

**IMPACT OF ADMINISTRATION OF FLUOXETINE WITH GALANGIN
AND ALLICIN AGAINST MILD STRESS INDUCED MAJOR
DEPRESSIVE DISORDER IN RODENTS**

Thesis Submitted

For the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

Pharmacology

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DECLARATION

I, hereby declared that the presented work in the thesis entitled “**Impact of Administration of Fluoxetine with Galangin and Allicin against mild stress induced major depressive disorder in rodents**” in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **Dr. Sanjeev Kumar Sahu, Professor**, Pharmaceutical Chemistry at School of Pharmaceutical Sciences, Lovely Professional University, Punjab, India. and **Dr. Nilesh Jain, Professor**, Pharmaceutical Chemistry at Sagar Institute of Research Technology & Sciences- Pharmacy- Bhopal, Madhya Pradesh, India in keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled “**Impact of Administration of Fluoxetine with Galangin and Allicin against mild stress induced major depressive disorder in rodents**” submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Pharmacology/School of Pharmaceutical Sciences, is a research work carried out by Ashish Mishra, 41900688, is bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

Background: Across the globe, during the previous decade depressive disorder has been depicted in a progressive manner and this leads to increasing mortality and morbidity rate. As stated by the World Health Organization (WHO), psychiatric disorders are believed to affect approximately 450 million individuals globally. Over 260 million individuals worldwide suffer from major depressive disorder (MDD), generally known as depression. In today's scenario, many synthetic drugs are available to overcome the symptoms of depression but unfortunately there were also some adverse events reported during the depression therapy which plays a negative role in person's life. The focus of present research was to evaluate effect of Galangin and Allicin as a flavonoid with Fluoxetine as standard anti-depressant drugs using chronic unpredictable mild stress (CUMS) model in rat. Using monitoring of important markers such as Brain derived neurotrophic factor (BDNF), Serotonin (5-HT), Dopamine (DA), assessment of drug efficacy was followed.

Aim: Impact of Administration of Fluoxetine with Galangin and Allicin against mild stress induced major depressive disorder in rodents

Method: Research was carried firstly through the induction of depression using CUMS model in rats. Chronic unpredictable mild stress (CUMS) procedure was applied in rats for continuously 6 weeks (42 days) based on established protocol. The protocol was consisting of eight stressors such as noise, cage tilting, overnight illumination, food deprivation, water deprivation, crowded housing, soiled cage, and food and water deprivation that may induce depressive-like behavior, which then evaluated by the combination of administration of Fluoxetine (10 mg/kg, p.o.) as well as Galangin and Allicin (10 mg/kg, p.o. and 9 mg/kg, p.o. respectively). All treatments were assessed for depression using Sucrose preference test – forced swim test - tail suspension test - open field test - clonidine induced aggressive behavior - hyponeophagia test. Further pharmacokinetic study was done to find out the peak concentration, concentration peak time, area under concentration time curve. After that, biochemical analysis of BDNF, serotonin and dopamine was evaluated using ELISA technique.

Result: Treatment of animals with Galangin at a dose of 10 mg/kg, p.o. and Allicin at a dose of 9 mg/kg, p.o. in alone and combination after Chronic unpredictable mild stress model, increased the BDNF, serotonin and Dopamine level.

Conclusion: In conclusion, on the basis of received results, it is suggested that Galangin and Allicin significantly decreases the depressive symptoms and also overcomes the adverse event reported with Fluoxetine . Fluoxetine + Galangin raised BDNF, dopamine and serotonin levels but were lower than Fluoxetine + Allicin. In summarized way, all the flavonoids such as Galangin and Allicin have showed better effects both alone and in combination which indicate their synergistic activity and depression. The present study was designed to overcome the side effects caused by SSRI drug Fluoxetine . With such objectives, we analyzed the neuroprotective effect of flavanoidal drugs such as Allicin and Galangin alone as well as in combination with Fluoxetine . Connection between Fluoxetine administration and suicidal thought linked with the dysregulation of serotonin metabolism. As serotonin metabolite 5-Hydroxyindoleacetic acid (5-HIAA) was found to be more in depressed patients. Thus, both drugs have a protective effect in CUMS model of depression.

Keywords: Major depressive disorder, Chronic unpredictable stress, Brain derived neurotropic factor

ACKNOWLEDGMENT

We do not accomplish anything in this world alone... and whatever happens is the result of the whole tapestry of one's life and all the weaving of individual threads from one to another that create something. The thread initiates with my supervisor **Dr. Sanjeev Kumar Sahu**, Professor, School of Pharmaceutical Sciences, Jalandhar, Lovely Professional University, Phagwara, Punjab as well as Ex-Supervisor **Dr. Dewesh Tewari**, Assistant Professor, Delhi Pharmaceutical Sciences and Research University (DPSRU) to whom I will always be grateful for embellishing me with all his knowledge and valuable guidance. His scientific approach, patient hearing, all his appreciation and constructive criticisms made this work, to reach the goal. I owe him lots of gratitude for providing all big or small facilities required for this project and for showing me the way to true research. I am immensely grateful to my co-supervisor **Dr. Nilesh Jain**, Professor, Sagar Institute of Research Technology & Sciences- Pharmacy, Bhopal, Madhya Pradesh for his excellent scientific guidance, valuable suggestions and constructive criticism and above all for his morale boosting attitude during the period of project work. I thank him wholeheartedly for trusting me and finding me worthy to assign a project and correcting my flaws throughout my project.

I express my sincere thanks to **Dr. Monica Gulati**, Sr. Dean, Lovely Professional University, Jalandhar for suggestions & cooperation. I am deeply grateful to honorable chancellor **Dr. Ashok Mittal** and Pro-chancellor **Mrs. Rashmi Mittal**, Lovely Professional University, Jalandhar. A special & well deserve token of thanks goes to **Dr. Mayank Tenguria** the person without him, my research project wouldn't be possible.

Now, it's time to say thanks to those who encouraged me during my work especially when my work was not running well or when I was not getting the result. My father **Mr. Uma Dutta Mishra** and mother **Mrs. Manjul Mishra** who soulfully provided me their moral support, unbounded love & affection and the right impetus to undertake the challenge of this proportion like all other spheres of life. My sincere thanks to my Paternal Grandfather and Grand Mother (**Late. Mr. Jamuna Prasad Mishra and Late. Mrs. Brijrani Mishra**) and Maternal Grandfather and Grand Mother (**Late. Mr. Vinod Shankar Shukla and Late Mrs. -Rani Shukla**) and my uncle, Aunty **Mr. Rama Dutta Mishra and Mrs. Mridula Mishra** for their continuous blessings.

I also gratitude to my wife **Mrs. Pooja Mishra**, siblings **Rishika, Devansh and Akshat** and My mentor **Prof. Dr. Sunil Mistry** and Senior colleague **Prof. Dr. Jalaluddin** and friends **Ms. Snehal Uttam Kashid, Ms. Deepa Lashkari, and Mr. Amit Gupta**, for their immense support.

I pay my homage to those animals who have sacrificed their lives in making research successful. May God grant them eternal Peace!

I acknowledge all my other nears & dears. I shall be failing in my duty without expressing deep sense of faith to GOD, the 'Almighty' who graced me in finishing this task.

Needless to say, errors and omissions are mine.

Date:

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LIST OF ABBREVIATIONS	
BA	Brodmann Area
BBB	Blood-Brain Barrier
BDNF	Brain Derived Neurotropic Factor
cAMP	cyclic Adenosine Mono Phosphate
COMT	Catechol-o-Methyl Transferase
COX-2	Cyclooxygenase-2
CY	Cytochrome
DA	Dopamine
DM	Diabetes Mellitus
DSM	Diagnostic and Statistical Manual of Mental Disorders
EAAT	Excitatory Amino-Acid Transporters
ED	Erectile Dysfunction
EMA	European Medicines Agency
FDA	Food and Drug Administration
FKBP5	FK506- Binding Protein 5
GABA	Gamma-Amino Butyric Acid
GBD	Global Burden of Diseases
GluRs	Glutamate Receptors
GRs	Glucocorticoid Receptors
GWAS	Genome-Wide Association Studies
HAMD	Hamilton Rating Scale for Depression
HPA	Hypothalamic-Pituitary-Adrenal
HPG	Hypothalamic-Pituitary-Gonad
ICD	International Classification of Diseases
ICPE	International Collaboration of Psychiatric Epidemiology
IL	Interleukin
iNOS	inducible Nitric Oxide Synthase
LC	Locus Coeruleus
LHb	Lateral Habenula
MADRS	Montgomery Asberg Depression Assessment Scale
MAOIs	Monoamine Oxidase Inhibitors

MMAS	Massachusetts Male Aging Study
MDD	Major Depressive Disorder
mRNA	messenger RNA
NaSSA	Noradrenergic and Specific Serotonergic Antidepressants
NDRIs	Norepinephrine-Dopamine Reuptake Inhibitors
NE	Norepinephrine
NFKB	Nuclear Factor kappa B
NIMH	National Institutes of Mental Health
NLRP3	Nod-Like Receptor Pyrin containing 3 inflammasome
NMDA	N-Methyl-D-aspartic Acid
NO	Nucleus Oralis
PB	Parabrachial Nuclei
PDEs	Phosphodiesterase's
PFC	Prefrontal Cortex
PGE2	Prostaglandin E2
PGC	Psychiatric Genomics Consortium
PHQ	Patient Health Questionnaire
PPT	Pedunculopontine
PRSs	Polygenic Risk Scores
PTSD	Posttraumatic Stress Disorder
SDI	Socio-Demographic Index
SNRIs	Serotonin-Norepinephrine Reuptake Inhibitors
SNPs	Single Nucleotide Polymorphisms
SSRIs	Selective Serotonin Reuptake Inhibitors
TCAs	Tricyclic Antidepressants
TMN	Tuberomammillary Nucleus
TNF	Tumor Necrosis Factor
TRD	Treatment-Resistant Depression
UGT	UDP-Glucuronosyl Transferase
WHO	World Health Organization

1. INTRODUCTION

1.1 Depression

In recent years, psychiatric diseases have gained prominence as a serious public health concern. Mental illness has horrible side consequences. Neurological illnesses are a worldwide problem, not depending on age, religion, culture, gender, etc. A prevalent mental ailment is major depressive disorder (MDD). Furthermore, to having severe dysregulation of affect and mood, MDD is also known to cause cognitive impairment, drowsiness and hunger disturbances, exhaustion, and other inflammatory, endocrine, or metabolic conditions changes (Villanueva, 2013). As the World Health Organization has said (WHO), psychiatric disorders are believed to affect approximately 450 million individuals globally. Major depressive illness, sometimes known as depression, affects around 260 million people worldwide. (Al-Jumaili et al., 2021). There have also been instances of brain structural abnormalities (Harrison, 2002). Fifth Version of the Diagnostic and Statistical Manual of Mental Disorders, states (DSM-5), the symptoms of depression include cognitive impairment, guilt feelings, fatigue, weight gain or loss, decreased concentration, and recurrent suicidal thoughts, with 10% of adults reporting symptoms preferably once a year (Guha, 2014). Hence, the WHO, depression will rank as the second most morbid condition during the next ten years. Currently, depression affects one in five women and one in twelve men. Depression has been the most prevalent cause for patients to see a psychiatrist, while the average person believes that all psychiatric issues are depression (James et al., 2018). Most patients exhibit the myth associated with depression. Some continue to assume that self-treatment is possible because of a personality flaw, or that medicine is permanent and only sedatives.

With newer medications and improved facilities, it is now simpler to treat depression, and the majority of patients react well to therapy and quickly regain optimal functioning (Harrison, 2002).

1.2 Signs and symptoms

Since these symptoms also pertain to other mental illnesses, they can be roughly divided into emotional, cognitive, and neuro vegetative symptoms, which are not specific to depression (Malhi et al., 2002). According to the study, females are more likely than males to struggle at work, feel depressed, or be unsure of themselves.

Considering research findings on antidepressants and electroconvulsive therapy, it may be feasible to identify distinct depression symptoms linked to a particular neurochemical process, for instance.

Norepinephrine and Dopamine are linked to motivation, focus, enjoyment, and reward. Along with anxiety, attention, and interest in life, it also has something to do with energy and focus. In contrast, serotonin has been linked to anxiety, obsessions, and compulsions. Different symptoms of depression were identified by Ettman et al. in 2020, including (Mayr et al., 2010; Gaspersz et al., 2017).

- **Emotional change**

Indicators of this include melancholy, guilt, anxiety, hopelessness, mood swings, and irritability.

- **A change in thinking**

A depressed individual has bad thoughts including suicidal thoughts, poor memory, lack of attention, and lack of decisiveness.

