.22 μm syringe filter	Merck, Germany

4.2. Computer programs and softwares

Table 12: List of computer softwares

Softwares	Manufacture
BioRender®	Crunchbase Pvt. Ltd. Toronto, Canada
Chem Draw Ultra 12.0 Software®	CambridgeSoft Corp, Cabridge, USA
Design Expert (Ver. 11.0.5.0) ®	Stat-Ease, Inc., Minneapolis, USA
DD Solver®	China Pharmaceutical University, Nanjing, China
GraphPad Prism® 8	GraphPad Inc., La Jolla, USA
Mendeley® Dekstop (ver. 1803)	Elsevier, London, UK
Triplot 4.1.2	Todd Thompson Software

4.3. Pre-formulation studies

4.3.1. Drug Identification

FS was purchased from Tokyo Chemical Industry Co. Ltd. The FS was characterized for its physicochemical properties such as melting point, log p, differential scanning calorimeter (DSC), Powder X-ray diffraction (PXRD) and SEM.

4.3.1.1. Melting point determination

The melting point was observed by using two methods: Capillary method and DSC.

4.3.1.1.1. Capillary method

The capillary was sealed at one end by heating on a flame. FS was placed in a thin-walled capillary tube of 10-15 cm length and a diameter of 1 mm. The capillary containing the drug and a thermometer was placed in the melting point apparatus and was heated evenly. The

capillary tube was observed through a glass window and the temperature at which the sample melted was noted as the drug's melting point [178].

4.3.1.1.2. DSC

The melting point of FS was also determined by DSC (DSC calorimeter, Universal V4.7A TA Instruments, India). Initially, 2 mg of the sample was placed in standard aluminum pans with lids, with a continuous supply of liquid nitrogen at a rate of 10 mL/min. The temperature range was set between 25°C and 300°C, and the temperature ramp rate was adjusted to 10°C/min [179].

4.3.1.2. PXRD

PXRD (Bruker AXS, D8 Advance, USA) is used to determine to analyzed nature of the compound such as amorphas and crystalline. Powdered FS inserted in an aluminum holder, and the spectra were scanned using copper Ka radiation. The test was conducted at a constant voltage and current (40 mA) over a two-angle range of 10-80 ℃, with a slow angle scan of 0.01/min and a sample interval of 0.02/s [180].

4.3.1.3. SEM

The SEM was used to analyze the morphological features of FS. A uniform distribution of FS was applied to one side of a double-sided adhesive tape, which was then affixed with an aluminum stub on the opposite side. In the case of pellets, a cross-sectional cut was made using a blade, and the section was secured with an aluminum stub. Subsequently, the aluminum stub underwent gold coating under high vacuum conditions. Scanning was performed at a voltage of 10 kV [181].

4.3.1.4. Scanning of adsorption maxima of FS

FS was dissolved in methanol and scanned at the concentration of 10 µg/mL for determination of absorption maxima (λ_{max}) in the range of 200-400 nm on UV spectrophotometer against methanol as blank [182].

4.3.1.5. Analytical method development using HPLC
4.3.1.5.1. Preparation of ortho-phosphoric acid (OPA) solution

A 0.2 % v/v solution of OPA was prepared by adding 200 µL OPA in 100 mL of distilled water and the final pH was checked using a pH meter and it was found 2.13. The solution was then filtered through 0.45 µm Nylon membrane filter and degassed in a sonicator for the duration of 15 min [183].

4.3.1.5.2. Preparation of standard stock solutions

FS (10mg) was weighed and diluted to 100 mL methanol. The solution was sonicated for 15 min to attain the concentration of 100 µg/mL ≡ 100000 ng/mL. From this solution, 10 mL was taken and diluted to 90 mL of methanol to attain the concentration of 10 µg/mL ≡ 10000 ng/mL. Furthermore, from this solution 10 mL was taken and diluted to 100 mL of methanol, to attain the concentration of 1 µg/mL ≡ 1000 ng/mL. The final concentration was maintained at 0.01 µg/mL ≡ 10 ng/mL following the same procedure as above. From the concentration of 0.01 µg/mL ≡ 10 ng/mL, 1 mL was taken, and further diluted to 9 mL of methanol to get the concentration of 0.001 µg/mL ≡ 1 ng/mL. Additionally, 2 mL was taken from 10 ng/mL, and further diluted to 8 mL of methanol to get the concentration of 0.002 µg/ml ≡ 2 ng/mL. Moreover, 4 mL, 6 mL, 8 mL and 10 mL were taken from 10 ng/ml and diluted to 6ml, 4ml and 2ml of methanol to attain the concentration of 0.004 µg/ml ≡ 4 ng/ml, 0.006 µg/ml ≡ 6 ng/ml, 0.008 µg/ml ≡ 8 ng/ml and 0.01 µg/ml ≡ 10ng/ml respectively [183]. The schematic diagram of serial dilution is presented in the figure 10.

4.3.1.5.3. Method development

The method development of FS was reported by Kumar *et al,* [184]. A method for quantitatively measuring FS was created and confirmed using HPLC. For analysis of the drug, it was injected to injection site of HPLC. Acetonitrile (ACN) and 0.2% OPA in distilled water at the ratio of 30:70 v/v were used as mobile phase and C-18 reverse phase column was used as stationary phase. The rate of flow was established at 1.0 mL/min, and the detection wavelength was configured to 362 nm. Standard solutions within the concentration range of 2–10 ng/mL were prepared [184].

4.3.1.5.4. Method validation

To validate method of FS various parameters were analysed such as linearity and range, system suitability, LOD, LOQ, accuracy, precision, and robustness. The validation process was

performed concordance with ICH Q2 (R1) guidelines. The following parameters are mentioned below [185].
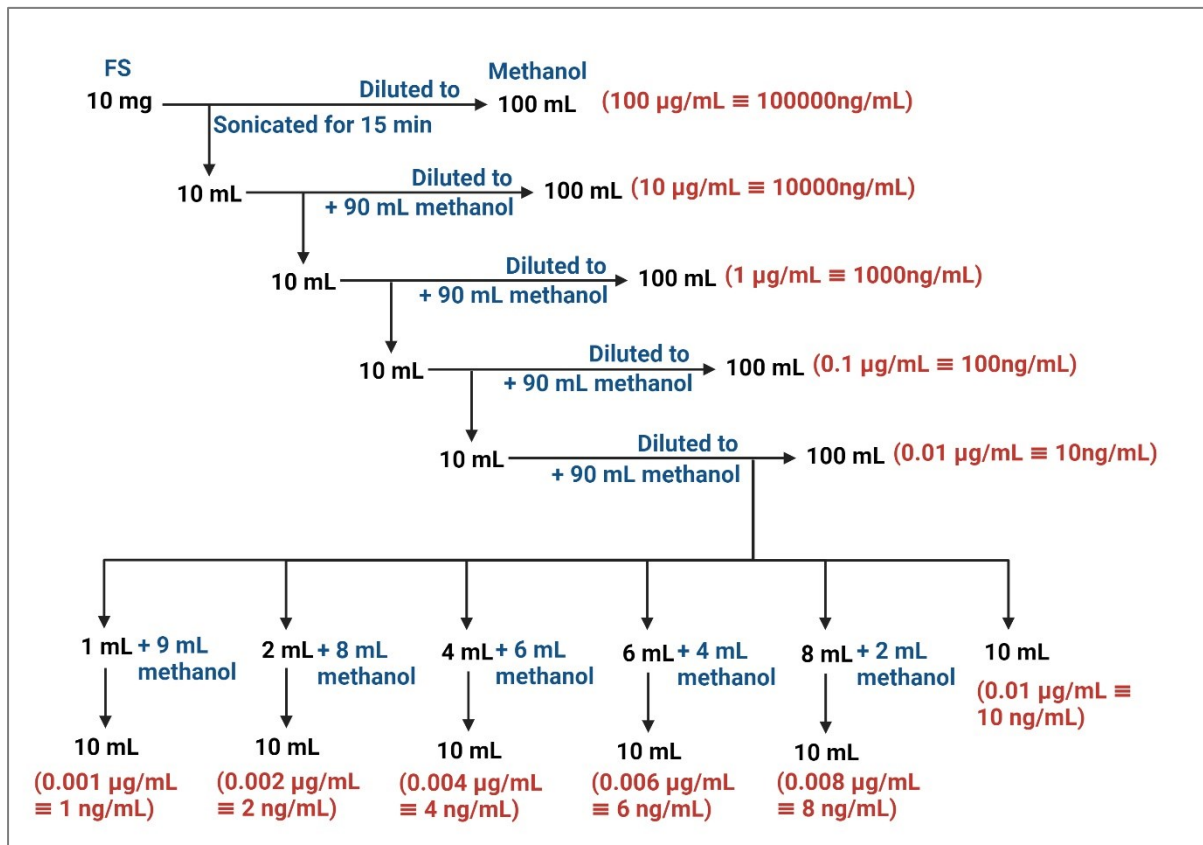
Figure 10: Preparation of standard stock solutions

4.3.1.5.5. *Linearity and range*

The range was selected by injecting minimum concentration of FS (10 ng/mL). Further, different dilutions of FS was injected to HPLC. Total five injections of each concentration of FS were analyzed and the mean area were recorded. Afterwards, a calibration curve was plotted in the range of 2-10 ng/mL. Slope, intercept and correlation coefficient of the calibration curves (peak area versus concentration) were determined to ensure linearity of the analytical method [186].

4.3.1.5.6. *System suitability*

In the system suitability study, various parameters were measured. These include peak area, peak hight, tailing factor, theoretical plates/meter, and peak purity index. According to the ICH Q2(R1) guidelines in the system suitability tailing factor, theoretical plates and resolution of the peaks must be under acceptance criteria such as tailing factor must be less than 2, peak purity index should be smaller than 0.5, and theoretical plates/meter should always be smaller than 20000 [182].

4.3.1.5.7. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of FS was calculated from the calibration curve. LOD is the quantity of analyte that can be detected at low concentration whereas, LOQ is the lowest concentration of analyte that can be determined by acceptable accuracy and repeatability [184]. The formula of both LOD and LOQ are mentioned in Eq. 1 and Eq.2.

$$LOD = \frac{3.3\sigma}{S} \qquad\qquad Eq.(1)$$

$$LOQ = \frac{10\sigma}{S} \qquad\qquad Eq.(2)$$

4.3.1.5.8. Accuracy

In order to evaluate the accuracy of the developed method three different concentration were taken i.e lower quantified concentration (LQC, 4.8 ng/mL) at 80 % level, middle quantified concentration. (MQC, 6 ng/mL) at 100 % level and higher quantified concentration (HQC, 7.2 ng/mL) at 120 % level and their % mean recovery was calculated. To prepare these samples an aliquot of 4.8 mL, 6 mL and 7.2 mL were taken from 10 ng/mL (solution A) and added separately in 10 mL volumetric flasks. The volume of each sample was made upto 10 mL by using methanol. The prepared samples were run for 5 times on the HPLC system and % recovery of each drug was calculated [184]. The formula to calculate absolute absolute recovery (%) is showed in Eq. 3.

$$Absolute\ recovery\ (\%)\frac{Actual\ concentration\ recovered}{Theoritical\ concentration} \times 100 \qquad Eq.(3)$$

4.3.1.5.9. Precision

The precision of the developed method was evaluated by checking repeatability and intermediate precision. In order to evaluate repeatability, the samples of 4.8 ng/mL, 6 ng/mL and 7.2 ng/mL were run on HPLC at the same day (intra-day) by following the same experimental conditions. Intermediate precision was determined by injecting six samples of each 4.8 ng/mL, 6 ng/mL and 7.2 ng/mL on different days (inter-day) as well as by different analyst (inter analyst). The average area of each six runs of 4.8 ng/mL, 6 ng/mL and 7.2 ng/mL were recorded and % relative standard deviation (% RSD) was calculated. The formula to calculate % RSD is shown in Eq. 4. Additionally, an inter-analyst study was conducted by having three different analysts each prepare and inject LQC, MQC, and HQC samples six times under identical experimental conditions. The mean data were recorded, and the percentage relative standard deviation (% RSD) was calculated [184].

$$\% \ relative \ standard \ deviation \ (\% \ RSD)$$
$$= \frac{Standard \ deviation \ of \ peak \ area}{Average \ peak \ area} \times 100 \qquad Eq.(4)$$

4.3.1.5.10. Robustness

Robustness of the analytical method was studied by varying flow rate (0.8, 1.0 and 1.2 mL/min), mobile phase ratio (28:72, 30:70 and 32:68) and pH (2.9, 3.1, and 3.3). The six replicates of MQC (6 ng/mL) sample were injected in the HPLC. Their average mean area, % recovery, retention time were recorded. In addition, their % RSD were also calculated [184].

4.3.1.5.11. Specificity

The effect of excipients used in the formulation on FS's peak was checked with the help of specifity study. The specificity study was carried out in order to check any possible interaction of excipients with the drugs. Each solubilizer and excipient used in formulation such as Capmul MCM EP/NF®, Tween 80, and Transcutol P, was diluted either with ethanol or hexane, depending upon their solubility, and injected into HPLC. The specificity of the proposed HPLC method for the determination of FS was established by injecting the mobile phase and placebo NE solution into the HPLC system [184]. Additionally, the black chromatograms of different solubilizers used during solubility study were recorded.

4.3.2. Solubility studies

The solubility of FS was determined in all excipients such as oil, surfactant, and co-surfactant. The oils used in the study were Labrafil M 1944CS®, Capmul MCM EP/NF®, Labrafac lipophile WL 1349®, cotton seed oil, eucalyptus oil, peanut oil, castor oil, and coconut oil. Furthermore, Tween 20, Transcutol P®, T80, Labrafac PG®, Lauroglycol FCC®, Captex 300 EP/NF®, and Maisine CC® were used as a surfactant and co-surfactant. One mL of above-mentioned emulsifiers were transferred in 5 mL glass vials individually. To this 5 mg of FS was added. The mixture was stirred using a cyclone mixer for 2 minutes. Samples were agitated in a shaking water bath for the duration of 48 h at 37 ± 2 °C. After 48 h, sample were centrifuged for 30 minutes at 4930 g. Further, the supernatant of the samples was separated carefully and filter through 0.22 μm syringe filter. The filtered supernatant was dilted with ethanol or hexane and the concentration of FS in various solubilizers were analyzed with the help of HPLC [187].

4.3.3. Formulation, development and optimization of FS-NE

4.3.3.1. Formulation of NE

The NE was formulated through the spontaneous emulsification method. The oil, surfactant, and co-surfactant were mixed in a cyclone mixture to develop a homogeneous isotropic mixture, and the aqueous phase was gradually added until a clear and transparent NE was achieved [188]. Method of preparation of FS-NE is shown in figure 11.

4.3.3.2. Pseudo-ternary phase diagram (PTPD) of FS-NE

Capmul MCM EP/NF®, Tween 80, and Transcutol P® were chosen as excipients for the formulation of the NE because the maximum solubility of FS was found in these emulsifiers. A total of 27 NE prototypes were formulated by varying the ratio of Tween 80, and Transcutol P® in proportions of 1:1, 1:2, and 2:1. The ratio of oil to Smix was used within the range of 1:9 to 9:1. The selected oil, surfactant, and co-surfactant were mixed to prepare the NE prototypes. The method of preparation of FS-NE are discussed in section 4.3.3.1. The PTPD of the FS-NE was prepared using triplot software version 4.1.2 (Todd Thompson Software). Samples that were transparent were classified as NE, while those that appeared white, milky, and opaque were classified as emulsions. The formulation underwent visual assessment for

upto 48 h to monitor potential phase separation, precipitation, and creaming of the drug molecule. The NE prototypes of FS were prepared, and the optimal batch was selected based on criteria such as drug loading, cloud point (°C), centrifugation stability, mean droplet size, polydispersity index (PDI), and zeta potential [189]. The procedure to conduct these studies are mentioned in the section 4.3.3. 5 and 4.3.3.8.
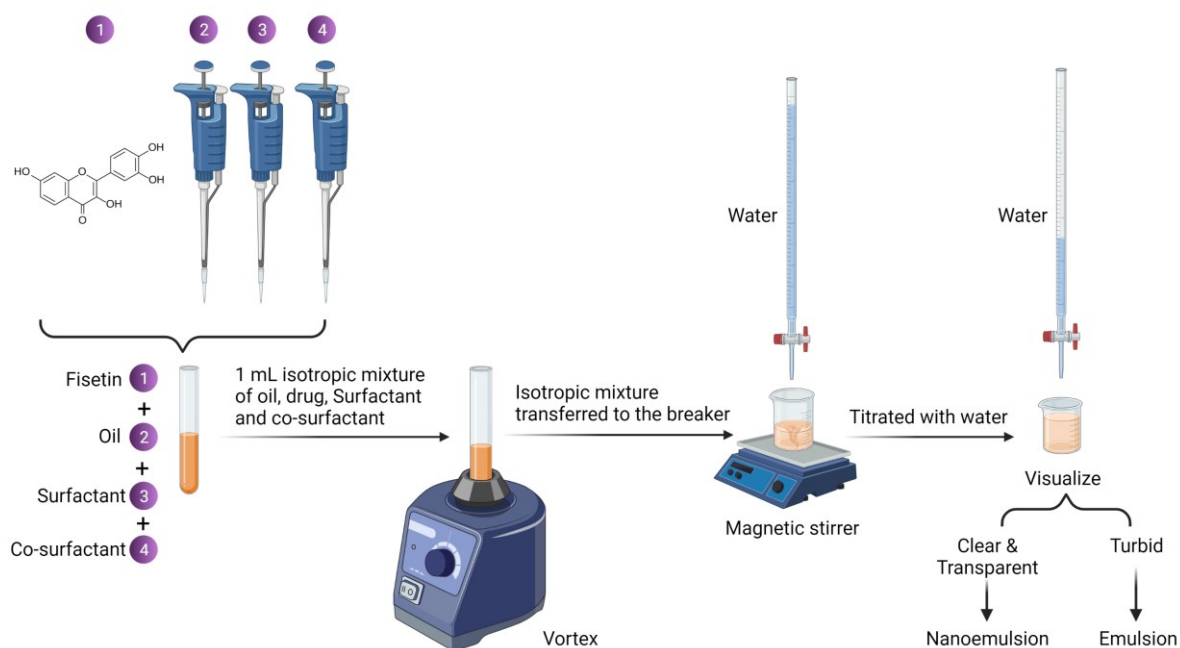
Figure 11: Method of preparation of FS-NE

4.3.3.3. Design of experiment by using box Behnken design (BBD)

The PTPD indicated the excellent NE region when oil (Capmul MCM EP/NF®), surfactant (Tween 80) and co-surfactant (Transcutol P®) utilized in the range of 10–20 %, 45–60, and 15–30 %, respectively. The three levels and three factors Box-Behnken design was used in Design Expert software 11 Stat-Ease Inc. 1300 Godward Street Northeast, Suite 6400 Minneapolis, MN 55413. In this design, the ratio of independent variables i.e. oil (X_1), surfactant (X_2), and co-surfactant (X_3) was radially varied by the software. The effect of each one of the selected factors at their coded levels were evaluated with respect to the response variables (dependent variables) such as droplet size (Y_1), zeta potential (Y_2), PDI (Y_3), and %

drug loading (Y_4). Considering the number of independent variables and their levels, 14 experimental runs were designed using BBD.

The experimental runs were utilised to assess the linear quadratic effects of independent variables on dependent variables. The impact of these independent variables was examined using DoE software. The software produced linear Eq. (5) and quadratic Eq. (6) models, representing the influence of each independent variable on the chosen dependent variables (responses). Statistical analysis through analysis of variance (ANOVA) was then conducted to analyze these effects.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 \ldots\ldots\ldots\ldots.\text{Eq. 5}$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_4 +$$
$$\beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 \ldots\ldots\ldots\ldots.\text{Eq. 6}$$

Y indicated measured response of the dependent variables; β_0 is the intercept; β_1 to β_{44} are the regression co-efficient; X_1, X_2, and X_3 are independent variables.

The statistical analysis of the obtained responses for the dependent variables was conducted using ANOVA. Based on the obtained responses, design expert software generated, perturbation plots, 2D-contour plots and 3D response surface plots, which was used to assess independent variables influenced the dependent variables. Further, it helped to create design space through the assessment of response parameters, the design generated an optimized composition of FS-NE, which was validated by checking droplet size, zeta potential, PDI and % drug loading [189]. The procedure to conduct these studies are mentioned in the section 4.3.3. 7 and 4.3.3.8.

4.3.3.4. Formulation and evaluation of optimized NE

Based on DoE, optimized formulation obtained and further numerous parameters were evaluated such as mean droplet size, zeta potential, drug loading, and PDI.

4.3.3.5. Estimation of thermodynamic and centrifugation stability, cloud point

In order to analyze thermodynamic stability, the NE prototypes of PTPD and optimized FS-NE were placed separately into two different temperature cycle viz. freeze thaw cycles (−21 °C

and +25 °C) and heating cooling cycle (4 °C and 40 °C). Centrifugation stability was evaluated by centrifuging aforementioned samples at 10000 g for the duration of 20 min. Furthermore, cloud point of the formulations was evaluated by diluting FS-NE in 10 mL of the distilled water and the samples were placed over water bath. The temperature was increased slowly from 10 °C to 100 °C and change in transparency of emulsion was observed. The temperature at which the cloudiness appeared in the emulsion was considered as cloud point. While performing experiment continues temperature were observed. The result of cloud point was recording by analyzing clear NE to opaque in nature [190].

4.3.3.6. Droplet size, zeta potential and PDI

All parameters i.e. droplet size, zeta potential and PDI were analyzed using Malvern zeta sizer nano ZS90 (Malvern Instruments Ltd., UK). FS-NE were placed into cuvettes constructed from polystyrene, positioned at a 90-degree angle, and subjected to laser light with an intensity of 50 mV. An isotropic mixture of 10 mL mixed with 10 mL of distilled water. Each experiment was replicated three times, and the average results were recorded [191].

4.3.3.7. Drug loading

To analyzed drug loading 10 mg of FS was dissolved in 5 mL of distilled water. To obtained clear solution sample was centrifuged for 15 min at 10000 g. The clear NE were injected to HPLC before passing though syringe filter (0.2 µm) [192]. The equation to calculate drug loading are shown in Eq. 7.

$$\text{Drug Loading capacity} = \frac{\text{Weight of the entrapped drug inside the formulation}}{\text{Total weight of formulation}} \times 100 \dots \text{Eq. 7}$$

4.3.3.8. TEM

TEM analysis of FS-NE was evaluated by mixing 10 mg of the formulation with 10 mL of double-distilled water. To negatively stain the sample, they applied a small amount of the sample into a copper grid coated with carbon, forming a thin film. Any excess solution was removed using filter paper. After 10 minutes, added 2% w/v solution of phosphor tungstic acid (PTA) to the copper grid for approximately 1 min, draining off any excess solution. The grid was allowed to air dry completely, and the sample was examined using TEM [191].

4.3.3.9. Optical microscopy of FS-NE

The optical microscopy of the NE method was used to analyze the nature of the NE, i.e., O/W and W/O. In this method, two drops of methylene blue (a cationic dye) were added to one mL of the formulation. Conversely, the emergence of a blue background with colorless globules indicated that the dispersed phase was the oil phase, while the continuous phase was composed of an aqueous phase [189].

4.3.3.10. Stability studies

The FS-NE was subjected to accelerated stability testing in a stability chamber maintained at $40°C \pm 2°C/ 75\% RH \pm 5\% RH$ for six months. The study was carried out as per ICH stability guideline Q1A(R2) [193]. Parameters such as droplet size, zeta potential, PDI, and drug loading were compared between fresh and aged samples of FS-NE after six months. The above study was conducted three times, and the mean responses ($\pm$S.D.) were documented [190].

4.4. In vitro studies

4.4.1. Cell line toxicity study (MTT assay)

MTT assay was utilized for major cellular metabolic activities. The cell culture was centrifuged to perform the MTT assay, and the supernatant was taken. Further from the supernatant, the cell count was standardized to 1.0×10^5 cells/ml using a dimethyl sulfoxide (DMEM) medium. Subsequently, 100 µl of the diluted cell suspension (approximately 10,000 cells/well) was added to each 96-well flat-bottom microtiter plate well. After 24 h, once the cell population reached a satisfactory level, cells were centrifuged, and the resulting pellets were reconstituted with 100µl of various concentrations of test samples in maintenance media. The plates were incubated at 37˚C for 48 h in a 5% CO_2 environment. The plates were examined under a microscope to check the condition of the cells. After the 48 h incubation period, 20 µl of MTT solution (2 mg/ml in MEM-PR, MEM without phenol red) was introduced to each well. The sample had mixed properties and was incubated for two hours at the same temperature. After incubation, 100 µl of DMSO media was introduced into the plate and mixed correctly. The absorbance was measured at 540 nm [194]. The percentage viability of the cells was calculated using the formula provided in Equation 8.

$$\% \ cell \ viability = \left(\frac{Mean \ OD \ of \ individual \ test \ group}{Mean \ OD \ of \ control \ group}\right) \times 100 \dots\dots\dots\dots Eq. \ 8$$

4.4.2. *In vitro* **permeation study**

In vitro permeation study was evaluated for naïve FS and FS-NE to observe rapid adsorption of the FS. The study was carried out on Caco-2 cell monolayer. Caco-2 cells were cultivated in 12 mm trans well polycarbonate membrane containing pore size of 0.4 mm, twenty one days before the study. Cells were provided with a transepithelial electrical resistance value of 300 Ω/cm^2 and then washed three times with Hank's balanced salt solution (pH 6.5) before assessing transcellular transport. Transport buffers of 0.5 mL and 1.5 mL were added to the apical (A) side and basolateral (B) side, respectively. A naïve FS solution was prepared by dissolving it in methanol. Both the naïve FS solution and FS-NE were added to the apical and basolateral sides of cell inserts, respectively, to achieve a concentration of 20 µM. Samples of 0.1 mL were taken from both the apical and basolateral sides at predetermined intervals of 1, 2, 3, and 4 hours. The withdrawn volume was replaced with an equal volume of transport buffer. The samples were then filtered and analyzed using HPLC, and the amount of drug that permeated over time was recorded [6].

4.5. *In vivo* **studies**

4.5.1. *Procurement and storage of animals*

Male SD rats, weighing between 220-270 g, were obtained from the National Institute of Pharmaceutical Education and Research (NIPER) in Mohali, India. These rats underwent a 10-day acclimatization period in plastic cages filled with rice husk bedding. During this period, they were provided with pellet feed and had unrestricted access to clean water. The housing conditions were maintained at a temperature of 24 ± 1 °C and a relative humidity of 45%. To regulate their circadian rhythm, the rats were exposed to a 12 h light and 12 h dark cycle using artificial lighting [190]. All experimental procedures were conducted in accordance with the approval of the Institutional Animal Ethical Committee, with the assigned approval number LPU/IAEC/2021/91.

4.5.2. *Bioanalytical method development*

The bioanalytical method development was previously reported by Kumar and coworkers [195]. The method was developed by using a C-18 reverse phase column. A combination of acetonitrile and orthophosphoric acid (0.2 % v/v) in the ratio of 30-70 v/v was used as the mobile phase. During HPLC analysis, one mL/min flow rate was used and chromatograms were analysed at 362 nm. The same analytical method was used to validate bioanalytical method development. The method for extraction of the plasma sample is described below.

4.5.2.1. Collection of blood and extraction of plasma

To perform the pharmacokinetic study, rat blood was taken through the tail vein method and transferred into radioimmunoassay (RIA) vials. RIA vials containing EDTA crystals as an anticoagulant. The blood sample was gently mixed with EDTA in vial and transferred to a centrifugation tube. The blood sample was centrifuged at 4932 *g* for the duration of 10 minutes. The resulting clear supernatant was plasma obtained which was carefully extracted using a micropipette and stored in a deep freezer at -20 °C for further processing.

4.5.2.2. Preparation of blank plasma

A 1 mL plasma sample was extracted from the blood and mixed with 1 mL of methanol. Further, the mixture was vortexed for a duration of 5 minutes to induce the precipitation of plasma proteins. Subsequently, the resulting supernatant was taken by centrifuging a precipitated plasma protein sample at 4932 *g* for 15 minutes. The supernatant was then transferred to a 100 mL volumetric flask, and the volume of methanol was adjusted up to 100 mL.

4.5.2.3. *Preparation of standard stock solution*

For the stock solution with plasma, 10 mg FS was added in 2 mL of plasma sample. FS solution was further vortexed for a duration of 10 minutes, followed by the addition of 2 mL methanol. Subsequently, vertexing was done to form the precipitation of plasma proteins. Samples were then centrifuged at 4932 g for 15 minutes. The resulting supernatant was collected in a volumetric flask, and the volume was adjusted to 100 mL methanol to develop a stock solution with a concentration of 100 μg/mL ≡ 100000 ng/mL. From this solution, 10 mL was taken and diluted to 90 mL of methanol to attain the concentration of 10 μg/mL ≡ 10000 ng/mL.

Furthermore, from this solution 10 mL was taken and diluted to 100 mL of methanol, to attain the concentration of 1 µg/mL ≡ 1000 ng/mL. The final concentration was maintained at 0.01 µg/mL ≡ 10 ng/mL following the same procedure as above.

4.5.2.4. Preparation of internal standard (IS)

Quercetin (Qu) (25 ng/mL) was used as the internal standard (IS). Considering the structural homology of Qu with FS, Qu was chosen as the IS. IS plays a crucial role in facilitating the quantification of an analyte, especially in situations with an anticipated loss of sample volume, such as in the case of biological samples with poor recovery from the matrix. To prepare the Qu stock solution, 10 mg of Qu was accurately weighed and added to a 100 mL volumetric flask containing 20 mL of methanol. The solution was subjected to sonication for 10 minutes to ensure complete dissolution of the drug. The final volume was adjusted to 100 mL using methanol, resulting in a stock solution with a 100 µg/mL ≡ 100000 ng/mL concentration. From this solution, 10 mL was taken and diluted to 90 mL of methanol to attain the concentration of 10 µg/mL ≡ 10000 ng/mL. Furthermore, from this solution 10 mL was taken and diluted to 100 mL of methanol, to attain the concentration of 1 µg/mL ≡ 1000 ng/mL. further 10 mL was taken and diluted to 100 mL of methanol to attain a concentration of 0.1 µg/mL ≡ 100 ng/mL. Finally, 25 mL was taken from 100 ng/mL solutions and diluted in 100 mL of methanol to attain the concentration of 0.25 µg/mL ≡ 25 ng/mL.

4.5.2.5. Method validation

Dilutions were made in the concentration range of 2-10 ng/mL from the stock solution of 10 µg/mL containing a FS in plasma. 1mL of Qu (25 ng/mL) was added to get the final volume of 10mL of IS Qu. Each dilution was injected into HPLC in five replicates and analyzed at 362 nm. Similarly, the calibration curve of FS was developed in the concentration range of 2-10 ng/mL in the mobile phase. Furthermore, the accuracy, precision, robustness, LOD, LOQ and system suitability were performed where IS was taken as Qu with the same method as described in analytical method development. The procedure is described in Section 4.3.1.5.4.

4.5.3. Pharmacokinetic Study

The pharmacokinetic study was performed on 12 rats. The rats were divided into two groups, and each group contained six rats. The first group of rats received 10 mg/kg naïve FS orally, and the second group rats received 10 mg/kg FS-NE orally. After administration of the drug, blood samples were collected at 0, 15, 30, 60, 120, and 240 minutes. After collection, the blood sample was processed as mentioned in sections 4.5.2.1 to 4.5.2.3. After a one-week washout period, the study was repeated in a crossover manner by reversing the treatment. The calculation of the area under the curve (Tmax, Cmax, MTR, AUC_{0-t}, and $AUC_{0-\infty}$) was conducted using PK Solver 2.0 software. Groups of rats that were used for the pharmacokinetic study are shown in Table 13.

Table 13 Pharmacokinetic study in blood

Group	Treatment	Treatment	Animals used	Dose
1	Naïve FS	Naïve FS + 0.5% CMC	6	10 mg/kg
2	FS-NE	FS-NE	6	10 mg/kg

Note: These animals were reused for the study of drug concentration in CSF.

4.5.3.1. Sample collection and pharmacokinetic studies of the brain cells

Hole brain was collected and homogenized at pre-determined time points. Brain samples was collected by centrifugation and internal standard was added. The study was carried out using 12 rats, which were divided into two groups, each containing an equal number of rats. The first group received FS 10 mg/kg orally, and the second group received FS-NE 10 mg/kg orally. After administering the drug, each rat was sacrificed at 0, 5, 10, 15, 30 and 60 minutes, and CSF (100-150 µL) was collected from rat's brain. CSF samples were stored frozen (-20 ºC) until analyzed for FS content. The group of animals used in the pharmacokinetic study (drug concentration in CSF) is tabulated in Table 14.

Table 14 Drug concentration in CSF

Group	Treatment	Treatment	Animals used	Dose

| 1 | Naïve FS | Naïve FS + 0.5% CMC | 6 | 10 mg/kg |
| 2 | FS-NE | FS-NE | 6 | 10 mg/kg |

Note: All Animals were scarified during CSF collection.

4.6. Pharmacodynamic studies

4.6.1. AlCl₃ induced AD in rats

For the pharmacodynamic study, 42 rats were divided into 7 equal groups (n = 6). All the groups, except groups I and II, received the assigned treatment for 8 to 12 weeks. The grouping of rats receiving various treatments is shown in Table 15. Rats of groups III, IV, V, VI, and VII received naïve FS (10 mg/kg, orally), donepezil (1 mg/kg, orally), FS-NE (5 mg/kg, orally), and FS-NE (10 mg/kg, orally), respectively. Simultaneously, all the groups, except group I, received $AlCl_3$ (100 mg/kg, orally) daily for a period of 0 to 8 weeks. During the study period (81-84 days), OFT and MWM tests were conducted as behavioral parameters. The biochemical parameters were evaluated at the end of the study by sacrificing the rats and isolating the tissue. The parameters include determination of AChE activity, determination of Aβ 1-42, determination of thiobarbituric acid reactive substances (TBARS) assay, CAT estimation, SOD assay, GSH assay, and enzyme-linked immunosorbent (ELISA) assays such as TNF-α and IL-1β. A schematic representation of the protocol is given in Figure 12.

Table 15: Pharmacodynamic Study

Group of the animals	Treatment	Dose (mg/kg), Route of administration (p.o.)
I	Vehicle Control	1 mL 0.2% CMC
II	Disease Control ($AlCl_3$)	100 mg/kg $AlCl_3$
III	NE Placebo+ $AlCl_3$	10 mg/kg 0.2% CMC + 100 mg/kg $AlCl_3$

IV	Standard Control + AlCl$_3$	1 mg/kg donepezil + 100 mg/kg AlCl$_3$
V	Naïve FS (High dose) + AlCl$_3$	10 mg/kg, naïve FS + 100 mg/kg
VI	FS-NE (Low dose) + AlCl$_3$	5 mg/kg, FS-NE + 100 mg/kg AlCl$_3$
VII	FS-NE (High dose) + AlCl$_3$	10 mg/kg FS-NE + 100 mg/kg AlCl$_3$

Note: AlCl$_3$, FS and donepezil were suspended in 0.2% CMC (Number of the animals, N=6)
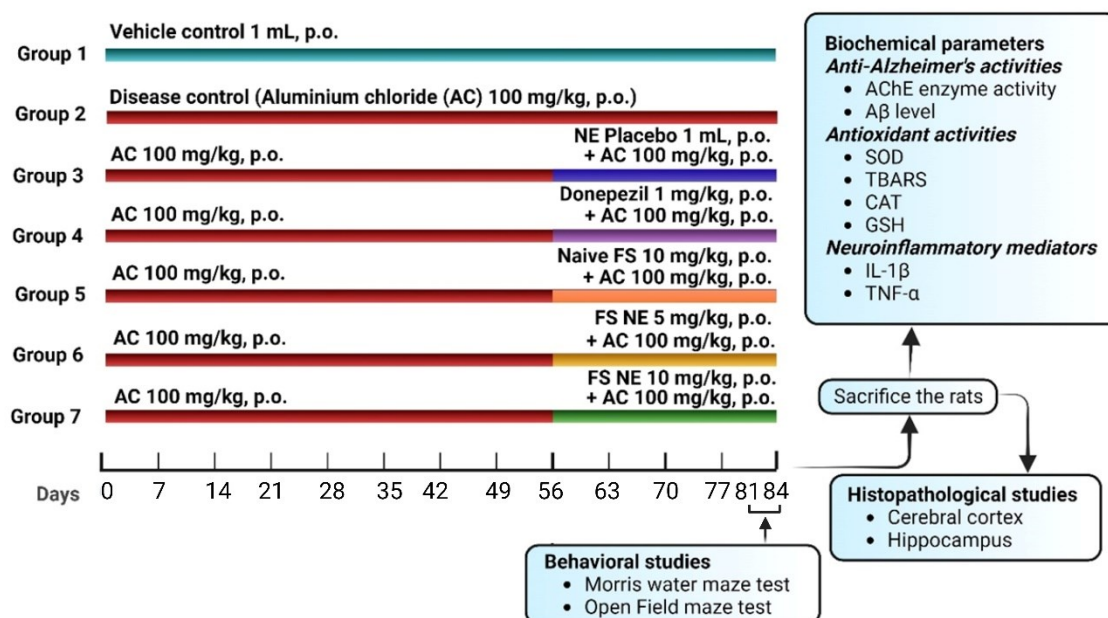
Figure 12: Study design for animal studies

4.6.1.1. MWM test

The MWM test was used to evaluate the level of working and spatial memory of the rodents. This method was first time coined by British neuroscientist Richard G. Morries in 1984. In MWM apparatus, four quadrants are present, where platforms are placed in the fourth quadrant. During acquisition, the platform was positioned one cm above the water level. Over five consecutive days, the rats underwent multiple trials. In each trial, each rat was placed in the pool in one of the quadrants, facing the wall. The drop location was varied for each trial. The rat was given 90 seconds to locate the platform, and if that rat failed to do so, it was guided towards the platform. On the platform, the rat was kept for 20 seconds. During the study, in the retrieval test, working and spatial memory were recorded and analysed.

4.6.1.2. OFT

The OFT was used to evaluate the behaviors and locomotor activities of the rodents. The rats' behavior was observed and recorded in a special wooden apparatus, which is divided into 16 squares and arranged in a 4x4 grid. The test lasted for 5 minutes. During the study, SD rats were placed in one of the external squares, and behavioral activities were recorded. In the OFT, various parameters were analyzed, including the number of squares crossed by the rats, time in the center, number of rearings, and number of groomings. All parameters are recorded in seconds.

4.6.2. Sample collection and preparation

On the last day of animal experiments i.e. 84 days, rats were kept in overnight fasting condition. The next day rats were sacrificed using cervical dislocation. The rats' skulls were opened to retrieve the entire brain. Careful isolation of the hippocampus and cerebral cortex region of the brain was done. The the hippocampus and cerebral cortex were rinsed in phosphate-buffered saline (PBS) to eliminate residual blood. Hippocampus was then divided into two segments: one for homogenization in ice-cold PBS for subsequent colorimetric and ELISA assays, and the other portion was kept in formalin solution for histological examination. The experimental protocol involved fasting, anesthesia, sacrifice, brain extraction, and specific processing steps

for the hippocampus and cerebral cortex, encompassing biochemical analysis and histological examinations utilizing various staining techniques.

4.6.3. Biochemical estimation

4.6.3.1. AChE activity

The determination of anti-cholinesterase activity involved measuring the levels of AChE in the hippocampus using Ellman's method. This method relies on measuring the rate at which thiocholine is produced during the hydrolysis of acetylcholine. This process involved the continuous reaction of thiocholine with 5,5'-diothiobis-2-nitrobenzoate ions, resulting in the formation of the yellow anion of 5-thio-2-nitrobenzoic acid. The rate of color production was then measured at 412 nm using a photometer.

4.6.3.2. Determination of $A\beta_{1-42}$ level

The brain (cerebral cortex and hippocampus) tissue weighing 100 mg was mixed with a solution containing 150 mM NaCl and 50 mM Tris HCl (pH = 7.6), along with protease inhibitors. The mixture was then thoroughly mixed. The resulting tissue mixture was subjected to centrifugation at 100,000 g and maintained at a temperature of 4 °C for a duration pried of 20 minutes. The concentration of $A\beta_{1-42}$ in the homogenized rat brain tissue was measured using the Wako Human/Rat $A\beta_{1-42}$ ELISA kit, following the manufacturer's instructions.

4.6.3.3. CAT estimation

For the assessment of CAT activity, a volume of 0.05 mL of rat's brain supernatant was obtained from homogenized cerebral cortex and hippocampus. This supernatant was combined with 1.95 mL of phosphate buffer (50 mM, pH 7.0). Following this, 1 mL of hydrogen peroxide (30 mM) was added to the mixture, and the variation in absorbance was monitored at 240 nm for a duration of 30 seconds with measurements taken at 15-second intervals. These measurements were conducted using a UV-spectrophotometer (UV-1800, Shimadzu Co. Ltd., Kyoto, Japan). The subsequent calculations were performed employing the following equation.

$$CAT = \{[(2.3 \times \log ODinitial \div ODfinal)/\Delta t \times 100]/0.693\}/mg\ of\ protein \ \dots \text{Eq. 9}$$

Where, OD – Optical density

4.6.3.4. Reduced glutathione (GSH) assay

The Beutler method was utilized to measure the concentration of GSH. To perform the measurement, one mL of supernatant obtained from homogenized brain tissues (cerebral cortex and hippocampus) was mixed with one mL of trichloroacetic acid (10% w/v in water) and subjected to centrifugation at 10000 g for a duration of 10 minutes. Following centrifugation, 0.5 mL of the resulting supernatant was extracted and mixed with 2 mL of disodium hydrogen phosphate (0.3M). Subsequently, 0.25 mL of freshly prepared 5,5,-dithio-bis-(2-nitrobenzoic acid (DTNB, 0.001 M in 1% w/v sodium citrate) was added to the mixture. The absorbance was then recorded at 412 nm using a UV-spectrophotometer, and the concentration of GSH was determined by referencing a standard plot (ranging from 10 to 100 µM) of reduced glutathione.