- **Change in behavior**

These symptoms include social exclusion, loss of interest, carelessness with one's duties, sobbing, and changes in one's physical appearance.

- **Physical change**

This alters when a depressed person has unusual sleeping patterns, persistent exhaustion, changes in weight, including increase or loss, and inexplicable discomfort or pains.

**Table 1.1: Rating Scale of depression based on symptoms from three scales
(Benazzi, 2000; Malhi, 2015)**

MAIER-6	HAMD-7	BECH-6
Mood	Mood	Mood
Guilt	Guilt	Guilt
jobs and hobbies	jobs and hobbies	jobs and hobbies
Psychic anxiety	Psychic anxiety	Psychic anxiety
Agitation	Energy	Energy
Retardation	Somatic anxiety	Retardation
	Suicide	

1.3 Depression and epigenetic factors

Constant emotional strain, severe circumstances in the environment or psychological stress are involved in a person's genetic makeup and cause various alterations on the epigenetic level that may be controlled utilizing epigenetic approaches. These changes in the epigenome lead to variations in gene expression that are passed down through generations (Fritz et al., 2017).

40% of people suffered with MDD is not necessarily related to genetic modification. An additional factor that is linked with MDD is environmental stresses including stressful life events and adversity experienced during childhood. Recent research revealed that the variety of epigenetic modifications mediate the biological effects of environmental factors as MDD and other stress related disorders (Yuan et al., 2023)

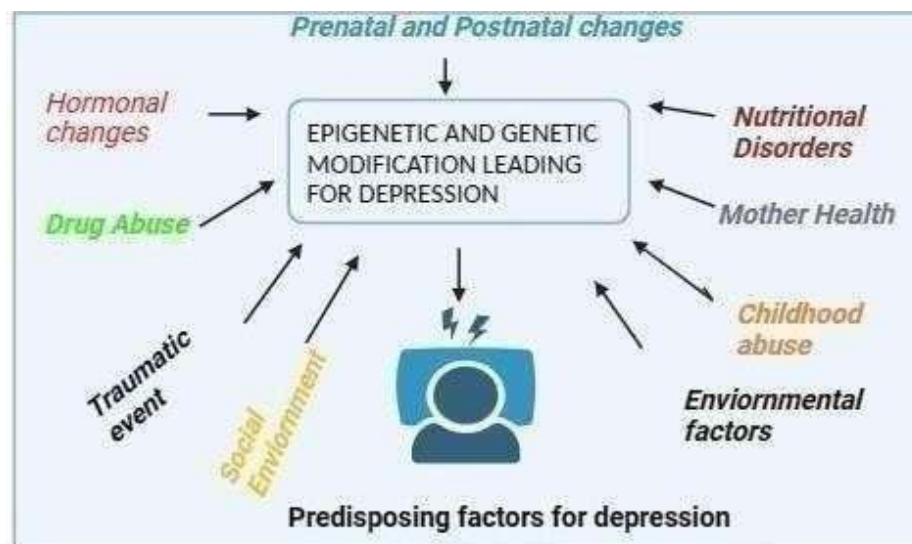


Fig. 1.1: Predisposing factors for depression

1.4 Etiology

1.4.1 Genetic causes of depression

Specific genetic modifications demonstrate a limited influence on the pathogenesis of complex diseases such as depression. Within the framework of the monoamine theory of depressive disorder development, an extensive examination of numerous candidate gene has been undertaken. Among these are genes encoding noradrenaline (SLC6A2) and dopamine receptors (HTR1A, HTR2A, HTR1B, HTR2C), enzyme genes encoding monoamine oxidase A (MAO-A), tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH1) and catechol-o- methyl transferase (COMT); and the piccolo presynaptic cytomatrix protein (PCLO). Point mutations were found to be associated with the identification of polymorphic variants for each of these genes.

This polymorphism was examined in samples collected from patients with DD of various ethnicity (Shadrina et al., 2018)

While the idea that depressive disorders run in families has been recognized a very long time, it was not clear until recently if a person's sensitivity to these diseases was inherited or due to another cause, such as their environment. Depressive illnesses may to some extent be inherited, according to researchers studying depression. A propensity for depression seems to be inherited. This indicates that if we have immediate family members who suffer from severe depression, we may inherit a propensity to do so as well. That does not imply that we will always experience depression (Rep et al., 2022).

1.4.2 Twin research

The bulk of our knowledge on the genetic component of clinical depression comes from twin research. Given that they share the same genetic code, identical twins are incredibly beneficial to scientists. When one of the identical twins develops depressive symptoms, the other will experience it 76% of the time, according to research. Approximately 67% of cases of depression have been seen among sets of identical twins who were reared apart. Because both twins suffer depression at such a high incidence, the conclusion is that there is a significant genetic component.

Clinical depression would presumably be totally hereditary if both twins constantly experience depression when one has clinical depression. Yet, given the chance of both identical twins being depressed is less than one hundred percent, this shows that additional factors affect how vulnerable a person is to depression. Past contextual variables including traumatic events, present-day demands, and health problems might all play a role (Benazzi, 2006). The inquiry also focuses on fraternal twins. In contrast to synonyms for "identical twins" "who possess the same genetic make-up, these siblings share around 50 percent of their genetic composition and may not appear alike. Studies indicate that there is a 19% risk that the second fraternal twin will suffer depression if the first did. Compared to rates in the general population, this is still a higher risk of depression, confirming the notion that clinical depression has a genetic component (Alshaya, 2022).

1.4.3 Depression's connection to the environment

Environmental variables including stress, traumatic events, and difficulties in childhood are a few instances of how they might cause depression. These are incidents that take place in our everyday lives and may affect everyone. They are thought of as unrelated variables. Since they represent a "meeting" or "combination" of social events and the operations of the human mind, some scholars refer to these occurrences as sociological or psychosocial variables. The impact of the experiences (events) we encounter in life on our mental health has long been recognized by researchers. People's past experiences in life have an impact on their current beliefs, feelings, and behaviors. Past relationships, early growth, and prior tragedies are only a few examples of these experiences. Some people's responses to the many environmental reasons or elements in their daily lives seem to have a major impact in the onset of clinical depression in such individuals (Alshaya, 2022).

1.4.4 Stress

Stressful situations, how one's mind and body handle stress, and the onset of clinical depression all seem to be intricately related. According to the majority of research, for certain people, going through a stressful situation and developing depression are associated in some way. It is essential to remember that stress may have both positive and negative effects. Negative stress examples include being divorced, losing your job, ending a relationship, and losing a loved one. Constructive stress may be caused by planning a wedding, moving to a new area, or preparing for a new job. External factors may cause both adverse and beneficial stress, which can then lead to depression (Keller et al., 1992).

1.4.5 Traumatic events

Indeed, several people endure a terrible incident prior to getting depression. The loss of a family member, a life-threatening disease, the breakdown of a marriage, and a substantial expense reduction are examples of traumatic life events. As it undermines a person's sense of security and control over their life, these kinds of events often cause emotional distress (Steinert, 2014).

1.4.6 Problems in childhood

It is well established that those who experienced substantial suffering as kids are more likely to have depressive symptoms. Parental divorce, emotional, sexual, or physical violence, poor parenting, and psychological disorders in either of the parents are among the most common childhood problems. Until the age of eleven, the separation from or passing of a parent is one of the most challenging emotional situations a child may endure. Also, this exposure increases a child's likelihood of developing depression (Verduijn et al., 2017).

1.4.7 Synthetic compounds

We consume synthetic chemicals from a multitude of sources every day. Preservatives, chemicals, and hormones which may be found in and added to a vast majority of our foods, as well as sprayed pesticides and air and water pollution, all contribute significantly to the degradation of human health. Air and water pollution may be carcinogenic and other ailments on their own, according to research. Current research investigates the link between exposure to industrial chemicals and pollution and severe depression episodes (Penninx et al., 2011).

1.4.8 Noise pollution

Aggressive behavior, high blood pressure, increased anxiety levels, tinnitus, hearing loss, and erratic sleeping patterns all been associated with noise pollution. Tinnitus is especially related with major depressive disorder, panic attacks, and amnesia. Long-term noise pollution exposure has been associated with hypertension and cardiovascular disease. Individuals who are already depressed are more prone to develop depression if they are continuously exposed to noise pollution (Spijker et al., 2002).

1.4.9 Electromagnetic pollution

Radio waves continually surround us wherever we go. According to study, Radio waves are a key component of many of the electrical devices we use, which have been linked to anger and melancholy. Electrical pollution, unlike other there are no physical senses that can be used to detect environmental causes of depression. The specific causes are yet unclear. Yet, it affects our brains and bodies (Burdusa & Iacono, 2007).

1.4.10 Natural and catastrophic disasters

Bombs, conflict, hurricanes, earthquakes, and flames can cause severe major depression in vulnerable people. Those who ordinarily wouldn't be candidates for depression have been

observed to develop symptoms, according to the national centre for environmental health, following significant life-altering events like having their home destroyed by a natural catastrophe.

1.5 Epidemiology with current scenario

A depressed person may attempt suicide. According to Kong L, Wu F, Tang Y, et al., among teenagers, suicide is the second largest cause of mortality, claiming the lives of more than 800,000 adolescents annually. Fronto subcortical volumetric abnormalities in a single episode, depressed individuals who have never taken medicine, and the consequences of an 8-week Fluoxetine therapy. The report from "Global, Regional, and National Burden of 12 Mental Disorders in 204 Countries and Territories, 1990- 2019: A Systematic Analysis for the Global Burden of Disease Study 2019" claims that, " 45.7 million people in India routinely suffer depression. According to past surveys, 10% of Americans reported experiencing depressive symptoms during the previous year, compared to 4.5% in Mexico, 0.3% in the Czech Republic, and 55 in West Germany. However according to Andrade L, the prevalence of depression throughout the course of a person's lifetime one percent in the czech republic, 17% in the US, 8.3% in canada, and 9% in chile. The ICPE surveys of the international collaboration of psychiatric epidemiology (ICPE) provide information on the epidemiology of clinically significant depression episodes (Andrade et al., 2003) (Kessler & Bromet, 2013). It is crucial to comprehend the brain underpinnings of major depressive disorder rather than only focusing on its symptoms (MDD). Due to complex interactions between a number of factors, such as among others, biological, behavioral, psychological, and cultural aspects, MDD is multifaceted and may progress from moderate to acute symptoms over the trajectory of a person's life (Clark et al., 2017; Disner et al., 2011; Park et al., 2019) provide information on the emotional imbalance in MDD. While the neurobiology of MDD is now better understood, its exact mechanism is still unclear, and there is currently no explanation that can completely account for the disease's symptoms.

1.5.1. Depression rates in european countries by gender

In the Western world, depression affects around two times as many women as men (Piccinelli & Wilkinson, 2000). Among the usual psychological and biological suspects, the research on the disparity between the sexes in depression alludes to a variety of social risk factors that are unique to each gender. Powerlessness and lower status levels are consequently more typical in female social situations (Connel, 1985; Collins, et al., 1993;

Blumerg, 1984). This is due to the perception that female roles are more vulnerable to constraints brought by reduction in opportunities as well their alternatives, too many burden tasks, combative societal responsibilities, as well as propensity considering females to be underestimated (Piccinelli & Wilkinson, 2000; Stiffler, 2000). They might have greater depression risk while working whereas it leads to tasks conflict moreover duty overload because of the mix of obligations related to work, the family, and caregiving, as well as an increased likelihood of economic prejudice and employment inequality than males. The conventional view of masculinity, which includes a refusal to ask for help and particular due to alterations to family structures and the job market, men regularly severely dispute their notions about parenthood, masculine expression of emotions, as well as the act of males the only breadwinners (Courtenay, 2000; Garfield, et al., 2006). It is mainly unknown how these cultural shifts may affect male risk and susceptibility variables for depression as well as male depression (Addis, 2008; Garfield et al., 2006). Yet, reliable data, a well-known one suggests that non-custodial parents who are divorced have a number of parenting-related difficulties, which might help to explain why they have greater levels of depression than males who are married (Umberson & Williams, 1993).

One related result is that when there are young children at preschool in the family, men are just as likely to have post-divorce sadness as moms (Williams & Dunne- Bryant, 2006).

1.5.2 Current scenario of depression in India

The most common mental illness identified in most community-based research is depression. According to Indian Council of Medical Research (ICMR) report, approximately 15% (198 million) of Indian population suffered with mental disorder, among them 45.7 million had depression and approx. 45 million affected with anxiety disorder. Studies conducted in India have also proved the significance of earlier life happens in the development of depression. Recognizing danger signs such interpersonal disputes, marital instability, and sexual coercion is crucial, according to studies of women. There is a need for further research on aspects including cost, treatment-related attitude, compliance, adherence, and neurobiological correlates. To assess the need and length of continued therapy, Moreover, study on the course of depressive illnesses in India is required. To effectively treat depression, research should evaluate cost-effective techniques for treating the condition rapidly in the initial care situation revealed by the report of DSM-5 (Fifth Edition) Diagnostic and Statistical Manual of Mental Disorders (Villanueva, 2013; Nestler et al., 2002).

1.6 Impact of various factors on depression

1.6.1 Sleep and depression

Depression and sleep have a complicated relationship. Sleep disturbances are a common sign of depression. Depression usually manifests as insomnia, and data suggests that major depressive episodes are typically preceded by sleep problems (MDE). Associated sleep with depression, the following traits are typically seen in EEG recordings: extended REM sleep durations, decreased REM sleep density and delay, especially in the early stages. Decreased delta ratio caused disruptions in the distribution of delta activity, decreased NREM sleep, disrupted sleep cycles, and reduced sleep efficiency (delta activity ratio between the first and second NREM phases). Since many mental illnesses often exhibit other sleep irregularities, it has been thought that depressive disorders of REM sleep are more distinctive. 1966 saw the work of Hartmann E., Green W.J., et al. who discovered that REM sleep had a shorter latency at sleep initiation and a higher percentage (Malhi et al., 2018). There have been theories that REM sleep latency is a sign of unhappiness as a result (Hasin et al., 2018).

1.6.1.1 Neurobiological reason for depression-related REM sleep abnormalities

- **Control of rapid eye movement sleep**

It is believed that brainstem cholinergic and monoaminergic neurons collaborate to govern when REM sleep begins (Parker et al., 2017; Keller, et al., 2007; Kaufmann *et al.*, 2017; Fink, 2013; Moller et al., 2016; Goldberg, 2012). It has been shown that locus coeruleus (LC's) noradrenergic neurons have a deleterious impact on REM sleep (Thombs, et al., 2014). These LC neurons are more active during wakefulness, much less NREM sleep is active, and completely during REM sleep inactive (H.Stuart, 2016). Some have hypothesized that cholinergic neurons in the peri-LC alpha tegmental nuclei, laterodorsally, and pedunculo pontine (PPT) are significant in terms of producing REM sleep (Mitchell, et al., 2016; Siu, 2016). The coordination of REM sleep production has been linked to the nucleus oral is (NPO). Previous study has shown that REM sleep reduces after a ventral NPO lesion (vNPO). In addition to GABA-ergic fibers from the posterolateral hypothalamus, cholinergic afferent fibers are sent from the rostral peri LC alpha, LDT, PPT, and parabrachial nuclei (PB) (Andrade et al., 2003). Thus, the interplay between the two systems is crucial to the control of REM sleep (Kessler & Bromet, 2013; Clark et al., 2017). To distinguish between neurons that begin to fire or significantly increase firing rate while

experiencing rapid eye movement sleep, those that quit firing or noticeably decrease firing rate concurrently, the terms "REM-on neurons" and "REM-off neurons" have been coined (Disner et al., 2011; Park et al., 2019). NE-ergic neurons in the LC, serotonergic (5-HT) neurons in the dorsal raphe, and neurons that make histamine in the hypothalamic tuberomammillary nucleus (TMN) make up the bulk of REM-off neurons (D. P. Goldberg, 2014; Joormann & Stanton, 2016; Stockmeier & Rajkowska, 2022; Maletic et al., 2017). Nonetheless, it has been shown that a subset of non-monoaminergic medulla neurons are REM-off neurons (Stein, et al., 2006). The PPT and LDT contain the majority of the cholinergic REM-on neurons (Stanley, et al., 1982; Grace, 2016; Herice et al., 2019). However there have also been a number of non-cholinergic REM-on brain neurons identified (Stein, et al., 2006; Perry, et al., 1983). How REM-off neurons stop firing during REM sleep, which is necessary for REM sleep to happen, remains unclear. One theory is that GABA prevents REM-off neurons from firing (Palmio et al., 2001). According to the flip switch hypothesis of Lu J et al., the mesopontine tegmentum of the brainstem contains both REM-on and REM-off areas that are mutually blocked (Seo et al., 2008).

Additionally, monoamine oxidase (MAO) inhibitors, which inhibit the breakdown of Nor epinephrine and 5-HT (Serotonin), may alleviate depressed symptoms, indicating that the Nor Epinephrine and 5-HT (Serotonin) schemes are crucial in the development of dullness, low spirits. Monoamine reuptake inhibitors may lessen REM sleep by raising extracellular levels of both NE and 5-HT, which may be helpful for addressing depression (Schenk et al., 2017). Selective lesions in the brainstem's monoaminergic or cholinergic nuclei, however, had no effect on REM sleep (Duman, et al., 2021; Martinowich, et al., 2007; Yang et al., 2020). Additionally, other data indicated that there was no connection between disturbed REM sleep and depressed behaviors (Goldberg & Fawcett, 2012). In order to govern REM sleep and depression, two parallel pathways that are connected to the monoaminergic system may exist.

The cholinergic system is essential in the abnormal REM sleep patterns connected to depression, together with the monoaminergic system. Major depressive disorder in short MDD and posttraumatic stress disorder (PTSD) are two stress-related illnesses having a link to REM sleep disruption (Duman & Monteggia, 2006; V Krishnan & Nestler, 2008; Vaishnav Krishnan & Nestler, 2010; Wohleb, et al., 2016). Although MDD is linked to waking and REM sleep hyper metabolism in these regions, PTSD is linked to more limbic and paralimbic metabolism during REM sleep (Raison, et al., 2006). Compared to those who weren't depressed, patients with depression had hippocampus that were 12% to 15%

smaller (M.Stuart & Baune, 2014). Theta oscillations, a feature of REM sleep, are discovered by Mizuseki K et al. to reduce spike synchrony in the entorhinal cortex and the hippocampal area (Liu et al., 2015; Müller, 2014). Stress may also cause Long-term depression and long-term potentiation loss, both of which have a substantial impact on cognition, anxiety, and depression (Treadway, 2016; Dendorfer, et al., 1994; Jeon & Kim, 2016; Gao & Hong, 2008). Because of this, the hippocampus is essential for managing REM sleep abnormalities, which can contribute to depressive illness. The lateral habenula (LHb), a brainstem nucleus, inhibits the activation of serotonergic neurons to negatively control the monoaminergic system (Banks, 1995). Aizawa et al studies indicate that synchronized LHb activity is essential for sustaining REM sleep through regulating the action of serotonin, which is necessary for the development of depression (McAfoose & Baune, 2009).

According to a research, abnormal REM sleep patterns in depression are influenced by the middle raphe has serotonergic neurons (Rial et al., 2016).

1.6.2 Fatigue and depression

Anxiety and depression are both closely related to fatigue, which is described as "a perceived lack of either physical and/or mental energy that interferes with regular and desirable activities" (Krishnan & Nestler, 2010; Nestler et al., 2002; Gray et al., 2020). The caliber of one's existence is also negatively correlated with fatigue (Benazzi, 2022; Alshaya, 2022).

Effects of inflammation on fatigue and depression

Patients who are depressed are more prone to complain of being worn out or unable to do a physical or mental activity, especially in primary care settings. The most prevalent depressed symptom in family practice settings was found to be weariness. In the large european collaborative study (DEPRES II), which included more than 2000 depressed patients from six different countries, 73% of participants reported feeling tired. This symptom was more common in women and was linked with the intensity of the episode (Kaufmann, et al., 2017; Fink, 2013). Relevance to diagnosis and treatment of the primary signs of serious depression disorder this construct is too generic to be classified as a major symptom, like how reduced interest, lack of energy, and even hesitation may overlap with apathy. 20% or more of patients using reuptake of selective serotonin antidepressant inhibitors have shown to experience apathy as a side effect more frequently (Möller et al., 2016).

1.6.3 Sexual Dysfunction and Depression

The following four stages of sexual maturation coexist: Arousal is indicated by an erection in men, lubrication and engorgement in women. The markers of release include orgasm and orgasmic ejaculation. Refractoriness is a necessary component of resolution. The process of normal sexual function is bio psychosocial. Every aspect of the sexual response may be affected in the context of a mental disorder. The most common symptom is a reduction in sexual desire, which may be related to more general anhedonia. Further proof that sadness may impact arousal or genital neurophysiology Vaso congestion results from the reduced nocturnal penile tumescence that occurs in around one-third of depressed males (NPT). It is crucial to take into account the baseline context— that is, elements like age, relationship status, and illness—when evaluating sexual function in depressed people, despite these shortcomings that seem to be exclusive to depression. While sexual problems and depression are directly connected, the specific reason why this happens is still a mystery. The five models may be utilized to comprehend how both scenarios could coexist since they are not mutually exclusive. Secondly, prolonged psychological distress brought on by sexual dysfunction may encourage the formation of subsequent mental disease in more delicate people. Second-sex dysfunction may manifest as signs of depression. Finally, using antidepressants may cause sexual dysfunction. Fourth, the causes of both ailments can be the same (such as alcohol, cigarettes, cardiovascular disease, or hypogonadism).

Lastly, despite the fact that both disorders are rather common, it is possible for depression and sexual dysfunction to coexist while originating from separate causes. The symptoms of major depressive disorder (MDD) include loss of libido, which has garnered considerable attention in both psychodynamic and alternative psychological explanations of the condition. Systematic study suggests that up to 75% of depressed individuals may have decreased libido (Angst et al., 2009; Carey, 2015).

1.6.4 Depression and erectile dysfunction

The co-occurrence of depression with erectile dysfunction (ED) is prevalent, especially in middle aged and older men. The existence or change of one of these diseases may be the cause, consequence, or moderator of the other, or there may be a bidirectional association

between ED and depression (Krawczyk & Świącicki, 2020). For instance, in men who are depressed, ED might be a sign of depression, or it may be an antidepressant drug adverse effect that appears during treatment. Due to the bio psychosocial stress that is often connected to the loss of sexual function, Men with erectile dysfunction may potentially develop secondary depression. Both population- based and clinical samples have been used to study the connection between depression and erectile dysfunction. The massachusetts male aging study provides the strongest evidence for a link between depression and erectile dysfunction (ED) (MMAS). A cross-sectional study, community-based known as the Males's Health and Aging Study (MMAS), guys between the ages of 40 and 70 had their health and ageing assessed. Between 1987 and 1989, it was done in 11 randomly chosen neighborhood's in the Boston region, with a 76% (n = 1290) response rate. Nine questions on biological signs of erectile response that were closely linked to the respondents 'replies to classified ED as zero (48%), mild (17%), moderate (25%), or total (10%). (Penninx et al., 2011). According to the CES-D threshold of 16, which shows a substantial connection with MDD, all males with minor, moderate, or complete depressive symptomatology developed ED. According to Spielberger's anger scales, men who had low levels of anger were twice as likely to develop ED as those who had high levels of anger, whether it was expressed or controlled (Penninx et al., 2011). Minimal research has been undertaken on the association between ED and depression among ED-afflicted males. Two noteworthy studies examining mental symptoms in male patients who attended the Johns Hopkins Sexual Behaviors Consultation Unit between 1976 and 1979 (n = 209) and 1984 and 1986 (n = 223) yielded intriguing results. (Malhi et al., 2018). Furthermore, very excessively high concentrations of general psychologic distress, males with erectile dysfunction also reported extremely high prevalence of physical illness, mental illness, and nervous system disorders

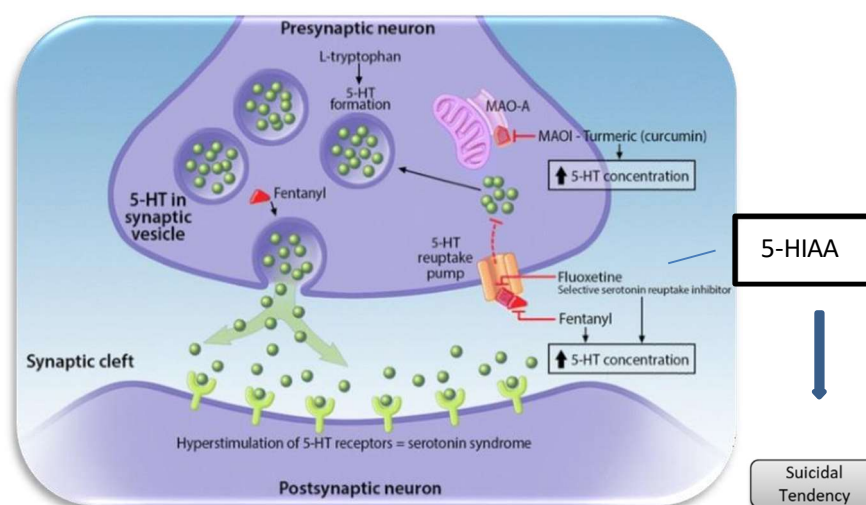


Fig. 1.2: Pathogenesis of depression

(e.g., these individuals had results that put them in the 92nd percentile of the sample group using one well-validated questionnaire that evaluates such distress).