4.6.3.5. Thiobarbituric acid reactive substances (TBARS) assay

The TBARS assay is a method used to assess the degree of lipid peroxidation by measuring the level of MDA. In this assay, 0.2 mL of the supernatant was combined with 0.2 mL of Tris HCl (pH 7.4) and incubated at a temperature of 37 °C for a period of 2 h. After incubation, 1 mL of 10% ice-cold trichloroacetic acid (TCA) was added, followed by centrifugation at 10000 g for 10 minutes. Subsequently, 1 mL of the resulting supernatant was mixed with 1 mL of 0.67% TBA and heated in a boiling water bath for 10 minutes. After this, the samples are allowed to cool, and 1 mL of distilled water is added. The absorbance was then measured at 532 nm. To perform the necessary calculations, the extinction coefficient of MDA (0.156 µM-1) is utilized, and the final concentration was expressed as nanomoles of MDA per milligram of protein.

4.6.3.6. Estimation of neuroinflammatory biomarker

The estimation of TNF-α, and IL-1β in the midbrain homogenate was conducted using ELISA assay kits. The procedures provided within each kit were followed. Initially, the wells of the provided plates were coated with their respective specific antibodies. Standard and sample solutions were dispensed into the wells and then incubated, facilitating the binding of proteins to the immobilized antibodies. Subsequently, a washing step was performed such as TNF-α, and IL-1β. After a specific incubation period, any unbound biotinylated antibodies were

washed away. Next, a haptoglobin-related protein (HRP)-conjugate was added, followed by another round of washing. Then, a (3,3',5,5'-Tetramethylbenzidine) TMB substrate was added, resulting in the generation of a blue color, which eventually turned yellow. The intensity of the resulting yellow color was measured at 450 nm using an ELISA plate reader (iMark Microplate Reader, BIORAD company).

4.7. Statical analysis

The results were reported as mean ± SD. Pharmacokinetic and pharmacodynamic parameter (behavioral, and biochemical parameters) were evaluated by using one way analysis of variance (ANOVA) using statical graph pad prism 8 software (GraphPad Inc., La Jolla, USA).

4.8. Histopathological examinations

All rats were sacrificed, and the sections of the cortex and hippocampus were separated. These sections were washed with cold phosphate-buffered saline and fixed using a 10% v/v formalin solution. Paraffin-embedded blocks were prepared for both types of tissues. Once sectioned, the paraffin sections were stained with congo red stain and observed under a light microscope. The entire experiment was conducted three times in order to ensure reproducibility. The histopathological assessment of the tissue sections was carried out in a blinded manner by the histopathologist.

Chapter 5
Results & discussion

5.0 Results and Discussion

5.1. Pre-formulation studies

5.1.1. Characterization of FS

FS appeared to be a pale yellowish, free-flowing crystalline powder with a pungent taste and slight aromatic odor. These observations were in concordance with properties given in the literature (120). Furthermore, determination of melting point was done wherein the melting of FS was found in the range of 346-356℃. Whereas, through DSC, the melting point was found to be 354℃ (Figure 13). Furthermore, the crystalline nature was also determined through DSC and PXRD. In the case of DSC, FS showed an endothermic nature with highly intense sharp peak. This was further confirmed by X-ray diffractogram where multiple sharp peaks were observed at angles of 3, 13,14,15,16,17,191, and 27 degrees, respectively with peak counts of 5800, 11500, 7700, 11400, 1600, 1200, 2000, and 5500, respectively (Figure 14a). The morphological appearance of the surface of FS was investigated through SEM imaging. The surface of FS appeared to be a flowery appearance with a reticulated non-uniform edge (Figure 14b).
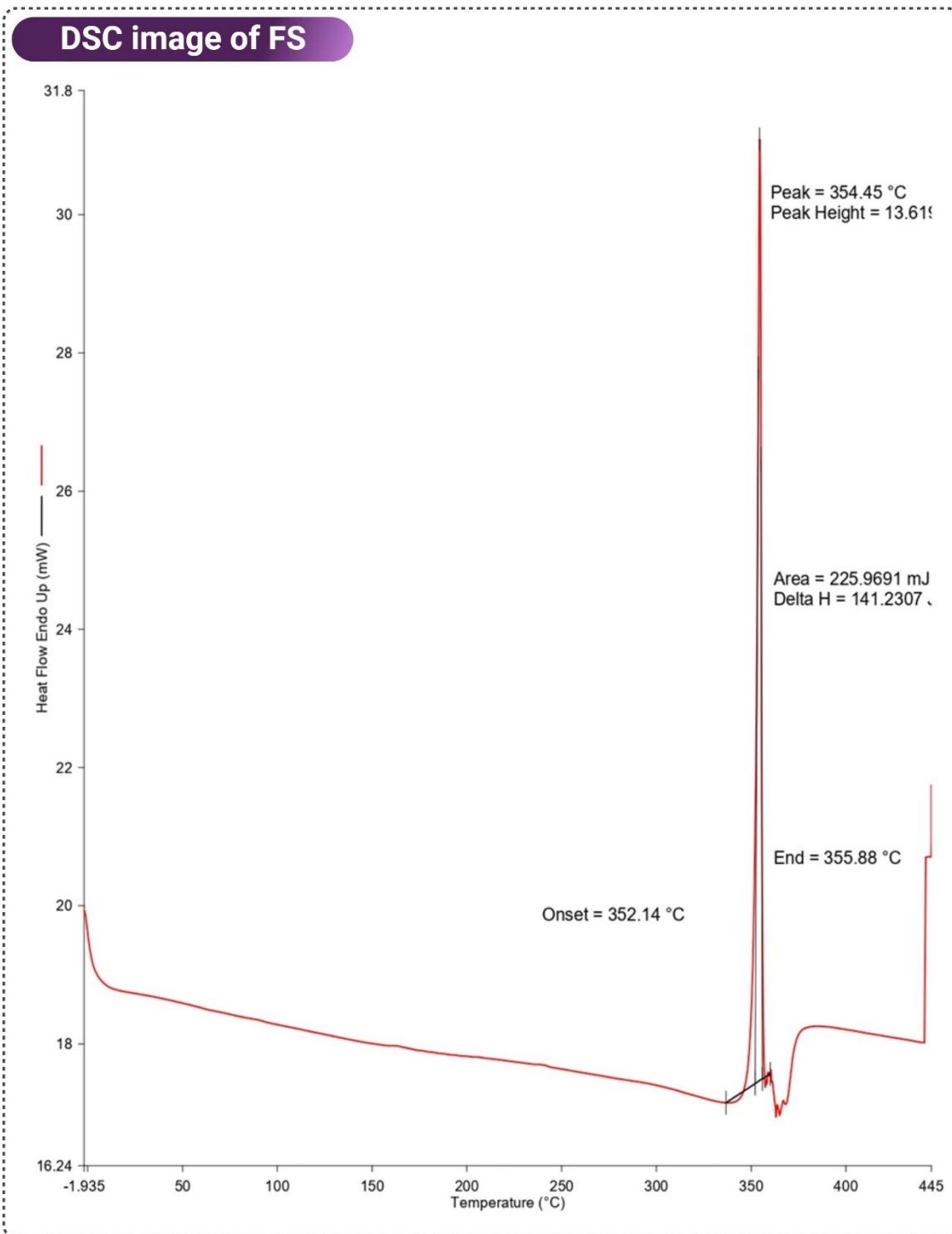
Figure 13: DSC image of FS
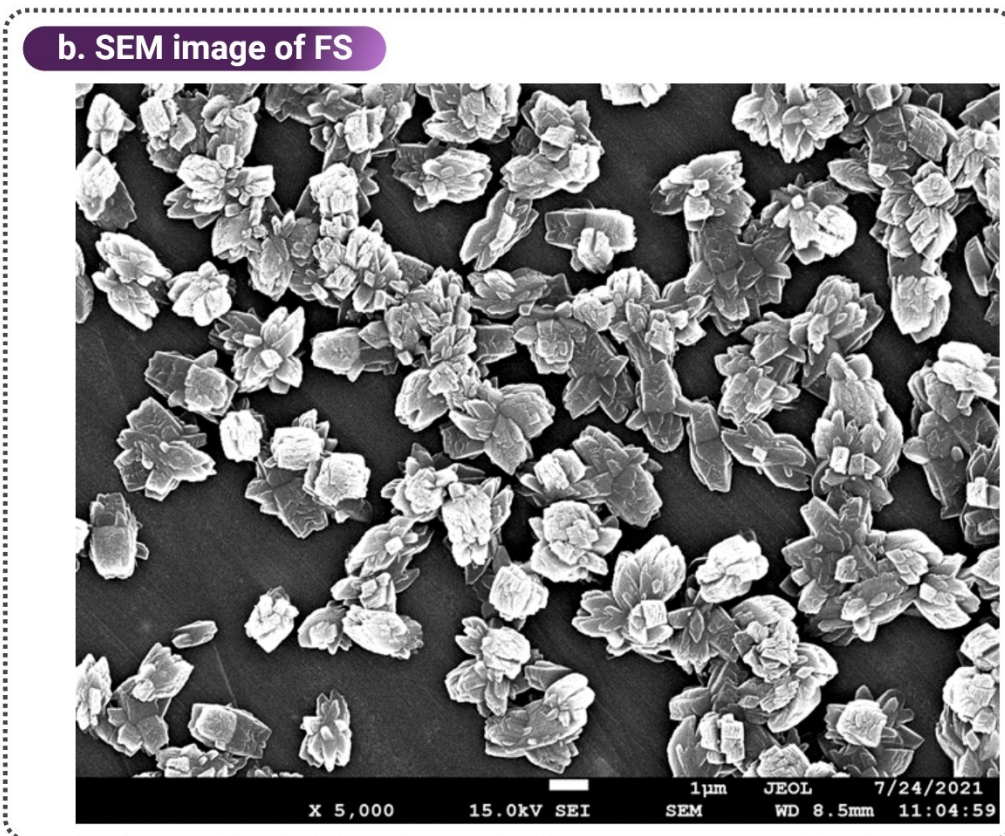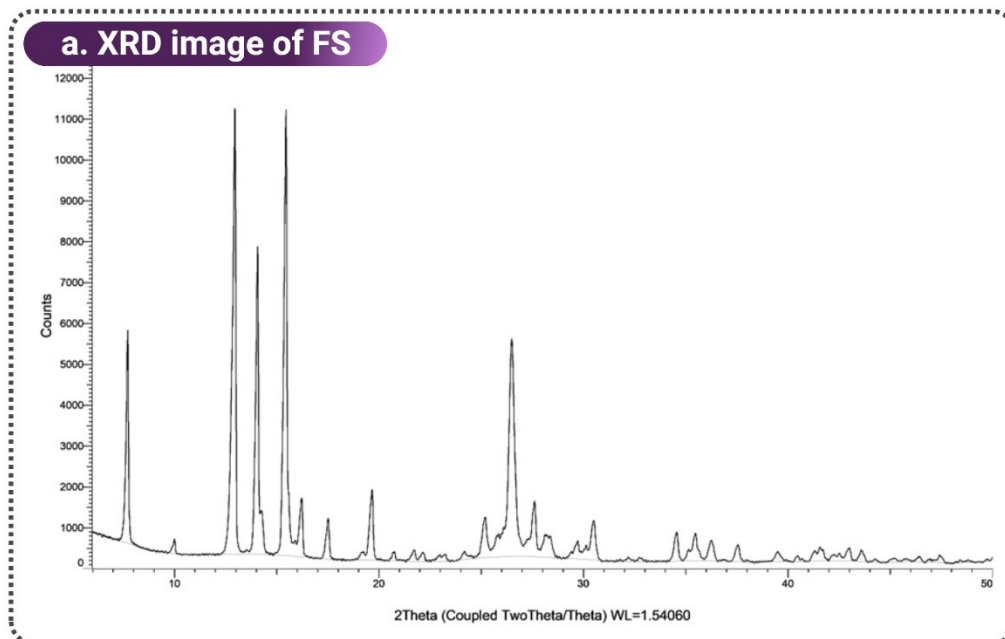
Figure 14: (a) PXRD image of FS (b) FESEM image of FS

5.1.2. Analytical method development of FS

The FS solution showed the absorption max (λ max) at 362 nm and this λ max was used for the analytical method development of FS through HPLC (Figure 15). The HPLC chromatogram of FS was developed using the method described in section 4.3.1.1. The images of blank sample, FS (10 μg/mL), FS (1 μg/mL), FS (0.1 μg/mL), FS (0.01 μg/mL), FS (0.001 μg/mL), and FS (0.0001 μg/mL) have been shown in figure 16 (a, b, c, d and f). The chromatograms clearly indicated the absence of any interference of mobile phase with the peak of FS. The FS peak was observed at 6.75 minutes. Furthermore, the distinctive peak easily detected in the ranges of 0.001 μg/mL (1 ng/mL) to 10 μg/mL (10000 ng/mL) indicating that the method or any other system generated noise is sensitive to detect the presence of FS in nanogram ng level without any interference of mobile phase. Based on these outcomes, the method was developed in the range of 2 ng/ mL to 10 ng/mL (0.0002 to 0.001 μg/mL).
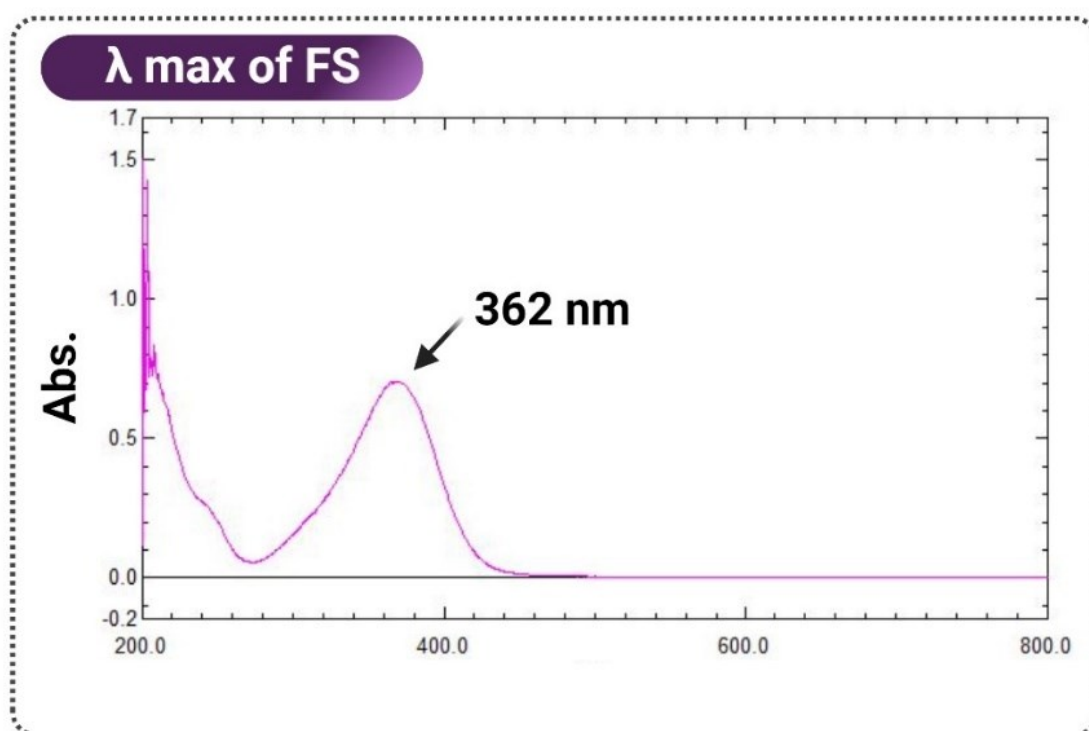
Figure 15: λ max of FS
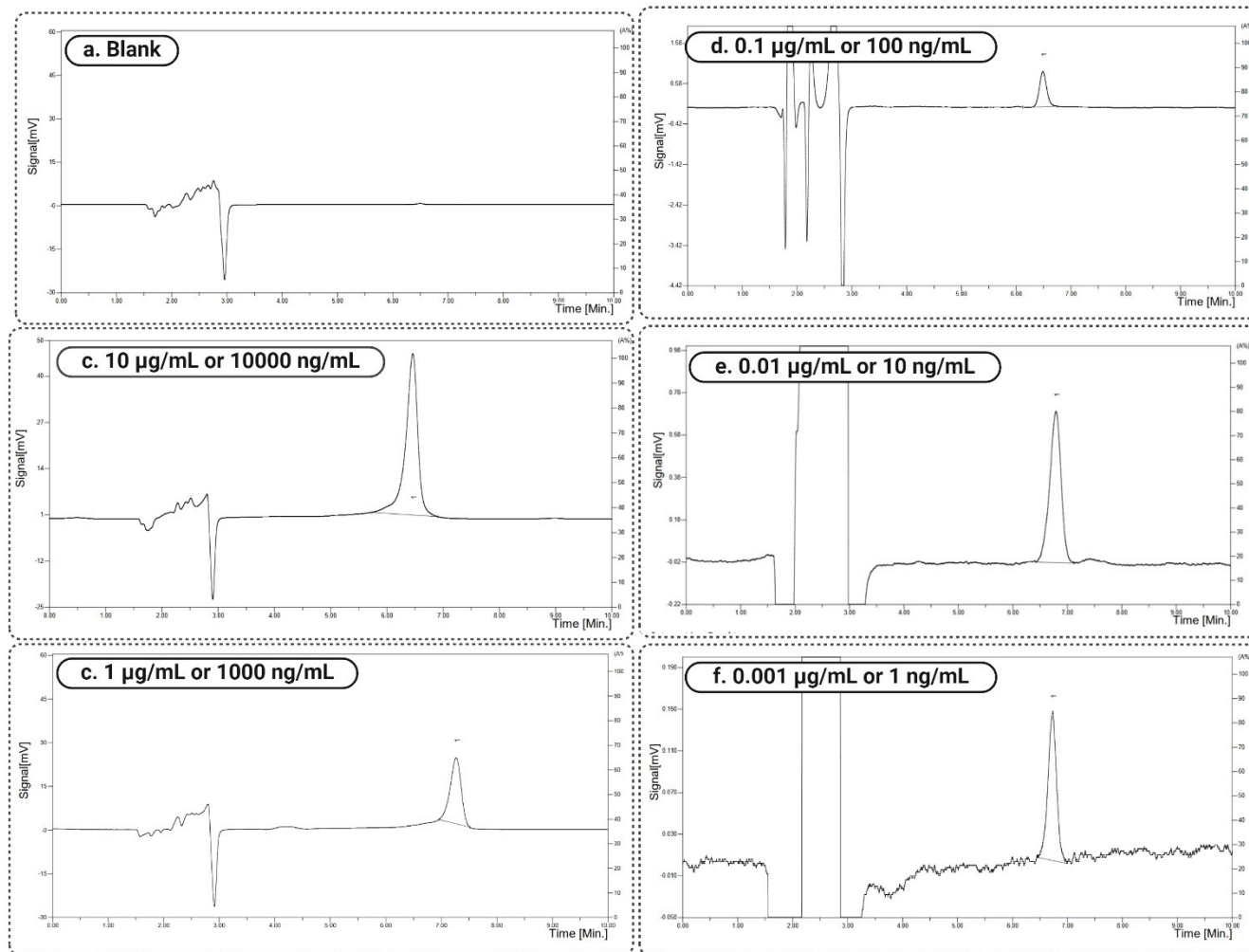
Figure 16: The chromatograms of blank sample, FS (10 µg/mL), FS (1 µg/mL), FS (0.1 µg/mL), FS (0.01 µg/mL), FS (0.001 µg/mL), and FS (0.0001 µg/mL)

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Five serial dilutions having concentrations of 2, 4, 6, 8 and 10 ng/mL were prepared to determine linearity and range. The data was found linear in this range with the correlation coefficient (r^2) of 0.9995. The slope of the curve was found to be 0.742X and intercept was 0.755. The calibration curve of FS is shown in figure 17.
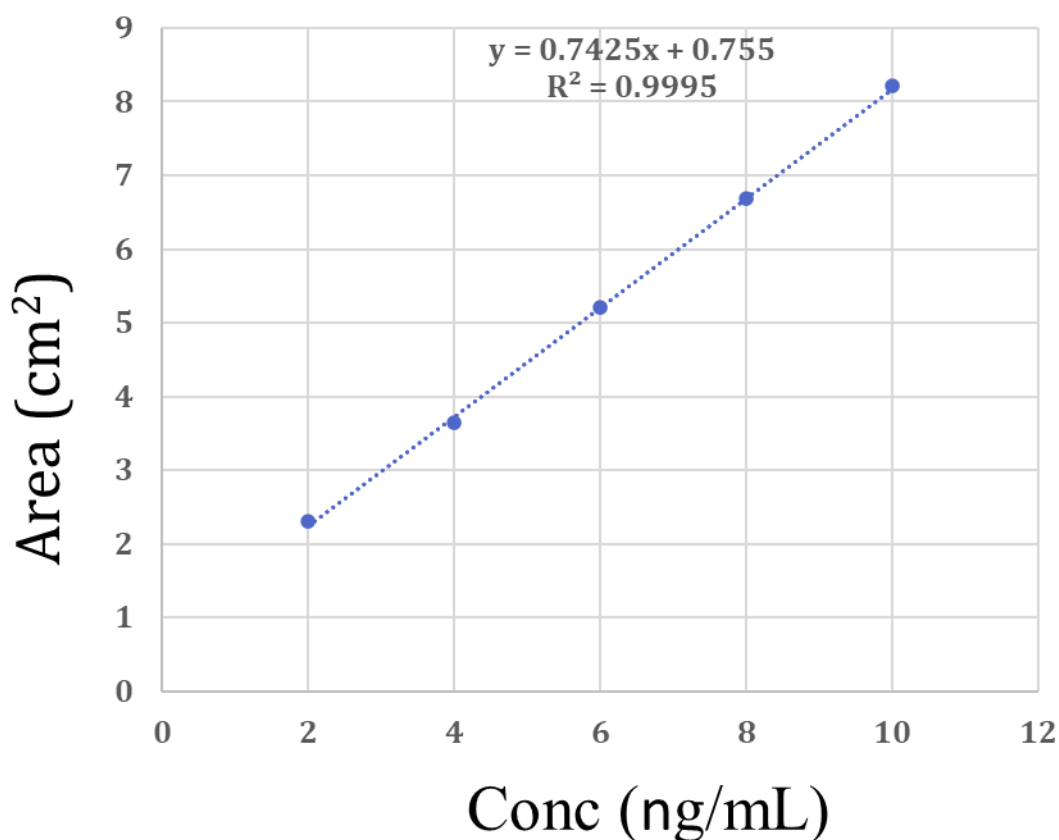
Figure 17: Calibration curve of FS

In the system suitability, the results indicated that the values of all parameters were found in the pharmacopeial limits and method was suitable for method development [196]. System suitability provides us information about the chromatographic system prior to use. The continuous use of chromatographic system affects their performance and affect the reliability of analytical results. The lower values of LOD and LOQ revealed that the method was enough sensitive to detect FS under specified chromatographic conditions. The value of theoretical plate more than 2,000, theoretical plate/meter more than 20,000 and tailing factor less than 1.5

indicated about better column efficiency. The obtained results in present study indicated about very good system suitability of developed method. The obtained results of system suitability indicated that method is reliable and suitable for further studies. The results of system suitability are mentioned in Table 16.

Table 16: System suitability testing results

Parameters	Value	Limits
Area	9.17	--
Height	0.73	--
Theoretical plate	2894.47	More than 2,000
Theoretical plate/meter	28944.72	More than 20,000
Tailing factor	0.62	Less than 1.5
Peak purity index	0.837	More than 0.5
LOD	0.26 ng/mL	--
LOQ	0.79 ng/mL	--

Accuracy study of FS is tabulated in Table 17. The study was carried out as per the procedure described in the section of 4.3.1.1.7. The % recovery was found in the range of 98-100 %, indicating about the accuracy of method.

Table 17: Accuracy study results

Level	Actual concentration of std solution (ng/mL)	Concentration of drug recovered (ng/mL), ($N=5$)	% recovery
LQC	4.8	4.74	98.76
MQC	6	5.89	98.29
HQC	7.2	7.13	99.15

Precision study was carried out as per the procedure mentioned in section 4.3.1.1.8. The three concentration levels (LQC, MQC and HQC) were used for repeatability and intermediate precision (interday and interanalyst precision). The % RSD was less than 2% which indicated the precision of the method. Intra-day precision and intermediate precision and their mean results are tabulated in Table 18. In the robustness study, The % RSD was observed to be less than 2%, which indicated that the method was found to be robust after slight changes in flow rate, mobile phase ratio, and pH. The results of the robustness study are shown in Table 19.

Table 18: Results of precision study

Parameter	Level	Concentration (ng/mL)	Area						Mean area	Standard deviation	% RSD
			1	**2**	**3**	**4**	**5**	**6**			
	LQC	4.80	4.38	4.31	4.35	4.37	4.39	4.30	4.35	0.04	0.81
	MQC	6.00	5.22	5.19	5.23	5.20	5.24	5.26	5.21	0.02	0.40
	HQC	7.20	6.17	6.11	6.12	6.14	6.16	6.12	6.13	0.03	0.52
Inter-day											
Day 1	LQC	4.80	4.32	4.35	4.32	4.34	4.30	4.31	4.33	0.02	0.40
	MQC	6.00	5.19	5.18	5.21	5.23	5.24	5.17	5.19	0.02	0.29
	HQC	7.20	6.20	6.17	6.14	6.19	6.13	6.16	6.17	0.03	0.49
Day 2	LQC	4.80	4.31	4.34	4.30	4.36	4.33	4.38	4.32	0.02	0.48
	MQC	6.00	5.21	5.19	5.23	5.21	5.25	5.27	5.21	0.02	0.38
	HQC	7.20	6.12	6.17	6.14	6.16	6.13	6.20	6.14	0.03	0.41
Day 3	LQC	4.80	4.34	4.39	4.40	4.32	4.31	4.30	4.38	0.03	0.73
	MQC	6.00	5.21	5.19	5.23	5.21	5.25	5.27	5.21	0.02	0.38
	HQC	7.20	6.19	6.15	6.18	6.16	6.17	6.20	6.17	0.02	0.34
Intermediate precision (inter analyst)											
Analyst 1	LQC	4.80	4.35	4.39	4.40	4.35	4.39	4.34	4.38	0.03	0.60
	MQC	6.00	5.20	5.24	5.27	5.22	5.22	5.27	5.24	0.04	0.67
	HQC	7.20	6.24	6.19	6.20	6.26	6.22	6.20	6.21	0.03	0.43

Analyst 2	LQC	4.80	4.37	4.38	4.38	4.35	4.37	4.32	4.38	0.01	0.13
	MQC	6.00	5.24	5.27	5.21	5.20	5.25	5.26	5.24	0.03	0.57
	HQC	7.20	6.16	6.19	6.17	6.15	6.13	6.20	6.17	0.02	0.25
Analyst 3	LQC	4.80	4.32	4.36	4.39	4.34	4.32	4.38	4.36	0.04	0.81
	MQC	6.00	5.19	5.26	5.20	5.27	5.25	5.29	5.22	0.04	0.73
	HQC	7.20	6.18	6.20	6.25	6.16	6.13	6.21	6.21	0.04	0.58
Analyst 1	LQC	4.80	4.35	4.39	4.40	4.35	4.39	4.34	4.38	0.03	0.60
	MQC	6.00	5.20	5.24	5.27	5.22	5.22	5.27	5.24	0.04	0.67
	HQC	7.20	6.24	6.19	6.20	6.26	6.22	6.20	6.21	0.03	0.43

Table 19: Robustness results of various parameters

Variable	Value	Conc (ng/mL)	Peak area (Mean ± SD) (*N=6)	Mean of peak area of three values (*N=3)	% Recovery (Mean ± SD) (*N=6)	Mean of % recovery of three values (*N=3)	Retention time (Mean ± SD) (*N=6)	Mean of retention time values of three (*N=3)
Flow rate (mL/min)	0.8	6	604403 ± 4811.75	5.32 ± 0.025	5.245	102.46± 0.56	100.78	7.76 ± 0.135
	1	6	500658.33 ± 2483.82	5.24 ± 0.027	SD = 0.025	100.748 ± 0.61	SD = 0.554	6.69 ± 0.09
	1.2	6	485780.5 ± 4072.30	5.172 ± 0.021	% RSD = 0.470	99.14 ± 0.48	% RSD = 0.549	5.663 ±0.071

Mobile phase Ratio (A:B) v/v	28:72	6	495749.91 ±2459.46	5.185 ±0.019	5.257	99.43 ± 0.42	101.06	7.90 ±0.089
	30:70	6	505664.91 ± 2508.65	5.252 ± 0.033	SD = 0.026	100.935 ±0.743	SD = 0.587	6.770 ± 0.064
	32:68	6	520684.66 ± 2583.17	5.335± 0.027	% RSD = 0.498	102.80 ± 0.59	% RSD = 0.581	5.765 ±0.100
pH	2.9	6	498720.16 ± 461215	5.165 ± 0.184	5.186	98.99 ± 4.13	99.45	6.883 ± 0.030
	3.1	6	503948.66 ± 3110.28	5.252 ± 0.033	SD = 0.082	100.93 ± 0.74	SD = 1.83	6.772 ±0.039
	3.3	6	503767 ± 1746.01	5.14 ± 0.028	% RSD = 1.57	98.42 ±0.61	% RSD =1.84	6.607 ± 0.088

In the specificity study, no peak corresponding to the retention time of FS was observed in the samples of all the excipients. Consequently, the method developed was deemed specific for FS. Figure 18 presents the chromatograms for the blank samples of these excipients.
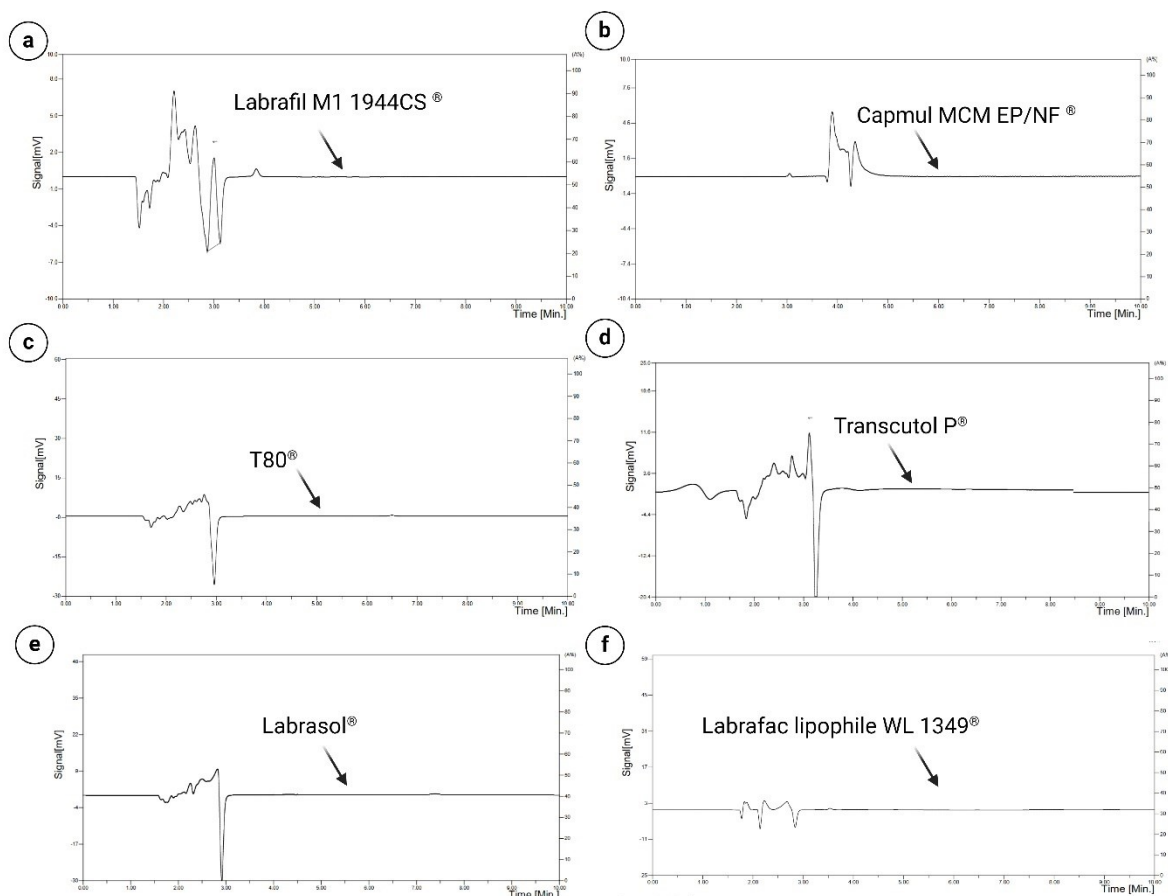
Fig. 18. Chromatograms of various solubilizers that are to be used for formulating FS-NE

5.1.3. Solubility studies

In a study of various oils, the solubility of FS was highest in Capmul MCM EP/NF® at 2.4 mg/mL, followed by Labrafil M1 1944CS® at 2.1 mg/mL, and Castor oil at 1.6 mg/mL. Lower solubility values were observed in Labrafac PG® and Coconut oil, both at 0.8 mg/mL, and Cottonseed oil at 0.7 mg/mL. Eucalyptus oil showed a solubility of 0.6 mg/mL, Maisine CC® at 0.5 mg/mL, and Peanut oil at 0.4 mg/mL. Similarly, the solubility of FS in different

surfactants and co-surfactants was found to be highest in Transcutol P® at 4.9 mg/mL, followed by PEG 200 at 4.8 mg/mL, T80 at 3.9 mg/mL, T20® at 3.4 mg/mL, Labrasol® at 2.5 mg/mL, Labrafac lipophile WL 1349® at 1.2 mg/mL, and Captex 300 EP/NF at 0.7® mg/mL. The solubility study of FS is presented in **figure 19**. Among these oil Capmul MCM EP/NF exhibited high solubility in oil.
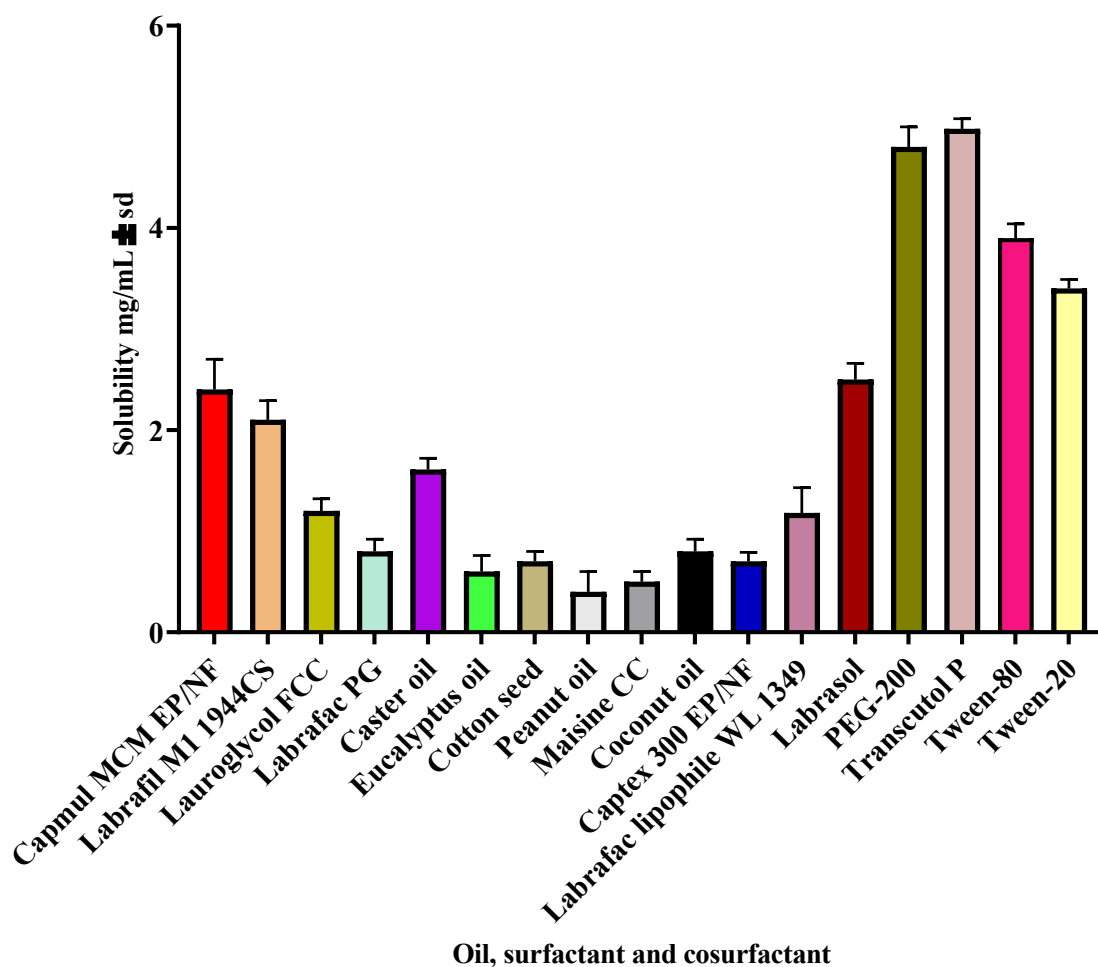
Fig. 19. Solubility of FS in different vehicles (each value represents the mean ± SD, n=3)

5.2. Formulation of development of FS-NE

5.2.1. PTPD analysis

Only four out of twenty-seven NE prototypes displayed clean and transparent emulsion. These were formulations F1, F10, F19 and F20. Rest all formulations showed emulsion (Table 20 and Figure 20). The oil concentration in the formulation was also shown to have a significant impact on the droplet size of the emulsion. An increase in oil concentration caused the increase in droplet size leading to transformation of emulsion whereas increase in concentration of surfactant and co-surfactant caused decrease in the droplet size leading to formation of NE. In order to further understand and select the best formulation, all the four formulations (i.e. F1, F10, F19 and F20) were subjected for size analysis, zeta potential, drug loading percentage, cloud point determination and centrifugation stress. The results are shown in Table 21. All these formulations passed the centrifugation stress as the emulsion remained transparent and clear in all these cases. The cloud point for all these formulations was found above 80ºC, indicating excellent thermodynamic stability. The droplet size of emulsion, drug loading in emulsion are important factors for getting better biopharmaceutical performance of drug. An ideal NE should have low droplet size and high drug loading. Similarly, the formulation should have high ± Zeta potential as it governs the stability of NE both *in vitro* and *in vivo*. Owing to this, the mean droplet size of 'F19' formulation was found to be least among all the formulation with highest drug loading and higher negative zeta potential than any other formulations. Images of all 27 batch are presented in Figure 20. Ternary phase diagram of FS-NE is shown in figure 21.