1.6.5 Depression and testosterone

Unknown is the absolute or relative values, if any, at which a certain ratio of hypothalamus to pituitary to gonad (HPG), such as testosterone, either free or total, may be connected to emotional distress. Low total testosterone levels, often between 200 and 300 ng/dL, are connected to the beginning of symptoms including reduced libido and the inability to get an erection at night. This is a result of diminishing testosterone levels in healthy adult guys. The intricate and bidirectional link between sexual dysfunction and depression remains poorly understood.

1.7 Control of depression

Prescription drugs and brief psychotherapy may alleviate symptoms of depression (cognitive behavioral and interpersonal therapy). Moreover, Better rates of depressive symptom reduction and enhanced treatment adherence have all been linked to combination therapy (Grande, et al., 2016). Moreover, electroconvulsive treatment may aid individuals who do not react well to medications or who are suicidal (D. Goldberg & Fawcett,2012) a summary of the standard instruments used to treat depression reported in Fig 1.2.

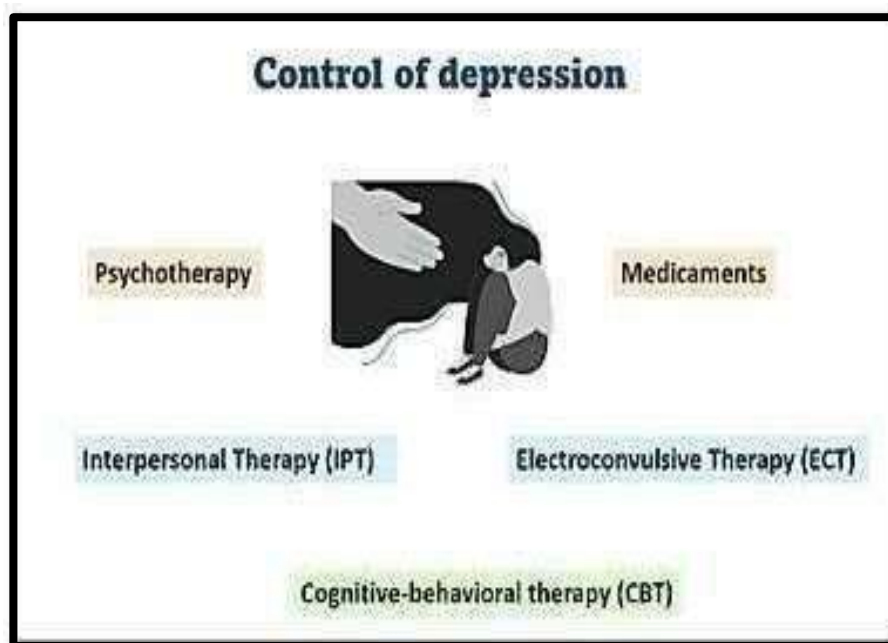


Fig. 1.3. Serious management depression tools

1.8 Anti-depressant medications

The most common time that depression manifests as a mental health problem is in early adulthood. The prevalence is higher in females. Yet, depression may affect anybody at any age. As depression has an effect on the brain, drugs that affect the brain may be beneficial. Even if there are a lot more choices, you could feel better if you use generic antidepressants. Each antidepressant works by restoring the neurotransmitters—brain chemicals—to their proper equilibrium. These medications lessen your depression symptoms in somewhat different ways.

The following pharmacological classes include a lot of popular medications for depression

Dependable sources: (Kupfer, 2022; Osuch, 2017) Dopamine reuptake blockers, Inhibitors of Enzyme Monoamine oxidase (MAOIs), Selective serotonin reuptake Inhibitors (SSRIs), Serotonin-norepinephrine reuptake Inhibitors (SNRIs), Tricyclic antidepressants (TCAs), Tetracyclic antidepressants.

Types

SSRIs are the class of antidepressants that are most often given. Serotonin imbalance may have a role in depression. These drugs work to reduce depressive symptoms by preventing the brain from reabsorbing serotonin. Your brain can now access more serotonin for utilization as a consequence of this activity.

Sertraline (Zoloft), Fluoxetine (Prozac, Sarafem), Citalopram (Celexa), Escitalopram (Lexapro), serotonin receptor antagonist like (5HT1A, 2, and 3). Paroxetine (Paxil, Pexeva, and Brisdelle), Fluvoxamine, and Paroxetine are examples of SSRIs (Luvox) SSRIs often cause side effects such as nausea, trouble sleeping, jitteriness, tremors, and sexual problems. Understanding SSRIs, or selective serotonin reuptake inhibitors, is essential.

The brain's serotonin and norepinephrine levels are raised by SNRIs. This could lessen the symptoms of depression. These medications include venlafaxine, duloxetine, levomilnacipran, and desvenlafaxine (Pristiq, Khedezla), among others (Effexor). In addition to treating depression, duloxetine has the potential to alleviate pain. This is crucial since persistent pain may develop or exacerbate depression. Depression may sometimes make a person more aware of their problems. Some patients may benefit from a medication like duloxetine that helps both depression and pain.

SNRIs often cause the following adverse effects

Dry mouth, fatigue, constipation, and drowsiness. When SSRIs or other antidepressants are ineffective, TCAs are often recommended. The exact mechanism through which these medications combat depression is unclear.

- Amitriptyline (Elavil),
- Amoxapine (Asendin),
- Clomipramine (Anafranil),
- Desipramine (Norpramin),
- Doxepin (Silenor),
- Imipramine (Tofranil),
- Nortriptyline (Pamelor)
- Protriptyline (Vivactil), and
- Trimipramine are some of the TCAs (Surmontil)

TCAs often cause adverse effects including

Constipation, dry lips, Tiredness, Slight vision haze some medications' more severe adverse effects include hypotension, irregular heartbeat, and seizures.

Tricyclic mood stabilizers.

Tetracyclic antidepressants are used to treat depression and anxiety, such as Maprotiline (Ludiomil). They also balance neurotransmitters to lessen depressive symptoms. Drowsiness, weakness, light-headedness, headaches, blurred vision, and dry mouth are typical side effects of this medicine. Bupropion dopamine and norepinephrine reuptake blocker is one kind of dopamine reuptake inhibitor. They are used to treat both seasonal affective disorder and depression. They may also be used to stop smoking.

Typical adverse consequences

Vomiting, diarrhea, dizziness, blurry vision, and nausea Vilazodone is the name of the medication in this class that is used in the treatment of depression (Viibryd). It functions by regulating the amounts of serotonin and other neurotransmitters. The first line of therapy for depression with this medication is uncommon. This implies that it is often only administered if other treatments have failed to help you or have given rise to unwelcome side effects.

Symptoms may include nausea; vomiting; and difficulty sleeping. Antidepressants like trazodone (Oleptro) and nefazodone (Serzone) work by blocking the 5-HT₂

receptor. These medicines are older. They change brain chemistry to treat depression. Typical adverse effects include drowsiness, dizziness, and dry mouth. Depression is treated with the serotonergic receptor blocker vortioxetine (Brintellix) by altering the action of certain substances in the brain.

Frequent side effects include nausea and sexual issues. Older medications that work against depression include MAOIs. They function by preventing the breakdown of dopamine, serotonin, and norepinephrine.

Since they interact with prescription medications, over-the-counter medications, and certain foods, they are more challenging for individuals to take than the majority of other antidepressants. Moreover, they cannot be used alongside other antidepressants or stimulants.

1.9 Complications associated with current treatment methods

1.9.1 Fluoxetine

The management of major depressive illness in individuals aged (eight years and above), bipolar depression (when combined with olanzapine), obsessive-compulsive disorder (in individuals seven years of age and older), anxiety disorders, disorders of premenstrual dysphoria, binge eating, and bulimia, and treatment-resistant depression with Fluoxetine has been ratified by FDA i.e., Food and Drug Administration (James et al., 2018; Shelton, 2007; Harrison, 2002), Raynaud phenomenon, selective mutism, and socially anxious disorder, Also referred to as social anxiety (Jacob, 2009).

Mechanism of action

Mood-related conditions have been related to the biological amines, serotonin and norepinephrine. Low serotonin levels in the cerebrospinal fluid have been linked to depression. Additionally, platelets of those with depression have fewer serotonin absorption sites. The dorsal raphe nucleus is linked to the prefrontal cortex through serotonin (5HT1A) receptors. By blocking the presynaptic reuptake transporter protein, Fluoxetine inhibits 5-HT from entering presynaptic serotonin neurons. Moreover, 5HT2A and 5HT2C receptors are little impacted by Fluoxetine .

The effect of Fluoxetine on noradrenergic reuptake is minimal. Since Fluoxetine reuptakes serotonin, it has an activating effect. Because to its prolonged half-life, the early antidepressant effects take two to four weeks to manifest. The cytochrome P450 enzyme (CYP2D6) creates nor Fluoxetine , which is the active metabolite of Fluoxetine .

Since Fluoxetine undergoes metabolism at the CYP2D6 isoenzyme, it is crucial to keep in mind that it has several drug- drug interactions. Nor Fluoxetine may also inhibit CYP3A4. Also, keep in mind that the active metabolite of Fluoxetine, nor Fluoxetine has an elimination half-life of 2–4 days and a half-life of 7to 9 days (Villanueva,2013; Weiner, et al., 2011).

Administration

Take 20 mg of Fluoxetine once day, preferably in the morning or evening. In the form of a delayed release capsule (10, 20, or 60 mg) and regular capsules (10, 20, or 40mg), as well as an oral solution (20 mg/5 ml), are the only four dosage forms that are available (90 mg). Taking into account its efficacy at doses as low as 5 milligrams and the profile of potential adverse effects, the medicine may be used at lower levels. For a patient who has difficulty tolerating side effects, the medication may be administered in 10 mg tablets as opposed to 20 mg tablets in order to mitigate adverse effects. For a large majority of people, a daily dose of 20 mg to 40mg is required for efficacy. Some persons may need 60 to 80 mg daily. The recommended daily dose for the treatment of bulimia is often between 60 and 80 mg.

Moreover, a 90 mg dose per week delayed-release capsule form is offered. The data that are available show that the efficacy of Fluoxetine 20 mg daily is equivalent to that of the delayed- release form (90 mg once weekly). As opposed to other SSRIs, Fluoxetine does not result in the abrupt start of withdrawal symptoms that are typical of other antidepressants (such as sleeplessness, dysphoria, fever, and nausea) (Eric et al., 2002).

Medication interactions need to be constantly monitored. Let's say you are starting a monoamine oxidase inhibitor to treat depression that is unresponsive to medication. Fluoxetine should be stopped five weeks prior to the administration of a monoamine oxidase blocker to prevent the development of serotonin syndrome. For instance, the non-steroid anti- inflammatory medicine ibuprofen may lessen the effectiveness of Fluoxetine.

Pregnant women's issues

It is classified as a Category C medication during pregnancy. Neonatal exposed to SSRIs, such as Fluoxetine, in the late third trimester needed longer hospitalization, tube feedings, and respiratory care. Problems that have been observed include fluctuations in body temperature, difficulty in eating, vomiting, breathing issues, apnea, cyanosis, low blood sugar, hypotonia, and hypertonia. These deleterious effects, which may manifest shortly after delivery, are reliable with each a drug withdrawal condition or the direct toxicity of

selective serotonin reuptake inhibitor. During the third trimester, the physician may consider decreasing the Fluoxetine dose (Gray, et al., 2020).

Thinking about breastfeeding

It is not recommended to breastfeed while using Fluoxetine since it is excreted in human milk (Gray et al., 2020). Most medical experts advise against switching medications while breastfeeding if a woman took Fluoxetine during pregnancy. If not, it may be preferable to use medications with lesser excretion into breast milk, particularly if you are breastfeeding a child or a premature new-born. It's also critical to watch out for behavioral side effects in breastfeeding babies, such as colic, anxiety, disturbances in mood, appetite, and weight gain.

Child health considerations

Fluoxetine has been accepted for use as a therapy by the FDA of MDD and OCD in pediatric patients. Like other SSRIs, Fluoxetine has a decreased risk of weight gain when given to young people. There aren't enough studies looking at Fluoxetine's long-term effects on patients' maturation, as well as pediatric growth and development.

Liver insufficiency

Due to slower medication clearance in people with liver cirrhosis, Fluoxetine and its active metabolite, nor Fluoxetine, have prolonged elimination half-lives. As a result, those who have cirrhosis should take Fluoxetine less often or at a lesser dose. Anyone who may have conditions or diseases that affect Fluoxetine's metabolism should take it cautiously.