Table 20 The 27 batch of PTPD

Batch Number	Oil (Capmul MCM EP/NF)	Surfactant: co-Surfactant (T80: Transcutol P)	Water	Appearance
F1	5.42	12.85	81.71	Transparent
F2	6.06	24.24	69.69	Opaque
F3	12	28	60	Opaque
F4	18.18	27.27	54.54	Opaque

F5	26.31	26.31	47.36	Opaque
F6	33.33	22.22	44.44	Opaque
F7	46.66	20	33.33	Opaque
F8	57.14	14.28	28.57	Opaque
F9	75	8.33	16.66	Opaque
F10	2.5	22.5	75	Transparent
F11	8	32	60	Opaque
F12	13.63	31.81	54.54	Opaque
F13	20	30	50	Opaque
F14	29.41	29.41	41.17	Opaque
F15	40	26.66	33.33	Opaque
F16	53.84	23.07	23.07	Opaque
F17	66.66	16.66	16.66	Opaque
F18	75	8.33	16.66	Opaque
F19	0.90	8.18	90.90	Transparent
F20	7.22	8.88	83.88	Transparent
F21	11.11	25.92	62.96	Opaque
F22	15.38	23.07	61.53	Opaque
F23	21.73	21.73	56.52	Opaque
F24	30	20	50	Opaque
F25	38.88	16.66	44.44	Opaque
F26	53.33	13.33	33.33	Opaque
F27	75	8.33	16.66	Opaque

Figure 20: The 29 batches of Pseudo ternary phase
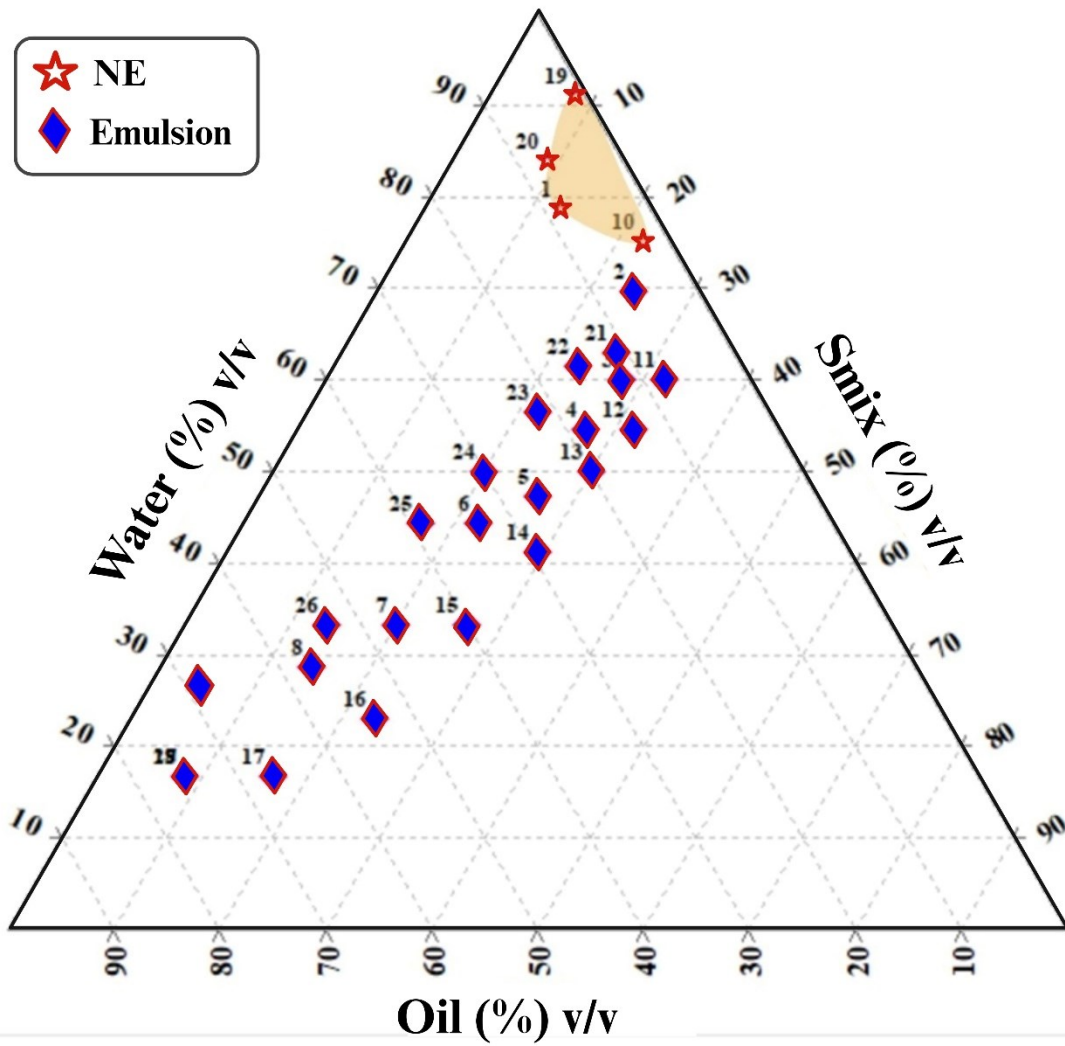
Figure 21: PTPD of FS-NE

Table 21 Drug loading, droplet size, zeta potential and PDI of optimized batches

S No.	Group	Capmul MCM EP/NF	Twe en 80	Transcut ol P	Drug loading (%)	Droplet size	Zeta potential	PDI	Cloud point (°C)	Centrifugation stability
1	1	100	450	450	98.23	17.00±0.058	-6.25±0.13	0.22±0.21	92.6	Pass
2	10	100	300	600	99.52	22.349±0.02	-7.25±1.03	0.328±0.15	89.1	Pass
3	19	100	600	300	100.23	24.04±0.20	-16.25±0.52	0.26±0.32	91.2	Pass
4	20	200	534	266	100.54	20.21±0.53	-10.52±1.52	0.24±0.52	87.1	Pass

5.2.2. Design of experiment (DOE)

A total of 14 formulations for FS-NE were developed using DoE to assess how variations in formulation factors impacted the average polydispersity index (Y_1), droplet size (Y_2), zeta potential (Y_3), and drug loading (Y_4) of FS-NE. The responses of all 14 formulations are tabulated in Table 22. The measured values for Y_1, Y_2, Y_3, and Y_4 fall were within the range of 0.14 to 0.56, 14.32 to 66.78 nm, -5 to -16 mV, and 80.02% to 101.19%. The ratio of the maximum to the minimum values for Y_1, Y_2, Y_3, and Y4 were found to be 4.67, 4.67, 3.20, and 1.26, respectively. Transformation wasn't necessary as the maximum to minimum ratio of all values was below 10. Statistical analysis of the model was conducted using DoE tools such as lack of fit test, sequential model sum of squares, and model summary statistics.The results obtained for the responses using these tools are summarized in Table 23. The results of the analysis of variance (ANOVA) indicated a significant influence ($p < 0.05$) of the variables on these responses. The final mathematical equations for Y_1, Y_2, Y_3, and Y_4 resulting from the experimental design are represented as equations 2, 3, 4, and 5, respectively. The coefficient of regression (R^2), F value, and probability suggested a linear model for Y_1, while a quadratic model was found to be more suitable for Y_2, Y_3, and Y_4.

$$Polydispersity\ Index\ (Y_1) = +0.3129 + 0.07258 * A - 0.1013 * B + 0.0813 * C$$
…..Eq. (2)

$$Droplet\ size\ (Y_2) = +54.03 + 6.35 * A - 14.29 * B + 12.33 * C - 6.02 * AB + 1.74 * AC - 8.44 * BC - 4.67 * A2 - 2.79 * B2 - 18.09 * C2$$ ….....
Eq. (3)

$$Zeta\ potential\ (Y_3) = -12.63 - 0.1050 * A + 0.3750 * B + 0.7900 * C - 4.00 * AB + 0.7900 * AC - 0.2500 * BC + 0.8625 * A2 + 1.28 * B2 - 2.39 * C2$$ …….Eq. (4)

$$Drug\ loading\ (Y_4) = +91.80 + 0.0937 * A + 2.43 * B - 0.8063 * C + 7.37 * AB - 1.93 * AC + 3.69 * BC + 2.46 * A2 - 4.24 * B2 + 4.93 * C2……Eq. (5)$$

The comparison of the factor coefficients in the coded equations is useful for the identification of the relative impact of the factors. In these equations, a positive sign indicates synergistic

effects, while a negative sign denotes antagonistic effects. Both PDI (Y_1) and mean droplet size (Y_2) displayed similar responses. However, PDI (Y_1) followed a linear equation, while mean droplet size (Y_2) exhibited a quadratic relationship with the selected variable. Both, PDI and droplet size increased with higher concentrations of A and C, and decreased with lower concentrations of B, indicating that A and C had synergistic effects, while B had antagonistic effects, leading to a decrease in both PDI and mean droplet size (equation 2 and 3) [127].

Zeta potential (Y_3) also demonstrated a quadratic relationship with the chosen variables. Response Y_3 showed an increase in zeta potential with reduced A concentration and higher B and C concentrations, as expressed in equation 4. On the other hand, the percentage of drug loading (Y_4) increased with higher concentrations of A and B but decreased with increased C concentration, as shown in equation 5. Along with this information obtained from the polynomial equation, it is important to identify the influence/interaction of each factor on the responses.

The perturbation plots for all responses are shown in Figures 22a, b, c, and d. In the case of PDI, all factors were found to have equal influence on PDI (figure 22 b). In case of mean droplet size, it is important to note that factor C is the main dominating factor affecting the droplet size of NE, whereas factor A showed slight effects the droplet size and factor B showed negligible effects on NE (Figure 22a). In the case of zeta potential, all factors showed equal influence (Figure 22b). Figure 22d revealed that factor B was having most domination effects, whereas factors C and A have the least influence (figure 22d). The polynomial equations derived from the BBD design aided in generation of 2D contour plots and 3D response surface plots. The images are presented in Figures 23a-23d (2D contour plots) and Figures 24a-24d (3D response surface plots). These plots further helped in understanding the design by visualizing the response surface with respect to the selected factors.

The optimization of the formulation graphically was carried out to find the levels of factors A-C within the obtained design space. The predicted values for responses obtained from the BBD were found to be in the range of 0.12 – 0.44 for PDI, 15.24 nm-50.21 nm for droplet size, -19.86 mV to -12.13 mV for zeta potencial and 95.33%-103% for % drug loading. The predicted values for factors A-C were 147 µL, 600 µL and 200 µL, respectively. In order to validate the

predicted values of factors and responses, the formulation was developed in triplicate using the predicted concentration of A, B, C for 1 mL of NE. The observed values for PDI was 0.26, droplet size was 32 nm, zeta potential was -15.99 mV and % drug loading was 99.5%. The value was observed as non-significant (P>0.05) when compared to the observed value. This indicated the reproducibility of the optimization method.

Table 22: Study design based on BBD to check the effect of variables (A-C) on responses (Y_1–Y_4)

S.N.	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
1	50	600	300	0.12	13.31	-6	83.29
2	150	600	300	0.42	43.03	-13	97.50
3	150	150	300	0.55	65.77	-9	80.02
4	100	600	450	0.32	23.45	-13	99.90
5	100	150	450	0.51	62.94	-12	85.56
6	100	600	150	0.23	18.22	-13	90.06
7	50	375	150	0.13	14.65	-14	95.44
8	150	375	150	0.27	18.64	-15	101.19
9	50	150	300	0.46	69.87	-14	96.28
10	100	150	150	0.37	23.98	-13	92.47
11	100	375	300	0.44	54.35	-12	9.80
12	100	375	300	0.45	52.6	-11.29	90.80
13	150	375	450	0.43	49.34	-10.76	90.10
14	50	375	450	0.39	38.40	-12.92	100.05

Table 23: Summary of ANOVA (Fit Summary)

Responses	Model	R^2	F-value	p-value	Lack of fit	Sum of Square	Df	Mean Square
Polydispersity Index (Y1)	Linear	0.52	10.23	0.01	0.06	1.95	3	0.06

Droplet size (Y2)	Quadratic	0.03	6.45	0.03	0.20	21765.40	3	351.51
Zeta potential (Y3)	Quadratic	0.04	6.19	0.06	0.24	32.38	3	10.77
Drug loading (Y4)	Quadratic	0.75	29.90	0.01	0.25	187.75	3	62.58
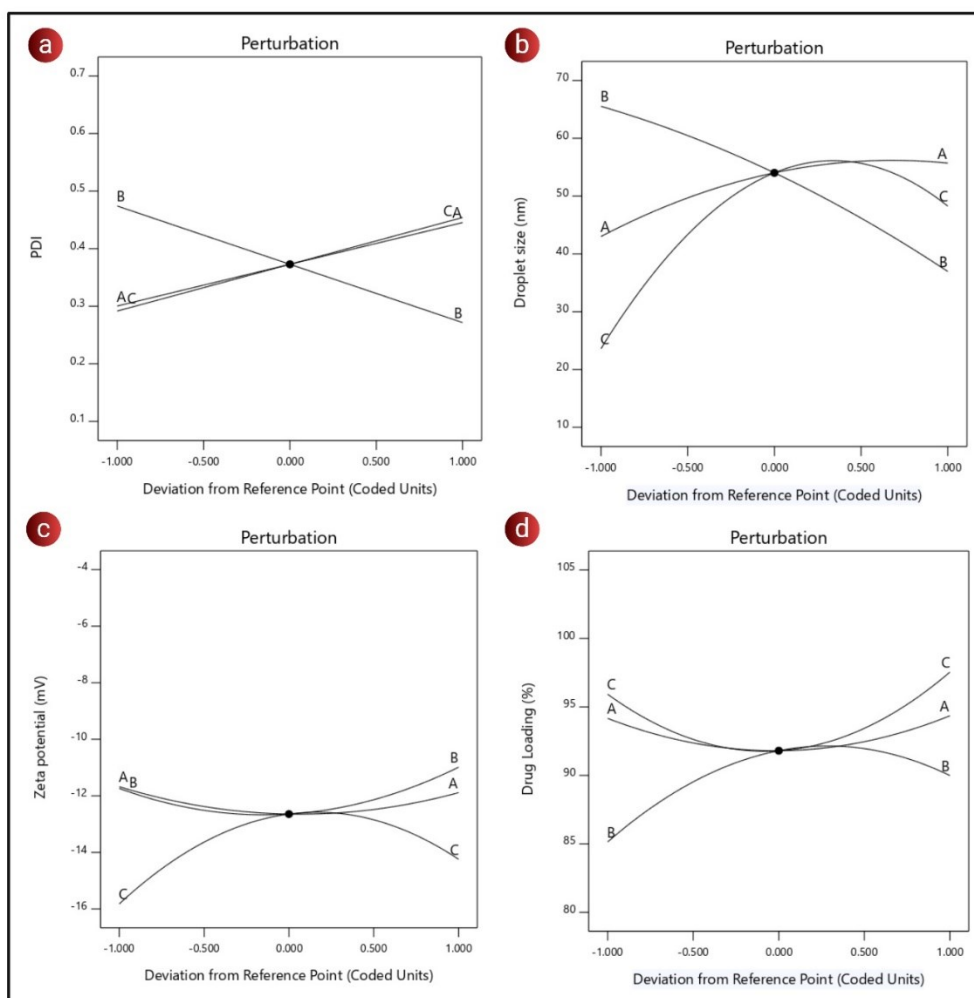
Figure 22: Perturbation plot a. PDI, b. Droplet size, c. Zeta potential, d. Drug loading
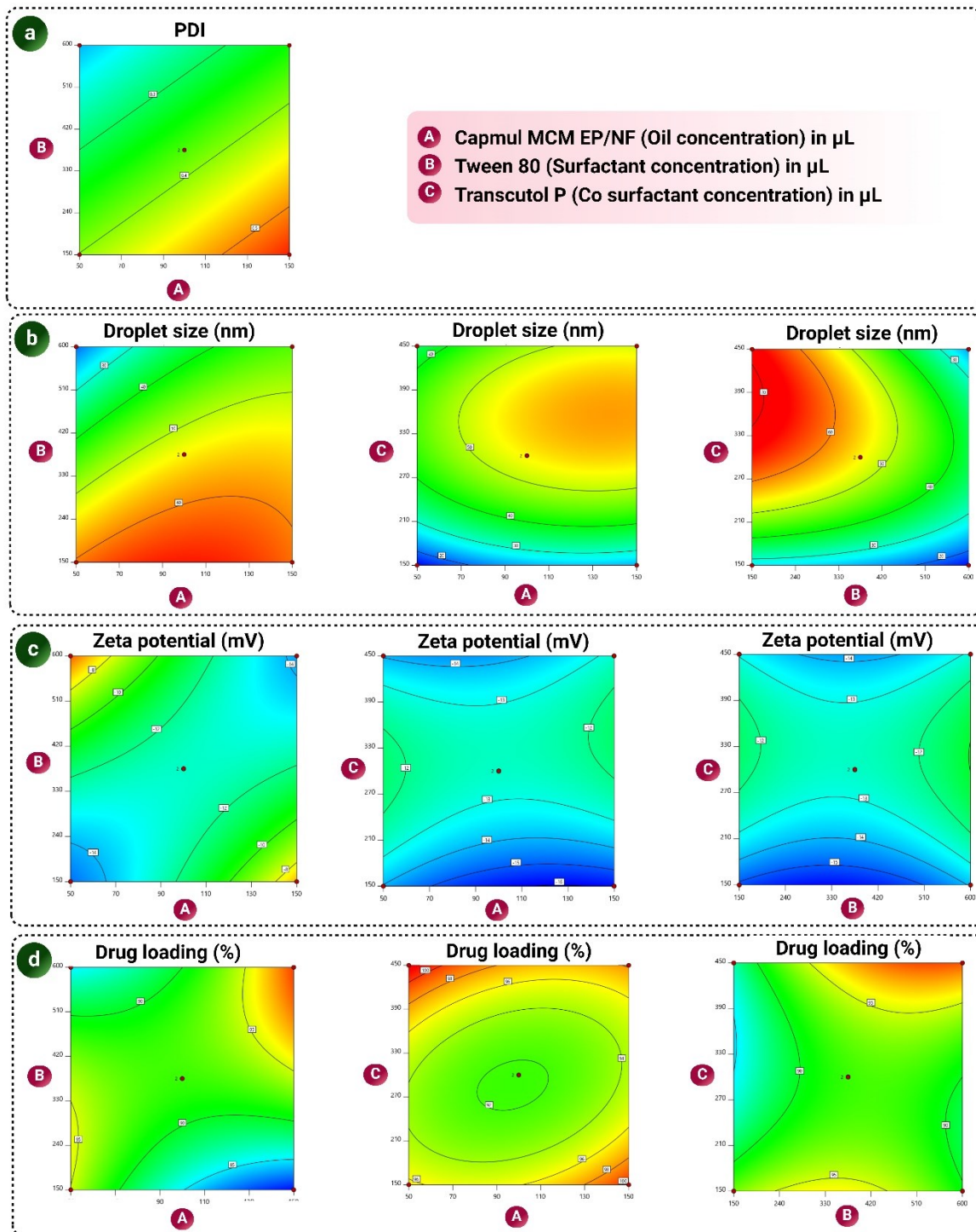
Figure 23: 2D-response surface plots representing effects of components of formulation a. PDI b.droplet size c. Zeta potential d. Drug loading
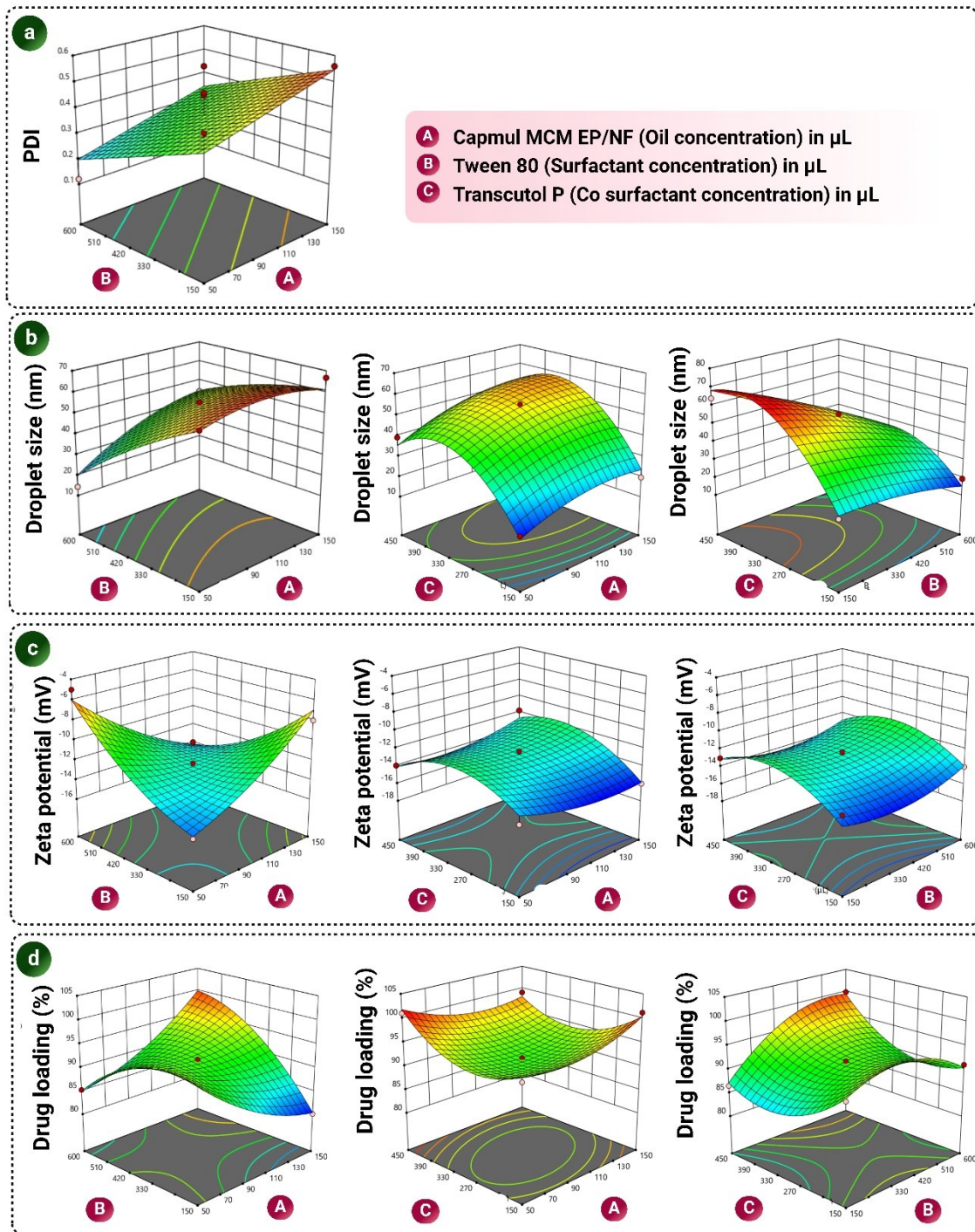
Figure 24: 3D-response surface plots representing effects of components of formulation a. PDI
b. droplet size c. Zeta potential d. Drug loading
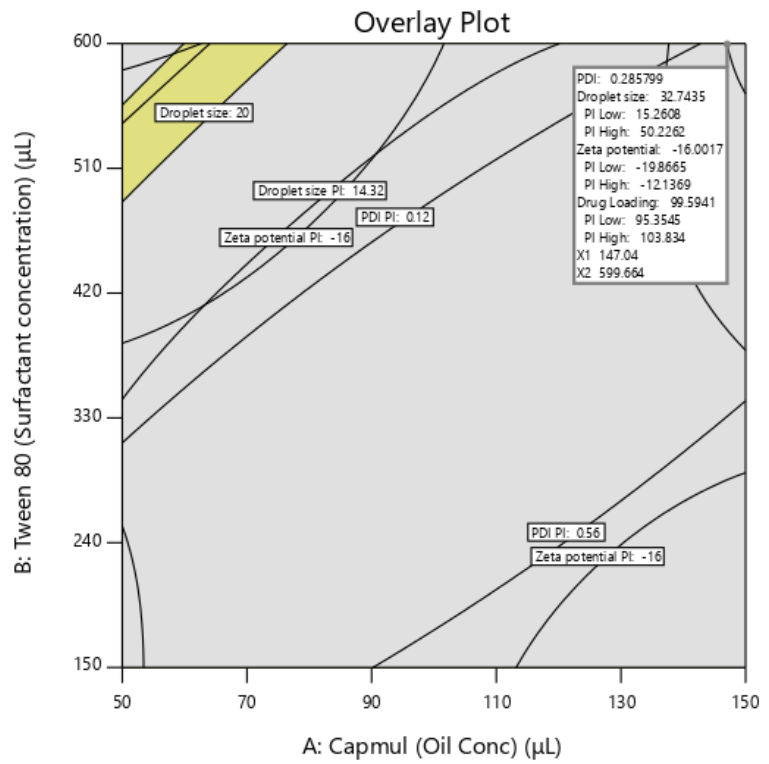
Figure 25: Overlay plot representing the optimized composition of the formulation
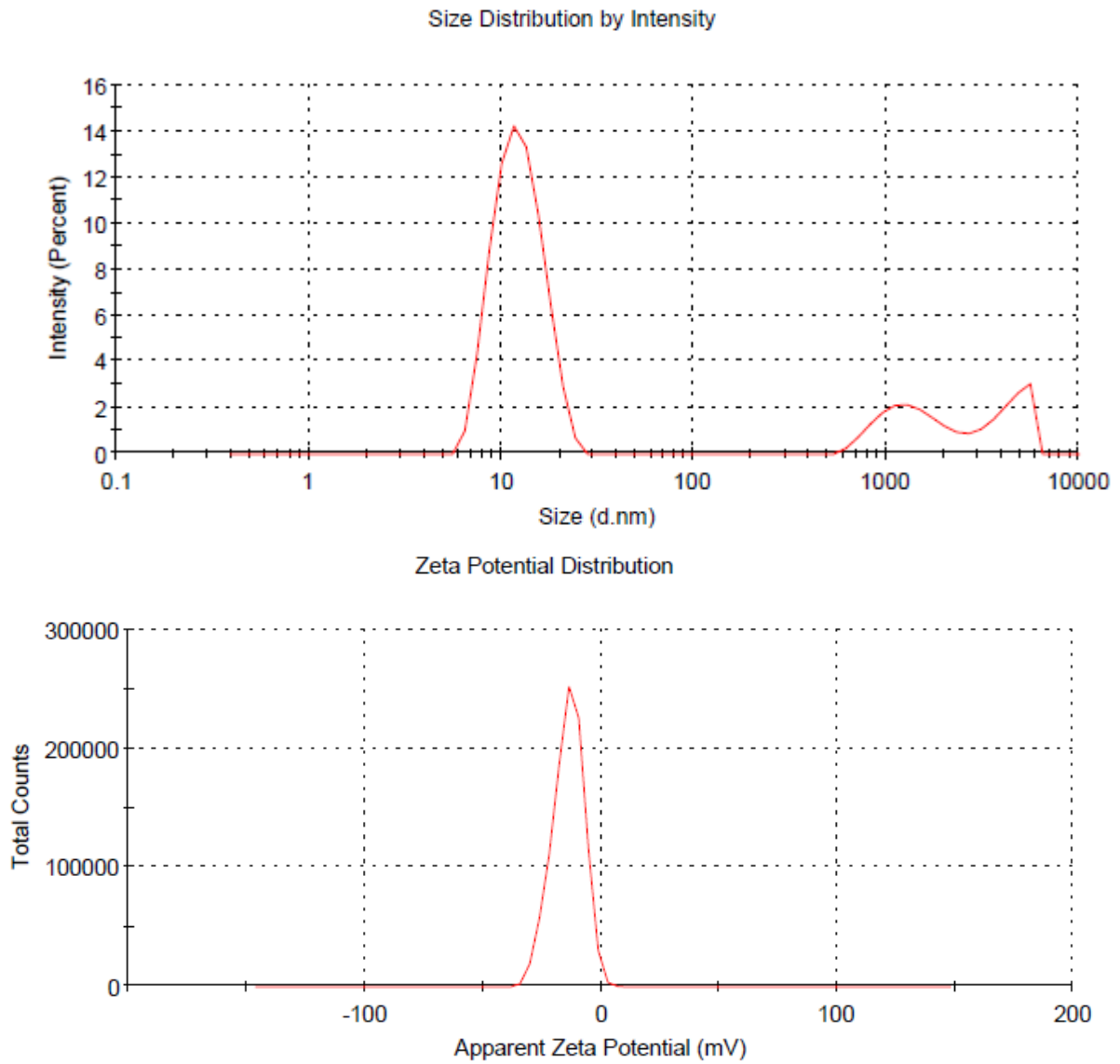
Figure 26: Results of droplet size, zeta potential and PDI
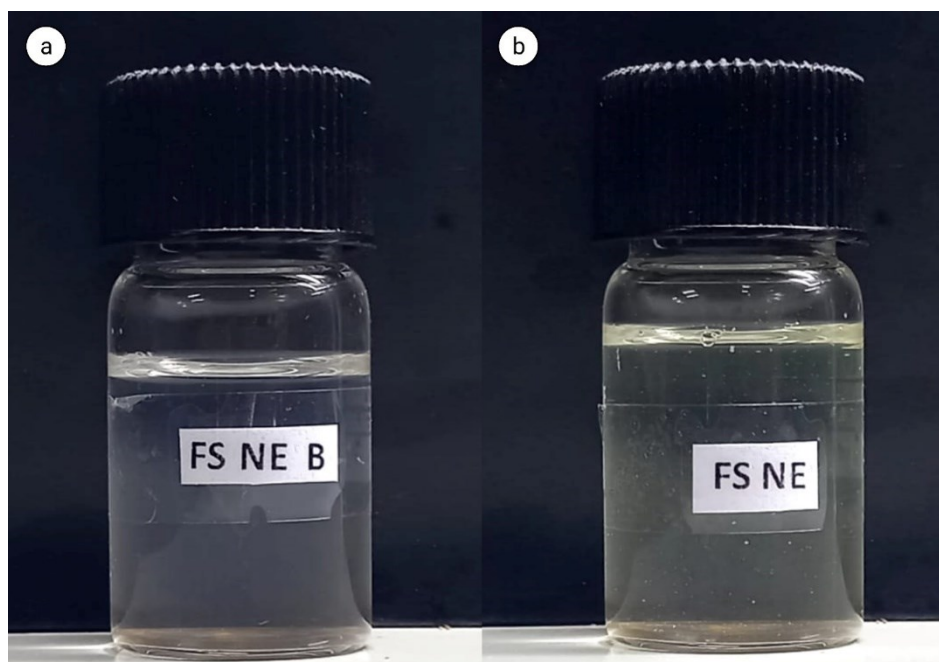
Figure 27: Images of blank NE (a) and FS-NE (b)

5.2.3. Thermodynamic and centrifugation stability

The optimized formulation demonstrated stability under diverse stress conditions, encompassing heating and cooling cycles as well as centrifugation, exhibiting no signs of drug precipitation or phase separation. Furthermore, the cloud point of the optimized formulation was identified at 85°C. Moreover, after the NE was diluted, there were no issues observed such as drug precipitation, creaming, or cracking for up to 7 days, demonstrating its sustained stability.

5.2.4. TEM analysis

The spherical droplets of FS-NE were visible in the TEM image of the developed formulation (Figure 28), with most of the droplets falling within the range of 30 to 20 nm in size. This observation indicates that the results obtained from TEM closely align with the droplet size analysis results for the optimized FS-NE.
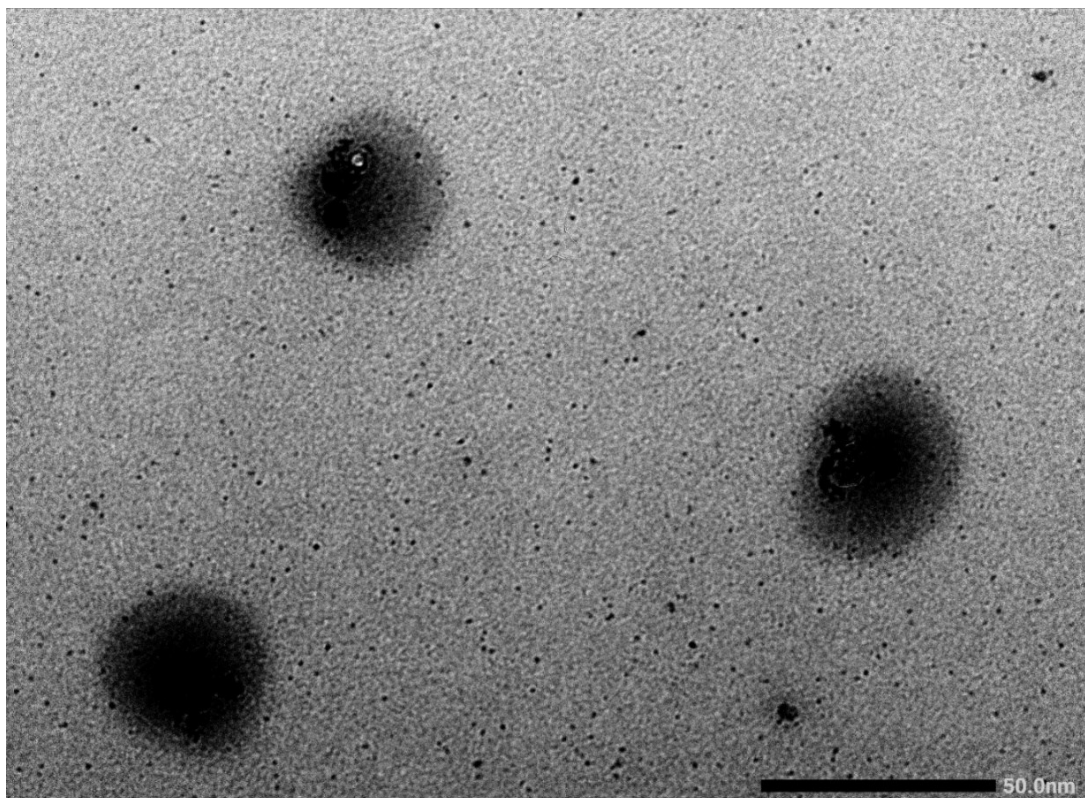
Figure 28: TEM image of FS-NE

5.2.5. Optical microscopy of FS-NE

Under optical microscopy, the image displayed a consistent dark blue background with colorless droplets dispersed throughout, confirming that the optimized NE features the oil phase as the dispersed phase and the aqueous phase as the continuous phase as dye is soluble in an aqueous medium.
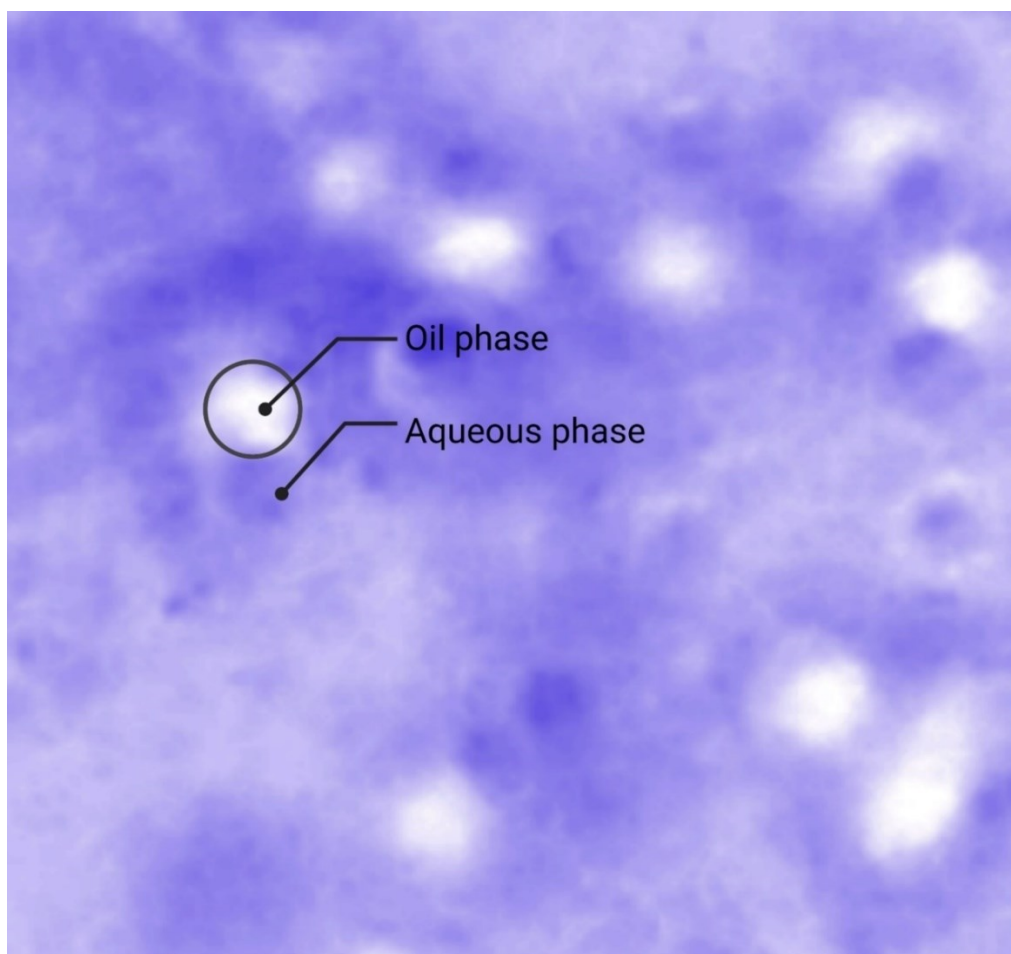
Figure 29: Dye test analysis of FS-NE

5.3. Stability studies

It was observed that the aged FS-NE after 6 months showed an increase in droplet size (33 ± 0.85 nm), a decrease in ZP (-14.42 ± 0.42 mV), an increase in PDI (0.31 ± 0.52), and decreased in drug loading (98.21 ± 1.52 %) compared to the initial analysis at 0 months. The initial droplet size of the FS-NE was recorded as 32 ± 1.21 nm, ZP (-15.99 ± 0.58 mV), PDI (0.28 ± 0.45) and drug loading (97.7 ± 1.47 %). The p-values were found to be 0.0739, 0.1001, 0.1161, and 0.5081 for droplet size, ZP, PDI, and % drug loading, which are higher than the p-value of 0.05. This indicates that no significant differences were observed between aged and fresh FS-NE.

5.4. Invitro studies

5.4.1. MTT assays

The findings from the cell line study indicated that naïve FS caused greater toxicity to the cells than FS-NE. The naïve FS at the concentration of 100 μg/mL resulted in the highest level of cell death, with only 49.74 of cells remaining viable. In case of the FS-NE, the identical concentration resulted in reduced cell death, with more than 68% of cells demonstrating viability. This observation suggests that the developed FS-NE formulation, upon emulsion formation, remained confined within the globule and exhibited limited interaction with the cells. Consequently, this lead to a decreased level of toxicity towards the cells.
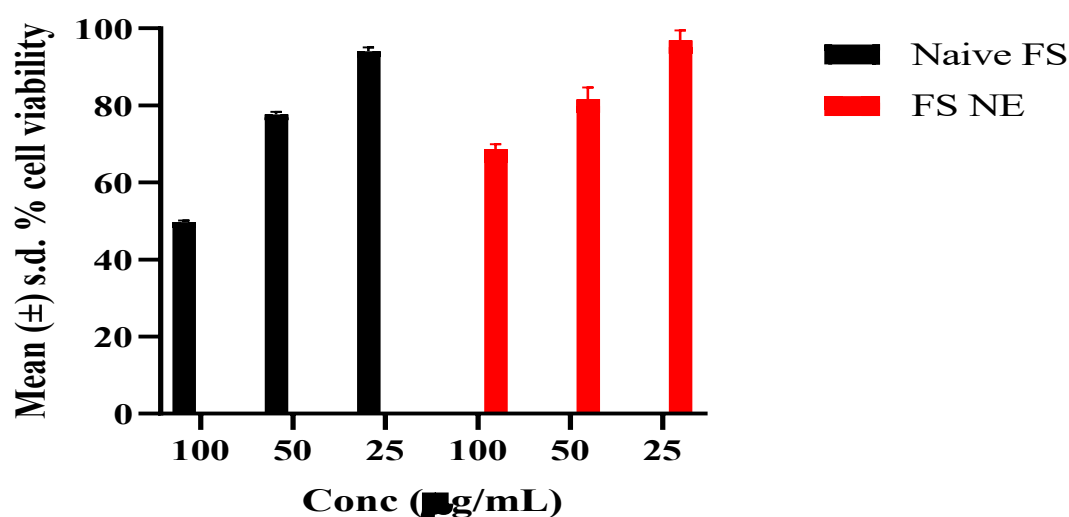
Figure 30: MTT assay

5.4.2. In vitro release

In vitro cell line permeability was conducted to check the permeation of FS around the intestinal membrane loaded in NE. Optimized FS-NE (A-B) showed an enhanced drug permeation (3.03 folds) compared to its naïve FS (A-B). Optimized FS-NE (B-A) showed lower drug excretion compared to naïve FS (B-A). The duration of the study was 4 hours. The *in vitro* release permeability study is shown in Figure 31. The drug permeability study is one of the very important parameters for checking the absorption and bioavailability of lipophilic drugs.
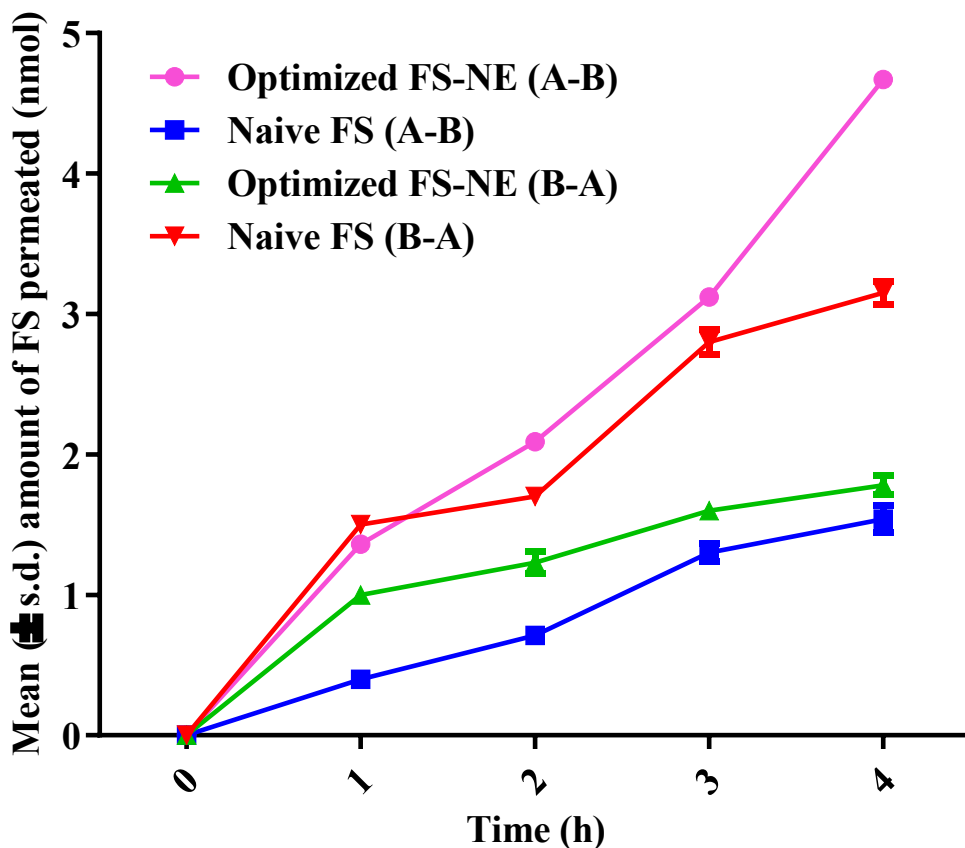
Figure 31: *In vitro* release studies

Figure 31: Permeation profile mean (mean±s.d.) of FS from naïve form and FS-NE. A to B represents permeation of drug from apical (A) side to basolateral (B) side and B to A represents permeation (excretion) of drug from basolateral (B) side to apical side (A) (n = 3).

5.5. Bioanalytical method development

Method validation includes several parameters like accuracy, precision, linearity, robustness, SST, specificity, LOD, and LOQ. These all-validation parameters have been performed systematically as per ICH Q2 (R1) guidelines.

5.5.1. Chromatograms of FS and Qu

The chromatogram of FS and QUE prepared in mobile phase is shown in Fig.32. The Rts of FS, Qu were found to be 4.2, 5.5 and 12.1 min respectively.
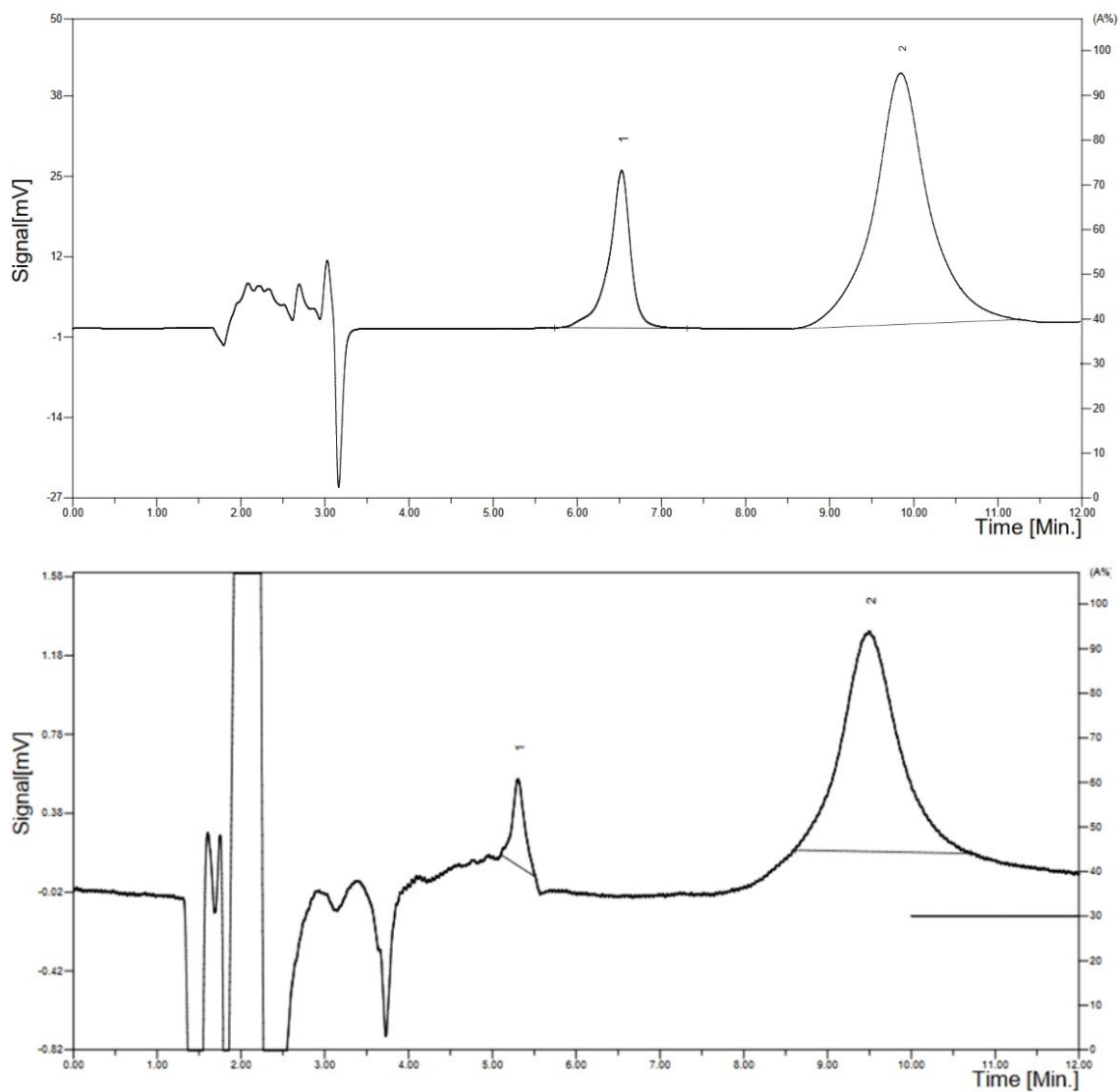
Figure 32: Chromatograms of FS and Qu

5.5.2. Specificity studies

Figure 33 displays the chromatogram of blank plasma. The lack of peaks at the retention times (Rts) of FS and Qu confirms that the plasma matrix does not interfere with the

quantitative analysis of these drugs when extracted from blood.
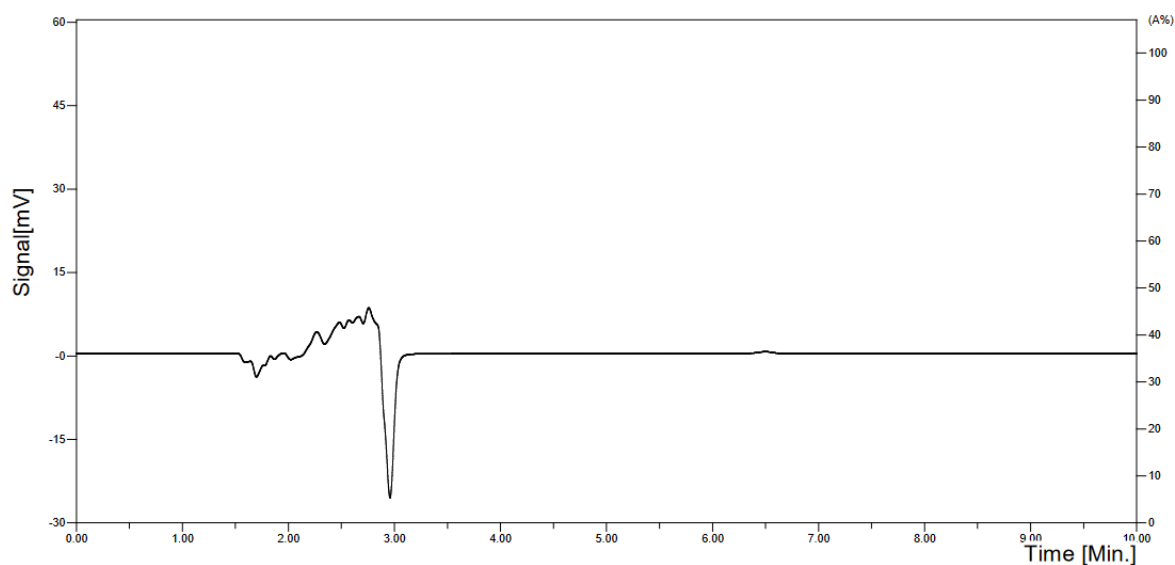
Figure 33: Chromatograms of blank plasma

5.5.3. Development of calibration curve

Five serial dilutions having concentrations of 2, 4, 6, 8 and 10 ng/mL were prepared to determine linearity and range. The data was found linear in this range with the correlation coefficient (r^2) of 0.9994. The slope of the curve was found to be 0.0655X and intercept was 0.093. The calibration curve of FS is shown in figure 34.
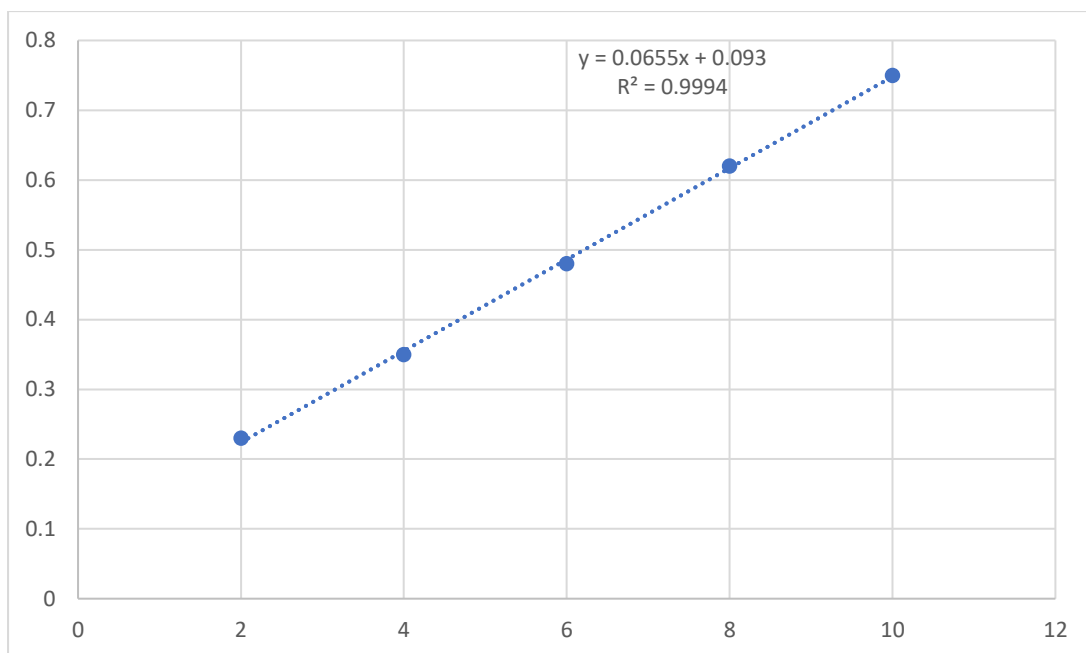
Fig. 34. Calibration curve of FS in please sample

5.5.4. Accuracy study

The study was carried out as per the procedure described in the section of 4.3.1.5.4. The % recovery was found in the range of 98-100 %, indicating about the accuracy of method. Accuracy study of FS is tabulated in Table 24.

Table 24: Accuracy study

Level	Actual concentration of std solution (µg/mL)	Concentration of drug recovered (ng/mL), (*N=5*)	% recovery
LQC	4.8	4.74	98.76
MQC	6	5.89	98.29
HQC	7.2	7.13	99.15

5.5.5. Precision studies

The intraday, interday, and interanalyst precision, studies were carried out for FS in plasma and mobile phase. The % RSD of the areas recorded for LQC, MQC and HQC samples was less than 2 indicating the precision of the method. (Table 25)

5.5.6. Robustness study

In the robustness study, The % RSD was observed to be less than 2%, which indicated that the method was found to be robust after slight changes in flow rate, mobile phase ratio, and pH. The results of the robustness study are shown in Table 26.

Table 25: Results of precision study

Parameter	Level	Concentration (ng/mL)	Area						Mean area	Standard deviation	% RSD
			1	2	3	4	5	6			
Intra day											
	LQC	4.80	0.426	0.422	0.425	0.424	0.428	0.427	0.424	0.0021	0.49
	MQC	6.00	0.514	0.518	0.519	0.517	0.523	0.520	0.517	0.0026	0.51
	HQC	7.20	0.601	0.604	0.597	0.602	0.599	0.604	0.601	0.0035	0.58
Inter-day											
Day 1	LQC	4.80	0.424	0.426	0.427	0.424	0.421	0.425	0.426	0.0015	0.36
	MQC	6.00	0.513	0.516	0.514	0.517	0.516	0.519	0.514	0.0015	0.30
	HQC	7.20	0.605	0.601	0.599	0.600	0.560	0.603	0.602	0.0031	0.51
Day 2	LQC	4.80	0.421	0.424	0.427	0.426	0.422	0.425	0.424	0.0030	0.71
	MQC	6.00	0.514	0.510	0.515	0.517	0.521	0.525	0.513	0.0026	0.52
	HQC	7.20	0.601	0.604	0.597	0.602	0.599	0.596	0.601	0.0035	0.58
Day 3	LQC	4.80	0.423	0.426	0.422	0.427	0.424	0.419	0.424	0.0021	0.49
	MQC	6.00	0.515	0.512	0.516	0.518	0.526	0.529	0.514	0.0021	0.40
	HQC	7.20	0.603	0.601	0.598	0.604	0.602	0.599	0.60	0.0025	0.42
Intermediate precision											
Analyst 1	LQC	4.80	0.421	0.425	0.422	0.426	0.428	4.225	0.42	0.0021	0.49

	MQC	6.00	0.513	0.510	0.514	0.516	0.520	0.518	0.51	0.0021	0.41
	HQC	7.20	0.601	0.604	0.597	0.602	0.599	0.596	0.60	0.0035	0.58
Analyst 2	LQC	4.80	0.422	0.425	0.423	0.424	0.421	0.425	0.42	0.0015	0.36
	MQC	6.00	0.514	0.517	0.519	0.516	0.514	0.518	0.52	0.0025	0.49
	HQC	7.20	0.604	0.601	0.597	0.599	0.597	0.603	0.60	0.0035	0.58
Analyst 3	LQC	4.80	0.422	0.425	0.421	0.422	0.427	0.424	0.42	0.0021	0.49
	MQC	6.00	0.514	0.512	0.516	0.516	0.519	0.517	0.51	0.0020	0.39
	HQC	7.20	0.602	0.601	0.598	0.603	0.599	0.601	0.60	0.0021	0.35

Table 26: Robustness results of various parameters

Variable	Value	Conc (ng/mL)	Peak area (Mean ± SD) (*N=6)	Mean of peak area of three values (*N=3)	% Recovery (Mean ± SD) (*N=6)	Mean of % recovery of three values (*N=3)	Retention time (Mean ± SD) (*N=6)	Mean of retention time values of three (*N=3)
Flow rate (mL/min)	0.8	6	0.53 ± 0.002	0.52	111.45 ± 0.611	109.78	7.76 ± 0.135	6.708
	1	6	0.524 ± 0.003	SD = 0.02	109.75 ± 0.695	SD =0.582	6.69 ± 0.09	SD = 0.101
	1.2	6	0.517 ± 0.002	% RSD = 0.436	107.84 ± 0.43	% RSD = 0.53	5.66 ± 0.071	% RSD = 1.508
Mobile phase Ratio (A:B) v/v	28:72	6	0.519 ±0.002	0.52	108.312 ± 0.526	110.13	7.90 ± 0.089	6.812
	30:70	6	0.525 ± 0.003	SD = 0.003	109.96 ± 0.84	SD = 0.662	6.77 ± 0.064	SD = 0.084
	32:68	6	0.534± 0.002	% RSD = 0.49	112.12 ± 0.616	% RSD = 0.601	5.76 ± 0.100	% RSD =1.23
pH	2.9	6	0.517 ± 0.018	0.519	107.76 ± 0.509	108.369	6.88 ± 0.030	6.75
	3.1	6	0.525 ± 0.003	SD = 0.008	109.96 ± 0.84	SD =2.01	6.77 ±0.039	SD = 0.05
	3.3	6	0.515 ± 0.002	% RSD = 1.524	107.37 ±0.50	% RSD =1.87	6.60 ± 0.088	% RSD = 0.77

5.5.7. System suitability

In the system suitability, the results indicated that the values of all parameters were found in the pharmacopeial limits and method was suitable for method development [196]. System suitability provides us information about the chromatographic system prior to use. The continuous use of chromatographic system affects their performance and affect the reliability of analytical results. The lower values of LOD and LOQ revealed that the method was enough sensitive to detect FS under specified chromatographic conditions. The value of theoretical plate more than 2,000, theoretical plate/meter more than 20,000 and tailing factor less than 1.5 indicated about better column efficiency. The results obtained in the current study suggest excellent system suitability for the developed method. The obtained results of system suitability indicated that method is reliable and suitable for further studies. The results of system suitability are mentioned in Table 27.

Table 27: System suitability of FS in plasma

Parameters	Value	Limits
Area	0.77	--
Height	0.39	--
Theoretical plate	2952.21	More than 2,000
Theoretical plate/meter	29520.21	More than 20,000
Tailing factor	0.59	Less than 1.5
Peak purity index	0.79	More than 0.5
LOD	0.03 ng/mL	--
LOQ	0.08 ng/mL	--

5.6. Pharmacokinetic studies

5.6.1. Pharmacokinetic studies in blood

A non-compartmental analysis was conducted to calculate the pharmacokinetic parameters, and the results are presented in Table 28 and Figure 35 (a). The findings revealed that naïve FS exhibited a Tmax in 5 minutes and FS-NE exhibited in 5 minutes. The P value was found to be 0.0048 which is smaller than 0.005 indicating significant differences ($p < 0.05$) in their area under the curve (AUC) and Cmax values. The Cmax for naïve FS was measured at 10.56 ng/mL, while FS-NE showed a Cmax of 96.93 ng/mL, indicating a 9.2-fold increase in Cmax for FS-NE. Similarly, the $AUC_{0-\infty}$ was determined to be 179.70 ng.mL*min for naïve FS and 1514.75 ng.mL*min for FS-NE. It is important to note that $AUC_{0-\infty}$ reflects the extent of drug absorption, while Cmax represents both the rate and extent of absorption. Therefore, the higher Cmax and AUC values for FS-NE compared to naïve FS indicate improved drug absorption and enhanced oral bioavailability. The relative bioavailability of FS-NE was calculated to be 842.93%. Additionally, the longer $t_{1/2}$ and mean residence time (MRT) of FS-NE indicated a greater retention of FS-NE in the plasma compared to naïve FS.

5.6.2. Pharmacokinetic studies (Brain)

FS-NE revealed a concentration of 22.21 ng/mL in the brain, whereas the availability of naïve FS in the brain was only 2.52 ng/mL. (Fig. 35b) About 8.81-fold enhanced availability of FS was found in rat's brain. This significantly ($P < 0.05$) increased concentration of FS in plasma as well as in brain indicated the potential of NE to enhance the absorption as well as brain permeability of lipophilic drugs.
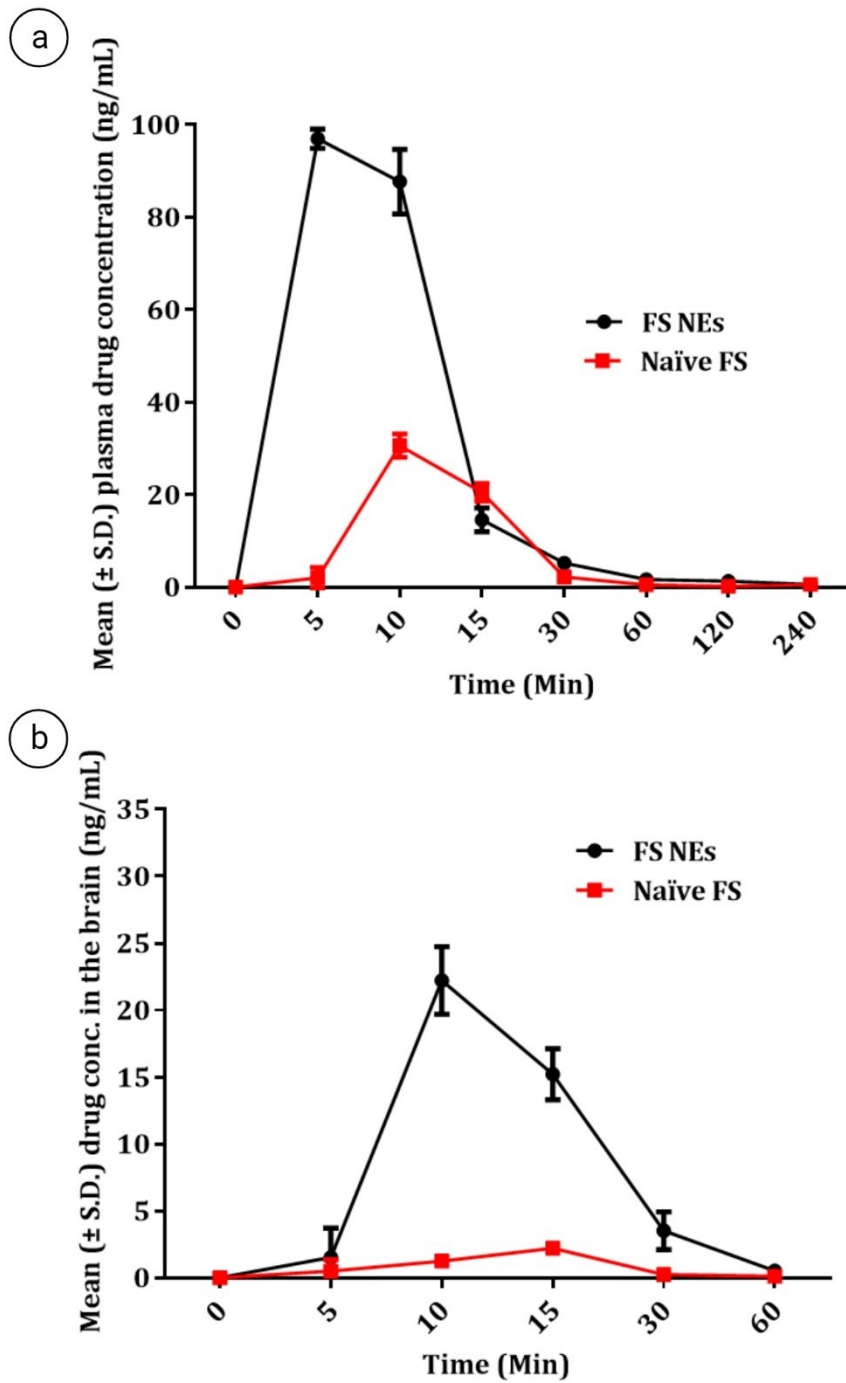
Figure 35 Pharmacokinetics data in blood (a) Pharmacokinetics data in brain (b)

Table 28: Pharmacokinetics parameters.

Parameters	FS-NE	Naïve FS
$T_{1/2}$ (min)	111.773	42.63
T_{max} (min)	5	10
C_{max} (ng/L)	96.93	10.56
AUC_{0-1} (ng.mL*min)	1414.67	177.86
$AUC_{0-\infty}$ (ng.mL*min)	1514.75	179.70
$MRT_{0-\infty}$ (Min)	53.22	35.17

5.7. Pharmacodynamic studies

Neurological disorders are the primary contributor to disability on a global level, and among these conditions, AD is witnessing a considerably more rapid growth in its prevalence compared to other neurodegenerative diseases [197]. AD pertains to a specific commencement and progression of cognitive and functional deterioration linked with aging, along with a particular neuropathology [198]. Contemporary clinical diagnostic standards have been formulated, and standards have also been suggested to identify preclinical (or presymptomatic) phases of the ailment through the application of biomarkers. AD acknowledges the coexisting neuropathologies that frequently contribute to clinical dementia. The early stages of the disease are characterized by impairments in the ability to encode and retain new memories. As the disease progresses, there are subsequent changes in cognition and behavior. The combination of alterations in APP cleavage, production of Aβ (Aβ) fragments, and aggregation of hyperphosphorylated tau protein contribute to synaptic strength reduction, synaptic loss, and degeneration of the neurons [199–201]. Metabolic, vascular, and inflammatory changes, as well as concurrent pathologies, play significant roles in the disease process [202]. While symptomatic treatment has a modest impact on cognition, there is an urgent need for disease-modifying therapies. The treatment for this disease comprises the administration of anti- AChE

medications, either as a standalone or in combination with supplementary drugs, as well as non-pharmacological approaches such as physical, occupational, and speech therapy. The degeneration AChE neurons and disruption in cholinergic pathways are responsible for the occurrence of most cognitive symptoms in AD. As a result, ongoing drug development endeavors are not only focused on symptom management but also on targeting molecules or pathways that can modify the disease, thereby safeguarding and restoring cholinergic neurons [202–205].

Several studies have revealed a strong association between heavy metals and NDs such as AD [206]. Aluminium (Al) is a significant heavy metal that plays a role in the onset and progression of NDs, as it directly impacts various metabolic pathways in the nervous system. The use of $AlCl_3$ is highly prevalent, as it is present in various commercially produced products such as toothpaste, food, medications, and packaged drinking water [207]. The onset of AD has been directly linked to the intake of certain metal toxins, such as Al, which enters the body through occupational exposure, food contamination, drinking water contamination, and the use of Al cookware [208]. Al has the ability to alter the b (BBB) and accumulate in the brain over time [209]. As a result, it is considered a risk factor for neurological disorders due to Al-induced brain intoxication [210]. Moreover, Al can impede the activities of antioxidant enzymes, disrupt brain neurochemistry, and lead to DNA damage in the brain[211]. Experimental evidence from several studies has demonstrated that prolonged exposure to Al directly influences neurological symptoms that resemble advanced neurodegeneration. Neurofilament changes have been observed in the hippocampus, spinal cord, and cerebral cortex, along with biochemical alterations in animals with Al-induced AD. As a result, AD induced by $AlCl_3$ in animals is widely regarded as the most extensively utilized animal model that replicates human AD [15,148,212–216]. Moreover, $AlCl_3$ potentiates the abnormal aggregation of protein synthesis, initiating major cascade of events resulting into cell death. One of the mechanisms by which $AlCl_3$ induces neurotoxicity is through oxidative stress [213]. As $AlCl_3$ accumulates in cholinergic neurons of the cerebral cortex and hippocampus, it triggers the production of free reactive radicals by the mitochondria of the cell. These reactive moeties include nitric oxide (NO), superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH). Furthermore, inflammation plays a crucial role in the neurotoxic effects caused by $AlCl_3$

[213,217]. Administrating the high dose of FS significantly reduced the AlCl$_3$-induced cognitive impairement and memory loss. Treatment with FS also notably reversed the reduction in acetylcholine levels generated by AlCl$_3$ in the brain. Moreover, the behavioural and biochemical alterations induced by AlCl$_3$ were ameliorated by the post treatment with high and low dose of FS in a dose dependent manner. Among all the treatments, the high dose of FS showed significant results followed by low dose of FS and high dose of naïve FS. Further results such as behavioural, biochemical and histopathological studies are discussed in subsequent sections.

Various behavioural alterations have been noted in the AlCl$_3$ induced AD in rats wherein the following tests have been carried out viz MWM and OFT. Early manifestations of anxiety-like behavior serve as a precursor to AD and can serve as an indicator for cognitive decline in older individuals. MWM is used to evaluate cognitive function of the rats wherein the escape latency and time spend in target quadrants in seconds were analyzed (Figure 36 a and b). The escape latency of rats under disease control conditions was notably elevated (P≤ $\mathbf{0.0001}$) on day 84 compared to standard control rats. The escape latency of rats treated with naïve FS (HD) on day 84 was lower when it was compared to positive control. The escape latency of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was notably decreased (P≤ $\mathbf{0.0001}$) when compared to the rats in the inducer group. Moreover, the escape latency of rats treated with naïve FS (HD) on day 84 was higher when it was compared to standard control. The escape latency of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when it was compared to standard control. Furthermore, the escape latency of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was significantly reduced (P≤ $\mathbf{0.001}$) and (P≤ $\mathbf{0.0001}$) respectively when compared to naïve FS (HD) as shown in (Figure 36 a).

The duration spent in the designated quadrant of disease control rats on day 84 was found to be notably decreased (P≤ $\mathbf{0.0001}$) when it was compared to standard control rats. The duration spent in the designated quadrant of rats induced with naïve FS (HD) on day 84 was increased when compared to disease control. The duration spent in the designated quadrant of rats given with FS-NE (LD) and FS-NE (HD) on day 84 was significantly increased (P≤ $\mathbf{0.0001}$) when compared to disease control. Moreover, the duration spent in the designated

quadrant of rats treated with naïve FS (HD) on day 84 was decreased when compared to standard control. The duration spent in the designated quadrant of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when compared to standard control. Furthermore, The duration spent in the designated quadrant of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was significantly increased (P≤ 0.001) and (P≤ 0.0001) respectively when compared to naïve FS (HD) as shown in (Figure 36 b).

The results of the current study concluded that the administration of FS mitigates the impact of $AlCl_3$ by reinstating locomotor and exploratory activity, enhancing cognitive abilities, and inducing anxiolytic effects through MWM. Several studies have been conducted where in MWM test was employed by researchers for the evaluation of anti- Alzheimers and the treatment with bromelain results showed significant changes in ameliorating the effects of $AlCl_3$ by restoring the cognitive function and improving the anxiolytic effects [12,13,75,218,219]
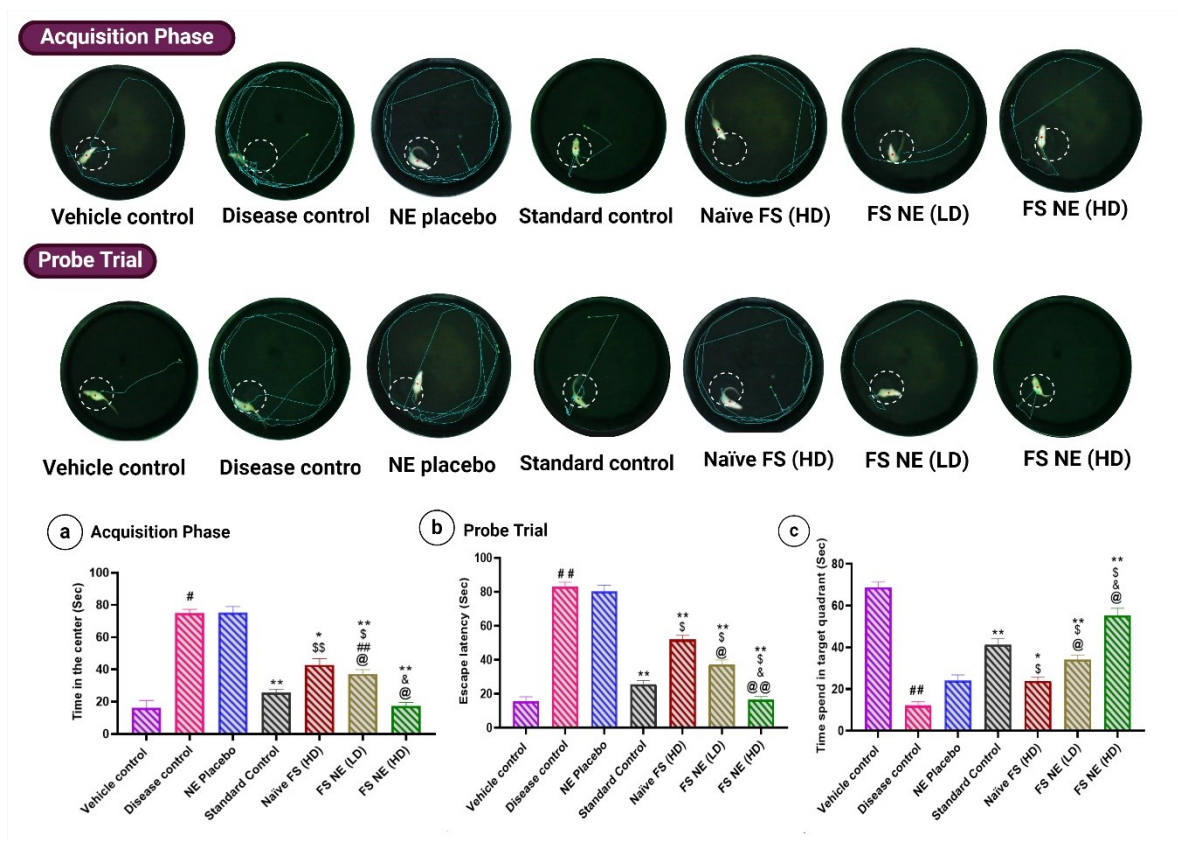
Figure 36. Effect on rats of different groups during MWM test. a. Escape latency and b. Tme spent in target quadrant. *In various treatments # P ≤ 0.0001, vehicle control vs disease control; ** P ≤ 0.0001, disease control vs treatment groups (NE placebo, standard control, naïve FS, FS-NE (LD) and FS-NE (HD)), * P ≤ 0.001, disease control vs treatment groups, $$ P ≤ 0.0001, standard control vs treatment groups, $ P ≤ 0.001, standard control vs treatment groups, & P ≤ 0.0001, FS-NE (LD) vs FS-NE (HD), @@ P ≤ 0.0001 Naïve FS vs FS-NE (LD) and FS-NE (HD). @ P ≤ 0.001, FS vs FS-NE (LD) and FS-NE (HD).*

Additionally, another behavioural study open field test (OFT) has been carried out for the evaluation of anxiety, locomotor and exploratory activity in $AlCl_3$ induced AD in rats wherein the time spent in the centre, number of rearing, number of squares crossed and number of grooming has been evaluated.

The period spent in the center by disease control rats on day 84 was notably reduced (P ≤ 0.0001) compared to standard control rats. The period spent in the centre of rats treated with naïve FS (HD) on day 84 was increased when compared to disease control. The time spent in the centre of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was significantly increased (P ≤ 0.0001) when compared to disease control. Moreover, the period spent in the centre of rats treated with naïve FS (HD) on day 84 was decreased when compared to standard control. The period spent in the centre of rats given with FS-NE (LD) and FS-NE (HD) on day 84 was or similar when compared to standard control. Furthermore, the period spent in the centre of rats given with FS-NE (LD) was not significant and FS-NE(HD) on day 84 was significantly increased (P ≤ 0.001) when compared to naïve FS (HD) as shown in (Figure 37 a).

The frequency of rearing in disease control rats on day 84 was notably higher (P ≤ 0.0001) compared to standard control rats. The number of rearing of rats treated with naïve FS (HD) on day 84 was increased when compared to disease control. The number of rearing of rats administered with FS-NE (LD) and FS-NE (HD) on day 84 was significantly increased (P ≤ 0.0001) when compared to disease control. Moreover, the number of rearing of rats treated with naïve FS (HD) on day 84 was decreased when it was compared to standard control. The

number of rearing of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when it was compared to standard control group. Furthermore, the number of rearing of rats treated with FS-NE (LD) was not significant and FS-NE(HD) on day 84 was significantly increased (P ≤ 0.001) when compared to naïve FS (HD) as shown in (Figure 37 b).

The number of squares crossed by disease control rats on day 84 was found to be significantly decreased (P ≤ 0.0001) when compared to standard control rats. The number of squares crossed by rats treated with naïve FS (HD) on day 84 was increased when compared to disease control. The number of squares crossed by rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was significantly increased (P ≤ 0.0001) when compared to disease control. Moreover, the number of squares crossed by rats treated with naïve FS (HD) on day 84 was decreased when compared to standard control. The number of squares crossed by rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was decreased when compared to standard control. Furthermore, the number of squares crossed by rats treated with FS-NE (LD) and FS-NE (HD) increased (P ≤ 0.0001) on day 84 when compared to naïve FS (HD) as shown in (Figure 37 c).

The grooming frequency of disease control rats on day 84 showed a marked increase (P ≤ 0.0001) compared to standard control rats. The number of grooming of rats treated with naïve FS (HD) on day 84 was increased when it was compared to disease control. The number of grooming of rats treated with FS-NE (LD) was not significant and FS-NE (HD) on day 84 was notably increased (P ≤ 0.001) when being compared to induced control group. Moreover, the number of rearing of rats treated with naïve FS (HD) on day 84 was decreased when it was compared to standard control. The number of rearing of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was similar when compared to standard control. Furthermore, the number of rearing of rats administered with FS-NE (LD) was not significant and FS-NE (HD) on day 84 was notably increased (P ≤ 0.001) when it was compared to naïve FS (HD) as shown in (Figure 37 d).

Similar studies have been conducted previously wherein OFT was employed by researchers for the evaluation of anxiety, locomotor and exploratory activity in AlCl$_3$ induced AD in rats and the treatment drugs showed significant changes in ameliorating the effects of AlCl$_3$ by

restoring the cognitive function, improving the anxiolytic effects and improving the locomotor and exploratory behaviour [12,75,220].
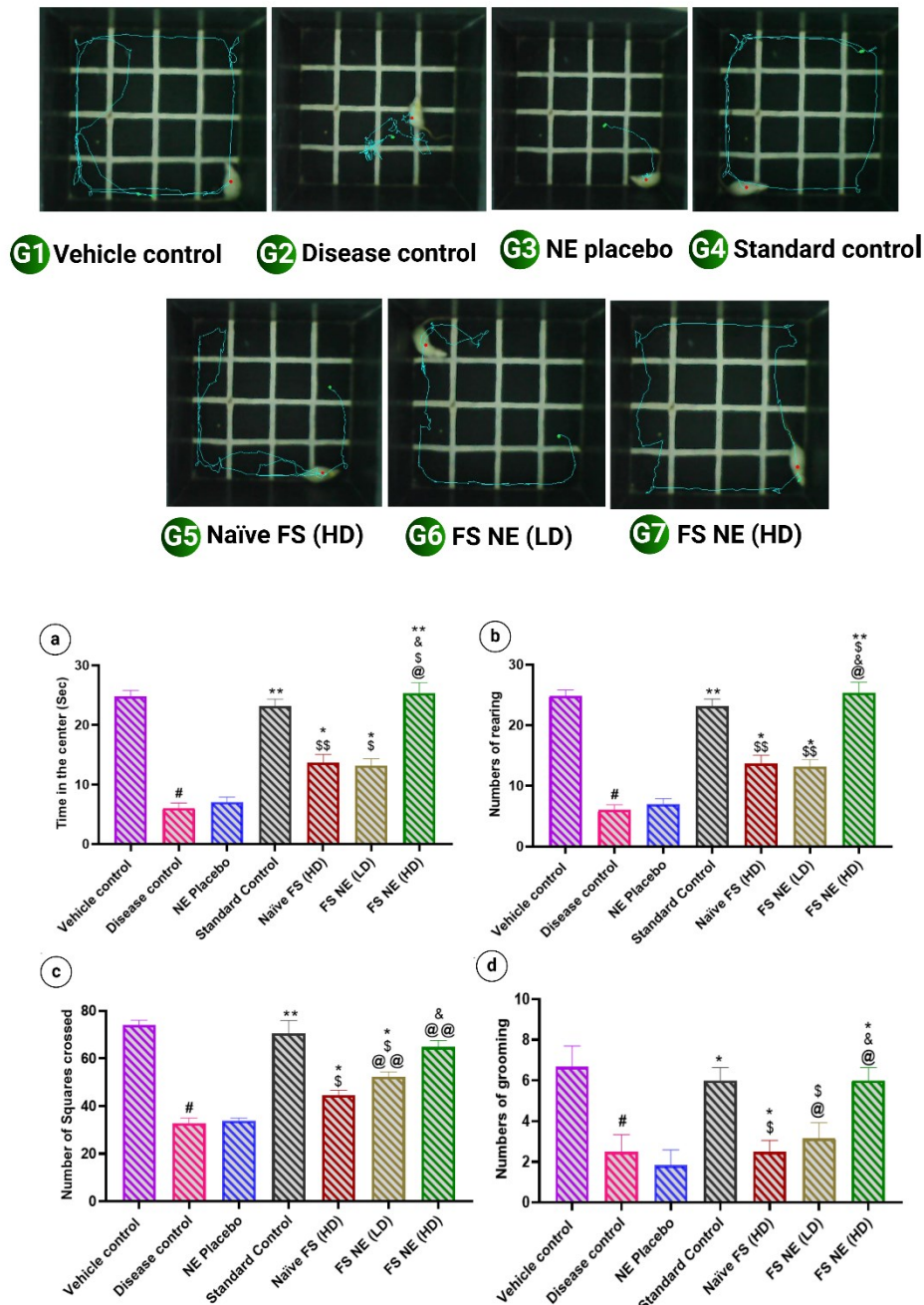

Figure 37. Effect of various treatments on rats during open field test, a. Time in the center; b. Numbers of rearing; c. Number of square crossed; d. Numbers of grooming. Here, *#P ≤ 0.0001,*

The emergence of AD is associated with an inadequate supply of cholinergic neurotransmission. The root cause of AD induced by $AlCl_3$ is connected to elevated activity of cerebral cortex and hippocampal AChE, resulting in the excessive depletion of acetylcholine [221]. This mechanism was substantiated in the present study by noting increased AChE activity in the $AlCl_3$ group, a condition mitigated by the administration of donepezil and FS. AChE is an enzyme present in extracellular fluid. It metabolizes acetylcholine into acetic acid and choline, thereby lowering the elevated levels of acetylcholine in the brain, especially in the cerebral cortex and hippocampus. The $AlCl_3$ is well known to enhance the level of AChE enzyme, which further reduces the level of ACh. In this study the AChE level in brain homogenate in cerebral cortex and hippocampal region of positive control rats on day 84 was observed to be notablys increased (P ≤ 0.0001) when it was compared to standard control rats. The AChE levels of rats treated with naïve FS (HD) on day 84 was decreased when compared to disease control. The AChE levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was significantly decreased (P ≤ 0.0001) when it was compared to positive control. Moreover, AChE levels of rats treated with naïve FS (HD) on day 84 was increased when compared to standard control. The AChE levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when compared to standard control. Furthermore, AChE levels of rats treated with FS-NE (LD) and FS-NE(HD) on day 84 was significantly decreased (P ≤ 0.0001) when compared to naïve FS (HD) as shown in (Figure 38 c). Inhibitor effects of AChE enzyme by FS-NE are presented in **Figure 38 (a).**

Moreover, FS-NE inhibited level of amyloid β in cereberal and hippocampal regions of the brain wherein the brain contents of Aβ1 in of disease control rats on day 84 was found to be notably increased (P ≤ 0.0001) when it was compared to standard control rats. The brain contents of Aβ1 of rats treated with naïve FS (HD) on day 84 was decreased when compared

to disease control. The brain contents of Aβ1 of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to disease control. Moreover, the brain contents of Aβ1 of rats treated with naïve FS (HD) on day 84 was increased when compared to standard control. The brain contents of Aβ1 of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when compared to standard control. Furthermore, the brain contents of Aβ1 of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to naïve FS (HD) as shown in (Figure 38 b).