Negative responses

Hyperhidrosis, bruising, bleeding (rarely), Some of the negative effects of antidepressant usage include yawning, a reduced libido, decreased arousal, anorexia, dry mouth, headaches, nausea, diarrhea, insomnia, and dry mouth (visible as diminished lubrication in females and problems with erectile dysfunction in males), anxiety, nervousness, and yawning are the most common adverse effects reported by adults (Malhi & Mann, 2018; Benazzi, 2022).

Fluoxetine users may experience agitation, sleeplessness, and anxiety. The antagonistic effects of 5HT_{2C} are assumed to be the reason for this. Patients who are taking Fluoxetine may have a panic attack as a side effect. Thus, educating the patient is the clinician's responsibility. The overwhelming majority of adverse consequences manifest immediately and diminish with time. Therefore, it is wise to wait until the negative symptoms pass before changing the treatment plan. The bulk of negative effects are based on dosage and duration.

Caution is warranted at the onset of agitation or activation since it may be a sign of bipolar disorder requiring the addition of a medication that stabilizes the mood or an atypical antipsychotic.

If you have trouble going to sleep, you might consider taking your dosage of Fluoxetine in the morning since it has an energizing effect. If the patient has too many undesirable side effects, the dose may be reduced. Patients should be informed of possible side effects; if they persist after a few weeks, it may be essential to switch to a different antidepressant (Alshaya, 2022). Prior to undergoing augmentation measures, it is suggested to explore other options such as trying different antidepressants. The implementation of this particular technique has the potential to mitigate the phenomenon of polypharmacy and improve adherence to psychiatric medications. For insomnia, you can consider trazodone, mirtazapine, or a hypnotic. Furthermore, Mirtazapine may be utilized as a therapeutic intervention for managing symptoms of agitation or gastrointestinal side effects. Anxiety may be treated with benzodiazepines. Sexual dysfunction may be treated with bupropion or a PDE inhibitor (such as sildenafil). For possible cognitive slowdown or apathy associated with Fluoxetine, bupropion may also be an alternative.

Contraindications

Fluoxetine hypersensitivity, or to any ingredient in its composition

MAOI inhibitors are used to treat mental health conditions (After two weeks of stopping MAOI medication, doctors shouldn't start Fluoxetine therapy). Never begin therapy with Fluoxetine in a patient who is on linezolid. Those who have had seizures in the past should take this drug cautiously. Those who are elderly should be dosed carefully. In addition, Fluoxetine users are cautioned to watch out for suicidal thoughts, especially in young children and adults between the ages of 18 and 24 (Steinert et al, 2014). In the first several weeks after beginning a new medication, parents and caregivers should be strongly reminded to keep a close eye on their children for any behavioral changes. While pregnant, Fluoxetine should not be taken. Nonetheless, depending on the situation, counselling could be needed when pregnant. There has to be a careful weighing of the pros and cons of using Fluoxetine while pregnant. Early prenatal Fluoxetine exposure may increase the risk of septal heart anomalies. While this relationship is not conclusive, using the medicine beyond the 20th week of pregnancy is linked to the fetus developing pulmonary hypertension. Preeclampsia and pregnancy related hypertension risk may rise if Fluoxetine is used throughout the third trimester of pregnancy. In addition, very minute levels of the drug were detectable in breast milk.

Monitoring

Particularly at the start of treatment or whenever dosages are modified, a comprehensive evaluation of depression, suicide risk, mania/mood liability, social interaction, anxiety/panic episodes, and serotonin syndrome symptoms should be carried out (Steinert et al., 2014).

Regular laboratory testing is not necessary for healthy individuals. Nonetheless, they may ask for blood glucose and liver function testing in elderly or members of a certain group. Prescribers may also ask for an ECG analysis for individuals who have ventricular arrhythmias and QT prolongation risk factors. Pediatric patients should have regular height and weight checks while using Fluoxetine .

Toxicity

When used in monotherapy, Fluoxetine overdose is seldom lethal. Nevertheless, it could cause ataxia and respiratory depression if coupled with alcohol. Intense dosing or co-administration with other serotonin-raising medicines may bring on serotonin syndrome, a group of symptoms including changes in mood and behavior as well as problems with the body's internal systems and the muscles (Burcusa & Iacono, 2007). Fluoxetine also reported to cause suicidal behavior. This mechanism is basically caused by the increased level of 5-Hydroxyindoleacetic acid (5-HIAA) i.e., metabolite of serotonin which in turn decline the serotonin level in post synaptic terminal and disturb the serotonergic system (Crundwell, 1993). SSRI overdose treatment is the goal of supportive therapy. A few examples of this support are airway preservation, frequent ECGs to monitor for cardio toxicity, benzodiazepine injections for drowsiness, and Gastrointestinal cleaning with activated charcoal. Serotonin syndrome may be treated with cyproheptadine (Angst, et al., 2009; Carey, 2016).

1.9.2 Herbal drug combination

A significant global health issue is posed by neuropsychiatric and neurodegenerative illness like dementia, PD, schizophrenia, epilepsy, mood disorders, and anxiety, which are also accompanied by a heavy illness, death, and physical complications, high financial costs, and a poor quality of life. There has been a recent increase in interest in traditional remedies on a worldwide scale, and phytomedicines are highly regarded by researchers because of their natural source and lack of negative side effects. Contrarily, it has been noted that traditional synthetic medications have unfavorable but unavoidable side effects and suffer from low patient compliance. Since they are a more effective treatment for many brain illnesses than

synthetic medication treatments, herbal remedies are chosen. Ayurveda offers a comprehensive approach to therapy in addition to a number of nootropic plants with multifaceted bioactivities in a range of illnesses. There is sporadic material available on traditional Ayurveda treatments for different mental diseases.

The current review includes: (i) prevalent brain conditions and the alterations they cause Ayurveda single herbs and polyherbal mixtures with descriptions of their traditional administration and use are significant Ayurvedic treatments. (ii) Ayurvedic holistic approach to treating neurodegenerative and depressive illnesses. It also provides a platform for further research and standardization of Ayurveda nootropic botanicals.

Antidepressants and suicide: ecological studies' reports on the connection

On a national level, antidepressant therapy may or may not reduce the likelihood of suicide or suicide attempts. According to research, the majority of Nordic nations and the United States have experienced modest overall declines in suicide rates over the past ten years. These declines have varied by sex and age groups (Isacsson, 2000; Grunebaum, et al., 2004; Baldessarini et al., 2007; Tómas, Helgi, & Tómas, 2004; Gibbons, et al., 2005; Parker et al., 2006; Ludwig & Marcotte, 2005; Sondergard, et al., 2006; Reseland, et al., 2006). But, analogous trends, including a drop in suicide rates, were also seen in the United States and a few Scandinavian countries a decade before Fluoxetine 's launch, the first clinically effective antidepressant of the twenty- first century (Baldessarini, et al., 2006; Reseland, et al., 2006; Sebestyen et al., 2010) examined the relationship between trends in antidepressant prescriptions and Rates of suicide in Hungary from 1998 to 2006 using a seasonality index and temporal trend analysis. (The level of suicide rates' seasonality). They discovered that only among guys who took more antidepressants did the seasonality of suicides significantly decrease. Just nine out of 29 ecological studies, according to recent research by Baldessarini et al., found significant negative associations between rising use of contemporary antidepressants and lowering trends in suicide rates (Baldessarini, et al., 2006). Six researchers found no connection despite the fact that (Nordstrom, et al., 1995) discovered intermittent associations between sexes or age groups. 54% of the 78 countries that gave information on suicide rates during the 1950s, when antidepressants were not yet available, and the early 2000s, a decade following their introduction, reported decreases, while 46% recorded increases. The trends in each region's suicide rates were also erratic (Asia, Western and Eastern Europe, and South America). From 1990 and the beginning of the 2000s, there was a comparable distribution of rising and falling suicide rates across all

countries (Ajdacic-Gross et al., 2008). According to these numbers, the growing suicide rate is a highly complicated issue caused by a variety of underlying variables. In Hungary, where there are around 10 million people, (Grossman & D'Augelli, 2007) examined the annual rates of antidepressant prescriptions and suicide rates between 1999 and 2005.

They found a negative correlation between the prevalence of suicide and growing antidepressant use among both men and women. Even after adjusting for a number of factors include jobless and divorce rates, the rate of antidepressant prescriptions, and the prevalence of alcohol consumption, the suicide rate remained correlated. Given a variety of restrictions, ecological research should be considered. First off, despite better accessibility to contemporary psychopharmacological therapy, it is likely that rising suicide rates are caused by advances in public health, modifications to social, cultural or legislative bans against suicide, as well as advancements in suicide detection and reporting. Socioeconomic variables, such the availability of healthcare and social support, might potentially affect the intricate findings of ecological research. Last but not least, the main problem with correlational research is that it is unable to establish a causal link between exposure to certain substances or other circumstances and a person's suicidal behavior (Harris, 2015). Unlike ecological research, however, demonstrate a direct, causal connection between antidepressant medication and decreased suicide risk at the individual level, more research using more accessible approaches is necessary (Pompili et al., 2010; Al-Harbi, 2012; Cuijpers, et al., 2020).

Flavonoids

Flavonoids, like fruits, cereals, vegetables, alcohol, and tea contain, can either prevent or reverse the consequences of stress (Harborne & Williams, 2000). Much research have been undertaken in current years to investigate the antidepressant properties of natural chemical substances, particularly flavonoids, which have diverse effects on the brain (Bakoyiannis et al., 2019). Certain flavonoids have been reported to restore depressed behavior in rodents in animal models, according to a few preclinical investigations. The proposed underlying mechanisms for anti-depressant activity include increased levels of different neurotransmitter expression, neurotrophic factors, and neurogenesis in the brain (German-Ponciano et al., 2018). We have concentrated on the ability of certain flavonoids to alleviate depression in this review and have described the route of activity found on pre-clinical research. The goal of the current appraisal is to gather possible bit with good antidepressant activity from the literature, which will aid in the development of efficient and secure nutraceuticals products to treat depression in people.

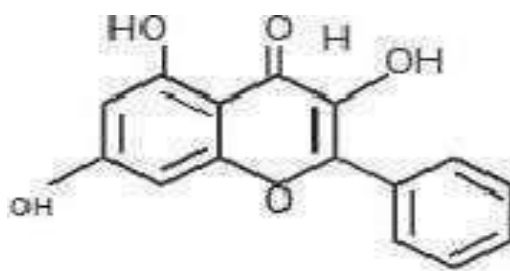


Fig 1.4: Structural representation of Galangin

Galangin

The development of breast cancer cells is inhibited by Galangin, a 7-hydroxyflavonol containing extra hydroxyl groups at positions 3 and 5, respectively. *Alpinia onchigera*, *Populus koreana*, and other species for which data are available all contain the natural substance Galangin. Galangin, also known as 3, 5, 7-trihydroxyflavone or 3, 5, 7-trihydroxy-2-phenyl, is a polyphenol that is primarily derived from a number of medicinal plants, including *Alpinia officinarum* Hance, *Alnus pendula* Matsum, and *Plantago major* L. (E umkeb, et al., 2010). It has been since long time from Asian cultures as a traditional remedy for diabetes, diarrhea, digestive problems, coughs, and colds (Aloud, et al., 2018). Galangin is the substance that provides propolis or "bee gum" with its antibacterial qualities. Propolis is often used in a variety of cosmetic products, such as toothpastes, gargles, face creams, creams, sugarless gum, elegance, and colours, as a result of its antioxidant properties, anti-inflammatory, astringent, and local anesthetic characteristics (Abbasi et al., 2018). Also, it act with mitochondrial enzyme and exerts neuroprotective effect (Jazvinscak Jembrek, 2023). Galangin is a flavanol derivative. The insufficient water solubility of Galangin is an issue that other flavonoids also have (Patel, et al., 2012). It is the most lipophilic of the flavonoid chemicals, which also include quercetin, kaempferol, morin, and myricetin (Kumar & Pandey, 2013). Galangin's chemical makeup is hypothesized to have antimutagenic, enzyme-regulating, and antioxidant properties (Mukherjee et al., 2021).

Galangin's bioavailability and kinetics

Regarding the distribution and absorption of Galangin, no information is available. Galangin is believed to be transformed in the liver to the antioxidants kaempferol and quercetin (Tuli et al., 2022). Galangin is converted to kaempferol in microsomes from human liver starting at the 40- position. This process is catalysed by the cytochrome enzymes CYP1A2 and CYP2C9 (Otake & Walle, 2002). Galangin is glucuronidated and sulfatated seven times more often than it is oxidised, according to certain research. The liver's UGT 1A9 isoform

seems to be principally in charge of catalyzing the very effective glucuronidation. It is interesting to notice that the 7- position of Galangin was glucuronidated by UGT1A9, UGT1A1, and UGT2B15 (Otake & Walle, 2002). Galangin and other flavonoids were glucuronidated with a high degree of efficiency, which may indicate interactions with other chemicals, medicines, and carcinogens. Typically, Galangin and its metabolites are eliminated in faeces (Zhao et al., 2022).