There are various compelling explanations for the cognitive impairments associated with AD, with oxidative stress being a prominent factor strongly linked to AD. Aluminum (Al) is known to effectively traverse the blood-brain barrier (BBB) and accumulate in various brain regions, potentially inducing the production of free radicals. This, in turn, can lead to brain injury, particularly in areas responsible for memory and learning [222,223]. Oxidative stress-induced neurotoxicity stands out as a primary pathological event in the fundamental neurodegenerative process of AD [224,225]. Antioxidants represent a promising element in preventing the onset and progression of AD. Al intoxication induces significant oxidative stress by heightening the pro-oxidant effects of iron in the brain and diminishing the activities of antioxidant enzymes [226]. The assessment of MDA serves as a crucial biomarker for oxidative stress. MDA accumulates as a result of lipid peroxidation induced by reactive oxygen species (ROS), leading to damage and degradation of membranes [227]. Aluminum (Al) accelerates lipid peroxidation, fostering increased accumulation of free radicals, thereby causing oxidative stress and ultimately resulting in neurotoxicity [228]. The brain is highly vulnerable to oxidative stress due to elevated levels of free radicals and concurrently diminished antioxidant status, leading to toxicity [229]. FS-NE enhance antioxidant effects which is represented by CAT, GSH and TBAR assays. Antioxidaint effects of FS-NE are presented in figure 38 (c and d).

In the present study the CAT activity in brain homogenate in cerebral cortex and hippocampal region of positive control rats on day 84 was found to be significantly increased (P ≤ 0.0001) when it was compared to standard control rats. The CAT activity of rats given with naïve FS (HD) on day 84 was decreased when it was compared to disease control. The CAT activity of

rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was notably decreased (P ≤ 0.0001) when it was compared to positive control. Moreover, CAT activity of rats treated with naïve FS (HD) on day 84 was increased when it was compared to standard control. The CAT activity of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when compared to standard control. Furthermore, CAT activity of rats treated with FS-NE (LD) and FS-NE(HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to naïve FS (HD) as shown in (Figure 38 c).

Additionally, GSH levels in brain homogenate in cerebral cortex and hippocampal region of positive control rats on day 84 was found to be notably decreased (P ≤ 0.0001) when compared to standard control rats. The GSH levels of rats given with naïve FS (HD) on day 84 was increased when compared to disease control. The GSH levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was significantly increased (P ≤ 0.0001) when compared to disease control. Moreover, GSH levels of rats treated with naïve FS (HD) on day 84 was decreased when compared to standard control. The GSH levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was decreased or similar when compared to standard control. Furthermore, GSH levels of rats treated with FS-NE (LD) and FS-NE(HD) on day 84 was significantly increased (P ≤ 0.001) and (P ≤ 0.0001) respectively when compared to naïve FS (HD) as shown in (Figure 38 d).

Moreover, the SOD levels in brain homogenate in cerebral cortex and hippocampal region of positive control rats on day 84 was observed to be significantly increased (P ≤ 0.0001) when it was compared to standard control rats. The SOD levels of rats administered with naïve FS (HD) on day 84 was decreased when compared to disease control. The SOD levels of rats given with FS-NE (LD) and FS-NE (HD) on day 84 was notably increased (P ≤ 0.0001) when it was compared to disease control. Moreover, SOD levels of rats administered with naïve FS (HD) on day 84 was increased when compared to standard control. The SOD levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when compared to standard control. Furthermore, SOD levels of rats treated with FS-NE (LD) and FS-NE(HD) on day 84 was notably increased (P ≤ 0.0001) when it was compared to naïve FS (HD) as shown in (Figure 38 e).

Moreover, TBARS level in brain homogenate in cerebral cortex and hippocampal region of positive control rats on day 84 was found to be significantly increased (P ≤ 0.0001) when it was compared to standard control rats. The TBARS level of rats administered with naïve FS (HD) on day 84 was decreased when compared to disease control. The TBARS level of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to disease control. Moreover, TBARS level of rats treated with naïve FS (HD) on day 84 was increased when compared to standard control. The TBARS level of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when compared to standard control. Furthermore, CAT activity of rats treated with FS-NE (LD) and FS-NE(HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to naïve FS (HD) as shown in (Figure 38 c)
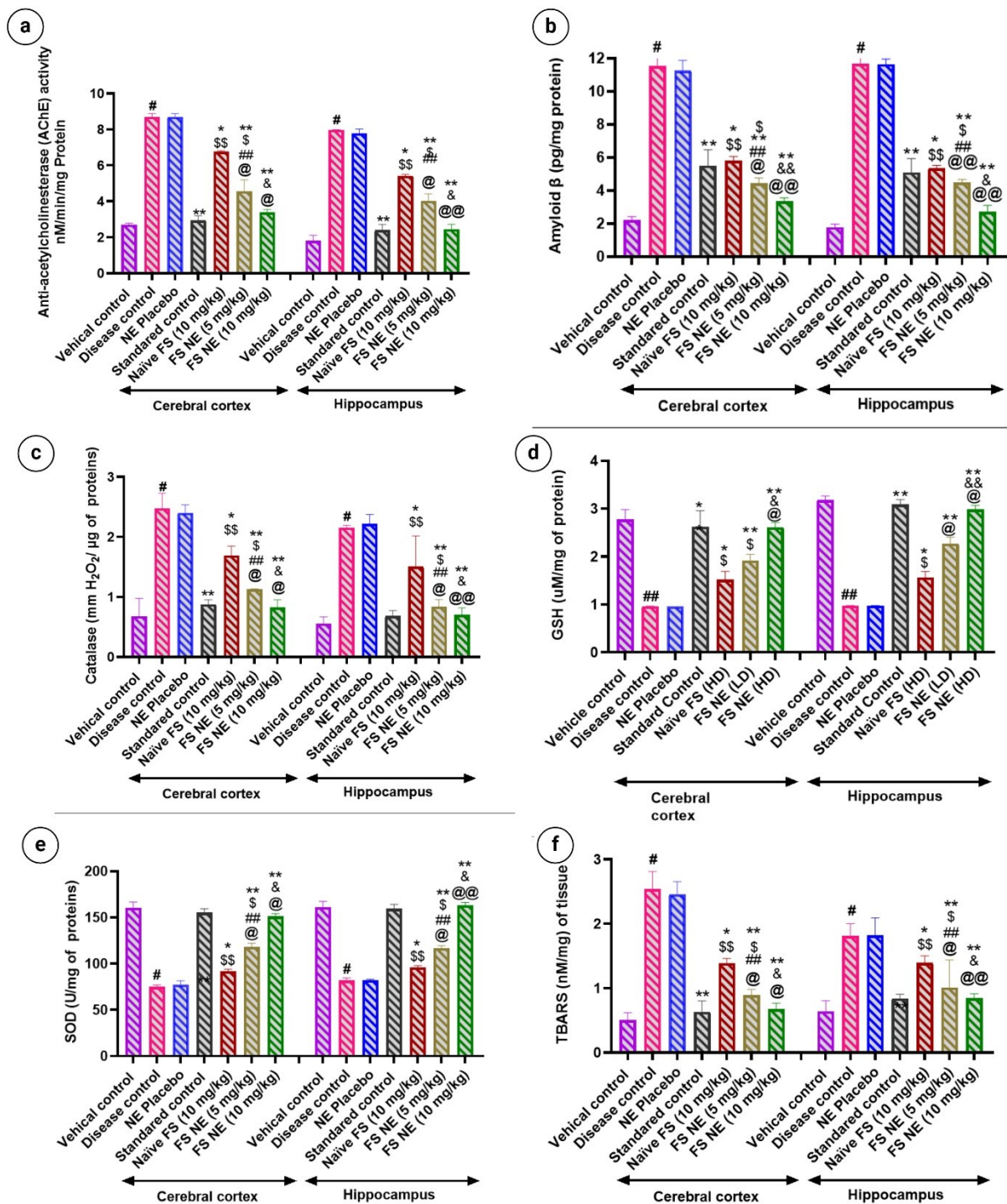
Figure 38. Anti-Alzheimer's (AChE and Aβ) and Antioxidant effects (CAT GSH, SOD and TBARS) of various treatments of rats of different groups. *# P ≤ 0.0001, vehicle control vs disease control; ** P ≤ 0.0001, disease control vs treatment groups (NE placebo, standard*

*control, naïve FS, FS-NE (LD) and FS-NE (HD)), * P ≤ 0.001, disease control vs treatment groups, $$ P ≤ 0.0001, standard control vs treatment groups, $ P ≤ 0.001, standard control vs treatment groups, & P ≤ 0.0001, FS-NE (LD) vs FS-NE (HD), @ P ≤ 0.0001 Naïve FS vs FS-NE (LD) and FS-NE (HD).*

Numerous studies conducted by different researchers have previously demonstrated that antioxidants can enhance neuroprotection. Various antioxidants have been documented to elevate antioxidant levels, consequently promoting neuroprotection in animal models induced AD [130], quercetin [230], curcumin [231], berberine [232], rutin [233], etc.

Role of FS-NE on neuroinflammation

Neuroinflammation plays a pivotal role in the onset and advancement of neuronal disorders, potentially leading to difficulties in memory and learning [234]. The significance of neuroinflammation is particularly pronounced in the pathological progression of neuronal disorders such as AD. In this context, interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) stand out as prominent inflammatory mediators, contributing to cellular dysfunction and regulating inflammation in both cells and organs [235]. We observed a significant increase in the levels of IL-1β and TNF- in the brain tissues of AD animals challenged with $AlCl_3$. Notably, this elevation was markedly attenuated by the administration of FS treatment.

In the present study, IL-1β levels in brain homogenate in cerebral cortex and hippocampal region of disease control rats on day 84 was found to be notably increased (P ≤ 0.0001) when it was compared to standard control rats. The IL-1β levels of rats treated with naïve FS (HD) on day 84 was decreased when compared to disease control. The IL-1β levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to positive control. Moreover, IL-1β levels of rats treated with naïve FS (HD) on day 84 was increased when compared to standard control. The IL-1β levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when compared to standard control. Furthermore, IL-1β levels of rats treated with FS-NE (LD) and FS-NE(HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to naïve FS (HD) as shown in (Figure 39 a).

Additionally, TNF-α levels in brain homogenate in cerebral cortex and hippocampal region of disease control rats on day 84 was found to be notably increased (P ≤ 0.0001) when compared to standard control rats. The TNF-α levels of rats treated with naïve FS (HD) on day 84 was decreased when compared to disease control. The TNF-α levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to positive control. Moreover, TNF-α levels of rats treated with naïve FS (HD) on day 84 was increased when compared to standard control. The TNF-α levels of rats treated with FS-NE (LD) and FS-NE (HD) on day 84 was increased or similar when compared to standard control. Furthermore, TNF-α levels of rats treated with FS-NE (LD) and FS-NE(HD) on day 84 was notably decreased (P ≤ 0.0001) when compared to naïve FS (HD) as shown in (Figure 39 b).

FS-NE also exhibited antiinflammatory effects by attenuating the level of IL-1β and TNF-α. The antiinflammatory effects of FS-NE are highlighted in Figure 39. Numerous studies conducted by different researchers have previously demonstrated that anti-inflammatory activity can enhance neuroprotection. Various anti-inflammatory substances have been documented to elevate antioxidant levels, consequently promoting neuroprotection in animal models induced with AD [13,75,216,220,236]
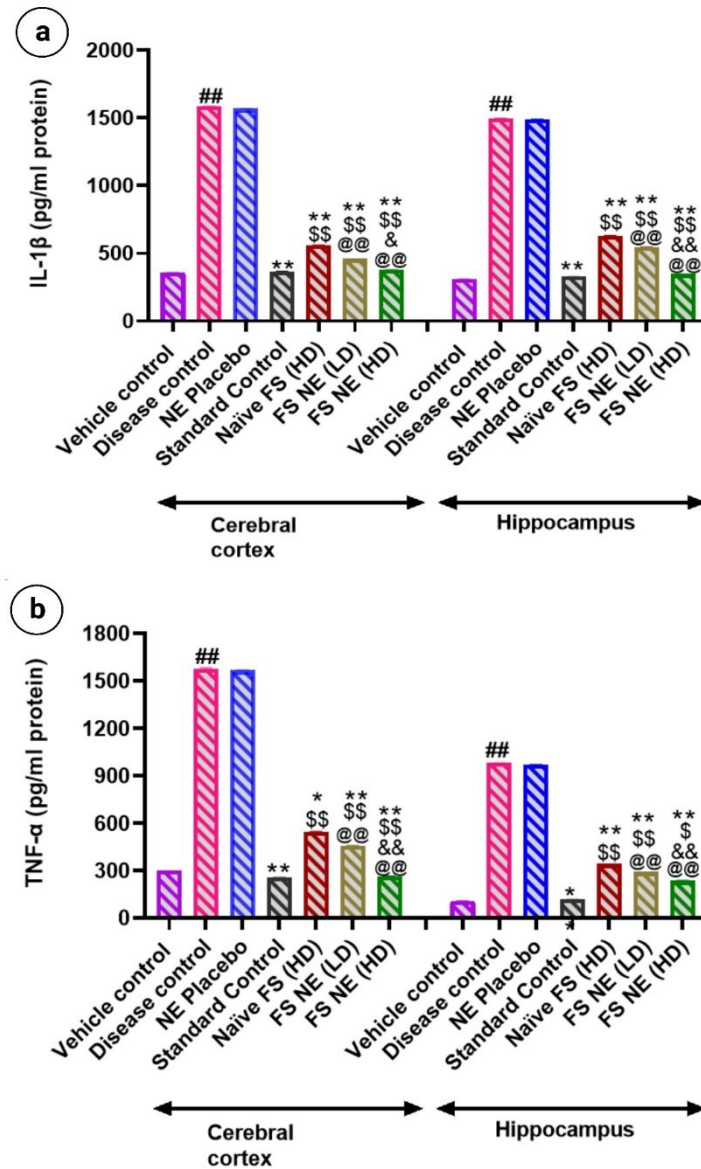
Figure 39. Anti-inflammatory (IL-1β and TNF-α) effects of various treatments given to rats belonging to different groups. *# P ≤ 0.0001, vehicle control vs disease control; ** P ≤ 0.0001, disease control vs treatment groups (NE placebo, standard control, naïve FS, FS-NE (LD) and FS-NE (HD)), * P ≤ 0.001, disease control vs treatment groups, $$ P ≤ 0.0001, standard control vs treatment groups, $ P ≤ 0.001, standard control vs treatment groups, & P ≤ 0.0001, FS-NE (LD) vs FS-NE (HD), @ P ≤ 0.0001 Naïve FS vs FS-NE (LD) and FS-NE (HD).*

5.8. Histopathological studies

During the histopathological examinations, the mid-brain of the vehicle control group exhibited normal intact cellular arrangements. Upon histological analysis of hippocampus sections from the disease control group, several histopathological alterations, notably significant neuronal degeneration, abnormal and eosinophilic lesions and shrinkage accompanied by nuclear pyknosis were observed. Likewise, prefrontal cortex sections from the $AlCl_3$ group exhibited neuronal loss concomitant with severe neuronal degeneration and apoptosis. Neurons within the pyramidal layer of the CA3 region and the granular layer of the dentate gyrus exhibited a characteristic appearance in both the treatment and control groups. Additionally, cortical sections from individuals treated with either FS-NE (HD) or donepezil exhibited an elevated neuron count. A minor occurrence of pyknotic and apoptotic nuclei was observed in the FS(NE) group and a slight presence of neuronal degeneration was observed in the donepezil-treated group. Furthermore, normal hippocampus structures with reduced histological alterations were seen with FS(HD) (Figure 40 a and b). Administering various doses of standard drugs, naïve FS, and FS-NE in succession alleviated these neuropathological changes, with FS-NE demonstrating the most significant impact. The compromised neurons in rats administered with FS-NE at both doses regained their distinctive morphology as shown in Fig 40 a and b.

Figure 40a: Effect of FS-NE on the hippocampus part of the brain histopathology of the AlCl$_3$ inducted AD in rats
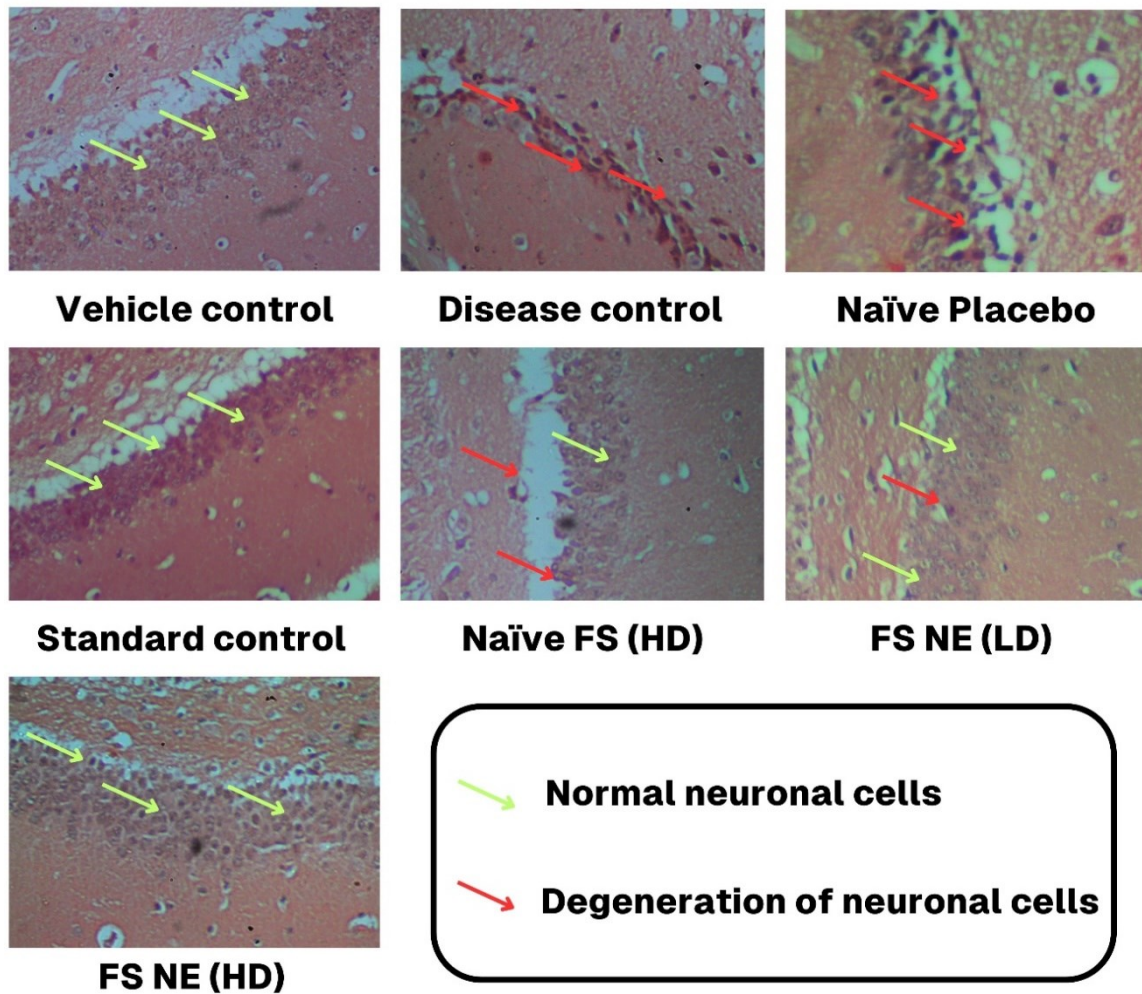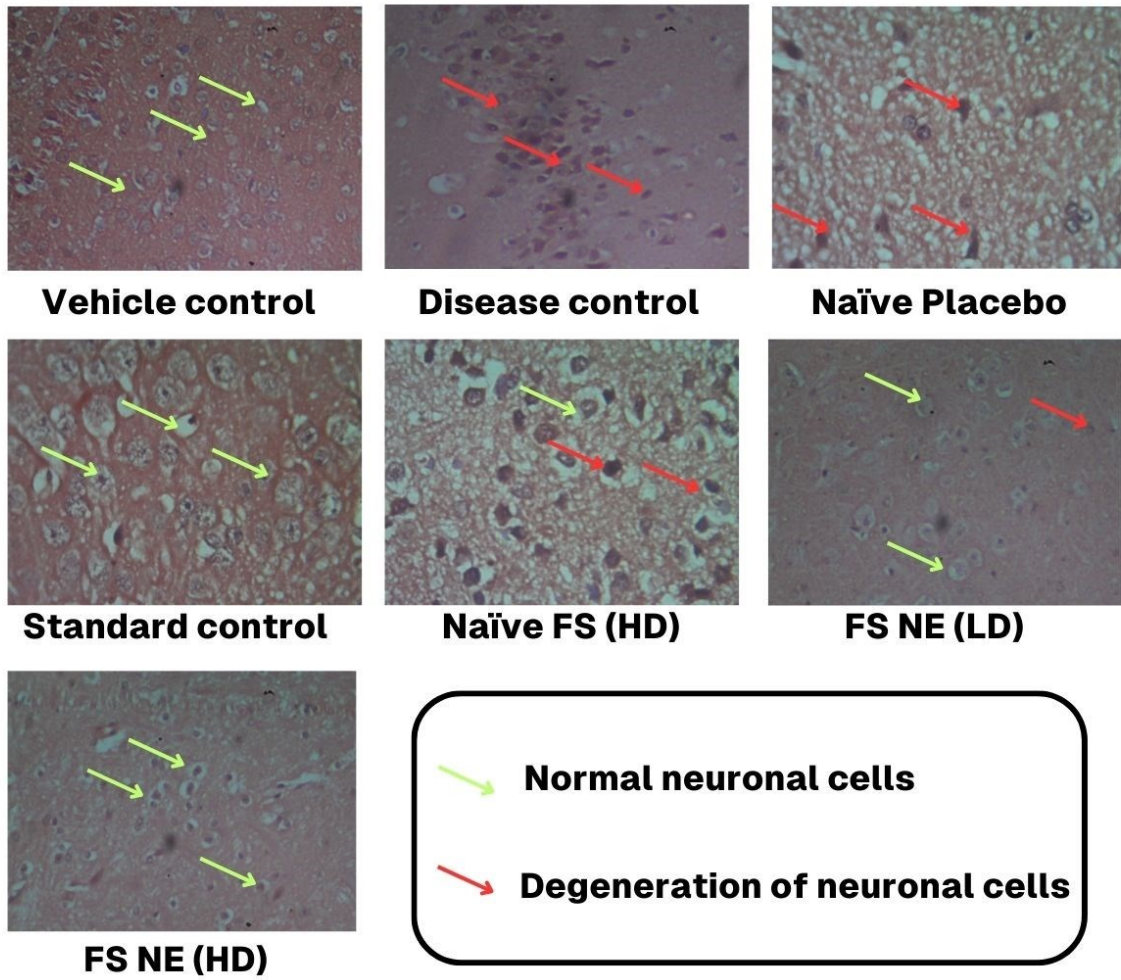
Figure 40b: Effect of FS-NE on the cerebral cortex part of the brain histopathology of the AlCl₃ inducted AD in rats

Chapter 6
Conclusion and Future perspectives

6.0. Conclusion and future prospective

In the present study, the effects of FS-NE were observed for the management of AD. FS-NE was prepared from a spontaneous emulsification method where Capmul MCM EP/NF was used as oil, Tween 80 was used as surfactant, and Transcutol P was used as co-surfactant. The developed formulation was optimized using a ternary phase diagram and QbD (BBD). The formulation was analyzed for droplet size, zeta potential, PDI, and drug loading. The droplet size of NE was further confirmed by TEM images. The thermodynamic stability and accelerated stability studies confirmed that FS-NE can be stored for more than six months duration, and during this time period, no major changes were observed in droplet size, zeta potential, PDI, and % drug loading. In the MTT assay, FS-NE revealed absence of any cytotoxicity.

Pharmacological studies were performed to evaluate the anti-Alzheimer effects of FS-NE. In pharmacokinetic study, FS-NE showed 9.2 folds higher concentration of FS in blood and 8.81 folds higher concentration of FS in the brain compared to its naïve form. Further pharmacodynamic studies were performed. In behavioural study, $AlCl_3$ induced AD model was used in rats. It was an 84-day study where the animal received 100 mg/kg $AlCl_3$ every day, and after 56 days the treatment was started. The results of pharmacodynamic studies showed that FS-NE at high doses and low doses showed significant responses for the treatment of AD. In the behavioral model, OFT and MWM revealed that FS-NE improved cognitive function and attenuated anxiety. On the 84 days of animal study, the animals were sacrificed, and biochemical and histopathological studies were conducted.

The results of biochemical studies showed that FS-NE (10 mg/kg) (group VII) possessed anti-Alzheimer's effects by attenuating the level of AChE enzyme and Aβ. FS-NE (10 mg/kg) (group VII) also exhibited antioxidant and anti-inflammatory effects. FS-NE produced non-significant and similar effects compared to the standard group (1mg/kg donepezil) (group III) and the normal control group (group I). The results of histopathological studies showed that FS-NE attenuated the degeneration of the neurons and helped in their regeneration. At both the doses of FS-NE (groups VI and VII) i.e. 5 mg/kg (low dose) and 10 mg/kg (high dose) improved regeneration of neurons was observed.

The neuroprotective effects of FS-NE, open up avenues for innovative strategies in AD therapy using phytoconstituents. The ability of NE to increase drug delivery to the brain could enable more precise and targeted interventions, addressing specific molecular pathways implicated in AD's progression. As preclinical studies continue to demonstrate the efficacy and safety of FS-NE, the translation to human clinical trials becomes a crucial next step. Future research should focus on designing robust clinical trials to evaluate the therapeutic potential of FS-NE in a diverse population of individuals with AD.

Future research should prioritize incorporating patient-centered outcomes, such as cognitive function, quality of life, and caregiver burden, into clinical trial assessments. Understanding the real-world impact of FS-NE on the lives of individuals with AD will be essential for its broader acceptance and adoption. FS can combine other therapeutic agents, such as existing FDA-approved drugs or emerging compounds, which may lead to synergistic effects. This approach could potentially enhance efficacy, mitigate side effects, and simultaneously target multiple aspects of AD pathology. The future perspectives on FS-NE for the management of AD are marked by a convergence of scientific advancements, personalized medicine approaches, and a commitment to improving patient outcomes. As we move forward, the collaboration between researchers, clinicians, and industry stakeholders will be instrumental in realizing the full potential of FS-NE as a transformative therapy for the management of AD.

Chapter 7
Bibliography

7. References

[1]   H.W. Querfurth, F.M. LaFerla, Alzheimer's Disease, N. Engl. J. Med. 362 (2010) 329–344. https://doi.org/10.1056/NEJMra0909142.

[2]   Alzheimer's and Dementia in India, Alzheimer's Assoc. (2024). https://www.alz.org/in/dementia-alzheimers-en.asp Accessed date 21/05/2024.

[3]   S. Vishwas, A. Awasthi, L. Corrie, S. Kumar Singh, M. Gulati, Multiple target-based combination therapy of galantamine, memantine and lycopene for the possible treatment of Alzheimer's disease, Med. Hypotheses. 143 (2020) 109879. https://doi.org/10.1016/j.mehy.2020.109879.

[4]   A. Teli, P. Dhande, Advancing Alzheimer's care: a novel therapy with lecanemab, Egypt. J. Neurol. Psychiatry Neurosurg. 59 (2023). https://doi.org/10.1186/s41983-023-00747-y.

[5]   S. Vishwas, S.K. Singh, M. Gulati, A. Awasthi, R. Khursheed, L. Corrie, R. Kumar, T. Collet, R. Loebenberg, O. Porwal, S. Gupta, N.K. Jha, P.K. Gupta, H.P. Devkota, D.K. Chellappan, G. Gupta, J. Adams, K. Dua, Harnessing the therapeutic potential of fisetin and its nanoparticles: Journey so far and road ahead, Chem. Biol. Interact. 356 (2022) 109869. https://doi.org/10.1016/j.cbi.2022.109869.

[6]   R. Kumar, R. Khursheed, R. Kumar, A. Awasthi, N. Sharma, S. Khurana, B. Kapoor, N. Khurana, S.K. Singh, K. Gowthamarajan, A. Wadhwani, Self-nanoemulsifying drug delivery system of fisetin: Formulation, optimization, characterization and cytotoxicity assessment, J. Drug Deliv. Sci. Technol. 54 (2019) 101252. https://doi.org/10.1016/J.JDDST.2019.101252.

[7]   S. Vishwas, R. Kumar, R. Khursheed, A.K. Ramanunny, R. Kumar, A. Awasthi, L. Corrie, O. Porwal, M.F. Arshad, M.K. Alshammari, A.A. Alghitran, A.N. Qumayri, S.M. Alkhaldi, A.K. Alshammari, D.K. Chellappan, G. Gupta, T. Collet, J. Adams, K. Dua, M. Gulati, S.K. Singh, Expanding arsenal against neurodegenerative diseases using quercetin based nanoformulations: breakthroughs and bottlenecks, Curr.

Neuropharmacol. 20 (2022). https://doi.org/10.2174/1570159X20666220810105421.

[8]   A. Kaur, N. Singh, H. Kaur, V. Kakoty, D.S. Sharma, R. Khursheed, M.R. Babu, V. Harish, G. Gupta, M. Gulati, P. Kumar, H. Dureja, N.S. Alharthi, F.R. Khan, Z. ur Rehman, M.A. Hakami, M. Patel, R. Patel, M. Zandi, S. Vishwas, K. Dua, S.K. Singh, Neurodegenerative disease and brain delivery of therapeutics: Bridging the gap using dendrimers, J. Drug Deliv. Sci. Technol. (2023) 104868. https://doi.org/https://doi.org/10.1016/j.jddst.2023.104868.

[9]   N. Singh, S. Vishwas, A. Kaur, H. Kaur, V. Kakoty, V. Harish, P. Yanadaiah, S. Gupta, A.A. Sayed, A. El-sayed, I. Ali, O.A. Kensara, N. Ghaboura, G. Gupta, A.M. Dou, M. Algahtani, A.F. El-kott, K. Dua, K. Singh, M.M. Abdel-daim, Biomedicine & Pharmacotherapy Harnessing role of sesamol and its nanoformulations against neurodegenerative diseases, Biomed. Pharmacother. 167 (2023) 115512. https://doi.org/10.1016/j.biopha.2023.115512.

[10]   E. Drummond, T. Wisniewski, Alzheimer's Disease: Experimental Models and Reality, Acta Neuropathol. 133 (2017) 155. https://doi.org/10.1007/S00401-016-1662-X.

[11]   M. Dey, R.K. Singh, Neurotoxic effects of aluminium exposure as a potential risk factor for Alzheimer's disease, Pharmacol. Rep. 74 (2022) 439–450. https://doi.org/10.1007/S43440-022-00353-4.

[12]   X. Chen, M. Zhang, M. Ahmed, K.M. Surapaneni, V.P. Veeraraghavan, P. Arulselvan, Neuroprotective effects of ononin against the aluminium chloride-induced Alzheimer's disease in rats, Saudi J. Biol. Sci. 28 (2021) 4232–4239. https://doi.org/10.1016/j.sjbs.2021.06.031.

[13]   D. Shunan, M. Yu, H. Guan, Y. Zhou, Neuroprotective effect of Betalain against AlCl3-induced Alzheimer's disease in Sprague Dawley Rats via putative modulation of oxidative stress and nuclear factor kappa B (NF-κB) signaling pathway, Biomed. Pharmacother. 137 (2021) 111369. https://doi.org/10.1016/j.biopha.2021.111369.

[14]   A.D. Kraft, D.A. Johnson, J.A. Johnson, Nuclear factor e2-related factor 2-dependent

antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult, j. neurosci. 24 (2004) 1101–1112. https://doi.org/10.1523/JNEUROSCI.3817-03.2004.

[15] A.J. Thenmozhi, T.R.W. Raja, U. Janakiraman, T. Manivasagam, Neuroprotective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats, Neurochem. Res. 40 (2015) 767–776.

[16] S. Vishwas, A. Awasthi, L. Corrie, S.K. Singh, M. Gulati, Multiple target-based combination therapy of galantamine, memantine and lycopene for the possible treatment of Alzheimer's disease, Med. Hypotheses. 143 (2020) 109879.

[17] S. Vishwas, R. Kumar, R. Khursheed, A.K. Ramanunny, R. Kumar, A. Awasthi, L. Corrie, O. Porwal, M.F. Arshad, M.K. Alshammari, Expanding Arsenal against Neurodegenerative Diseases using Quercetin based Nanoformulations: Breakthroughs and Bottlenecks, Curr. Neuropharmacol. (2022). https://doi.org/https://doi.org/10.2174/1570159x20666220810105421.

[18] Alzheimer's and Dementia in India, Alzheimer's Assoc. (2024). https://www.alz.org/in/dementia-alzheimers-en.asp Accessed date 21/05/2024..

[19] Alzheimer Association, 2022 Alzheimer's Disease Facts and Figures, Alzheimer's Dement. 2016. 12 (2022) 1–117. https://doi.org/10.1016/j.jalz.2016.03.001.

[20] Dementia statistics, Dement. Aust. (2022). https://www.dementia.org.au/statistics Accessed date 25/7/2023.

[21] Japan will have the fastest growing prevalent cases of Alzheimer's, Clin. Trials Arena. (2022). https://www.clinicaltrialsarena.com/comment/japan-will-fastest-growing-prevalent-cases-alzheimers/#:~:text=In Japan%2C 7%25 of men,years and older have Alzheimer's. Accessed date 25/7/2023.

[22] L. Jia, M. Quan, Y. Fu, T. Zhao, Y. Li, C. Wei, Y. Tang, Q. Qin, F. Wang, Y. Qiao, S. Shi, Y.J. Wang, Y. Du, J. Zhang, J. Zhang, B. Luo, Q. Qu, C. Zhou, S. Gauthier, J. Jia, Dementia in China: epidemiology, clinical management, and research advances, Lancet

Neurol. 19 (2020) 81–92. https://doi.org/10.1016/S1474-4422(19)30290-X.

[23] F. Zhang, L. Jiang, Neuroinflammation in Alzheimer's disease, Neuropsychiatr. Dis. Treat. (2015) 243–256.

[24] A. Sobue, O. Komine, K. Yamanaka, Neuroinflammation in Alzheimer's disease: microglial signature and their relevance to disease, Inflamm. Regen. 43 (2023) 4–9. https://doi.org/10.1186/s41232-023-00277-3.

[25] L. Cassidy, F. Fernandez, J.B. Johnson, M. Naiker, A.G. Owoola, D.A. Broszczak, Oxidative stress in alzheimer's disease: A review on emergent natural polyphenolic therapeutics, Complement. Ther. Med. 49 (2020) 102294. https://doi.org/10.1016/J.CTIM.2019.102294.

[26] I. Onyango, S. Khan, Oxidative Stress, Mitochondrial dysfunction, and stress signaling in Alzheimers Disease, Curr. Alzheimer Res. (2006). https://doi.org/10.2174/156720506778249489.

[27] W.J. Huang, X. Zhang, W.W. Chen, Role of oxidative stress in Alzheimer's disease, Biomed. Reports. 4 (2016) 519. https://doi.org/10.3892/BR.2016.630.

[28] D.J. DiSabato, N. Quan, J.P. Godbout, Neuroinflammation: the devil is in the details, J. Neurochem. 139 (2016) 136–153.

[29] Y.-J. Jung, J.S. Isaacs, S. Lee, J. Trepel, L. Neckers, IL-1β mediated up-regulation of HIF-lα via an NFkB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis, FASEB J. 17 (2003) 1–22.

[30] A.F. Naeimi, M. Alizadeh, Antioxidant properties of the flavonoid fisetin: An updated review of in vivo and in vitro studies, Trends Food Sci. Technol. 70 (2017) 34–44.

[31] Z.R. Chen, J.B. Huang, S.L. Yang, F.F. Hong, Role of Cholinergic signaling in Alzheimer's Disease, Mol. 2022, Vol. 27, Page 1816. 27 (2022) 1816. https://doi.org/10.3390/MOLECULES27061816.

[32] C.Y. Ye, Y. Lei, X.C. Tang, H.Y. Zhang, Donepezil attenuates Aβ-associated

mitochondrial dysfunction and reduces mitochondrial Aβ accumulation in vivo and in vitro, Neuropharmacology. 95 (2015) 29–36. https://doi.org/10.1016/J.NEUROPHARM.2015.02.020.

[33] Alzheimer's Facts and Figures Report, Alzheimer's Assoc. (2022). https://www.alz.org/alzheimers-dementia/facts-figures (accessed September 25, 2022).

[34] C. Gilles, S. Ertlé, Pharmacological models in Alzheimer's disease research, Https://Doi.Org/10.31887/DCNS.2000.2.3/Cgilles. 2 (2022) 247–255. https://doi.org/10.31887/DCNS.2000.2.3/CGILLES.

[35] R. Wang, P.H. Reddy, Role of glutamate and NMDA receptors in Alzheimer's disease, J. Alzheimers. Dis. 57 (2017) 1041. https://doi.org/10.3233/JAD-160763.

[36] G. Khanna, R. Bhandari, A. Kuhad, A. Kuhad, Aducanumab, Drugs Future. 44 (2022) 115–121. https://doi.org/10.1358/dof.2019.44.2.2895649.

[37] R. Ali, G. Das Gupta, P.A. Chawla, Aducanumab: A new hope in Alzheimer's disease, Heal. Sci. Rev. 4 (2022) 100039. https://doi.org/10.1016/J.HSR.2022.100039.

[38] P. Agostinho, R. A. Cunha, C. Oliveira, Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease, Curr. Pharm. Des. 16 (2010) 2766–2778. https://doi.org/10.2174/138161210793176572.

[39] M. Mhatre, R.A. Floyd, K. Hensley, Oxidative stress and neuroinflammation in Alzheimer's disease and amyotrophic lateral sclerosis: Common links and potential therapeutic targets, IOS Press, 2004.

[40] W. Yang, Z.K. Tian, H.X. Yang, Z.J. Feng, J.M. Sun, H. Jiang, C. Cheng, Q.L. Ming, C.M. Liu, Fisetin improves lead-induced neuroinflammation, apoptosis and synaptic dysfunction in mice associated with the AMPK/SIRT1 and autophagy pathway, Food Chem. Toxicol. 134 (2019). https://doi.org/10.1016/J.FCT.2019.110824.

[41] L. Zhang, H. Wang, Y. Zhou, Y. Zhu, M. Fei, Fisetin alleviates oxidative stress after traumatic brain injury via the Nrf2-ARE pathway, Neurochem. Int. 118 (2018) 304–313. https://doi.org/10.1016/J.NEUINT.2018.05.011.

[42] J.A. Godoy, C.B. Lindsay, R.A. Quintanilla, F.J. Carvajal, W. Cerpa, N.C. Inestrosa, Quercetin exerts differential neuroprotective effects against h2o2 and aβ aggregates in hippocampal neurons: the role of mitochondria, Mol. Neurobiol. 54 (2017) 7116–7128. https://doi.org/10.1007/S12035-016-0203-X.

[43] T.J. Chen, J.Y. Jeng, C.W. Lin, C.Y. Wu, Y.C. Chen, Quercetin inhibition of ROS-dependent and-independent apoptosis in rat glioma C6 cells, Toxicology. 223 (2006) 113–126.

[44] W. Danysz, C.G. Parsons, H.-Jö. MÖbius, A. StÖffler, Gü. Quack, Neuroprotective and symptomatological action of memantine relevant for Alzheimer's disease—a unified glutamatergic hypothesis on the mechanism of action, Neurotox. Res. 2 (2000) 85–97.

[45] A. Prema, U. Janakiraman, T. Manivasagam, J.T. Arokiasamy, Neuroprotective effect of lycopene against MPTP induced experimental Parkinson's disease in mice, Neurosci. Lett. 599 (2015) 12–19. https://doi.org/10.1016/J.NEULET.2015.05.024.

[46] C. Bin Liu, R. Wang, Y.F. Yi, Z. Gao, Y.Z. Chen, Lycopene mitigates β-amyloid induced inflammatory response and inhibits NF-κB signaling at the choroid plexus in early stages of Alzheimer's disease rats, J. Nutr. Biochem. 53 (2018) 66–71. https://doi.org/10.1016/J.JNUTBIO.2017.10.014.

[47] A. Prakash, A. Kumar, Implicating the role of lycopene in restoration of mitochondrial enzymes and BDNF levels in β-amyloid induced Alzheimer′s disease, Eur. J. Pharmacol. 741 (2014) 104–111. https://doi.org/10.1016/J.EJPHAR.2014.07.036.

[48] C. Huang, D. Gan, C. Fan, C. Wen, A. Li, Q. Li, J. Zhao, Z. Wang, L. Zhu, D. Lu, The Secretion from Neural Stem Cells Pretreated with Lycopene Protects against tert-Butyl Hydroperoxide-Induced Neuron Oxidative Damage, Oxid. Med. Cell. Longev. 2018 (2018). https://doi.org/10.1155/2018/5490218.

[49] J. V. Formica, W. Regelson, Review of the biology of quercetin and related bioflavonoids, Food Chem. Toxicol. 33 (1995) 1061–1080. https://doi.org/10.1016/0278-6915(95)00077-1.

[50] V. Mehta, A. Parashar, M. Udayabanu, Quercetin prevents chronic unpredictable stress induced behavioral dysfunction in mice by alleviating hippocampal oxidative and inflammatory stress, Physiol. Behav. 171 (2017) 69–78. https://doi.org/10.1016/J.PHYSBEH.2017.01.006.

[51] P.H. Reddy, M. Manczak, X. Yin, M.C. Grady, A. Mitchell, S. Tonk, C.S. Kuruva, J.S. Bhatti, R. Kandimalla, M. Vijayan, S. Kumar, R. Wang, J.A. Pradeepkiran, G. Ogunmokun, K. Thamarai, K. Quesada, A. Boles, A.P. Reddy, Protective Effects of Indian Spice Curcumin Against Amyloid-β in Alzheimer's Disease, J. Alzheimers. Dis. 61 (2018) 843–866. https://doi.org/10.3233/JAD-170512.

[52] J.H. Lu, M.T. Ardah, S.S.K. Durairajan, L.F. Liu, L.X. Xie, W.F.D. Fong, M.Y. Hasan, J.D. Huang, O.M.A. El-Agnaf, M. Li, Baicalein inhibits formation of α-synuclein oligomers within living cells and prevents Aβ peptide fibrillation and oligomerisation, Chembiochem. 12 (2011) 615–624. https://doi.org/10.1002/CBIC.201000604.

[53] X.H. Gu, L.J. Xu, Z.Q. Liu, B. Wei, Y.J. Yang, G.G. Xu, X.P. Yin, W. Wang, The flavonoid baicalein rescues synaptic plasticity and memory deficits in a mouse model of Alzheimer's disease, Behav. Brain Res. 311 (2016) 309–321. https://doi.org/10.1016/J.BBR.2016.05.052.

[54] M. Ramezani, N. Darbandi, F. Khodagholi, A. Hashemi, Myricetin protects hippocampal CA3 pyramidal neurons and improves learning and memory impairments in rats with Alzheimer's disease., Neural Regen. Res. 11 (2016) 1976–1980. https://doi.org/10.4103/1673-5374.197141.

[55] M. Asai, N. Iwata, A. Yoshikawa, Y. Aizaki, S. Ishiura, T.C. Saido, K. Maruyama, Berberine alters the processing of Alzheimer's amyloid precursor protein to decrease Abeta secretion, Biochem. Biophys. Res. Commun. 352 (2007) 498–502. https://doi.org/10.1016/J.BBRC.2006.11.043.

[56] M.K. Dewi, A.Y. Chaerunisaa, M. Muhaimin, I.M. Joni, Improved Activity of Herbal Medicines through Nanotechnology, Nanomater. (Basel, Switzerland). 12 (2022). https://doi.org/10.3390/NANO12224073.

[57]   Y.S. Ho, M.S. Yu, X.F. Yang, K.F. So, W.H. Yuen, R.C.C. Chang, Neuroprotective effects of polysaccharides from wolfberry, the fruits of Lycium barbarum, against homocysteine-induced toxicity in rat cortical neurons, J. Alzheimers. Dis. 19 (2010) 813–827. https://doi.org/10.3233/JAD-2010-1280.

[58]   Y.A.R. Mahaman, F. Huang, M. Wu, Y. Wang, Z. Wei, J. Bao, M.T.M. Salissou, D. Ke, Q. Wang, R. Liu, J.Z. Wang, B. Zhang, D. Chen, X. Wang, Moringa oleifera alleviates homocysteine-induced Alzheimer's Disease-like pathology and cognitive impairments, j. Alzheimers. Dis. 63 (2018) 1141–1159. https://doi.org/10.3233/JAD-180091.

[59]   K. Blecharz-Klin, A. Piechal, I. Joniec, J. Pyrzanowska, E. Widy-Tyszkiewicz, Pharmacological and biochemical effects of Ginkgo biloba extract on learning, memory consolidation and motor activity in old rats, Acta Neurobiol. Exp. (Wars). 69 (2009) 217–231.

[60]   K.K. Chakravarthi, R. Avadhani, Beneficial effect of aqueous root extract of Glycyrrhiza glabra on learning and memory using different behavioral models: An experimental study, J. Nat. Sci. Biol. Med. 4 (2013) 420–425. https://doi.org/10.4103/0976-9668.117025.

[61]   P.H. Reddy, M. Manczak, X. Yin, M.C. Grady, A. Mitchell, S. Tonk, C.S. Kuruva, J.S. Bhatti, R. Kandimalla, M. Vijayan, S. Kumar, R. Wang, J.A. Pradeepkiran, G. Ogunmokun, K. Thamarai, K. Quesada, A. Boles, A.P. Reddy, Protective effects of indian spice curcumin against amyloid beta in Alzheimer's Disease, J. Alzheimers. Dis. 61 (2018) 843. https://doi.org/10.3233/JAD-170512.

[62]   S. Akhondzadeh, M. Noroozian, M. Mohammadi, S. Ohadinia, A.H. Jamshidi, M. Khani, Salvia officinalis extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial, J. Clin. Pharm. Ther. 28 (2003) 53–59. https://doi.org/10.1046/J.1365-2710.2003.00463.X.

[63]   F. Yang, G.P. Lim, A.N. Begum, O.J. Ubeda, M.R. Simmons, S.S. Ambegaokar, P. Chen, R. Kayed, C.G. Glabe, S.A. Frautschy, G.M. Cole, Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo, J.

Biol. Chem. 280 (2005) 5892–5901. https://doi.org/10.1074/JBC.M404751200.

[64] M. Moss, J. Cook, K. Wesnes, P. Duckett, Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults, Int. J. Neurosci. 113 (2003) 15–38. https://doi.org/10.1080/00207450390161903.

[65] M.S. Uddin, A. Al Mamun, M.S. Hossain, M. Ashaduzzaman, M.A.A. Noor, M.S. Hossain, M.J. Uddin, J. Sarker, M. Asaduzzaman, M.S. Uddin, A. Al Mamun, M.S. Hossain, M. Ashaduzzaman, M.A.A. Noor, M.S. Hossain, M.J. Uddin, J. Sarker, M. Asaduzzaman, Neuroprotective effect of phyllanthus acidus l. on learning and memory impairment in scopolamine-induced animal model of dementia and oxidative stress: natural wonder for regulating the development and progression of Alzheimer's Disease, Adv. Alzheimer's Dis. 5 (2016) 53–72. https://doi.org/10.4236/AAD.2016.52005.

[66] X. Liu, J. Rubio, W. Qiong, Z. Jiang, H. Dang, S.L. Chen, G.F. Gonzales, Aqueous extract of black maca (lepidium meyenii) on memory impairment induced by ovariectomy in mice, Evid. Based. Complement. Alternat. Med. 2011 (2011). https://doi.org/10.1093/ECAM/NEN063.

[67] I. Kiris, W. Kukula-Koch, M. Karayel-Basar, B. Gurel, J. Coskun, A.T. Baykal, Proteomic alterations in the cerebellum and hippocampus in an Alzheimer's disease mouse model: Alleviating effect of palmatine, Biomed. Pharmacother. 158 (2023) 114111. https://doi.org/10.1016/J.BIOPHA.2022.114111.

[68] W. Kleawyothatis, P. Jattujan, K. Chumpoochai, P. Chalorak, P. Sobhon, K. Meemon, Holothuria scabra extracts confer neuroprotective effect in C. elegans model of Alzheimer's disease by attenuating amyloid-β aggregation and toxicity, J. Tradit. Complement. Med. 13 (2023) 93–104. https://doi.org/10.1016/J.JTCME.2022.10.006.

[69] E. Elmorsy, E. Elsharkawy, F.A. Alhumaydhi, M. Salama, The protective effect of Indian Catechu methanolic extract against aluminum chloride-induced neurotoxicity, A rodent model of Alzheimer's disease, Heliyon. 7 (2021) e06269. https://doi.org/10.1016/J.HELIYON.2021.E06269.

[70] T. Hase, S. Shishido, S. Yamamoto, R. Yamashita, H. Nukima, S. Taira, T. Toyoda, K. Abe, T. Hamaguchi, K. Ono, M. Noguchi-Shinohara, M. Yamada, S. Kobayashi, Rosmarinic acid suppresses Alzheimer's disease development by reducing amyloid β aggregation by increasing monoamine secretion, Sci. Reports 2019 91. 9 (2019) 1–13. https://doi.org/10.1038/s41598-019-45168-1.

[71] F.J. Mirza, S. Amber, Sumera, D. Hassan, T. Ahmed, S. Zahid, Rosmarinic acid and ursolic acid alleviate deficits in cognition, synaptic regulation and adult hippocampal neurogenesis in an Aβ1-42-induced mouse model of Alzheimer's disease, Phytomedicine. 83 (2021) 153490. https://doi.org/10.1016/J.PHYMED.2021.153490.

[72] Z. Wang, C. Gao, L. Zhang, R. Sui, Hesperidin methylchalcone (HMC) hinders amyloid-β induced Alzheimer's disease by attenuating cholinesterase activity, macromolecular damages, oxidative stress and apoptosis via regulating NF-κB and Nrf2/HO-1 pathways, Int. J. Biol. Macromol. (2023) 123169. https://doi.org/10.1016/J.IJBIOMAC.2023.123169.

[73] S. Ahmad, A. Khan, W. Ali, M.H. Jo, J. Park, M. Ikram, M.O. Kim, Fisetin rescues the mice brains against d-galactose-induced oxidative stress, neuroinflammation and memory impairment, front. Pharmacol. 12 (2021). https://doi.org/10.3389/FPHAR.2021.612078.

[74] S.R. Temitope, The role of lycopene on the hippocampus of rat model of lipopolysaccharide-induced Alzheimer's disease, Alzheimer's Dement. 17 (2021) e058528. https://doi.org/10.1002/ALZ.058528.

[75] S.M. Eraky, N.M. Ramadan, N.F. Abo El-Magd, Ameliorative effects of bromelain on aluminum-induced Alzheimer's disease in rats through modulation of TXNIP pathway, Int. J. Biol. Macromol. 227 (2023) 1119–1131. https://doi.org/10.1016/J.IJBIOMAC.2022.11.291.

[76] L. Nazari, S. Komaki, I. Salehi, S. Raoufi, Z. Golipoor, M. Kourosh-Arami, A. Komaki, Investigation of the protective effects of lutein on memory and learning using behavioral methods in a male rat model of Alzheimer's disease, J. Funct. Foods. 99 (2022) 105319.

https://doi.org/10.1016/J.JFF.2022.105319.

[77] A. dos Santos, F.C. Teixeira, D.S. da Silva, T.A. Veleda, J.E. de Mello, K.P. Luduvico, R.G. Tavares, F.M. Stefanello, W. Cunico, R.M. Spanevello, Thiazolidin-4-one prevents against memory deficits, increase in phosphorylated tau protein, oxidative damage and cholinergic dysfunction in Alzheimer disease model: Comparison with donepezil drug, Brain Res. Bull. 193 (2023) 1–10. https://doi.org/10.1016/J.BRAINRESBULL.2022.11.015.

[78] S. Chavoshinezhad, V. Safari, E. Izadpanah, Intranasal interferon-beta as a promising alternative for the treatment of Alzheimer's disease, Med. Hypotheses. 170 (2023) 110996. https://doi.org/10.1016/J.MEHY.2022.110996.

[79] A. Valverde, J. Dunys, T. Lorivel, D. Debayle, A.S. Gay, C. Caillava, M. Chami, F. Checler, Dipeptidyl peptidase 4 contributes to Alzheimer's disease–like defects in a mouse model and is increased in sporadic Alzheimer's disease brains, J. Biol. Chem. 297 (2021) 100963. https://doi.org/10.1016/J.JBC.2021.100963.

[80] T.N.M. Nguyen, L.J. Chen, K. Trares, H. Stocker, B. Holleczek, K. Beyreuther, H. Brenner, B. Schöttker, Long-term low-dose acetylsalicylic use shows protective potential for the development of both vascular dementia and Alzheimer's disease in patients with coronary heart disease but not in other individuals from the general population: results from two larg, Alzheimer's Res. Ther. 14 (2022) 1–14. https://doi.org/10.1186/S13195-022-01017-4/TABLES/3.

[81] K. Abu-Elfotuh, A.H. Al-Najjar, A.A. Mohammed, A.S. Aboutaleb, G.A. Badawi, Fluoxetine ameliorates Alzheimer's disease progression and prevents the exacerbation of cardiovascular dysfunction of socially isolated depressed rats through activation of Nrf2/HO-1 and hindering TLR4/NLRP3 inflammasome signaling pathway, Int. Immunopharmacol. 104 (2022). https://doi.org/10.1016/J.INTIMP.2021.108488.

[82] X. Li, Q. Wang, T. Hu, Y. Wang, J. Zhao, J. Lu, G. Pei, A tricyclic antidepressant, amoxapine, reduces amyloid-β generation through multiple serotonin receptor 6-mediated targets, Sci. Rep. 7 (2017). https://doi.org/10.1038/S41598-017-04144-3.

[83] ClinicalTrials.gov, (2023). https://clinicaltrials.gov/ (accessed January 13, 2023).

[84] W. Yang, Z.K. Tian, H.X. Yang, Z.J. Feng, J.M. Sun, H. Jiang, C. Cheng, Q.L. Ming, C.M. Liu, Fisetin improves lead-induced neuroinflammation, apoptosis and synaptic dysfunction in mice associated with the AMPK/SIRT1 and autophagy pathway, Food Chem. Toxicol. 134 (2019) 110824.

[85] A. Ahmad, T. Ali, H.Y. Park, H. Badshah, S.U. Rehman, M.O. Kim, Neuroprotective effect of fisetin against amyloid-beta-induced cognitive/synaptic dysfunction, neuroinflammation, and neurodegeneration in adult mice, Mol. Neurobiol. 54 (2017) 2269–2285.

[86] N. Cho, K.Y. Lee, J. Huh, J.H. Choi, H. Yang, E.J. Jeong, H.P. Kim, S.H. Sung, Cognitive-enhancing effects of Rhus verniciflua bark extract and its active flavonoids with neuroprotective and anti-inflammatory activities, Food Chem. Toxicol. 58 (2013) 355–361.

[87] J.Y. Chuang, P.C. Chang, Y.C. Shen, C. Lin, C.F. Tsai, J.H. Chen, W.L. Yeh, L.H. Wu, H.Y. Lin, Y.S. Liu, Regulatory effects of fisetin on microglial activation, Molecules. 19 (2014) 8820–8839.

[88] C. Chen, L. Yao, J. Cui, B. Liu, Fisetin protects against intracerebral hemorrhage-induced neuroinflammation in aged mice, Cerebrovasc. Dis. 45 (2018) 154–161.

[89] N. Ahmad, R. Ahmad, A.A. Naqvi, M.A. Alam, R.A. Rub, F.J. Ahmad, Enhancement of quercetin oral bioavailability by self-nanoemulsifying drug delivery system and their quantification through ultra high performance liquid chromatography and mass spectrometry in cerebral ischemia, Drug Res. (Stuttg). 67 (2017) 564–575.

[90] Y. Liu, H. Zhou, T. Yin, Y. Gong, G. Yuan, L. Chen, J. Liu, Quercetin-modified gold-palladium nanoparticles as a potential autophagy inducer for the treatment of Alzheimer's disease, J. Colloid Interface Sci. 552 (2019) 388–400. https://doi.org/10.1016/J.JCIS.2019.05.066.

[91] B. Wilson, M.K. Samanta, M.S. Muthu, G. Vinothapooshan, Design and evaluation of

chitosan nanoparticles as novel drug carrier for the delivery of rivastigmine to treat Alzheimer's disease, Ther. Deliv. 2 (2011) 599–609. https://doi.org/10.4155/TDE.11.21.

[92] M.S. Almuhayawi, W.S. Ramadan, S. Harakeh, S.K. Al Jaouni, D.J. Bharali, S.A. Mousa, S.M. Almuhayawi, The potential role of pomegranate and its nano-formulations on cerebral neurons in aluminum chloride induced Alzheimer rat model, Saudi J. Biol. Sci. 27 (2020) 1710–1716. https://doi.org/10.1016/J.SJBS.2020.04.045.

[93] X. Huo, Y. Zhang, X. Jin, Y. Li, L. Zhang, A novel synthesis of selenium nanoparticles encapsulated PLGA nanospheres with curcumin molecules for the inhibition of amyloid β aggregation in Alzheimer's disease, J. Photochem. Photobiol. B. 190 (2019) 98–102. https://doi.org/10.1016/J.JPHOTOBIOL.2018.11.008.

[94] G. Liu, P. Men, W. Kudo, G. Perry, M.A. Smith, Nanoparticle-chelator conjugates as inhibitors of amyloid-beta aggregation and neurotoxicity: a novel therapeutic approach for Alzheimer disease, Neurosci. Lett. 455 (2009) 187–190. https://doi.org/10.1016/J.NEULET.2009.03.064.

[95] J.F. Poduslo, K.L. Hultman, G.L. Curran, G.M. Preboske, R. Chamberlain, M. Marjańska, M. Garwood, C.R. Jack, T.M. Wengenack, Targeting vascular amyloid in arterioles of Alzheimer disease transgenic mice with amyloid β protein antibody-coated nanoparticles, J. Neuropathol. Exp. Neurol. 70 (2011) 653–661. https://doi.org/10.1097/NEN.0B013E318225038C.

[96] S. Palle, P. Neerati, Quercetin nanoparticles attenuates scopolamine induced spatial memory deficits and pathological damages in rats, Bull. Fac. Pharmacy, Cairo Univ. 55 (2017) 101–106. https://doi.org/10.1016/J.BFOPCU.2016.10.004.

[97] E. Conti, M. Gregori, I. Radice, F. Da Re, D. Grana, F. Re, E. Salvati, M. Masserini, C. Ferrarese, C.P. Zoia, L. Tremolizzo, Multifunctional liposomes interact with Abeta in human biological fluids: Therapeutic implications for Alzheimer's disease, Neurochem. Int. 108 (2017) 60–65. https://doi.org/10.1016/J.NEUINT.2017.02.012.

[98] C. Balducci, S. Mancini, S. Minniti, P. La Vitola, M. Zotti, G. Sancini, M. Mauri, A. Cagnotto, L. Colombo, F. Fiordaliso, E. Grigoli, M. Salmona, A. Snellman, M. Haaparanta-Solin, G. Forloni, M. Masserini, F. Re, Multifunctional liposomes reduce brain β-amyloid burden and ameliorate memory impairment in Alzheimer's disease mouse models, J. Neurosci. 34 (2014) 14022–14031. https://doi.org/10.1523/JNEUROSCI.0284-14.2014.

[99] R.G.R. Pinheiro, A. Granja, J.A. Loureiro, M.C. Pereira, M. Pinheiro, A.R. Neves, S. Reis, Quercetin lipid nanoparticles functionalized with transferrin for Alzheimer's disease, Eur. J. Pharm. Sci. 148 (2020). https://doi.org/10.1016/J.EJPS.2020.105314.

[100] M. Yusuf, M. Khan, M.M. Alrobaian, S.A. Alghamdi, M.H. Warsi, S. Sultana, R.A. Khan, Brain targeted Polysorbate-80 coated PLGA thymoquinone nanoparticles for the treatment of Alzheimer's disease, with biomechanistic insights, J. Drug Deliv. Sci. Technol. 61 (2021) 102214. https://doi.org/10.1016/J.JDDST.2020.102214.

[101] F. Gao, J. Zhao, P. Liu, D. Ji, L. Zhang, M. Zhang, Y. Li, Y. Xiao, Preparation and in vitro evaluation of multi-target-directed selenium-chondroitin sulfate nanoparticles in protecting against the Alzheimer's disease, Int. J. Biol. Macromol. 142 (2020) 265–276. https://doi.org/10.1016/J.IJBIOMAC.2019.09.098.

[102] T. Dara, A. Vatanara, M. Sharifzadeh, S. Khani, M.A. Vakilinezhad, F. Vakhshiteh, M. Nabi Meybodi, S. Sadegh Malvajerd, S. Hassani, M.H. Mosaddegh, Improvement of memory deficits in the rat model of Alzheimer's disease by erythropoietin-loaded solid lipid nanoparticles, Neurobiol. Learn. Mem. 166 (2019). https://doi.org/10.1016/J.NLM.2019.107082.

[103] D. Sun, W. Zhang, Q. Yu, X. Chen, M. Xu, Y. Zhou, J. Liu, Chiral penicillamine-modified selenium nanoparticles enantioselectively inhibit metal-induced amyloid β aggregation for treating Alzheimer's disease, J. Colloid Interface Sci. 505 (2017) 1001–1010. https://doi.org/10.1016/J.JCIS.2017.06.083.

[104] A. Vilella, D. Belletti, A.K. Sauer, S. Hagmeyer, T. Sarowar, M. Masoni, N. Stasiak, J.J.E. Mulvihill, B. Ruozi, F. Forni, M.A. Vandelli, G. Tosi, M. Zoli, A.M. Grabrucker,

Reduced plaque size and inflammation in the APP23 mouse model for Alzheimer's disease after chronic application of polymeric nanoparticles for CNS targeted zinc delivery, J. Trace Elem. Med. Biol. 49 (2018) 210–221. https://doi.org/10.1016/J.JTEMB.2017.12.006.

[105] L. Yang, T. Yin, Y. Liu, J. Sun, Y. Zhou, J. Liu, Gold nanoparticle-capped mesoporous silica-based H2O2-responsive controlled release system for Alzheimer's disease treatment, Acta Biomater. 46 (2016) 177–190. https://doi.org/10.1016/J.ACTBIO.2016.09.010.

[106] K.K. Cheng, P.S. Chan, S. Fan, S.M. Kwan, K.L. Yeung, Y.X.J. Wáng, A.H.L. Chow, E.X. Wu, L. Baum, Curcumin-conjugated magnetic nanoparticles for detecting amyloid plaques in Alzheimer's disease mice using magnetic resonance imaging (MRI), Biomaterials. 44 (2015) 155–172. https://doi.org/10.1016/J.BIOMATERIALS.2014.12.005.

[107] R. Barbara, D. Belletti, F. Pederzoli, M. Masoni, J. Keller, A. Ballestrazzi, M.A. Vandelli, G. Tosi, A.M. Grabrucker, Novel Curcumin loaded nanoparticles engineered for Blood-Brain Barrier crossing and able to disrupt Abeta aggregates, Int. J. Pharm. 526 (2017) 413–424. https://doi.org/10.1016/J.IJPHARM.2017.05.015.

[108] T. Yin, L. Yang, Y. Liu, X. Zhou, J. Sun, J. Liu, Sialic acid (SA)-modified selenium nanoparticles coated with a high blood-brain barrier permeability peptide-B6 peptide for potential use in Alzheimer's disease, Acta Biomater. 25 (2015) 172–183. https://doi.org/10.1016/J.ACTBIO.2015.06.035.

[109] J.A. Loureiro, S. Andrade, A. Duarte, A.R. Neves, J.F. Queiroz, C. Nunes, E. Sevin, L. Fenart, F. Gosselet, M.A.N. Coelho, M.C. Pereira, N. Latruffe, Resveratrol and Grape Extract-loaded Solid Lipid Nanoparticles for the Treatment of Alzheimer's Disease, Molecules. 22 (2017). https://doi.org/10.3390/MOLECULES22020277.

[110] E. Manek, F. Darvas, G.A. Petroianu, Use of Biodegradable, Chitosan-Based Nanoparticles in the Treatment of Alzheimer's Disease, Molecules. 25 (2020). https://doi.org/10.3390/MOLECULES25204866.

[111] A. Kaur, K. Nigam, I. Bhatnagar, H. Sukhpal, S. Awasthy, S. Shankar, A. Tyagi, S. Dang, Treatment of Alzheimer's diseases using donepezil nanoemulsion: an intranasal approach, Drug Deliv. Transl. Res. 10 (2020) 1862–1875. https://doi.org/10.1007/S13346-020-00754-Z.

[112] K.-L. Chen, P.-H. Tsai, C.-W. Lin, J.-M. Chen, Y.-J. Lin, P. Kumar, C.-C. Jeng, C.-H. Wu, L.-M. Wang, H.-M. Tsao, Sensitivity enhancement of magneto-optical Faraday effect immunoassay method based on biofunctionalized γ-Fe2O3@ Au core-shell magneto-plasmonic nanoparticles for the blood detection of Alzheimer's disease, Nanomedicine Nanotechnology, Biol. Med. 46 (2022) 102601.

[113] X. Zhang, X. Kang, L. Du, L. Zhang, Y. Huang, J. Wang, S. Wang, Y. Chang, Y. Liu, Y. Zhao, Tanshinone IIA loaded chitosan nanoparticles decrease toxicity of β-amyloid peptide in a Caenorhabditis elegans model of Alzheimer's disease, Free Radic. Biol. Med. 193 (2022) 81–94. https://doi.org/10.1016/J.FREERADBIOMED.2022.09.030.

[114] E.S. Gil, L. Wu, L. Xu, T.L. Lowe, β-cyclodextrin-poly(β-amino ester) nanoparticles for sustained drug delivery across the blood-brain barrier, Biomacromolecules. 13 (2012) 3533–3541. https://doi.org/10.1021/BM3008633.

[115] C. Praça, A. Rai, T. Santos, A.C. Cristovão, S.L. Pinho, R. Cecchelli, M.P. Dehouck, L. Bernardino, L.S. Ferreira, A nanoformulation for the preferential accumulation in adult neurogenic niches, J. Control. Release. 284 (2018) 57–72. https://doi.org/10.1016/J.JCONREL.2018.06.013.

[116] Y. Kim, J.-H. Park, H. Lee, J.-M. Nam, How Do the Size, Charge and Shape of Nanoparticles Affect Amyloid β Aggregation on Brain Lipid Bilayer?, Sci. Rep. 6 (2016) 19548. https://doi.org/10.1038/srep19548.

[117] Y.C. Kuo, C.W. Tsao, Neuroprotection against apoptosis of SK-N-MC cells using RMP-7- and lactoferrin-grafted liposomes carrying quercetin, Int. J. Nanomedicine. 12 (2017) 2857–2869. https://doi.org/10.2147/IJN.S132472.

[118] A. Gupta, H.B. Eral, T.A. Hatton, P.S. Doyle, Nanoemulsions: formation, properties and

applications, Soft Matter. 12 (2016) 2826–2841.

[119] L. Rigano, N. Lionetti, Nanobiomaterials in galenic formulations and cosmetics, Nanobiomaterials Galen. Formul. Cosmet. Appl. Nanobiomaterials. (2016) 121–148. https://doi.org/10.1016/B978-0-323-42868-2.00006-1.

[120] H. Jasmina, O. Džana, E. Alisa, V. Edina, R. Ognjenka, Preparation of nanoemulsions by high-energy and lowenergy emulsification methods, in: C. 2017 Proc. Int. Conf. Med. Biol. Eng. 2017, Springer, 2017: pp. 317–322.

[121] S. Kotta, A.W. Khan, S.H. Ansari, R.K. Sharma, J. Ali, Formulation of nanoemulsion: a comparison between phase inversion composition method and high-pressure homogenization method, Drug Deliv. 22 (2015) 455–466.

[122] P. Izquierdo, J. Esquena, T.F. Tadros, J.C. Dederen, J. Feng, M.J. Garcia-Celma, N. Azemar, C. Solans, Phase behavior and nano-emulsion formation by the phase inversion temperature method, Langmuir. 20 (2004) 6594–6598.

[123] Q. Ma, J. Cao, Y. Gao, S. Han, Y. Liang, T. Zhang, X. Wang, Y. Sun, Microfluidic-mediated nano-drug delivery systems: from fundamentals to fabrication for advanced therapeutic applications, Nanoscale. 12 (2020) 15512–15527.

[124] S. Deen, S. Sajjadi, Dilute nanoemulsions via separation of satellite droplets, J. Colloid Interface Sci. 407 (2013) 354–360.

[125] G.T. Vladisavljević, Preparation of microemulsions and nanoemulsions by membrane emulsification, Colloids Surfaces A Physicochem. Eng. Asp. 579 (2019) 123709.

[126] J.T. Park, G. Paneru, M. Iwamatsu, B.M. Law, H.K. Pak, Nonclassical Surface Nucleation of 6CB at the Air–Liquid Interface of a 6CB Oil-in-Water Nanoemulsion, Langmuir. 37 (2021) 9588–9596.

[127] A.K. Ramanunny, S. Wadhwa, S. Kumar Singh, B. Kumar, M. Gulati, A. Kumar, S. Almawash, A. Al Saqr, K. Gowthamarajan, K. Dua, H. Singh, S. Vishwas, R. Khursheed, S. Rahana Parveen, A. Venkatesan, K.R. Paudel, P.M. Hansbro, D. Kumar Chellappan, Topical non-aqueous nanoemulsion of Alpinia galanga extract for effective

treatment in psoriasis: In vitro and in vivo evaluation, Int. J. Pharm. 624 (2022). https://doi.org/10.1016/J.IJPHARM.2022.121882.

[128] R. Khursheed, S.K. Singh, S. Wadhwa, M. Gulati, A. Awasthi, Therapeutic potential of mushrooms in diabetes mellitus: Role of polysaccharides, Int. J. Biol. Macromol. 164 (2020) 1194–1205.

[129] D.J. McClements, H. Xiao, Potential biological fate of ingested nanoemulsions: influence of particle characteristics, Food Funct. 3 (2012) 202–220. https://doi.org/10.1039/C1FO10193E.

[130] R. Kumar, R. Kumar, N. Khurana, S.K. Singh, S. Khurana, S. Verma, N. Sharma, B. Kapoor, M. Vyas, R. Khursheed, A. Awasthi, J. Kaur, L. Corrie, Enhanced oral bioavailability and neuroprotective effect of fisetin through its SNEDDS against rotenone-induced Parkinson's disease rat model, Food Chem. Toxicol. 144 (2020) 111590. https://doi.org/10.1016/J.FCT.2020.111590.

[131] S.M. Đorđević, N.D. Cekić, M.M. Savić, T.M. Isailović, D. V Ranđelović, B.D. Marković, S.R. Savić, T.T. Stamenić, R. Daniels, S.D. Savić, Parenteral nanoemulsions as promising carriers for brain delivery of risperidone: Design, characterization and in vivo pharmacokinetic evaluation, Int. J. Pharm. 493 (2015) 40–54.

[132] L. Nothnagel, M.G. Wacker, How to measure release from nanosized carriers?, Eur. J. Pharm. Sci. 120 (2018) 199–211. https://doi.org/10.1016/J.EJPS.2018.05.004.

[133] C. Chung, D.J. McClements, Characterization of Physicochemical Properties of Nanoemulsions: Appearance, Stability, and Rheology, in: S.M. Jafari, D.J.B.T.-N. McClements (Eds.), Academic Press, 2018: pp. 547–576. https://doi.org/https://doi.org/10.1016/B978-0-12-811838-2.00017-5.

[134] L.A. Fielding, J.A. Lane, M.J. Derry, O.O. Mykhaylyk, S.P. Armes, Thermo-responsive Diblock Copolymer Worm Gels in Non-polar Solvents, J. Am. Chem. Soc. 136 (2014) 5790–5798. https://doi.org/10.1021/ja501756h.

[135] Q. Liu, H. Huang, H. Chen, J. Lin, Q. Wang, Food-grade nanoemulsions: Preparation,

stability and application in encapsulation of bioactive compounds, Molecules. 24 (2019) 1–37. https://doi.org/10.3390/molecules24234242.

[136] C. Jadhav, V. Kate, S.A. Payghan, Investigation of effect of non-ionic surfactant on preparation of griseofulvin non-aqueous nanoemulsion, J. Nanostructure Chem. 5 (2015) 107–113. https://doi.org/10.1007/s40097-014-0141-y.

[137] L. Zhang, Z.-Q. Zhang, W.-C. Dong, S.-J. Jing, J.-F. Zhang, Y. Jiang, Accuracy assessment on the analysis of unbound drug in plasma by comparing traditional centrifugal ultrafiltration with hollow fiber centrifugal ultrafiltration and application in pharmacokinetic study, J. Chromatogr. A. 1318 (2013) 265–269. https://doi.org/https://doi.org/10.1016/j.chroma.2013.09.078.

[138] A. Guillot, A.-C. Couffin, X. Sejean, F. Navarro, M. Limberger, C.-M. Lehr, Solid Phase Extraction as an Innovative Separation Method for Measuring Free and Entrapped Drug in Lipid Nanoparticles, Pharm. Res. 32 (2015) 3999–4009. https://doi.org/10.1007/s11095-015-1761-8.

[139] P. Khatri, J. Shao, Separation of external aqueous phase from o/w nanoemulsions, Eur. J. Pharm. Sci. 96 (2017) 171–175. https://doi.org/10.1016/J.EJPS.2016.09.021.

[140] A.K.F. Dyab, L.A. Mohamed, F. Taha, Non-aqueous olive oil-in-glycerin (o/o) Pickering emulsions: Preparation, characterization and in vitro aspirin release, J. Dispers. Sci. Technol. 39 (2018) 890–900. https://doi.org/10.1080/01932691.2017.1406368.

[141] S. Helvig, I.D.M. Azmi, S.M. Moghimi, A. Yaghmur, S. Helvig, I.D.M. Azmi, S.M. Moghimi, A. Yaghmur, Recent Advances in Cryo-TEM Imaging of Soft Lipid Nanoparticles, AIMS Biophys. 2015 2116. 2 (2015) 116–130. https://doi.org/10.3934/BIOPHY.2015.2.116.

[142] T.M. Ho, F. Abik, K.S. Mikkonen, An overview of nanoemulsion characterization via atomic force microscopy, Crit. Rev. Food Sci. Nutr. 62 (2022) 4908–4928. https://doi.org/10.1080/10408398.2021.1879727.

[143] R. Khursheed, S.K. Singh, M. Gulati, S. Wadhwa, B. Kapoor, N.K. Pandey, D.K. Chellappan, G. Gupta, N.K. Jha, K. Dua, D.N. Kapoor, V.V.S.R. Karri, P. Pattanayak, A. Sharni, S. Mondal, Exploring role of polysaccharides present in Ganoderma lucidium extract powder and probiotics as solid carriers in development of liquisolid formulation loaded with quercetin: A novel study, Int. J. Biol. Macromol. 183 (2021) 1630–1639. https://doi.org/10.1016/J.IJBIOMAC.2021.05.064.

[144] L. Corrie, A. Awasthi, J. Kaur, S. Vishwas, M. Gulati, I.P. Kaur, G. Gupta, N. Kommineni, K. Dua, S.K. Singh, Interplay of Gut Microbiota in Polycystic Ovarian Syndrome: Role of Gut Microbiota, Mechanistic Pathways and Potential Treatment Strategies, Pharmaceuticals. 16 (2023) 197.

[145] A. Kadukkattil Ramanunny, S.K. Singh, S. Wadhwa, M. Gulati, B. Kapoor, R. Khursheed, G. Kuppusamy, K. Dua, H. Dureja, D.K. Chellappan, Overcoming hydrolytic degradation challenges in topical delivery: non-aqueous nano-emulsions, Expert Opin. Drug Deliv. 19 (2022) 23–45.

[146] V.R. Bitra, D. Rapaka, N. Mathala, A. Akula, Effect of wheat grass powder on aluminum induced Alzheimer's disease in Wistar rats, Asian Pac. J. Trop. Med. 7 (2014) S278–S281.

[147] D. Rapaka, V.R. Bitra, T.C. Vishala, A. Akula, Vitis vinifera acts as anti-Alzheimer's agent by modulating biochemical parameters implicated in cognition and memory, J. Ayurveda Integr. Med. 10 (2019) 241–247.

[148] K.A. Khan, N. Kumar, P.G. Nayak, M. Nampoothiri, R.R. Shenoy, N. Krishnadas, C.M. Rao, J. Mudgal, Impact of caffeic acid on aluminium chloride-induced dementia in rats, J. Pharm. Pharmacol. 65 (2013) 1745–1752.

[149] S. Nakamura, N. Murayama, T. Noshita, H. Annoura, T. Ohno, Progressive brain dysfunction following intracerebroventricular infusion of beta1–42-amyloid peptide, Brain Res. 912 (2001) 128–136.

[150] C. Guela, C.-K. Wu, D. Saroff, A. Lorenzo, M. Yuan, B.A. Yankner, Aging renders the

brain vulnerable to amyloid β-protein neurotoxicity, Nat. Med. 4 (1998) 827–831.

[151] X. Wang, X. Zhou, B. Uberseder, J. Lee, C.S. Latimer, C.M. Furdui, C.D. Keene, T.J. Montine, T.C. Register, S. Craft, Isoform-specific dysregulation of AMP-activated protein kinase signaling in a non-human primate model of Alzheimer's disease, Neurobiol. Dis. 158 (2021) 105463.

[152] Z. Hruska, G.P. Dohanich, The effects of chronic estradiol treatment on working memory deficits induced by combined infusion of β-amyloid (1–42) and ibotenic acid, Horm. Behav. 52 (2007) 297–306.

[153] H. Lannert, S. Hoyer, Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats., Behav. Neurosci. 112 (1998) 1199.

[154] N.A. Noor, E.N. Hosny, Y.A. Khadrawy, I.M. Mourad, A.I. Othman, H.S. Aboul Ezz, H.S. Mohammed, Effect of curcumin nanoparticles on streptozotocin-induced male Wistar rat model of Alzheimer's disease, Metab. Brain Dis. 37 (2022) 343–357.

[155] H.-G. Yeo, Y. Lee, C.-Y. Jeon, K.-J. Jeong, Y.B. Jin, P. Kang, S.-U. Kim, J.-S. Kim, J.-W. Huh, Y.-H. Kim, Characterization of cerebral damage in a monkey model of Alzheimer's disease induced by intracerebroventricular injection of streptozotocin, J. Alzheimer's Dis. 46 (2015) 989–1005.

[156] A.H. Abu Almaaty, R.M. Mosaad, M.K. Hassan, E.H.A. Ali, G.A. Mahmoud, H. Ahmed, N. Anber, S. Alkahtani, M.M. Abdel-Daim, L. Aleya, Urtica dioica extracts abolish scopolamine-induced neuropathies in rats, Environ. Sci. Pollut. Res. 28 (2021) 18134–18145.

[157] K. Khurana, M. Kumar, N. Bansal, Lacidipine prevents scopolamine-induced memory impairment by reducing brain oxido-nitrosative stress in mice, Neurotox. Res. 39 (2021) 1087–1102.

[158] G. Riedel, S.H. Kang, D.Y. Choi, B. Platt, Scopolamine-induced deficits in social memory in mice: reversal by donepezil, Behav. Brain Res. 204 (2009) 217–225.

[159] H. Sain, B. Sharma, A.S. Jaggi, N. Singh, Pharmacological investigations on potential of peroxisome proliferator-activated receptor-gamma agonists in hyperhomocysteinemia-induced vascular dementia in rats, Neuroscience. 192 (2011) 322–333.

[160] S.A. Khodir, M.A. Faried, H.I. Abd-Elhafiz, E.M. Sweed, Sitagliptin attenuates the cognitive deficits in L-methionine-induced vascular dementia in rats, Biomed Res. Int. 2022 (2022).

[161] V. Mani, K. Ramasamy, A. Ahmad, M. Parle, S.A.A. Shah, A.B.A. Majeed, Protective effects of total alkaloidal extract from Murraya koenigii leaves on experimentally induced dementia, Food Chem. Toxicol. 50 (2012) 1036–1044.

[162] D. Dhingra, M. Parle, S.K. Kulkarni, Memory enhancing activity of Glycyrrhiza glabra in mice, J. Ethnopharmacol. 91 (2004) 361–365.

[163] A. Fisher, I. Hanin, Potential animal models for senile dementia of Alzheimer's type, with emphasis on AF64A-induced cholinotoxicity, Annu. Rev. Pharmacol. Toxicol. 26 (1986) 161–181.

[164] K. Yamada, S. Furukawa, T. Iwasaki, Y. Ichitani, Nicotine improves AF64A-induced spatial memory deficits in Morris water maze in rats, Neurosci. Lett. 469 (2010) 88–92.