Health and Galangin

Galangin may be able to prevent mutagenesis, function as an antioxidant, and prevent muscular contractions. In human liver microsomes, cytochrome p450 hydroxylase inhibition and metabolic enzyme modulation actions have also been described. According to reports, Galangin promoted the transcription of COX-2 and messenger RNA (mRNA) during an inflammatory response and inhibited the growth of bacterial cells and tumors.

1.9.5 Allicin

The aroma and flavour of freshly cut or crushed garlic are attributed to Allicin, a naturally occurring sulphur-containing molecule having many positive biological effects. One of the reasons for their expanding consumer demand as well as their growing usage in health and agriculture is the prevalent, if unfounded, notion that natural goods are gentle and essentially safe in contrast to their chemically manufactured competitors (Borlinghaus, et al., 2014). Allicin is an important defense molecule in Garlic known for its anti-cancer, antioxidant, anti-inflammatory, neuroprotection, cardioprotective, hepatoprotective property (Savairam, 2023). Despite the pharmaceutical industry's current emphasis on the basis of extensive biochemical testing techniques for the development and discovery of novel medications, natural compounds have a long history of usage in medicinal and antibacterial applications. For example, the earliest medical source still in existence may include evidence of the medicinal and preventative usage of varieties of plant and plant-derived chemicals.

This ancient Mesopotamian compendium contains instructions for treating common diseases and parasite problems using *Papaver somniferum* (poppy juice), *Glycyrrhiza glabra* (liquorice), Cedrus species, etc. At 2600 BC, it was penned in cuneiform on clay tablets. The Chinese Materia Medica, Greek "magical papyra," and Egyptian Codex Ebers are only a few of the ancient works that include significant documentation the medicinal use of plants and plant extracts (Ebers Papyrus). Virgil, a well-known a poet who lived in the first century and was Roman BC, emphasized the use of garlic extracts in the management of snake bites in his Second Idyll, despite the fact that the "magical papyri" and the Ebers

Papyrus clearly mentions their usage for therapeutic reasons. In his Corpus Hippocraticum, the renowned Greek physician Hippocrates discusses their effectiveness in curing pneumonia and wounds. Although garlic, the more potent sibling of onions and one of the most eaten foods globally, is well known for its dynamic interaction with human olfactory receptors, Onions are one of the most eaten foods and the most popular Allium. From its early usage as a remedy for dog bites and a medication for vampire bites to its more modern use by Greek midwives to ward off "the evil eye" in labor rooms, garlic's voyage Over and again throughout history has been both colorful and eventful. There are several possibilities on the domestication of the Allium genus, which includes garlic (*Allium sativum*), leeks (*Allium porrum*), onions (*Allium cepa*), and chives (*Allium schoenoprasum*), as well as about 700+ additional species. However, the situation is still unclear (Borlinghaus, et al., 2014), despite initial reports from Turkmenistan, Afghanistan, Kazakhstan, Kyrgyzstan, Pakistan, Tajikistan, and Uzbekistan.

It wasn't until 1944 that Cavallito and Bailey (Cavallito, et al., 1945) Allicin, the chemical liable intended for garlic's pungent odour, was isolated and its qualities characterized. This discovery led to decades of more research and improved scientists' understanding of the chemical miracle that nature had preserved in the composite bulbs of this exquisite Allium (Bhatwalkar et al., 2021)

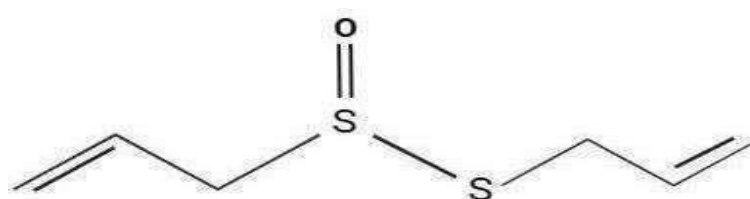


Fig 1.5: Structural representation of Allicin Biosynthesis of Allicin

In 1948, Stoll and Seebeck discovered the thio sulfinate structure of Allicin. (Stoll & Seebeck, 1948). In the natural world, an enzymatic activity that damages the plant tissue results in the production of Allicin. The non-proteinogenic amino acid alliin (S-allyl-L-cysteine sulfoxide) is the precursor of Allicin (Granroth & Sarnesto, 1974). The enzyme alliinase catalyses the hydrolysis of alliin as well as S-alkyl-L-cysteine sulfoxides, resulting in the formation of dehydroalanine and allyl sulfenic acid. Two allyl sulfenic acid molecules spontaneously join to generate Allicin. Both garlic (*Allium sativum*) and ramsons (*Allium ursinum*) contain the compound known as alliin (Stoll & Seebeck, 1948). The onion

(*Allium cepa*) only produces isoalliin (Trans-(+)-S-(1-propenyl)-l-cysteine sulfoxide), not alliin (Artturi & EJ, 1959). It is still unclear how alliin is biosynthesized. Up to this point, no one has improved on Granroth's ground-breaking work (Granroth & Sarnesto, 1974), which described two potential biosynthetic routes based on radioactive labelling tests.

2. REVIEW OF LITERATURE

An exhaustive literature work was done on different topics as per given below:

2.1 Depression

Author	Year	Title	Findings
Liu	2023	The impact of aerobic exercise on depressive-like behavior and the TLR4/NLRP3 pathway in the CA1 area of the hippocampus in mice subjected	The findings indicate that exercise has the potential to enhance depression-like symptoms in mice with chronic unpredictable mild stress (CUMS) via modulating the TLR4/NLRP3 inflammatory signaling system.
Lin	2023	Latest updates on the serotonergic system in depression and anxiety	Authors reported about new therapeutics developed during recent years against psychiatric disorders, including non-competitive NMDA receptors, anti-cholinergic
Patil	2023	Targeting inflammatory pathways for major depressive disorder therapy	This article discusses the role of inflammation in major depressive disorder and its connection to the immune system. It explores the impact of cytokines, damage associated molecular
Rajssouni	2023	Correlation between the use of antidepressants and the suicide risk among infants and adolescents	The results revealed that in children and adolescents, antidepressant especially SSRI exposure, substantially increase the risk of suicide and suicide attempts compared to no antidepressant use.

Alqurashi	2022	The Impact of Chronic Unpredictable Mild Stress- Induced Depression on Spatial, Recognition, and Reference Memory Tasks in Mice: Behavioral and Histological Study	CUMS is frequently used animal model for depression. In this paradigm, animals are subjected to continuous and unexpected stressors of both environmental and psychological nature, aiming to replicate the stresses encountered in everyday human life.
Abdoli	2022	The worldwide incidence of major depressive disorder (MDD) in the elderly: Ameta- analysis and systematic review	The study analyzed the global prevalence of major depressive disorder in the elderly, revealing a 13.3% prevalence. The study found that elderly women had an 11.9% prevalence of major depression, while men had a 9.7% prevalence. Australia had the highest prevalence at 21.1%, followed by Europe at 12.9%.
Liu	2022	Chronic stress and smoke exacerbated changes in A/J- rodents that resemble depression and the expression of lung cancer factor; these alterations involve inflammation and BDNF dysfunction	Epidemiological surveys show that cancer patients are more likely to experience depressive symptoms, which is positively correlated with the high incidence of cancer and low survival rates, especially lung cancer. CUMS or smoke can decrease sucrose consumption and increase immobility time which are exacerbated by stress +smoke. CUMS decreased brain derived neurotropic factor (BDNF), while CUMS or smoke increases tyrosine kinase B and p75 concentrations, which are exacerbated by stress +smoke.

Greenberg	2021	The Economic impact of major depressive disorder on the adult population in the United States between 2010 and 2018.	This research article reported that the financial burden of individuals MDD increased by 37.9% apart from each other
Li	2021	The global prevalence of major depressive disorder (MDD) among the elderly: A systematic review and meta- analysis	Depression is a multifactorial disease involving social, psychological, and biological factors. The most accepted hypothesis is BDNF, which is reduced by stress and attenuated by antidepressants. BDNF like effects have been reported by agents involved in BDNF system.
Ignacio	2019	Physical exercise	Physical exercise can trigger MDD by increasing PGC1 α gene expression, a transcriptional co- activator that reduces pro- inflammatory cytokine synthesis and release and increasing anti- inflammatory cytokine release. Studies have shown that exercise can also alter monoaminergic neurotransmission circuitry by releasing Proinflammatory cytokines.
Milenkovic	2019	The involvement of chemokines in the pathogenesis of major depressive disorder	This review summarizes experimental and clinical studies on the role of chemokines in the CNS and their regulation of the inflammatory response. The CX3CL1 chemokine and its

			receptor, CX3CR1 modulate microglial inflammatory responses by reducing TNF- α and NO levels. Clinical studies show that chemokines regulate neurobiological processes relevant to psychiatric disorders, and dysregulation of chemokines could contribute to the pathophysiology of MDD.
Pan	2019	Cognitive impairment in major depressive disorder	Zihang Pan and colleagues highlighted the cognitive impairments associated with MDD, which significantly impact functional disability. Improving cognitive function could improve psychosocial and workplace outcomes. However, current clinical paradigms and a lack of mechanism-oriented drug development hinder cognitive recovery. Further research is needed to understand neural substrates and metabolites in cognitive symptoms.
Juruena	2018	A biomarker for atypical and non-atypical depression: Does the function of the HPA axis play a role in this regard? An exhaustive review	According to this article, major depression is a significant healthcare issue in the 21 st century, contributing to years of disability worldwide. Stress response system abnormalities are linked to depression, with melancholic depression causing increased cortisol levels and atypical depression affecting decreased HPA axis activity. Differences in HPA axis

			function between melancholic and atypical depression are stronger with stronger associations with biological or vegetative symptoms.
Kennedy	2018	Unpacking major depressive disorder: From Classification to treatment selection	The text discusses the limitations of current diagnostic approaches in mental illness, particularly major depressive disorder (MDD), and suggests the use of precision medicine and biomarkers. The text emphasizes the need for personalized and precise approaches to diagnosing and treating mental illness.
Belzung	2015	Depression: From Psychopathology to Pathophysiology	The study investigates the link between abnormal spontaneous brain activity and cognitive function in major depressive disorder. Results suggest that cognitive deficits in MDD may be linked to abnormal spontaneous brain activity. XYS can inhibit chronic-stress induced miR-200a/b-3p expression regulate signaling and reduce stress-related behaviors.
Mann	2013	The serotonergic system in mood disorders and suicidal behavior	This article presents a stress-diathesis model of suicidal behavior, integrating clinical and neurobiological components. It identifies a deficiency in serotonin input in brain areas like the anterior cingulate and ventromedial prefrontal cortex, linked to suicidal and non-fatal suicidal behavior.

2.2 Herbal Drug Formulations for Anti-Depressant Activity

Author	Year	Title	Findings
Fadzil	2023	The capacity for application of honey as a neurologically protective agent for the management of neurodegenerative diseases.	The text explores the potential use of honey as a neuroprotective agent for managing neurodegenerative diseases. It discusses various studies on honey's effects on neurodegenerative diseases including <i>in-vitro</i> and <i>in-vivo</i> studies. Active compounds in honey such as TUH, THH, quercetin and gallic acid have shown promising neurodegenerative agents. Galangin has potential as a pharmaceutical medication or food supplement for treating neuroinflammatory processes.
Jikah	2023	Mechanism of Action by sulphur compounds in <i>Allium Sativum</i>	Several sulphur compounds found in <i>Allium Sativum</i> have been identified as the source of its therapeutic properties, which include anti-bacterial, neuroprotective, anti-viral, fungicidal etc. These compounds are diallyl disulphide, Allicin, diallyl trisulphide, ajoene, vinyl-dithiin, micronutrient selenium, and S-allyl cysteine.