[165] D.L. Sparks, Cholesterol, copper, and accumulation of thioflavine S-reactive Alzheimer's-like amyloid β in rabbit brain, J. Mol. Neurosci. 24 (2004) 97–104.

[166] D.L. Sparks, B.G. Schreurs, Trace amounts of copper in water induce β-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease, Proc. Natl. Acad. Sci. 100 (2003) 11065–11069.

[167] J. Shi, Y. Dong, M.-Z. Cui, X. Xu, Lysophosphatidic acid induces increased BACE1 expression and Aβ formation, Biochim. Biophys. Acta (BBA)-Molecular Basis Dis. 1832 (2013) 29–38.

[168] C.L. Sayas, J. Ávila, F. Wandosell, Regulation of neuronal cytoskeleton by lysophosphatidic acid: role of GSK-3, Biochim. Biophys. Acta (BBA)-Molecular Cell

Biol. Lipids. 1582 (2002) 144–153.

[169] X.-Y. Song, J.-F. Hu, S.-F. Chu, Z. Zhang, S. Xu, Y.-H. Yuan, N. Han, Y. Liu, F. Niu, X. He, Ginsenoside Rg1 attenuates okadaic acid induced spatial memory impairment by the GSK3β/tau signaling pathway and the Aβ formation prevention in rats, Eur. J. Pharmacol. 710 (2013) 29–38.

[170] Z. Zhang, J.W. Simpkins, An okadaic acid-induced model of tauopathy and cognitive deficiency, Brain Res. 1359 (2010) 233–246.

[171] G.M. Abu-Taweel, J.S. Ajarem, M. Ahmad, Neurobehavioral toxic effects of perinatal oral exposure to aluminum on the developmental motor reflexes, learning, memory and brain neurotransmitters of mice offspring, Pharmacol. Biochem. Behav. 101 (2012) 49–56.

[172] G. Qi-Hai, W. Qin, H. Xie-Nan, S. An-Sheng, N. Jing, S. Jing-Shan, Protective effect of Ginkgo biloba leaf extract on learning and memory deficit induced by aluminum in model rats, Chin. J. Integr. Med. 12 (2006) 37–41.

[173] K. Amberla, A. Nordberg, M. Viitanen, B. Winblad, Long-term treatment with tacrine (THA) in Alzheimer's disease—evaluation of neuropsychological data, Acta Neurol. Scand. 88 (1993) 55–57.

[174] S.-Z. Zhong, Q.-H. Ge, R. Qu, Q. Li, S.-P. Ma, Paeonol attenuates neurotoxicity and ameliorates cognitive impairment induced by d-galactose in ICR mice, J. Neurol. Sci. 277 (2009) 58–64.

[175] J.L. Cummings, D. Kaufer, Neuropsychiatric aspects of Alzheimer's disease: the cholinergic hypothesis revisited, Neurology. 47 (1996) 876–883.

[176] Y. Luo, F. Niu, Z. Sun, W. Cao, X. Zhang, D. Guan, Z. Lv, Y. Xu, Altered expression of Aβ metabolism-associated molecules from d-galactose/AlCl3 induced mouse brain, Mech. Ageing Dev. 130 (2009) 248–252.

[177] D. Shunan, M. Yu, H. Guan, Y. Zhou, Neuroprotective effect of Betalain against AlCl3-induced Alzheimer's disease in Sprague Dawley Rats via putative modulation of

oxidative stress and nuclear factor kappa B (NF-κB) signaling pathway, Biomed. Pharmacother. 137 (2021) 111369. https://doi.org/10.1016/J.BIOPHA.2021.111369.

[178] S. Rohilla, R. Awasthi, M. Mehta, D.K. Chellappan, G. Gupta, M. Gulati, S.K. Singh, K. Anand, B.G. Oliver, K. Dua, H. Dureja, Preparation and Evaluation of Gefitinib Containing Nanoliposomal Formulation for Lung Cancer Therapy, Bionanoscience. 12 (2022) 241–255. https://doi.org/10.1007/s12668-022-00938-6.

[179] D. Ghosh, S.K. Singh, R. Khursheed, N.K. Pandey, B. Kumar, R. Kumar, Y. Kumari, G. Kaur, A. Clarisse, A. Awasthi, M. Gulati, S.K. Jain, O. Porwal, E. Bayrakdar, M. Sheet, K. Gowthamarajan, S. Gupta, L. Corrie, P. Gunjal, R.K. Gupta, T.G. Singh, S. Sinha, Impact of solidification on micromeritic properties and dissolution rate of self-nanoemulsifying delivery system loaded with docosahexaenoic acid, Taylor & Francis, 2020. https://doi.org/10.1080/03639045.2020.1742143.

[180] L. Corrie, M. Gulati, A. Awasthi, S. Vishwas, J. Kaur, R. Khursheed, R. Kumar, A. Kumar, M. Imran, D.K. Chellappan, Polysaccharide, fecal microbiota, and curcumin-based novel oral colon-targeted solid self-nanoemulsifying delivery system: formulation, characterization, and in-vitro anticancer evaluation, Mater. Today Chem. 26 (2022) 101165.

[181] R. Khursheed, S.K. Singh, M. Gulati, S. Wadhwa, B. Kapoor, N.K. Pandey, D.K. Chellappan, G. Gupta, N.K. Jha, K. Dua, D.N. Kapoor, V.V.S.R. Karri, P. Pattanayak, A. Sharni, S. Mondal, Exploring role of polysaccharides present in Ganoderma lucidium extract powder and probiotics as solid carriers in development of liquisolid formulation loaded with quercetin: A novel study, Int. J. Biol. Macromol. 183 (2021) 1630–1639. https://doi.org/10.1016/j.ijbiomac.2021.05.064.

[182] A. Awasthi, A. Kumar, R. Kumar, S. Vishwas, R. Khursheed, J. Kaur, L. Corrie, B. Kumar, M. Gulati, D. Kumar, M. Kaushik, G. Gupta, P. Prasher, D.K. Chellappan, A.P. Kumar, K. Dua, S.K. Singh, RP-HPLC method development and validation for simultaneous estimation of mesalamine and curcumin in bulk form as well as nanostructured lipid carriers, South African J. Bot. 151 (2022) 529–537.

https://doi.org/10.1016/J.SAJB.2022.05.044.

[183] R. Kumar, R. Kumar, R. Khursheed, B. Kapoor, N. Sharma, S. Khurana, N. Khurana, S.K. Singh, M. Vyas, Development and validation of UV spectroscopic method for estimation of fisetin in self nanoemulsifying drug delivery system, Res. J. Pharm. Technol. 13 (2020) 1179–1182. https://doi.org/10.5958/0974-360X.2020.00217.6.

[184] R. Kumar, R. Kumar, R. Khursheed, A. Awasthi, A.K. Ramanunny, J. Kaur, N. Khurana, S.K. Singh, S. Khurana, N.K. Pandey, B. Kapoor, N. Sharma, Validated reverse phase-high-performance liquid chromatography method for estimation of fisetin in self-nanoemulsifying drug delivery system, Assay Drug Dev. Technol. 18 (2020) 274–281. https://doi.org/10.1089/adt.2020.983.

[185] VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY Q2(R1), ICH Harmon. Tripart. Guidel. (2005). https://doi.org/chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://database.ich.org/sites/default/fil es/Q2%28R1%29%20Guideline.pdf.

[186] R. Khursheed, S.K. Singh, B. Kapoor, M. Gulati, S. Wadhwa, S. Gupta, Development and Validation of RP-HPLC Method for Simultaneous Determination of Curcumin and Quercetin in Extracts , Marketed Formulations , and Self-Nanoemulsifying Drug Delivery System, 1 (2021) 43–52. https://doi.org/10.1089/regen.2021.0021.

[187] S.R. Parveen, S. Wadhwa, M.R. Babu, S. Vishwas, L. Corrie, A. Awasthi, F.R. Khan, M.M. Al-Bazi, N.S. Alharthi, F. Alotaibi, Formulation of chrysin loaded nanostructured lipid carriers using Box Behnken design, its characterization and antibacterial evaluation alone and in presence of probiotics co-loaded in gel, J. Drug Deliv. Sci. Technol. 84 (2023) 104411.

[188] S. Sood, K. Jain, K. Gowthamarajan, Optimization of curcumin nanoemulsion for intranasal delivery using design of experiment and its toxicity assessment, Colloids Surfaces B Biointerfaces. 113 (2014) 330–337. https://doi.org/10.1016/J.COLSURFB.2013.09.030.

[189] A. Kadukkattil Ramanunny, S. Wadhwa, S. Kumar Singh, B. Kumar, M. Gulati, A. Kumar, S. Almawash, A. Al Saqr, K. Gowthamrajan, K. Dua, H. Singh, S. Vishwas, R. Khursheed, S. Rahana Parveen, A. Venkatesan, K.R. Paudel, P.M. Hansbro, D. Kumar Chellappan, Topical non-aqueous nanoemulsion of Alpinia galanga extract for effective treatment in psoriasis: in vitro and in vivo evaluation, Int. J. Pharm. (2022) 121882. https://doi.org/10.1016/j.ijpharm.2022.121882.

[190] R. Khursheed, S.K. Singh, B. Kumar, S. Wadhwa, M. Gulati, A. A, A. Awasthi, S. Vishwas, J. Kaur, L. Corrie, K.R. Arya, R. Kumar, N.K. Jha, P.K. Gupta, F. Zacconi, K. Dua, N. Chitranshi, G. Mustafa, A. Kumar, Self-nanoemulsifying composition containing curcumin, quercetin, Ganoderma lucidum extract powder and probiotics for effective treatment of type 2 diabetes mellitus in streptozotocin induced rats, Int. J. Pharm. 612 (2022) 121306. https://doi.org/10.1016/J.IJPHARM.2021.121306.

[191] A. Awasthi, B. Kumar, M. Gulati, S. Vishwas, L. Corrie, J. Kaur, R. Khursheed, R.A. Muhammed, D. Kala, O. Porwal, M.R. Babu, M.V.N.L. Chaitanya, A. Kumar, N.K. Pandey, H. Dureja, D.K. Chellappan, N.K. Jha, G. Gupta, P. Prasher, D. Kumar, K. Dua, S.K. Singh, Novel Nanostructured Lipid Carriers Co-Loaded with Mesalamine and Curcumin: Formulation, Optimization and In Vitro Evaluation, Pharm. Res. 39 (2022). https://doi.org/10.1007/S11095-022-03401-Z.

[192] M. Hanmantrao, S. Chaterjee, R. Kumar, S. Vishwas, V. Harish, O. Porwal, M. Alrouji, O. Alomeir, S. Alhajlah, M. Gulati, G. Gupta, K. Dua, S.K. Singh, Development of Guar Gum-Pectin-Based Colon Targeted Solid Self-Nanoemulsifying Drug Delivery System of Xanthohumol, Pharm. 2022, Vol. 14, Page 2384. 14 (2022) 2384. https://doi.org/10.3390/PHARMACEUTICS14112384.

[193] International Conference on Harmonisation (ICH). Guidance for industry: Q1A(R2) Stability Testing of New drug Substances and Products, ICH Harmon. Tripart. Guidel. 4 (2003) 24.

[194] S.Y. Rajesh, S.K. Singh, N.K. Pandey, P. Sharma, P. Bawa, B. Kumar, M. Gulati, S.K. Jain, K. Gowthamarajan, S. Singh, Impact of various solid carriers and spray drying on

pre/post compression properties of solid SNEDDS loaded with glimepiride: in vitro-ex vivo evaluation and cytotoxicity assessment, Drug Dev. Ind. Pharm. 44 (2018) 1056–1069. https://doi.org/10.1080/03639045.2018.1431656.

[195] R. Kumar, R. Kumar, R. Khursheed, A. Awasthi, N. Khurana, S.K. Singh, S. Khurana, N. Sharma, P. Gunjal, J. Kaur, L. Corrie, Development and validation of RP-HPLC method for estimation of fisetin in rat plasma, South African J. Bot. 140 (2021) 284–289. https://doi.org/10.1016/j.sajb.2020.05.010.

[196] A. Awasthi, A. Kumar, R. Kumar, S. Vishwas, R. Khursheed, J. Kaur, L. Corrie, B. Kumar, M. Gulati, D. Kumar, RP-HPLC method development and validation for simultaneous estimation of mesalamine and curcumin in bulk form as well as nanostructured lipid carriers, South African J. Bot. 151 (2022) 529–537.

[197] G.M. Babulal, Y.T. Quiroz, B.C. Albensi, E. Arenaza-Urquijo, A.J. Astell, C. Babiloni, A. Bahar-Fuchs, J. Bell, G.L. Bowman, A.M. Brickman, G. Chételat, C. Ciro, A.D. Cohen, P. Dilworth-Anderson, H.H. Dodge, S. Dreux, S. Edland, A. Esbensen, L. Evered, M. Ewers, K.N. Fargo, J. Fortea, H. Gonzalez, D.R. Gustafson, E. Head, J.A. Hendrix, S.M. Hofer, L.A. Johnson, R. Jutten, K. Kilborn, K.L. Lanctôt, J.J. Manly, R.N. Martins, M.M. Mielke, M.C. Morris, M.E. Murray, E.S. Oh, M.A. Parra, R.A. Rissman, C.M. Roe, O.A. Santos, N. Scarmeas, L.S. Schneider, N. Schupf, S. Sikkes, H.M. Snyder, H.R. Sohrabi, Y. Stern, A. Strydom, Y. Tang, G.M. Terrera, C. Teunissen, D. Melo van Lent, M. Weinborn, L. Wesselman, D.M. Wilcock, H. Zetterberg, S.E. O'Bryant, Perspectives on ethnic and racial disparities in Alzheimer's disease and related dementias: Update and areas of immediate need, Alzheimer's Dement. 15 (2019) 292–312. https://doi.org/https://doi.org/10.1016/j.jalz.2018.09.009.

[198] J.A. Soria Lopez, H.M. González, G.C. Léger, Chapter 13 - Alzheimer's disease, in: S.T. Dekosky, S. Asthana (Eds.), Geriatr. Neurol., Elsevier, 2019: pp. 231–255. https://doi.org/https://doi.org/10.1016/B978-0-12-804766-8.00013-3.

[199] B.R. Hoover, M.N. Reed, J. Su, R.D. Penrod, L.A. Kotilinek, M.K. Grant, R. Pitstick, G.A. Carlson, L.M. Lanier, L.-L. Yuan, K.H. Ashe, D. Liao, Tau Mislocalization to

Dendritic Spines Mediates Synaptic Dysfunction Independently of Neurodegeneration, Neuron. 68 (2010) 1067–1081. https://doi.org/https://doi.org/10.1016/j.neuron.2010.11.030.

[200] G.G. Glenner, C.W. Wong, Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein, Biochem. Biophys. Res. Commun. 122 (1984) 1131–1135. https://doi.org/https://doi.org/10.1016/0006-291X(84)91209-9.

[201] G.G. Glenner, C.W. Wong, Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein, Biochem. Biophys. Res. Commun. 120 (1984) 885–890. https://doi.org/https://doi.org/10.1016/S0006-291X(84)80190-4.

[202] A. John, P.H. Reddy, Synaptic basis of Alzheimer's disease: Focus on synaptic amyloid beta, P-tau and mitochondria, Ageing Res. Rev. 65 (2021) 101208.

[203] M.S. Uddin, M.M. Rahman, M. Jakaria, M.S. Rahman, M.S. Hossain, A. Islam, M. Ahmed, B. Mathew, U.M. Omar, G.E. Barreto, Estrogen signaling in Alzheimer's disease: molecular insights and therapeutic targets for Alzheimer's dementia, Mol. Neurobiol. 57 (2020) 2654–2670.

[204] G.D. Rabinovici, R. La Joie, Amyloid-targeting monoclonal antibodies for Alzheimer disease, Jama. (2023).

[205] Q. Li, X. Li, B. Tian, L. Chen, Protective effect of pterostilbene in a streptozotocin-induced mouse model of Alzheimer's disease by targeting monoamine oxidase B, J. Appl. Toxicol. 42 (2022) 1777–1786.

[206] H.M. Hussien, A. Abd-Elmegied, D.A. Ghareeb, H.S. Hafez, H.E.A. Ahmed, N.A. El-moneam, Neuroprotective effect of berberine against environmental heavy metals-induced neurotoxicity and Alzheimer's-like disease in rats, Food Chem. Toxicol. 111 (2018) 432–444. https://doi.org/https://doi.org/10.1016/j.fct.2017.11.025.

[207] Z. Cao, F. Wang, C. Xiu, J. Zhang, Y. Li, Hypericum perforatum extract attenuates behavioral, biochemical, and neurochemical abnormalities in Aluminum chloride-

induced Alzheimer's disease rats, Biomed. Pharmacother. 91 (2017) 931–937. https://doi.org/https://doi.org/10.1016/j.biopha.2017.05.022.

[208] C. Exley, T. Vickers, Elevated brain aluminium and early onset Alzheimer's disease in an individual occupationally exposed to aluminium: a case report, J. Med. Case Rep. 8 (2014) 1–3.

[209] A. Mirza, A. King, C. Troakes, C. Exley, Aluminium in brain tissue in familial Alzheimer's disease, J. Trace Elem. Med. Biol. 40 (2017) 30–36. https://doi.org/https://doi.org/10.1016/j.jtemb.2016.12.001.

[210] E. Inan-Eroglu, A. Ayaz, Is aluminum exposure a risk factor for neurological disorders?, J. Res. Med. Sci. Off. J. Isfahan Univ. Med. Sci. 23 (2018).

[211] L. Liaquat, S. Sadir, Z. Batool, S. Tabassum, S. Shahzad, A. Afzal, S. Haider, Acute aluminum chloride toxicity revisited: Study on DNA damage and histopathological, biochemical and neurochemical alterations in rat brain, Life Sci. 217 (2019) 202–211.

[212] T. Garcia, J.L. Esparza, M.R. Nogués, M. Romeu, J.L. Domingo, M. Gómez, Oxidative stress status and RNA expression in hippocampus of an animal model of Alzheimer's disease after chronic exposure to aluminum, Hippocampus. 20 (2010) 218–225.

[213] Y. Zhao, M. Dang, W. Zhang, Y. Lei, T. Ramesh, V.P. Veeraraghavan, X. Hou, Neuroprotective effects of Syringic acid against aluminium chloride induced oxidative stress mediated neuroinflammation in rat model of Alzheimer's disease, J. Funct. Foods. 71 (2020) 104009.

[214] D.B. Mathiyazahan, A.J. Thenmozhi, T. Manivasagam, Protective effect of black tea extract against aluminium chloride-induced Alzheimer's disease in rats: A behavioural, biochemical and molecular approach, J. Funct. Foods. 16 (2015) 423–435.

[215] B.V.S. Lakshmi, M. Sudhakar, K.S. Prakash, Protective effect of selenium against aluminum chloride-induced Alzheimer's disease: behavioral and biochemical alterations in rats, Biol. Trace Elem. Res. 165 (2015) 67–74.

[216] X. Chen, M. Zhang, M. Ahmed, K.M. Surapaneni, V.P. Veeraraghavan, P. Arulselvan,

Neuroprotective effects of ononin against the aluminium chloride-induced Alzheimer's disease in rats, Saudi J. Biol. Sci. 28 (2021) 4232–4239. https://doi.org/https://doi.org/10.1016/j.sjbs.2021.06.031.

[217] D. Prakash, K. Gopinath, G. Sudhandiran, Fisetin enhances behavioral performances and attenuates reactive gliosis and inflammation during aluminum chloride-induced neurotoxicity, Neuromolecular Med. 15 (2013) 192–208.

[218] R. Jadhav, Y.A. Kulkarni, Effects of baicalein with memantine on aluminium chloride-induced neurotoxicity in Wistar rats, Front. Pharmacol. 14 (2023) 1–12. https://doi.org/10.3389/fphar.2023.1034620.

[219] L. Liu, Y. Liu, J. Zhao, X. Xing, C. Zhang, H. Meng, Neuroprotective Effects of D-(-)-Quinic Acid on Aluminum Chloride-Induced Dementia in Rats, Evidence-Based Complement. Altern. Med. 2020 (2020). https://doi.org/10.1155/2020/5602597.

[220] S.J. Gilani, M.N. Bin-Jumah, F.A. Al-Abbasi, S.S. Imam, S. Alshehri, M.M. Ghoneim, M. Shahid Nadeem, M. Afzal, S.I. Alzarea, N. Sayyed, I. Kazmi, Antiamnesic Potential of Malvidin on Aluminum Chloride Activated by the Free Radical Scavenging Property, ACS Omega. 7 (2022) 24231–24240. https://doi.org/10.1021/acsomega.2c01406.

[221] A. Justin Thenmozhi, M. Dhivyabharathi, T.R. William Raja, T. Manivasagam, M.M. Essa, Tannoid principles of Emblica officinalis renovate cognitive deficits and attenuate amyloid pathologies against aluminum chloride induced rat model of Alzheimer's disease, Nutr. Neurosci. 19 (2016) 269–278.

[222] V. Kumar, A. Bal, K.D. Gill, Aluminium-induced oxidative DNA damage recognition and cell-cycle disruption in different regions of rat brain, Toxicology. 264 (2009) 137–144.

[223] K. Saba, N. Rajnala, P. Veeraiah, V. Tiwari, R.K. Rana, S.C. Lakhotia, A.B. Patel, Energetics of excitatory and inhibitory neurotransmission in aluminum chloride model of Alzheimer's disease: Reversal of behavioral and metabolic deficits by Rasa Sindoor, Front. Mol. Neurosci. 10 (2017) 323.

[224] G.H. Kim, J.E. Kim, S.J. Rhie, S. Yoon, The role of oxidative stress in neurodegenerative diseases, Exp. Neurobiol. 24 (2015) 325.

[225] M. Qu, Z. Jiang, Y. Liao, Z. Song, X. Nan, Lycopene prevents amyloid [beta]-induced mitochondrial oxidative stress and dysfunctions in cultured rat cortical neurons, Neurochem. Res. 41 (2016) 1354–1364.

[226] D. Praticò, K. Uryu, S. Sung, S. Tang, J.Q. Trojanowski, V.M. Lee, Aluminum modulates brain amyloidosis through oxidative stress in APP transgenic mice, FASEB J. 16 (2002) 1138–1140.

[227] C.J. Busch, C.J. Binder, Malondialdehyde epitopes as mediators of sterile inflammation, Biochim. Biophys. Acta (BBA)-Molecular Cell Biol. Lipids. 1862 (2017) 398–406.

[228] M. Kawahara, M. Kato-Negishi, Link between aluminum and the pathogenesis of Alzheimer's disease: the integration of the aluminum and amyloid cascade hypotheses, Int. J. Alzheimers. Dis. 2011 (2011). https://doi.org/10.4061%2F2011%2F276393

[229] V. Kumar, K.D. Gill, Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: a review, Neurotoxicology. 41 (2014) 154–166.

[230] H. Khan, H. Ullah, M. Aschner, W.S. Cheang, E.K. Akkol, Neuroprotective effects of quercetin in Alzheimer's disease, Biomolecules. 10 (2019) 59.

[231] A. Ataie, M. Sabetkasaei, A. Haghparast, A.H. Moghaddam, B. Kazeminejad, Neuroprotective effects of the polyphenolic antioxidant agent, Curcumin, against homocysteine-induced cognitive impairment and oxidative stress in the rat, Pharmacol. Biochem. Behav. 96 (2010) 378–385.

[232] N.-N. Yuan, C.-Z. Cai, M.-Y. Wu, H.-X. Su, M. Li, J.-H. Lu, Neuroprotective effects of berberine in animal models of Alzheimer's disease: a systematic review of pre-clinical studies, BMC Complement. Altern. Med. 19 (2019) 109. https://doi.org/10.1186/s12906-019-2510-z.

[233] M.S. Ola, M.M. Ahmed, R. Ahmad, H.M. Abuohashish, S.S. Al-Rejaie, A.S. Alhomida, Neuroprotective Effects of Rutin in Streptozotocin-Induced Diabetic Rat Retina, J. Mol.

Neurosci. 56 (2015) 440–448. https://doi.org/10.1007/s12031-015-0561-2.

[234] X. Cheng, J. Gu, Y. Pang, J. Liu, T. Xu, X. Li, Y. Hua, K.A. Newell, X.-F. Huang, Y. Yu, Tacrine–hydrogen sulfide donor hybrid ameliorates cognitive impairment in the aluminum chloride mouse model of Alzheimer's disease, ACS Chem. Neurosci. 10 (2019) 3500–3509.

[235] A. Trovato Salinaro, M. Pennisi, R. Di Paola, M. Scuto, R. Crupi, M.T. Cambria, M.L. Ontario, M. Tomasello, M. Uva, L. Maiolino, Neuroinflammation and neurohormesis in the pathogenesis of Alzheimer's disease and Alzheimer-linked pathologies: modulation by nutritional mushrooms, Immun. Ageing. 15 (2018) 1–8.

[236] H.A. Elreedy, A.M. Elfiky, A.A. Mahmoud, S. Khadiga, M.A. Ghazy, H.A. Elreedy, A.M. Elfiky, A.A. Mahmoud, S. Khadiga, Neuroprotective effect of quercetin through targeting key genes involved in aluminum chloride induced Alzheimer's disease in rats, Egypt. J. Basic Appl. Sci. 10 (2023) 174–184. https://doi.org/10.1080/2314808X.2022.2164136.

ANNEXURES

Annexure 1	:	Candidacy letter of Ph.D.
Annexure 2	:	Approval by Institutional Animal Ethics Committee
Annexure 3	:	List of publications, patents, awards, certificates

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LPU/CRDP/PHD/EC/20210803/001247
Dated: 09th Aug 2021

Sukriti Vishwas

Registration Number: 42000319

Programme Name: Doctor of Philosophy (Pharmacology)

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 22nd May 2021 by accepting your research proposal entitled: "Formulation development and evaluation of fisetin loaded oral nanoemulsion for the treatment of Alzheimer s disease in rats" under the supervision of Dr. Sachin Kumar Singh and Co-supervision of Dr. Monica Gulati.

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.

Head

Centre for Research Degree Programmes

CERTIFICATE

This is to certify that the project titled *"Development and Evaluation of Fisetin Loaded Oral Nanoemulsion for the Treatment of Alzheimer's Disease"* has been approved by the IAEC.

Name of Principal Investigator: Dr. Sachin Kumar Singh

IAEC approval number: LPU/IAEC/2021/91

Date of Approval: 29th October 2021

Animals approved: 54 SD Rats

Remarks if any: - NA

Dr. Monica Gulati	**Dr. Navneet Khurana**	**Dr. Bimlesh Kumar**
Biological Scientist, Chairperson IAEC	Scientist from different discipline	Scientist In-Charge of Animal House, Member Secretary IAEC

African Journal of Biological Sciences

Development and Validation of an RP-HPLC Technique for Quantifying Fisetin in Rat Plasma

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Abstract

Fisetin (FS) is a phyto-flavonoid with antioxidant, neuroprotective, and anticancer properties. A bioanalytical method was developed and validated to estimate FS levels in rat plasma using reverse phase ultra-fast liquid chromatography with a C-18 reverse phase column. Quercetin (Qu) served as an internal standard. The mobile phase consisted of acetonitrile and orthophosphoric acid (0.2% v/v) in a 30:70 v/v ratio, with a flow rate of 1 mL/min, and detection was performed at a wavelength of 362 nm. Protein precipitation was employed to extract the drug from plasma samples. The retention times for FS and Qu were 5.6 min and 10.3 min, respectively. The method demonstrated linearity in the range of 2-10 ng/mL with a regression coefficient (r^2) of 0.9994. Validation followed the ICH Q2 (R1) guidelines, with percentage recovery between 98-100%, indicating accuracy. The percentage relative standard deviation was below 2%, showing precision. The limit of detection (LOD) and limit of quantification (LOQ) were 0.03 ng/mL and 0.08 ng/mL, respectively. The method proved robust, showing no significant response changes with variations in flow rate and mobile phase composition. These results indicate that the developed method meets all validation criteria and is suitable for estimating fisetin in rat plasma.

Keywords: Fisetin; Bioanalytical method development; Validation; rat plasma; Quercetin

ORIGINAL ARTICLE

Enhancing the oral bioavailability of fisetin: polysaccharide-based self nano-emulsifying spheroids for colon-targeted delivery

Pradnya Gunjal[1] · Sukriti Vishwas[1] · Rajan Kumar[1] · Bushra Bashir[1] · Bimlesh Kumar[1] · Navneet Khurana[1] · Monica Gulati[1,2] · Gaurav Gupta[3,4] · Parteek Prasher[5] · Popat Kumbhar[6] · John Disouza[6] · Gowthamarajan Kuppusamy[7] · Yousuf Mohammed[8,9] · Harish Dureja[10] · Kamal Dua[2,11] · Sachin Kumar Singh[1,2]

Abstract

Fisetin (FS) is a flavonoid that possesses antioxidant and anti-inflammatory properties against ulcerative colitis. FS shows poor dissolution rate and permeability. An attempt has been made to develop colon-targeted solid self-nanoemulsifying drug delivery systems (S-SNEDDS) of FS. Initially, liquid (L) SNEDDS were prepared by loading FS into isotropic mixture of L-SNEDDS was prepared using Labrafil M 1944 CS, Transcutol P, and Tween 80. These L-SNEDDS were further converted into solid (S) SNEDDS by mixing the isotropic mixture with 1:1:1 ratio of guar gum (GG), xanthan gum (XG) and pectin (PC) [GG:XG:PC (1:1:1)]. Aerosil-200 (A-200) was added to enhance their flow characteristics. Further, they were converted into spheroids by extrusion-spheronization technique. The solid-state characterization of S-SNEDDS was done by SEM, DSC, and PXRD, which revealed that the crystalline form of FS was converted into the amorphous form. In the dissolution study, S-SNEDDS spheroids [GG:XG:PC (1:1:1)] exhibited less than 20% drug release within the first 5 h, followed by rapid release of the drug between the 5th and 10th h, indicating its release at colonic site. The site-specific delivery of FS to colon via FS-S-SNEDDS spheroids was confirmed by conducting pharmacokinetic studies on rats. Wherein, results showed delay in absorption of FS loaded in spheroids up to 5 h and achievement of Cmax at 7h, whereas L-SNEDDS showed rapid absorption of FS. Furthermore, FS-L-SNEDDS and FS-S-SNEDDS spheroids [GG:XG:PC (1:1:1)] increased oral bioavailability of FS by 6.86-fold and 4.44-fold, respectively, as compared to unprocessed FS.

Keywords Fisetin · Self-nanoemulsifying drug delivery system · Colon targeting · Spheroids · Pharmacokinetics · Polysaccharides

Introduction

The rapid increase in the prevalence rate of colonic diseases such as ulcerative colitis, colon cancer, and Crohn's disease has drawn the interest of researchers for developing an oral colon targeted delivery system. Various oral drug delivery systems have been developed as immediate-release dosage forms, where the rapid dissolution of drugs takes place in the upper gastrointestinal tract (GIT) environment. This further causes efficient absorption of these drugs. However, this strategy is suitable for drugs whose target site is either the stomach or, the small intestine [1]. For drugs acting at other target sites such as large intestine, this approach may not be suitable. Hence, developing an oral colon-targeted delivery system for effective treatment of colonic diseases could offer certain advantages over immediate release or, modified release delivery systems as all the amount of drug can be directly delivered at local site of colon for their action. Furthermore, the colonic route also offers delivery of drugs meant for systemic use and drugs that are unstable at stomach or, upper intestine [2].

However, developing an oral colon-targeted delivery system is a challenging task. The challenges include traversing of formulation through the entire gut in the intact form, variable pH of GIT, anaerobicity of the colon, transient occurrence of mobility, and variability in fermentation activity in different regions [3]. Nevertheless, in the past, various colon-targeted formulations have been developed. These include timed-release system, pH-dependent polymer coated formulations, pressure-controlled systems, prodrugs, osmotically controlled systems, and colonic microbiota-activated system [4]. These delivery systems have been well explored

REVIEW ARTICLE

Neuroprotective Role of Phytoconstituents-Based Nanoemulsion for the Treatment of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is the most prevalent form of neurodegenerative disorder (ND), affecting more than 44 million individuals globally as of 2023. It is characterized by cognitive dysfunction and an inability to perform daily activities. The progression of AD is associated with the accumulation of amyloid beta (Aβ), the formation of neurofibrillary tangles (NFT), increased oxidative stress, neuroinflammation, mitochondrial dysfunction, and endoplasmic reticulum stress. Presently, various phytomedicines and their bioactive compounds have been identified for their neuroprotective effects in reducing oxidative stress, alleviating neuroinflammation, and mitigating the accumulation of Aβ and acetylcholinesterase enzymes in the hippocampus and cerebral cortex regions of the brain. However, despite demonstrating promising anti-Alzheimer's effects, the clinical utilization of phytoconstituents remains limited in scope. The key factor contributing to this limitation is the challenges inherent in traditional drug delivery systems, which impede their effectiveness and efficiency. These difficulties encompass insufficient drug targeting, restricted drug solubility and stability, brief duration of action, and a lack of control over drug release. Consequently, these constraints result in diminished bioavailability and insufficient permeability across the blood-brain barrier (BBB). In response to these challenges, novel drug delivery systems (NDDS) founded on nanoformulations have emerged as a hopeful strategy to augment the bioavailability and BBB permeability of bioactive compounds with poor solubility. Among these systems, nanoemulsion (NE) have been extensively investigated for their potential in targeting AD. NE offers several advantages, such as ease of preparation, high drug loading, and high stability. Due to their nanosize droplets, NE also improves gut and BBB permeability leading to enhanced permeability of the drug in systemic circulation and the brain. Various studies have reported the testing of NE-based phytoconstituents and their bioactives in different animal species, including transgenic, Wistar, and Sprague-Dawley (SD) rats, as well as mice. However, transgenic mice are commonly employed in AD research to analyze the effects of Aβ. In this review, various aspects such as the neuroprotective role of various phytoconstituents, the challenges associated with conventional drug delivery, and the need for NDDS, particularly NE, are discussed. Various studies involving phytoconstituent-based NE for the treatment of AD are also discussed.

Keywords: Alzheimer's disease, Phytoconstituents, Nanoemulsion, Bioavailability, Neuroprotective effects.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that predominantly impacts the brain, representing one of the main contributors to cognitive decline, commonly manifested as dementia. Initial indications of AD include difficulty recalling recently acquired information, difficulties in problem-solving, temporal or spatial confusion, and alterations in mood and personality. As the disease progresses, individuals may experience notable memory deterioration, disorientation, speech difficulties, and a decline in motor function [1-3]. The exact etiopathogenesis of AD is

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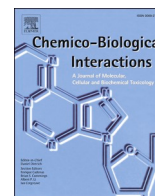
Harnessing the therapeutic potential of fisetin and its nanoparticles: Journey so far and road ahead

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ARTICLE INFO

ABSTRACT

Fisetin (FS) is a bioactive flavonoid obtained mostly from apple and strawberry and classified under the category of food supplements due to numerous pharmacological effects against various diseases through multiple mechanistic pathways. It acts as excellent neuroprotective, cardioprotective, anti-invasive, anti-tumorigenic, anti-angiogenic, anticancer, antidiabetics, antioxidant, anti-inflammatory agent. Despite having excellent safety and efficacy profile, FS is very less explored to clinical research either as food supplement or, as therapeutic agent due to its poor aqueous solubility, low bioavailability and reduced blood brain barrier permeability. Multiple mechanistic pathways through which FS elicits its pharmacological actions and the challenges associated with FS that compromises therapeutic efficacy are described in this article. The nanoformulations developed to enhance the bioavailability and therapeutic efficacy of FS are also covered with detailed description of research works carried by various researchers. These include nanoemulsions, liposomes, ethosomes, glycerosomes, polymeric micelles, self-nanoemulsifying drug delivery system and polymeric nanoparticles. Various patents pertaining to extraction/isolation, formula composition and therapeutic uses of FS as well as some clinical studies conducted using FS as active moiety are also enlisted.

1. Introduction

Fisetin (FS) is a polyphenolic flavonoid obtained from various fruits and vegetables such as strawberry, apple, persimmon, grape, onion, and cucumber [1]. Chemically, it is known as 3,3′,4′,7-tetrahydroxyflavone [2] (Fig. 1). It has been reported that FS is associated with pharmacological activities such as neuroprotective effects, anti-cancer, anti-oxidant, anti-inflammatory, anticonvulsant, antidepressant, hepatoprotective, nephroprotective, antiulcer and neurotropic effects [1,3, 4]. Despite being such a potential candidate, oral bioavailability of this drug is strongly limited (44%) due to its poor aqueous solubility having Log P value of 2.03 [5]. Novel drug delivery system (NDDS) based formulations are extensively reported to enhance the solubility,

REVIEW ARTICLE

Expanding Arsenal against Neurodegenerative Diseases Using Quercetin Based Nanoformulations: Breakthroughs and Bottlenecks

Sukriti Vishwas[1], Rajesh Kumar[1], Rubiya Khursheed[1], Arya Kadukkattil Ramanunny[1], Rajan Kumar[1], Ankit Awasthi[1], Leander Corrie[1], Omji Porwal[2], Mohammed F. Arshad[3], Mohammed Kanan Alshammari[4], Abdulrahman A. Alghitran[5], Ashwaq N. Qumayri[5], Saif M. Alkhaldi[6], Abdulaziz Khalaf Alshammari[7], Dinesh Kumar Chellappan[8,*], Gaurav Gupta[9,10,14], Trudi Collet[11], Jon Adams[12], Kamal Dua[12,13], Monica Gulati[1,12] and Sachin Kumar Singh[1,12,**]

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Abstract: Quercetin (Qu), a dietary flavonoid, is obtained from many fruits and vegetables such as coriander, broccoli, capers, asparagus, onion, figs, radish leaves, cranberry, walnuts, and citrus fruits. It has proven its role as a nutraceutical owing to numerous pharmacological effects against various diseases in preclinical studies. Despite these facts, Qu and its nanoparticles are less explored in clinical research as a nutraceutical. The present review covers various neuroprotective actions of Qu against various neurodegenerative diseases (NDs) such as Alzheimer's, Parkinson's, Huntington's, and Amyotrophic lateral sclerosis. A literature search was conducted to systematically review the various mechanistic pathways through which Qu elicits its neuroprotective actions and the challenges associated with raw Qu that compromise therapeutic efficacy. The nanoformulations developed to enhance Qu's therapeutic efficacy are also covered. Various ongoing/completed clinical trials related to Qu in treating various diseases, including NDs, are also tabulated. Despite these many successes, the exploration of research on Qu-loaded nanoformulations is limited mostly to preclinical studies, probably due to poor drug loading and stability of the formulation, time-consuming steps involved in the formulation, and their poor scale-up capacity. Hence, future efforts are required in this area to reach Qu nanoformulations to the clinical level.

1. INTRODUCTION

Neurodegenerative diseases (NDs) are a group of conditions defined by gradual loss of neuronal function and accumulation of proteins with altered physicochemical characteristics in the central and peripheral nervous system [1]. In addition, genetic influences, oxidative stress [2], neuroinflammation [3], and abnormalities in mitochondrial functions can also lead to NDs. These NDs can affect the body's sensory and motor neurons markedly, causing cognitive, behavioural and psychotic abnormalities that affect the lifestyle with increased mental stress [4].

Quercetin (Qu), a dietary flavonoid, is obtained from many fruits and vegetables such as coriander, broccoli, capers, asparagus, onion, figs, radish leaves, cranberry, walnuts, and citrus fruits [5]. It is a secondary polyphenolic me-

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REVIEW ARTICLE

Expanding the Arsenal Against Huntington's Disease-Herbal Drugs and Their Nanoformulations

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Abstract: Huntington's disease (HD) is an autosomal fatal genetic disease in which degeneration of neuronal cells occurs in the central nervous system (CNS). Commonly used therapeutics are clude-monoamine depletors, antipsychotics, antidepressants, and tranquilizers. However, these drugs cannot prevent the psychotic, cognitive, and behavioral dysfunctions associated with HD. In addition to this, their chronic use is limited by their long-term side effects. Herbal drugs offer a plausible alternative to this and have shown substantial therapeutic effects against HD. Moreover, their safety profile is better in terms of side effects. However, due to limited drug solubility and permeability to reach the target site, herbal drugs have not been able to reach the stage of clinical exploration. In recent years, the paradigm of research has been shifted towards the development of herbal drugs based nanoformulations that can enhance their bioavailability and blood-brain barrier permeability. The present review covers the pathophysiology of HD, available biomarkers, phytomedicines explored against HD, ongoing clinical trials on herbal drugs exclusively for treating HD and their nanocarriers, along with their potential neuroprotective effects.

1. INTRODUCTION

The term HD was coined by Ohio based physician George Huntington in 1872, who described this disease for the first time [1]. HD is an autosomal fatal genetic disorder which is a progressive, genetically programmed ND that leads to depletion of psychological, cognitive, and motor functions. As per Huntington's disease Society of America (HDSA), HD patients show symptoms similar to those of Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [2-7]. The symptoms associated with HD are "chorea" (abnormal autonomic movements), loss of rational abilities, and psychological disturbances. This abnormality occurs due to the mutation of the Huntingtin genes. Healthy neurons contain 6-35 repeats of units of cytosine-adenine-guanine (CAG) trinucleotide, while accumulation of mutant Huntingtin (mHTT) genes changes the translation process (more than 36 CAG repeats) [8, 9]. This process may lead to neuronal cell death and cause degeneration of neurotransmitters within the central nervous system (CNS) [10]. After the first appearance of symptoms in an affected person, death usually occurs within 15 to 20 years [11]. Various biochemical alterations such as downregulation of γ-aminobutyric acid (GABA) and acetylcholine (ACh), along with a decrease in their production enzymes, glutamate decarboxylase (GAD) and choline-acetyl transferase (CAT), respectively are seen in patients with HD [11-13].

Globally, 5 to 8 people in a population of 0.1 million are diagnosed with HD [14, 15]. The disease is reported to be more prevalent in Europe as compared to that in the USA, China, and India. A number of patients diagnosed with HD are extrapolated to increase from 58,176 in 2019 to 60,743 in 2024 [14].

2. ETIOPATHOGENESIS

2.1. Neuropsychiatric Disturbance

There is a broad range of HD neuropsychiatric symptoms, involving irritation, obsessive- compulsive behavior, depression, psychosis, and apathy. Prior to the knowledge of HD, this disease was categorized under psychiatric disorder because its symptoms were similar to psychiatric diseases. Later on, based on mechanistic studies, it was understood

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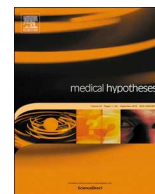
Multiple target-based combination therapy of galantamine, memantine and lycopene for the possible treatment of Alzheimer's disease

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ABSTRACT

Alzheimer's disease is type of dementia in which cognitive functions get declined. More than 50 million people are affected by this disease across the world. Many clinical and preclinical studies have been conducted on the treatment of AD but very limited number of drugs have found a clinical application. Because regeneration of neuron is a complicated process due to the involvement of multiple pathways, a combination of drugs that can work through multiple pathways could prove to be effective in treating AD. Based on prior studies and different mechanisms involved in the treatment, a new hypothesis has been proposed that a combination of galantamine, memantine and lycopene is anticipated to produce better activity as compared to the current therapies available in market for the treatment of this disease.

Introduction

Globally, more than 50 million people are suffering for dementia at present. It is expected to increase upto 152 million by 2050 [1]. It has been reported that most of the symptoms of dementia are caused by Alzheimer's disease (AD) [2]. AD is usually diagnosed at the stage of dementia. Unfortunately, till that time, most of the neuronal cells in the brain of AD's patients have already got degenerated [3]. Due to this disease, vulnerability of hippocampal subfields gets developed [4]. Two unusual changes are mainly observed in the microscopic evaluation of brain of Alzheimer's patients. These are: formation of abnormal clusters of protein fragments called β-amyloid peptide (Aβ) near the neuronal cells and appearance of neurofibrillary tangles (NFTs) formed due to hyperphosphorylation of microtubules associated tau protein [5–7]. The cholinergic hypothesis states that the loss of acetylcholine (ACh) in the various parts of the central nerves system (CNS) is responsible for the decline in cognitive function of the brain that can lead to AD [8]. As per clinical evidences, diminished activity of choline acetyltransferase has been observed in amygdala, hippocampus, and cortex parts of the CNS [9]. ACh binds with two different receptors i.e. the nicotinic ACh receptors (nAChRs) and the muscarinic ACh receptors (mAChRs) in postsynaptic neurons. Biochemical and morphological studies reported that loss of subtype receptor (a7 or a4b2) of nAChRs in presynaptic and postsynaptic neurons occurs in the brains of patients suffering from AD [10]. Some studies also showed loss of mAChRs in post-mortem of brain tissue of AD patients [11]. Inflammatory mediators, reactive oxygen species (ROS), bacterial lipopolysaccharides (LPS), ionizing radiation, tumor necrosis factor alpha (TNF-α), interleukin 1-beta (IL1β) are reported to be responsible for the activation of Nuclear factor-kappa B (NF-κB). Activation of NF-κB in CNS activates growth factors and excitatory neurotransmitter such as glutamate [12,13]. Mitochondrial dysfunction and increase in ROS can lead to free radicals' accumulation in presynaptic and postsynaptic receptor in CNS [14–16]. This will further increase the oxidative stress to the brain.

The proposed hypothesis

In this manuscript, the possible treatment and multiple targets of AD have been discussed. FDA has approved five medicines for the treatment of AD. These medicines reduce the symptoms by two mechanism i.e. AChE inhibition and NMDA glutamate antagonism. Tacrine, donepezil, galantamine, rivastigmine are AChE inhibitors, whereas, memantine is NMDA glutamate antagonist [17]. The combination therapy of AChE and NMDA glutamate inhibitor is very helpful in treating the disease. As per literature, phytoconstituents are also helpful in treating AD via one or more of the aforementioned mechanisms. Some of these

The Overview of Drugs Used in Alzheimer's Disease and Their Molecular Targets

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Abstract Alzheimer's disease (AD) is a progressive condition in which degeneration of neuronal cells is observed in the brain. There are many drugs approved by the

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Chapter 13

Advanced drug delivery systems to treat Huntington's disease: challenges and opportunities

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13.1 Introduction

Huntington's disease (HD) is a type of neurodegenerative diseases (NDs) in which there is the formation of an autosomal fatal genetic dysfunction. It produces depletion of psychological, motor, and cognitive functions (Gupta et al., 2021). The main symptoms of HD are hallucinations, mood swings, loss of rational abilities, speech difficulties, involuntary movements, chorea, and various psychological disturbances such as difficulty in concentrating, memory loss, feelings of hopelessness, lack of interest in works performed on daily life (Vishwas, Gulati, et al., 2020). As per the observations of previous studies, these abnormalities occur due to genetic mutation of Huntingtin (mHtt) genes. In healthy humans, there are nearly 6−35 repeats of cytosine-adenine-guanine (CAG) trinucleotide, while in the diseased condition these repeats increase by more than 36 pairs (Szlachcic et al., 2015; Warby et al., 2009). This process may lead to enhanced oxidative stress (OS) and neuroinflammation which causes degeneration of neurotransmitters within the central nervous system (CNS) (Li et al., 2010). Moreover, various biochemical alterations have been reported now such as the reduction of acetylcholine (ACh) and γ-aminobutyric acid (GABA), along with the downregulation of enzymes such as choline-acetyl transferase and glutamate decarboxylase respectively are seen in patients with HD (Choudhary et al., 2013; Dey et al., 2020; Pareek et al., 2011).

Many bioactive substances such as *Panax ginseng, Bacopa monnieri*, etc., and synthetic drugs such as benzodiazepines, riluzole, amantadine, and other neuroleptics have been reported to show very good neuroprotective effects. However, they have some limitations such as low aqueous solubility resulting in reduced bioavailability, and poor blood-brain barrier (BBB) permeability. Advanced drug delivery systems [liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), self-nano emulsifying drug delivery system (SNEDDS), and nanoemulsions (NEs)] have been reported to increase bioavailability, therapeutic efficacy (Kamble et al., 2013), stability and brain permeability (Khursheed et al., 2020; Lockman et al., 2002) of the bioactive substances and synthetic drugs and decrease their side effects, which, on the other hand, is difficult to be achieved through conventional drug delivery systems (Garbayo et al., 2012; Ou et al., 2018; Wang et al., 2014; Zhang et al., 2016). Advanced drug delivery systems based on nanoparticles are reported to reduce first-pass metabolism and enhance their bioavailability because their small particle size (less than 200 nm) enables them to cross endothelial cells of BBB by transcytosis (Betzer

Review article

Neurodegenerative diseases and brain delivery of therapeutics: Bridging the gap using dendrimers

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ARTICLE INFO

ABSTRACT

Neurodegenerative diseases (NDs) continue to burden human lives and economic conditions. They continue to challenge the healthcare system due to the associated physiological barriers. Traditional treatment approaches are associated with symptomatic relief and are ineffective in the long run. Dendrimers stand out amongst other nanocarriers due to ease of surface modifications, internal encapsulation, and nanoscale uniformity of the molecule. Moreover, their internal core can encapsulate drug through electrostatic interactions. These are stable carriers in the nanometer size range. These either act as therapeutic agents *per se* or deliver the target drug across the blood-brain barrier while minimizing toxicity. Emerging as a promising non-invasive approach, they demonstrate the capability to interfere with *in-vivo* protein aggregation, typically associated with neuro-degeneration. They assist via exerting various neuroprotective roles, such as in oxidative stress, neuro-inflammation, inhibiting certain biochemical parameters, altering protein misfolding and aggregation, etc. However, certain limitations are associated with their elimination and cytotoxicity. The investigation revealed the masking of exposed cationic surfaces of dendrimers with inert substances, such as polyethylene glycol to limit their cytotoxicity. This review describes the incidences and pathophysiology of several NDs, properties, and methods of dendrimer synthesis, followed by various research to explore dendrimers potential to treat NDs.

1. Introduction

Neurodegenerative diseases (NDs) are distinguished by the continuous loss or deterioration of a potentially active but vulnerable population of neurons in the central and peripheral nervous system (CNS and PNS respectively). These may include numerous diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), spinocerebral ataxias, Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS),

Review

Harnessing role of sesamol and its nanoformulations against neurodegenerative diseases

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ABSTRACT

Sesamol is a lignan of sesame seeds and a natural phenolic molecule that has emerged as a useful medical agent. Sesamol is a non-toxic phytoconstituent, which exerts certain valuable effects in the management of cancer, diabetes, cardiovascular diseases, neurodegenerative diseases (NDs), etc. Sesamol is known to depict its neuroprotective role by various mechanisms, such as metabolic regulators, action on oxidative stress, neuroinflammation, etc. However, its poor oral bioavailability, rapid excretion (as conjugates), and susceptibility to gastric irritation/toxicity (particularly in rats' forestomach) may restrict its effectiveness. To overcome the associated limitations, novel drug delivery system-based formulations of sesamol are emerging and being researched extensively. These can conjugate with sesamol and enhance the bioavailability and solubility of free sesamol, along with delivery at the target site. In this review, we have summarized various research works highlighting the role of sesamol on various NDs, including Alzheimer's disease, Huntington's disease, Amyotrophic lateral sclerosis, and Parkinson's disease. Moreover, the formulation strategies and neuroprotective role of sesamol-based nano-formulations have also been discussed.

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Unravelling role of crocin for the treatment of Alzheimer's and Parkinson's disease: sojourn from food to nanomedicine

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Kamal Dua · Sachin Kumar Singh

Abstract Flavonoids have a longstanding tradition of being employed in traditional medicine to improve human health. Crocin has been suggested as a potential candidate for addressing a range of ailments, including neurodegenerative diseases (NDs). In the modern era, diverse pharmacological activities of crocin have been explored due to its bioactive constituents like kaempferol, picrocrocin, safranal, and crocetin. It demonstrates pharmacological and therapeutic efficacy against various diseases such as cancer, kidney diseases, NDs, and measles, attributable to several mechanisms. Various studies evidence that it exerts a neuroprotective effect in conditions like Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis, and others, by modulating relevant parameters. Despite harboring numerous pharmacological benefits, it exhibits low bioavailability due to poor solubility and limited ability to cross the blood–brain barrier (BBB). To address this issue, diverse approaches involving nanoformulations have been developed to enhance their bioavailability, aqueous solubility, and BBB permeability. Various types of

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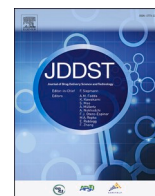
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Review article

An overview of biomedical applications for gold nanoparticles against lung cancer

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ARTICLE INFO

ABSTRACT

Lung cancer (LC) is the commonest class of cancer worldwide and is the reason for more deaths than any other type. Treatment of LC using conventional therapy has some limitations such as poor bioavailability and non-site release of drug causing various side effects. However, the use of nanotechnology makes it possible to offer efficacious treatment of LC by offering nanometer sized formulation that helps in better penetration of drug inside tumor cells and site specific drug release. Among various nanoparticles, gold nanoparticles (GNPs) has found unique and important application in the biomedical application of nanoparticles in various diseases, especially cancer. Owing to their high biocompatibility and stability against oxidation in vivo, tunable nano size, ease of functionalization with chemotherapeutic agents, capacity to enhance bioavailability and site specificity of entrapped drug, capacity to interact with visible light, make GNPs as special carrier for biomedical application in LC. GNPs can absorb infrared radiation and have optical qualities as well. In the present review, various aspects of LC and theranostic role of GNPs are comprehensively covered. These include prevalence, classification, economic burden, pathophysiology as well as commonly available therapies of LC. This was followed by advantages, synthesis, biomedical application of GNPs in treating LC as well as their fate in the body. Overall, the uniqueness of review relies on the fact that it covers all aspects of LC and all possibilities by which GNPs can offer their best role in management of LC.

Review article

Opening avenues for treatment of neurodegenerative disease using post-biotics: Breakthroughs and bottlenecks in clinical translation

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ABSTRACT

Recent studies have indicated the significant involvement of the gut microbiome in both human physiology and pathology. Additionally, therapeutic interventions based on microbiome approaches have been employed to enhance overall health and address various diseases including aging and neurodegenerative disease (ND). Researchers have explored potential links between these areas, investigating the potential pathogenic or therapeutic effects of intestinal microbiota in diseases. This article provides a summary of established interactions between the gut microbiome and ND. Post-biotic is believed to mediate its neuroprotection by elevating the level of dopamine and reducing the level of α-synuclein in substantia nigra, protecting the loss of dopaminergic neurons, reducing the aggregation of NFT, reducing the deposition of amyloid β peptide plagues and ameliorating motor deficits. Moreover, mediates its neuroprotective activity by inhibiting the inflammatory response (decreasing the expression of TNFα, iNOS expression, free radical formation, overexpression of HIF-1α), apoptosis (i.e. active caspase-3, TNF-α, maintains the level of Bax/Bcl-2 ratio) and promoting BDNF secretion. It is also reported to have good antioxidant activity. This review offers an overview of the latest findings from both preclinical and clinical trials concerning the use of post-biotics in ND.

1. Introduction

In recent years, there has been a growing interest in research on gut microorganisms. A significant volume of research has shown very good connections between disruptions in the intestinal microbiome and various medical conditions. These include inflammatory bowel diseases such as Crohn's disease (Dubinsky et al., 2006), irritable bowel syndrome(Frank et al., 2007), as well as colon cancer (O'Keefe et al., 2009).
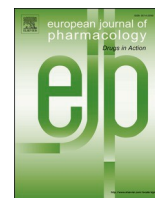
Harnessing the neuroprotective effect of oral administration of benfotiamine in MPTP induced Parkinson's disease in rats

Bushra Bashir [a], Swati Mittal [b,**], A. Muthukumar [c], Sukriti Vishwas [a], Narendra Kumar Pandey [a], Monica Gulati [a,d], Gaurav Gupta [e,f,n], Muralikrishnan Dhanasekaran [g], Puneet Kumar [h], Harish Dureja [i], Francisco Veiga [j,k], Ana Cláudia Paiva-Santos [j,k], Jon Adams [d], Kamal Dua [d,l,m], Sachin Kumar Singh [a,d,*]

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ABSTRACT

The study was performed to evaluate the neuroprotective effects of Benfotiamine (BFT) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease (PD) in rats. The rats were given daily doses of BFT (100 mg/kg, 200 mg/kg) through oral administration for 42 days. The rats were given a single bilateral dosage of MPTP (0.1 mg/nostril) intranasally once before the drug treatment to induce PD. On day 42, the animals were subjected to various behavioral paradigms. Post-treatment with BFT for 42 days significantly improved the motor and nonmotor fluctuations of MPTP. The results demonstrated that treatment with BFT ameliorated MPTP-induced disorders in behavior, body balance, and dopamine levels in the mid-brain. Among the post-treated groups, a high dose of BFT was the most effective treatment. Mean values are indicated in ±SEM, n = 5***($p < 0.001$) when compared with the vehicle control, n = 5 ### ($p < 0.001$) when compared with the disease control; ($p < 0.001$) when compared with the BFT *per se*; ($p < 0.001$) when compared with the low dose of BFT; ($p < 0.001$) when compared with the high dose of BFT. Our finding suggests that BFT contributed to superior antioxidant, and anti-inflammatory and could be a novel therapeutic method for PD management. In conclusion, BFT could be a potential drug candidate for curbing and preventing PD.

1. Introduction

Neurodegenerative diseases are one of the most prevalent health concerns in today's society. Parkinson's disease (PD) accounts for the second most common neurodegenerative disease, with four primary cardinal manifestations: rigidity, postural instability, tremor, and akinesia. Deterioration of dopaminergic neurons in the midbrain, notably in the area of substantia nigra pars compacta (SNpc) (essentially governs movements and muscle tone), creates a dysbalance in dopamine (DA) and acetylcholine concentrations in the brain, resulting in the dreadful

Self-nanoemulsifying composition containing curcumin, quercetin, *Ganoderma lucidum* extract powder and probiotics for effective treatment of type 2 diabetes mellitus in streptozotocin induced rats

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ABSTRACT

Liquid self-nanoemulsifying drug delivery system (L-SNEDDS) of curcumin and quercetin were prepared by dissolving them in isotropic mixture of Labrafil M1944CS®, Capmul MCM®, Tween-80® and Transcutol P®. The prepared L-SNEDDS were solidified using *Ganoderma lucidum* extract, probiotics and Aerosil-200® using spray drying. These were further converted into pellets using extrusion-spheronization. The mean droplet size and zeta potential of L-SNEDDS were found to be 63.46 ± 2.12 nm and -14.8 ± 3.11 mV while for solid SNEDDS pellets, these were 72.46 ± 2.16 nm and -38.7 ± 1.34 mV, respectively. The dissolution rate for curcumin and quercetin each was enhanced by 4.5 folds while permeability was enhanced by 5.28 folds (curcumin) and 3.35 folds (quercetin) when loaded into SNEDDS pellets. The Cmax for curcumin and quercetin containing SNEDDS pellets was found 532.34 ± 5.64 ng/mL and 4280 ± 65.67 ng/mL, respectively. This was 17.55 and 3.48 folds higher as compared to their naïve forms. About 50.23– and 5.57-folds increase in bioavailability was observed for curcumin and quercetin respectively, upon loading into SNEDDS pellets. SNEDDS pellets were found stable at accelerated storage conditions. The developed formulation was able to normalize the levels of blood glucose, lipids, antioxidant biomarkers, and tissue architecture of pancreas and liver in streptozotocin induced diabetic rats as compared to their naïve forms.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder of elevated blood glucose level (BGL) that is attributed to non-utilization of insulin, because of insulin resistance (IR) (Khursheed et al., 2019). The oral antihyperglycemic drugs used to manage T2DM are divided into various classes such as sulfonylureas (SUs), biguanides, dipeptidyl peptidase 4 (DPP-4) inhibitors, meglitinides, sodium glucose

Topical non-aqueous nanoemulsion of *Alpinia galanga* extract for effective treatment in psoriasis: *In vitro* and *in vivo* evaluation

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ABSTRACT

Non-aqueous nanoemulsion (NANE) of *Alpinia galanga* extract (AGE) was prepared using Palmester 3595 (MCT oil) as oil phase, Cremophor RH 40-Transcutol P® as surfactant-co-surfactant (S_{mix}), and glycerin as non-aqueous polar continuous phase. The composition was optimized by applying three-level, four factor Box-Behnken design (BBD). The mean droplet size and zeta potential of the optimized AGE NANE was found to be 60.81 ± 18.88 nm and -7.99 ± 4.14 mV, respectively. The *ex vivo* permeation studies of AGE NANE and AGE *per se* on porcine skin reported flux of 125.58 ± 8.36 μg/cm^2 h^{-1} and 12.02 ± 1.64 μg/cm^2 h^{-1}, respectively. Therefore, the enhancement ratio has shown 10-folds increase in the flux for AGE NANE when compared to extract *per se*. Later, confocal laser scanning microcopy confirmed that AGE NANE were able to penetrate into skin's stratum by *trans*-follicular transport mechanism. The stability studies of AGE NANE confirmed its stability at 30 ± 2 °C/75 ± 5 % RH and 5 ± 3 °C. The efficacy of AGE NANE was evaluated *in vivo* on imiquimod (IMQ) induced mouse model. The mice treated with low and high doses of AGE NANE (groups VI and VII) showed significant ($p < 0.05$) amelioration of psoriasis. Results of histopathology indicated reduction in psoriasis area severity index in AGE NANE treated mice (group VI and group VII).

ORIGINAL RESEARCH ARTICLE

Novel Nanostructured Lipid Carriers Co-Loaded with Mesalamine and Curcumin: Formulation, Optimization and *In Vitro* Evaluation

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Abstract

Purpose The aim of current study is to formulate, optimize and characterize the developed formulation of Mesalamine-Curcumin Nanostructured Lipid Carriers (Mes-Cur NLCs).

Methods It was formulated using high pressure homogenization followed by probe sonication and formulation variables were optimized using Central Composite Design. The particle size (PS), zeta potential (ZP), entrapment efficiency (EE), drug release, cytotoxicity on NIH 3T3 fibroblasts cells and HaCaT keratinocytes cells and efficacy on RAW264.7 cells for optimized formulation was determined.

Results The PS, ZP and EE were found to be 85.26 nm, -23.7 ± 7.45 mV, 99.2 ± 2.62 % (Mes) and 84 ± 1.51 % (Cur), respectively. The good correlation between predicted and obtained value indicated suitability and reproducibility of experimental design. NLCs showed spherical shape as confirmed by TEM. In vitro drug release profile of prepared formulation showed that Mes exhibited 100 % release at 48 h, whereas Cur exhibited 82.23 ± 2.97% release at 120 h. Both the drugs exhibited sustained release upon incorporation into the NLCs. The absence of any significant cell death during MTT assay performed on NIH 3T3 fibroblasts cells and HaCaT keratinocytes cells indicated that NLCs' were safe for use. Furthermore, significant reduction in nitric oxide level during anti-inflammatory evaluation of formulation on RAW264.7 cells showed excellent potential for the formulation to treat inflammation. The formulation was found stable as no significant difference between the PS, ZP and EE of the fresh and aged NLCs was observed.

Conclusion The outcomes of study deciphered successful formulation of Mes-Cur NLCs.

Keywords curcumin · central composite design · mesalamine · nanostructured lipid carriers · stability studies

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RP-HPLC method development and validation for simultaneous estimation of mesalamine and curcumin in bulk form as well as nanostructured lipid carriers

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ARTICLE INFO

ABSTRACT

The aim of this study was to develop simple, advance, accurate and precise method for the quantification of mesalamine (Mes) and curcumin (Cur) in nanostructured lipid carriers (NLCs) by using high Performance Liquid Chromatography (HPLC) technique. The analysis of the method was done by using C-18 Nucleodur column with 250 mm x 4.6 mm dimensions and having i.d. of 5μ. The quantification of Mes and Cur on HPLC was done by using mobile phase acetonitrile and water in the different ratios at different time intervals (gradient method), flow rate kept 1 mL/min and wavelength was set up at isosbestic point 365 nm. By using gradient method of ACN and water the peak of Mes and Cur was found to be at 1.7 min and 9.6 min respectively. The developed method was validated in accordance with ICH Q2 (R1) guidelines. The developed method showed linearity in the range of 2-10 ppm with r^2 equals to 1 i.e 0.9998 for Mes and 0.9998 for Cur. The system suitability parameters of the method were also measured which were found to be tailing factor less than 2, resolution between Mes and Cur peaks was more than two and theoretical plates more than 2000. The LOD and LOQ of the developed method for Mes was found to be 0.14 μg/mL and 0.45 μg/mL. Whereas, LOD and LOQ of the developed method for Cur was found to be 0.17 μg/mL and 0.52 μg/mL respectively. The relative standard deviation of the developed method for both Mes and Cur were below 2 and percentage recovery was found to be between 95-105%. The robustness of the developed method was also checked by doing small changes in the flow rate and wavelength. The results of robustness study revealed that relative standard deviation and percentage recovery for both Mes and Cur were under acceptance criteria of ICH Q2 (R1) guidelines. This indicated that the method is precise, accurate, simple and robust. In addition, the developed method was effectively used for the estimation of entrapment efficiency (EE), drug loading (DL) and *in vitro* drug release study of Mes and Cur in the prepared Mes-Cur based NLCs. The EE of Mes and Cur in NLCs was found to be 99% and 84% respectively. Whereas, DL of Mes and Cur in the formulation was found to be 0.0019% and 0.0033% respectively. The *in vitro* drug release study showed that within 48h 100% Mes was released from NLCs. Whereas, 82.23% Cur was released from the NLCs.
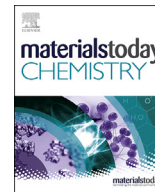
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Polysaccharide, fecal microbiota, and curcumin-based novel oral colon-targeted solid self-nanoemulsifying delivery system: formulation, characterization, and in-vitro anticancer evaluation

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ARTICLE INFO

ABSTRACT

The use of polysaccharides in preparation of microbially triggered colon-targeted delivery system has been explored extensively to deliver drugs at the colonic site. This type of targeting is attributed to inherent property of polysaccharides by virtue of which they are digested in the gut only by the colonic microflora. In last two decades, the use of fecal microbiota transplantation (FMT) has been reported widely to treat colonic diseases such as Crohn's disease, irritable bowel syndrome, and recurrent *Clostridium difficile* infection. It has been established that FMT downregulates inflammatory proliferation and pro carcinogenic pathways. Although a few reports exist on the use of colon-targeted FMT capsules in increasing the microbial diversity of gut microflora, they have not been able to establish clinical superiority of the colon-targeted capsules over conventional orally administered capsules. Hence, in the present study, an attempt has been made to utilize a multipronged strategy through a single formulation, wherein an anticancer phytoconstituent, polysaccharides, fecal microbiota extract (FME), and nano-delivery systems have been designed for the effective treatment of colon cancer. Overall, the study is aimed toward the development of an oral colon-targeted solid self-nanoemulsifying drug delivery system (S-SNEDDS) in which curcumin (CCM) was loaded. These SNEDDS were added to polysaccharide mixture of guar gum, pectin, lactose along with aerosol 200 (A-200) and then to the slurry of fecal microbiota extract. The liquid was then freeze dried and developed into solid SNEDDS powder. The process of formulating liquid SNEDDS was optimized using ternary phase diagram and Box Behnken Design. The developed formulation was characterized for physicochemical, physicomechanical, drug release and cytotoxic potential studies. The droplet size of reconstituted S-SNEDDS was found to be 78.46 ± 0.87 nm. The site-specific release of S-SNEDDS powder was confirmed by dissolution studies in medium containing rat caecal contents, wherein, the formulation containing guar gum and pectin in 1%

Formulation of chrysin loaded nanostructured lipid carriers using Box Behnken design, its characterization and antibacterial evaluation alone and in presence of probiotics co-loaded in gel

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ARTICLE INFO

ABSTRACT

The present study deciphers formulation, optimization and characterization of chrysin nanostructured lipid carriers with probiotics (PB) loaded gel (*Chrysin* (CS)- Nanostructured lipid carriers (NLCs) + PB loaded gel) for topical application. Hot homogenization-probe sonication method was used to formulate NLCs. Formulation parameters were optimized using Box Behnken Design. The optimized formulation was characterized for particle size (PS), zeta potential (ZP), % entrapment efficiency (%EE), % drug loading (%DL). The optimized values were found to be 199.99 mg, 33.92 mg, 700 mg and 376.86 mg of solid lipid, liquid lipid, surfactant and co-surfactant respectively. The PS, ZP, % EE and % DL of optimized CS-NLCs + PB loaded gel were found to be 66.45 ± 5.62 nm, -22 ± 5.21 mV, 97.25 ± 0.15 and 82.3 ± 0.104, respectively. Transmission electron microscopic images revealed that NLCs loaded with CS were spherical in shape. The *in vitro* diffusion studies revealed that $98 \pm 0.06\%$ of CS got released from CS-NLCs + PB loaded gel at the end of 48 h. For initial 8h, release of CS was about 6-fold higher in case of CS-NLCs + PB loaded gel than that of naive CS gel and thereafter the release got reduced, which indicated the sustained release of CS from NLCs. The zone of inhibition of CS-NLCs + PB loaded gel was 0.5-fold, 0.2-fold and 0.54-fold higher than naive PB gel alone, naïve CS gel alone and CS-PB gel combination, respectively. It indicated significantly higher antibacterial activity of CS–NLCs + PB loaded gel as that of any other treatment group.

Quality by design-oriented formulation optimization and characterization of guar gum-pectin based oral colon targeted liquisolid formulation of xanthohumol

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ABSTRACT

In the present study a colon targeted liquisolid powder of xanthohumol was developed using Central Composite Design (CCD). Xanthohumol is a drug obtained from hops plants that can be effective in the treatment of UC. It possesses poor aqueous solubility, permeability and dissolution rate limited oral bioavailability. Hence, it was thought to enhance its dissolution rate by formulating liquisolid powder. Liquisolid technology is simple, single step and cost effective techniques to enhance dissolution rate of lipophilic drugs. Further, the formulation was targeted to colon using optimized combination of guar gum and pectin. Liquisolid powder of xanthohumol was formulated using Transcutol P as non-volatile solvent, polysaccharide mixture of guar gum and pectin as carrier, Syloid XDP as coating agent. The ratio of guar gum, pectin and Syloid XDP affecting angle of repose, drug loading and dissolution of drug in 5 h were optimized using CCD. The optimized formulation showed angle of repose of 26.78°, drug loading of 89.34% and dissolution of drug in 5 h of 5.23%, respectively. The formulation showed site-specific release of liquisolid with less than 10% drug release in initial 5 h due to the release restricting property of guar gum and pectin followed by a burst release of xanthohumol between 5th and 12th hour indicating colon specific release of xanthohumol. The powder X-ray diffraction studies, differential scanning calorimetry and scanning electron microscopy revealed about complete solubility of xanthohumol in the Transcutol P and complete adsorption of the liquid on surface of Syloid XDP, guar gum and pectin. The accelerated stability studies indicated that the formulation was stable. The overall results of study indicated about successful development of colon targeted liquisolid formulation of xanthohumol that can be further explored for pre-clinical studies.

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Article

Development of Guar Gum-Pectin-Based Colon Targeted Solid Self-Nanoemulsifying Drug Delivery System of Xanthohumol

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Abstract: Present study deciphers development of oral polysaccharide-based colon targeted solid self-nanoemulsifying drug delivery system (S-SNEDDS) of xanthohumol (XH). Several studies have shown that XH has anti-inflammatory and antioxidant properties, suggesting that it could be a good candidate for the treatment of colorectal diseases (CRD). Despite its potential, XH has a low aqueous solubility. As a result, its bioavailability is constrained by the dissolution rate. The liquid (L)-SNEDDS was constituted using Labrafac PG as oil, Tween 80 as surfactant and Transcutol P as co-surfactant. The L-SNEDDS was then adsorbed onto the surface of guar gum and pectin and developed into S-SNEDDS powder. Ternary phase diagram was used to optimize the process of developing L-SNEDDS. The formulation showed mean droplet size of 118.96 ± 5.94 nm and zeta potential of -19.08 ± 0.95 mV and drug loading of $94.20 \pm 4.71\%$. Dissolution studies carried out in medium containing rat caecal contents (RCC) represented the targeted release of S-SNEDDS powder. It was observed that S-SNEDDS showed less than 10% release XH in initial 5 h and rapid release occurred between the 5th and 10th hour. Results of cytotoxicity studies revealed good cytotoxicity of XH loaded S-SNEDDS for Caco2 cells as compared to raw-XH.

Keywords: xanthohumol; solid self-nanoemulsifying drug delivery system; guar gum; colon targeted delivery system; quality by design

1. Introduction

Xanthohumol (XH) is a prenylated flavonoid extracted from the female flowers of the hops plant (*Humulus lupulus* L.), which is primarily found in Germany and China [1]. XH is chemically known as 30-[3,3-dimethyl allyl]-20,40,4-trihydroxy-60-methoxychalcone [2]. The main constituent of the hop plant, which belongs to the Humulus genus and is a member of the Cannabaceae family, is XH. Because of its aroma and bitter taste, it is one of the ingredients used in beer [3]. This herbal drug shows promising anti-inflammatory [4], antioxidant [5], and anti-cancer [6] properties that can be used in the treatment of CRD.

The oral route is the most preferred route for the administration of drugs due to its advantages such as ease of administration, controllable dosage regimen, flexibility

ELSEVIER

Harnessing the role of microneedles as sensors: current status and future perspectives

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In recent years, microneedles (MNs) have been transformed to serve a wide range of applications in the biomedical field. Their role as sensors in wearable devices has provided an alternative to blood-based monitoring of health and diagnostic methods. Hence, they have become a topic of research interest for several scientists working in the biomedical field. These MNs as sensors offer the continuous monitoring of biomarkers like glucose, nucleic acids, proteins, polysaccharides and electrolyte ions, which can therefore screen for and diagnose disease conditions in humans. The present review focuses on types of MN sensors and their applications. Various clinical trials and bottlenecks of MN R&D are also discussed.

Keywords: Microneedles; sensors; wearable devices; glucose monitoring; diagnosis

Introduction

The discovery of microneedles (MNs) has provided a new opportunity in healthcare diagnostics as a technology that enables minimally invasive diagnosis of biofluids using a portable and wearable device. MNs are needle-shaped devices of micron size (length and diameter) that can be delivered through transdermal patches. In recent years, MN-based devices have played a major part in biomedical engineering as sensors and diagnostic devices exploiting transdermal patches and external wearable devices. MNs offer minimal invasion and painless delivery of drugs because their penetration into the dermis layer of skin avoids contact with nerve cells.[p1] The research on MNs is constantly attracting the attention of scientists across the globe. It is reported that the market value of MN-based devices in 2022 was US$722.2 million, which increased to US$768.9 million in 2023 and it is forecasted that by 2033 the market value could rise up to US$1459.1 million with a compound annual growth (CAGR) of 6.6%.[p2]

Historically, in the clinic, blood serum and plasma was withdrawn by pricking of fingers to understand biochemical changes. The other method which is used to withdraw blood includes puncturing of a vein using a hypodermic needle. These techniques are painful and can cause infection and bruising.[p3] To avoid blood-withdrawal-based invasive techniques, withdrawal of interstitial fluid (ISF), saliva or urine is being practiced as a minimally or even noninvasive technique. ISF has been considered as the best alternative biofluid to blood and offers precise detection of biomarkers.[p4] The ISF is present beneath the skin surface and can be collected by conventional sampling techniques. However, these techniques suffer from problems that include insufficient volume as well as poor filtration.[p5]

The other techniques such as biopsy sampling can cause pain, skin damage and suction blisters[p6],[p7] that can last for several

Review article

Treatment avenues for age-related macular degeneration: Breakthroughs and bottlenecks

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ARTICLE INFO

ABSTRACT

Age-related macular degeneration (AMD) is a significant factor contributing to serious vision loss in adults above 50. The presence of posterior segment barriers serves as chief roadblocks in the delivery of drugs to treat AMD. The conventional treatment strategies use is limited due to its off-targeted distribution in the eye, shorter drug residence, poor penetration and bioavailability, fatal side effects, etc. The above-mentioned downside necessitates drug delivery using some cutting-edge technology including diverse nanoparticulate systems and microneedles (MNs) which provide the best therapeutic delivery alternative to treat AMD efficiently. Furthermore, cutting-edge treatment modalities including gene therapy and stem cell therapy can control AMD effectively by reducing the boundaries of conventional therapies with a single dose. This review discusses AMD overview, conventional therapies for AMD and their restrictions, repurposed therapeutics and their anti-AMD activity through different mechanisms, and diverse barriers in drug delivery for AMD. Various nanoparticulate-based approaches including polymeric NPs, lipidic NPs, exosomes, active targeted NPs, stimuli-sensitive NPs, cell membrane-coated NPs, inorganic NPs, and MNs are explained. Gene therapy, stem cell therapy, and therapies in clinical trials to treat AMD are also discussed. Further, bottlenecks of cutting-edge (nanoparticulate) technology-based drug delivery are briefed. In a nutshell, cutting-edge technology-based therapies can be an effective way to treat AMD.

1. Introduction

Age-related macular degeneration (AMD) is one of the chronic degenerative disorders of the macula and serves as a prevalent root of vision loss affecting above 30 million individuals across the globe (Yadav et al., 2020). AMD is expected to affect over 5 million Americans by 2050 (Streets et al., 2020). AMD compromises the optic nerve's normal vision processing, leading to hazy and impaired vision. Clinically, AMD is categorized into dry (atrophic) AMD and wet (exudative) AMD, with dry AMD accounting for around 90% of AMD cases (Tang

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REVIEW ARTICLE

Unravelling the success of transferosomes against skin cancer: Journey so far and road ahead

Popat S. Kumbhar[1] · Vikas Kamble[1] · Sukriti Vishwas[2] · Pranav Kumbhar[1] · Kaustubh Kolekar[1] · Gaurav Gupta[3,4] · Francisco Veiga[5,6] · Ana Cláudia Paiva-Santos[5,6] · Bey Hing Goh[7,9] · Sachin Kumar Singh[2,7,8] · Kamal Dua[8,9] · John Disouza[1] · Vandana Patravale[10]

Abstract

Skin cancer remains one of the most prominent types of cancer. Melanoma and non-melanoma skin cancer are commonly found together, with melanoma being the more deadly type. Skin cancer can be effectively treated with chemotherapy, which mostly uses small molecular medicines, phytoceuticals, and biomacromolecules. Topical delivery of these therapeutics is a non-invasive way that might be useful in effectively managing skin cancer. Different skin barriers, however, presented a major obstacle to topical cargo administration. Transferosomes have demonstrated significant potential in topical delivery by improving cargo penetration through the circumvention of diverse skin barriers. Additionally, the transferosome-based gel can prolong the residence of drug on the skin, lowering the frequency of doses and their associated side effects. However, the choice of appropriate transferosome compositions, such as phospholipids and edge activators, and fabrication technique are crucial for achieving improved entrapment efficiency, penetration, and regulated particle size. The present review discusses skin cancer overview, current treatment strategies for skin cancer and their drawbacks. Topical drug delivery against skin cancer is also covered, along with the difficulties associated with it and the importance of transferosomes in avoiding these difficulties. Additionally, a summary of transferosome compositions and fabrication methods is provided. Furthermore, topical delivery of small molecular drugs, phytoceuticals, and biomacromolecules using transferosomes and transferosomes-based gel in treating skin cancer is discussed. Thus, transferosomes can be a significant option in the topical delivery of drugs to manage skin cancer efficiently.

Keywords Skin cancer · Topical delivery · Transferosomes · Small molecular drugs · Biomacromolecules

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Patent Search

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Abstract:

NOVEL NANOEMULSION COMPOSITION OF FISETIN AND PROCESS THEREOF In this study, an endeavor was made to develop nanoemulsions (NEs) loaded with a pharmacologically active compound, FS (naïve drug), in response to the challenges associated with the oral delivery of FS. Nanoemulsions offer several advantages, su straightforward and single-step preparation, enhanced drug loading capacity, and convenient oral administration. Upon oral ingestion, these NEs reach the gastrointe (GIT) and form micelles, effectively shielding the encapsulated drug from the harsh conditions of the GIT, including pH fluctuations and enzymatic degradation. Additi absorption of the drug-loaded NEs occurs through the lymphatic route, thereby circumventing hepatic metabolism and enhancing the oral bioavailability of the drug, easily achievable with other nanoformulations. Furthermore, the nanometer-sized droplets of these NEs facilitate their systemic circulation and enable them to cross brain barrier (BBB), allowing for the therapeutic action of FS in the brain. This innovative approach holds promise for improving the oral delivery and efficacy of FS, ma potential solution for enhancing the bioavailability and therapeutic impact of this compound.

Complete Specification

Description:Field of the invention
The primary objective of this invention is novel nanoemulsion composition of fisetin and process thereof.
Background of the Invention
References which are cited in the present disclosure are not necessarily prior art and therefore their citation does not constitute an admission that such references prior art in any jurisdiction. All publications, patents and patent applications herein are incorporated by reference to the same extent as if each individual or patent application was specifically and individually indicated to be incorporated by reference.
Several patents have been issued for nanoemulsion but none of these are related to the present invention. For example, WO2008051186A2 discloses nanoemulsion compositions with low toxicity that demonstrate broad spectrum inactivation of microorganisms or prevention of diseases are described. The nanoemulsions conta aqueous phase, an oil phase comprising an oil and an organic solvent, at least one anti-inflammatory agent, and one or more surfactants, and particles having an av diameter of less than or equal to 250 nm. Methods of making nanoemulsions and inactivating pathogenic microorganisms are also provided.
Another patent, WO2009131995A1 relates to methods for inducing an immune response to influenza in a subject comprising administering a nanoemulsion vaccine composition comprising an influenza immunogen or protein. A method for inducing an immune response to influenza in a subject comprising administering to a su nanoemulsion vaccine, wherein the nanoemulsion vaccine comprises: (a) droplets having an average diameter of less than about 1000 nm; (b) an aqueous phase; (c least one oil; (d) at least one surfactant; and (e) no organic solvent, or at least one organic solvent; wherein the nanoemulsion further comprises at least one influen immunogen, recombinant influenza protein, or a combination thereof; or the nanoemulsion is sequentially administered with such an influenza immunogen, or the

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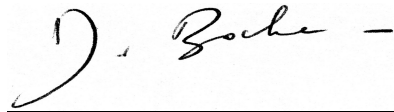Registrar of Copyrights

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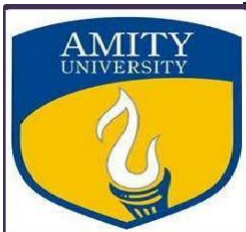
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