Yener	2023	Effect of Chronic Unpredictable Mild Stress on Hippocampus and Serum Markers in Rats	The result examined the impact of CUMS on rodent hippocampus morphology and serum BDNF GFAP markers. Result showed changes in the gyrus dentatus region crucial for memory function, under stress conditions. The study also revealed an increase in cell count in the gyrus dentatus peripheral blood lymphatic system (PCL) in the stress group.
Zhang	2023	The therapeutic potential and pharmacological activities of Galangin, an emerging natural flavone is age-related maladies.	This review discusses the effects and mechanisms of Galangin in treating age-related diseases. The accumulated data showed that Galangin has various pharmacological activities in treating neurodegenerative diseases.
Huang	2022	Garlic essential oil mediates acute and chronic mild stress-induced depression in rats <i>via</i> modulation of monoaminergic neurotransmission and brain-derived neurotrophic factor levels	GEO and DADS significantly increasing the 5-HT and DA levels, with no hippocampal effects. Chronic GEO treatment increased hippocampal brain-derived neurotrophic factor (BDNF), the BDNF-related signaling pathway

Tripathy	2022	Evaluation of Anti-Depressant Potential of Medicinal Ghrita.	The study collected fresh leaves from various plants and used ghee from a local dairy farm to create Brahmi Ghrita, an ancient medicinal herb with varying compositions and therapeutic claims. The results showed that Ghrita has significant antidepressant potential, making it a potential nutraceuticals or adjuvant medication for improving mental health.
Yadav	2022	Chemo profiling of Ashvagandharishta and its antidepressant activity using high performance thin layer chromatography	Ashvagandharishta, a traditional herb, was analyzed using HPTLC and found to have high levels of withanolide-A and β -Sitosterol. It also showed an antidepressant effect and antioxidant properties, making it a potential treatment for depression.
Iftikhar	2022	Potential Therapeutic Benefits of Honey in Neurological Disorders: The Role of Polyphenols	Honey has been recognized as a powerful medicine for treating neurological disorders due to its abundance of antioxidant and polyphenols.

Chen	2022	The potential of quercetin and its glycoside derivative as antidepressants	The present review provides an in-depth review of the available information regarding the pharmacological use of quercetin as an alternative therapy for the management of depression. The methods by which quercetin modulates neurotransmitter levels, enhances hippocampus neuron regeneration, improves HPA-axis dysfunction, and lowers anti-inflammatory states and oxidative stress were explained by the authors.
Kabra	2022	An examination of the mechanistic aspects underlying the therapeutic effects of nano-formulated plant polyphenols on depression.	A condition characterized by apathy towards activity and a depressed mood, depression restricts daily activities, impairs quality of life and induces behavioral issues. SSRIs are Selective Serotonin Reuptake Inhibitors, conventional treatments for depression include tricyclic antidepressants (TCAs), monoamine oxidase (MAO) inhibitors and atypical antidepressants. Standard antidepressants are associated with several limitations including adverse effects on cholinergic function, interactions between drugs and foods and drug-drug interactions. These concerns have compelled researchers to explore alternative strategies for managing depression. Utilizing plant derived polyphenols comprise a significant

			<p>category of compounds that are widely dispersed throughout plants. The purpose of this study is to provide a concise summary of the chemical, pharmacological and neurological evidence that polyphenols may have a therapeutic effect on depression.</p>
Zhao	2022	<p>Is it feasible to utilize dopamine receptors as a therapeutic target in the treatment of depression?</p>	<p>Research has focused on the involvement of dopamine receptors in depression, particularly the role of dopamine receptor D1-heterodimers. The brain regions involved in depression include the ventral tegmental area, nucleus accumbens, prefrontal cortex, and limbic network. The role of dopamine receptor heterodimers and their downstream signaling events may provide new opportunities for depression treatment.</p>
Peng	2022	<p>Review of Herbal Medicines for the treatment of Depression</p>	<p>The primary emphasis of this review was on herbal medicines that have demonstrated clinical effects. It provided descriptions of herbal medicines for of antidepressant efficacy is relatively robust, excluding results from randomized controlled trials.</p>

Nadeem	2021	Allicin, a neuroprotective and antioxidant compound ameliorates activity.	This review reported that Allicin, an antioxidant and neuroprotective agent, ameliorates cognitive impairment. Allicin, a compound found in garlic, has been studied for its antioxidant and neuroprotective properties
Asif	2021	The hydro alcoholic extract of <i>Cissampelos pareira</i> Linn. exhibits anti-depressant, anxiolytic, and muscle relaxant properties. The topic of discussion pertains to leaves.	The article reported that the study found that <i>Cissampelos pareira</i> L. has traditional medicinal uses for psychopharmacological disorders. The extract showed anxiolytic, antidepressant, and muscle-relaxant effects in mice.
Chai, 2021	2021	Zhi-zi-chi decoction's antidepressant effect on CUMS mice and the discovery of its signaling route	The findings suggested that the potential antidepressant properties of ZZCD could be attributed to alterations in the Metabolic pathway comprising PKACREB-BDNFTrKB-PSD-95.

Zhang	2021	Natural volatile oils extracted from herbal remedies: an auspicious therapeutic approach to address depressive disorder	Depression is a common mental disorder that is increasing in incidence, and while there is a lack of effective drugs, some natural volatile oils, such as <i>Lavender</i> and <i>Acorus tatarinowii</i> , have been found to have good antidepressant effects with minimal side effects.
Rani	2020	Evaluation of antidepressant activity of <i>Calotropis gigantea</i> by using experimental rats.	The aqueous extract of <i>Calotropis gigantea</i> leaf has shown promising results in experimental depression and can be used as a potential adjuvant in the treatment of depressive disorder. The extract possesses antidepressant activity and contains alkaloids, flavonoids, saponins, phenolic compounds, and triterpenoids.
Ahmad	2020	Medicinal plant-based functional foods for the management of neurological health.	The article reports that neurological disorders are a major global health concern, affecting millions of people. There is growing interest in using plant-based functional foods and bioactive phytochemicals for the prevention and treatment of these disorders. Phytochemicals from fruits, vegetables, herbs, and spices have been shown to have immunomodulatory and anti-inflammatory effects on brain.

Qi	2019	The nasal administration of curcumin-loaded guanidine-chitosan thermo-sensitive hydrogel and its potential anti-depressant effect	This study investigated the use of curcumin through nasal delivery and found that a curcumin/hydroxypropyl- β -cyclodextrin complex was obtained. The study also showed that a thermo-sensitive hydrogel delivery system could be promising formulation for the treatment of depression.
Li	2012	By enhancing the blood flow in the regional cortex, mitigating mitochondrial malfunction, and inhibiting sequential apoptosis. Galangin exhibits promising neuroprotective properties following the occurrence of acute ischemic stroke.	The findings of the study indicated that Galangin mitigated neurologic deficits, prevented cerebral infarction 24 hours following occlusion of the middle cerebral artery, and protected the mitochondria by reducing the production of reactive oxygen

2.3 Flavonoids Herbal Drug Combination with Different Drugs

Author	Year	Title	Findings
Melrose	2023	The Potential of flavonoids and flavonoid metabolites in the therapeutic management of neurodegenerative pathology in cognitive decline disorders	Flavonoids are a diverse group of compounds with various health benefits including antioxidant and anti-inflammatory properties and they have potential as therapeutic agents in biomedicine. The gut microbiome plays a role in generating bioactive flavonoid metabolites, which have positive effect on brain health and neurological disorders
Ramezani	2023	A review of clinical and preclinical studies on the potential role of flavonoids in the prevention and/or treatment of cognitive dysfunction, learning, and memory deficits	This article reports that flavonoids have shown potential for preventing or treating cognitive deficits, learning and memory loss, and neuroinflammation. Flavonoids contribute to these improvements through the regulation of neurotransmission systems and the enhancement of neurogenesis, synaptic plasticity, and neuronal survival.
Firoozeei	2021	A double- blind, randomized comparison of lavender and dodder herbal syrup and citalopram in the treatment of major depressive disorder with anxious distress	The efficacy and tolerability of the herbal syrup as a supplement for the treatment of major depressive disorder with anxious distress have been demonstrated.

Devi	2021	Flavonoids: Neurodegenerative disease therapy candidates	This review offers an analysis of the potential involvement of flavonoids in the cellular stress response, which serve to impede the development of neurodegenerative diseases.
Pannu	2021	Flavonoids' emerging utility in the management of depression	Based on pre-clinical experiments, the article discussed flavonoids' antidepressant properties and mechanisms by increasing neurogenesis, neurotransmitter, and BDNF, flavonoids can fight depression.
Prasanna	2021	The Potential of Flavonoid- Based Nano medicines in the Therapeutic Treatment of Alzheimer's Disease: Current Progress and Future Prospects	Alzheimer's disease is characterized by cognitive decline due to the accumulation of amyloid beta-42 and neurofibrillary tangles. Flavonoids have potential therapeutic benefits for Alzheimer's disease due to their antioxidant, anti-inflammatory, and anti-amyloidogenic properties.
Ravula	2021	Fisetin: A Comprehensive Review of its Potential as a Flavonoid Compound for the Treatment of Neurological Disorders	The author reported that fisetin, a flavonoid found in plants, has shown beneficial effects in preclinical models of various neurological disorders. Fisetin has been studied for its potential in treating Alzheimer's disease, Parkinson's disease, stroke, depression, etc.

Kansara and Jani's	2017	Possible interactions between garlic and conventional drugs: A review	Herbs can interact with drugs, which is especially for drugs with a narrow therapeutic index, as herbal medicines often contain a mixture of active constituents that can affect the ADME of drugs as well as their pharmacodynamics. This article focuses on the interaction between garlic and conventional drugs.
Ren	2016	The synergistic anti-cancer properties of Galangin and berberine in oesophageal carcinoma cells are achieved by inducing apoptosis and inhibiting proliferation.	The combination of Galangin and berberine has been shown to have a synergistic effect in oesophageal carcinoma cells, inhibiting cell growth, including apoptosis and cell cycle arrest and suppressing the expression of Wnt3a and β -catenin. This combination also inhibited tumor growth in mice without causing toxicity, suggesting it could be a promising treatment for esophageal carcinoma patients.
Srinivas	2015	Flavonoids' preclinical drug–drug interaction trends—case studies, challenges, and viewpoints	The consumption of herbal products and health supplements containing flavonoids is increasing due to their health benefits, and there is potential for combining flavonoids with anticancer drugs to improve their effectiveness and reduce dosage. This review discusses recent trends in areas such as oncology, immunosuppression, hypertension and provides a framework for designing pre-clinical studies.

Alissa	2014	Medicinal herbs and therapeutic drugs interactions	The Concurrent use of botanicals and medications may compromise the efficacy of both, presenting an issue for medical professionals and a risk to patient safety.
Jager & Saaby	2011	Flavonoids and the CNS	Flavonoids, which are found in plants and consumed by humans, have various effects on health and mood, including protection, anti-inflammatory properties, and the ability to bind to receptors in the central nervous system.
Dhingra	2008	Evidence for the involvement of monoaminergic and GABAergic systems in anti-depressant like activity of garlic extract in mice.	According to previous study garlic significantly improved the monoaminergic and GABAergic system in mice.

2.4 Fluoxetine and Their Herbal Combinations

Author	Year	Title	Findings
Yan	2020	Co-administering <i>Chaihu Shugan San</i> and Fluoxetine induces cognitive enhancement and anti-depressant-like effects; these effects are dependent on the BDNF-ERK-CREB Signaling pathway in hippocampus and prefrontal cortex.	The article found that both <i>Chaihu Shugan San</i> and the co-administration of CSS and FLU play an antidepressant role, which may be due to the regulation of the BDNF/ERK/CREB Signaling pathway in the hippocampus and frontal cortex. different doses having different effects. The LC-MS/MS method was used to analyze the plasma concentration of FLU and NOF in rats.

Zakerin	2019	Antidepressant effect of a polyherbal syrup based on Iranian traditional medicine	A polyherbal syrup containing various plants was formulated and found to be stable, with potential antidepressant activity demonstrated in mice.
Ahmed-F	2016	In an acute mouse depression model, resveratrol and Fluoxetine prevent oxidative DNA breakage and monoamine breakdown	Combining half the dose of resveratrol with Fluoxetine showed antidepressant activity comparable to Fluoxetine alone, suggesting the potential use of RSV and FLX combinations in Treating depression.
Kaur	2015	Assessment of the antidepressant properties of <i>Moringa oleifera</i> : A single ingestion and in combination with Fluoxetine	Combining modest doses of MOE with Fluoxetine or other SSRIs appears to have promising potential, according to the findings of this

2.5 Anti-Depressant Activity

Author	Year	Title	Finding
Jazvinscak	2023	The Mechanism of Action of Flavonols: Mitigating Neuroinflammation and Oxidative Stress in Major Depressive Disorder	According to the author, flavonoids play a positive role in maintaining the neuroendocrine control of the HPA axis, promoting neurogenesis, as well restoring monoamine depletion which further alleviate depressive like behavior.

Wenqi	2019	Allicin attenuated chronic social defeat stress induced depressive-like behaviors through suppression of NLRP3 suppression.	Allicin, a primary bioactive component obtained from garlic has been previously shown to have several pharmacological properties. Various natural dietary products including Allicin have been shown to have anti-inflammatory and neuroprotective properties. It has ability to penetrate blood-brain barrier leading to neuroprotective effects in the brain.
Mundugaru	2018	Neuroprotective Functions of <i>Alpinia galanga</i> in Forebrain Ischemia Induced Neuronal Damage and Oxidative Insults in Rat Hippocampus	The rhizomes of <i>Alpinia galanga</i> sometimes known as larger alangal, are often used as a replacement for ginger in meals. Traditionally, it has been used as nervine tonic and stimulant. <i>Alpinia galangal</i> treatment shows promise as a protective agent against forebrain ischemic brain damage.
Sohrabi	2017	Repeated systemic cinnamon essential oil treatment reduces anxiety and depression in mice.	The study found that cinnamon essential oil particularly its main component trans cinnamaldehyde, may have anti-depressant and anti-anxiety effects making it potential adjuvant therapy for depression and anxiety disorders.
Singh	2011	Neuroprotective efficacy of <i>Alpinia galanga</i> (L.) fractions on mice with A β - induced amnesia.	Various fractions of <i>Alpinia galangal</i> have shown an anti-amnesic effect, with the chloroform fraction being particularly effective.

3. AIM AND OBJECTIVES

- **Aim of research work**

Impact of administration of Fluoxetine with Galangin and Allicin against mild stress induced major depressive disorder in rodents.

- **Objectives of research work**

There were the following objectives to complete the proposed aim.

1. To evaluate the antidepressant effect of Galangin and Allicin alone and in combination.
2. To estimate the impact of co-administration of Galangin and Allicin with Fluoxetine on pharmacokinetic parameters.

3.1 Research gap

- Many patients suffered with MDD may discontinue anti-depressant drugs due to their serious adverse events such as incidence of suicidal risks, aggressive behavior. Fluoxetine as selective serotonin reuptake inhibitor (SSRIs) also has a tendency to cause suicidal behavior that causes a negative impact in depressed patients.
- Galangin is a flavonoidal drug derived from *Alpinia officinarum* that has tendency to maintain serotonergic system which further reduce suicidal tendency.
- Allicin is also a flavanoidal drug derived from *Allium Sativum* that significantly inhibits MAO-A and MAO-B levels resulting the enhanced monoaminergic transmission such as serotonin in brain.
- Hence, when given the Galangin and Allicin in combination with Fluoxetine, it decreases the suicidal tendency caused by SSRI and also works in widespread manner against major depressive disorder.

3.2 Plan of work

- Review of literature, designing of research work & procurement of animals & chemicals
- Identification and characterization of drug with the help of
 - ✓ Physical Evaluation
 - ✓ Solubility
 - ✓ Melting Point
 - ✓ UV Spectroscopy
 - ✓ FTIR Spectroscopy
- Development of MDD-induced model
- Depression assessment utilizing many factors like
 - ✓ Sucrose preference
 - ✓ Forced swim,
 - ✓ Actophotometer,
 - ✓ Open field test
 - ✓ Hyponeophagia test
 - ✓ Clonidine-induced aggression.
- *In-vivo* pharmacokinetic study
- Evaluation of antidepressant activity through neurochemical estimation
 - ✓ Estimation of Brain BDNF
 - ✓ Estimation of Brain Dopamine
 - ✓ Estimation of Brain Serotonin
 - ✓ Statistical Analysis and Compilation of the data and thesis

4. MATERIALS & METHODS

4.2 Materials

4.2.1 List of chemicals

Following chemicals were used for the completion of research work.

Table 4.1: Chemicals list

S.N.	List of Chemicals	Manufacturer
1.	Fluoxetine	Sigma Aldrich
2.	Allicin	Sigma Aldrich
3.	Galangin	Sigma Aldrich
4.	Ethanol	Merck Ltd.
5.	HCl	Merck Ltd
6.	NaOH	Merck Ltd.
7.	Methanol(HPLC Grade)	Merck Ltd
8.	Water(HPLC Grade)	Merck Ltd.
9.	Phosphoric acid (HPLC Grade)	Merck Ltd

4.12 List of instruments

Instruments employed during research were detailed below.

Table 4.2: Instruments list

S.N.	List of Instruments	Manufacturer
1.	UV-Vis Spectrophotometer	Lab India 3000+
2.	HPLC System	Shimadzu LC10
3.	FTIR System	Thermo Fischer
4.	ELISA kit	Elab Sciences
5.	Melting Point Apparatus	Chemline CL-725

4.13 Animals

- ✓ Species/Common name: Wistar Albino rat
- ✓ Age/weight/Size: Adult/200-250g
- ✓ Gender: Male
- ✓ Number of Animals: 48

Animals used for research purchased from the CCSEA approved facility. The animals were housed normally prior to the experiment's commencement, with 12 hours illumination period and unrestricted availability of food and water. One hour before the experiments, the animals were acclimated to the lab setting. The Institutional Animal Ethics Committee (IAEC) (1429/PO/a/11/CPCSEA) approved the experimental technique in Sagar Institute of Research & Technology-Pharmacy, Bhopal, India. Animal experimentation was conducted in accordance with the guidelines set out by the "Committee for the Control and Supervision of Experiments on Animals." recommendations (CCSEA).

4.3 Methodology

4.3.1 Identification and characterization of drug

4.3.1.1 Physical evaluation

Physical evaluation of a drug was done through examination of its taste, appearance, odor, and texture, among other sensory characteristics.

4.3.1.2 Solubility

To measure the medication's solubility, a little amount of the drug (approximately 1-2 mg) was placed in a test tube with 5 ml of solvents (water, methanol, ethanol, 0.1N HCl, and 0.1N NaOH) and violently shaking the tube for a length of time. The drug's solubility in several solvents at room temperature was then ascertained.

4.3.1.3 Melting point

In a capillary tube, a little quantity of powder was placed. This tube was inserted into the device for measuring melting point (Chemline CL-725). Invert and tap the tube on bench top and then insert the capillary tube in melting point apparatus, adjust the heating rate and then monitor the sample through the viewfinder (Nichols, 2021).

4.3.1.4 UV spectroscopy

Using a double-beam UV spectrophotometer, the λ max of Fluoxetine was measured by analyzing the spectra of drug solution. A volumetric flask containing 10 ml was used to dissolve 10 mg of the medication in 10 ml of methanol. From the resulting solution of 1000g/ml, remove 1 ml with a pipette and transfer it to a 100-ml volumetric flask. Next, using methanol, dilute the solution to a concentration range of 10 g/ml. This solution's spectrum was measured in the 200- 400 nm range using an ultraviolet spectrophotometer (Labindia-3000+). Figure depicts the spectrum peak point graph of absorbance of Fluoxetine vs. wavelength.

4.3.1.5 FTIR spectroscopy

A compound's infra-red spectrum is a crucial record that provides adequate information about its structure. This approach produces a spectrum with several absorption bands, from which a plethora of evidence about the arrangement of an organic chemical may be extracted. FTIR Spectroscopy was used to identify Fluoxetine relative to the reference spectrum. Fluoxetine was available in the form of a powder that was either crystalline white or virtually white. According to the results of the IR spectrum, it was identified.

4.2.2. Methods used for MDD-induced model

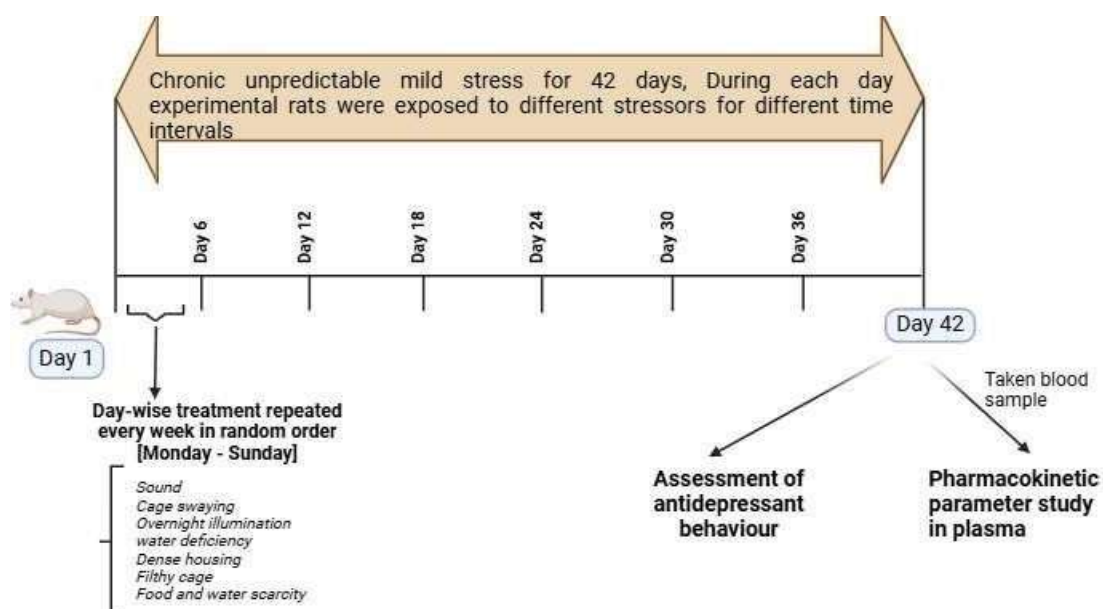


Fig 2.1: Flow Chart representation of present study

4.2.2.1. Development of MDD-Induced Model

According to protocol, rats underwent six weeks (42 days) of chronic unexpected mild stress (CUMS). The procedure included stressors, including sound, cage swaying, overnight illumination, food and drink deprivation, dense housing, filthy cage, and depressive-like behavior. From a drug development point of view, the most significance characteristic of the CUMS model is its potential for predicting the rapid commencement of antidepressant effects due to its chronic time course (Willner, 1997; Stekalova, 2022).

Table 4.3: The CUMS procedure followed as per schedule (Abdul et al., 2016)

Week	Day	Prophylaxis	Time frame
1	Sunday morning sucrose test	NA	NA
	Sunday evening	Sound	3 hours
	Monday	Cage swaying	7 hours
	Tuesday	Overnight illumination	12 hours
	Wednesday	Water deficiency	24 hours
	Thursday	Dense housing	24 hours
	Friday	Filthy cage	24 hours
	Saturday	Food and Water Scarcity	24 hours
2	Sunday morning sucrose test	NA	NA
	Sunday evening	Overnight Illumination	12 hours
	Monday	Filthy cage	24 hours
	Tuesday	Sound	3 hours
	Wednesday	Food deprivation	24 hours

	Thursday	Cage swaying	7 hours
	Friday	Dense housing	24 hours
	Saturday	Food and Water Scarcity	24 hours
3	Sunday morning sucrose test	NA	NA
	Sunday evening	Sound	3 hours
	Monday	Cage swaying	7 hours
	Tuesday	Filthy cage	24 hours
	Wednesday	Water deficiency	24 hours
	Thursday	Overnight Illumination	12 hours
	Friday	Dense housing	24 hours
	Saturday	Food and water Scarcity	24 hours
4	Sunday morning sucrose test	NA	NA
	Sunday evening	Filthy cage	24 hours
	Monday	Overnight Illumination	12 hours
	Tuesday	Food deprivation	24 hours
	Wednesday	Dense housing	24 hours
	Thursday	Cage swaying	7 hours
	Friday	Sound	3 hours
	Saturday	Food and Water Scarcity	24 hours
5	Sunday morning sucrose test	NA	NA
	Sunday evening	Dense housing	24 hours
	Monday	Cage swaying	7 hours

	Tuesday	Filthy cage	24 hours
	Wednesday	Water deficiency	24 hours
	Thursday	Sound	3 hours
	Friday	Overnight Illumination	12 hours
	Saturday	Food and Water Scarcity	24 hours
6	Sunday morning sucrose test	NA	NA
	Sunday evening	Sound	3 hours
	Monday	Dense housing	24 hours
	Tuesday	Food deprivation	24 hours
	Wednesday	Filthy cage	24 hours
	Thursday	Overnight Illumination	12 hours
	Friday	Cage swaying	7 hours
	Saturday	Food and Water Scarcity	24 hours

4.3.2 Depression assessment utilizing many factors like

4.3.2.1 Sucrose preference test

The Sucrose preference test was conducted as per protocol. Firstly, the animals were separately placed in cage with double bottles of 1% w/v solution 72 hrs before the actual test. Then after 24 hrs, one bottle of sucrose solution was exchanged with a bottle holding tap water for next 24 hrs, to adapt sucrose solution. After the adaptation period, in next 24 hrs, animals were deprived of food and water. Further fresh pre weighed two bottles of sucrose and tap water respectively given to animals and were permissible to drink for 1 hr. After that the weight of both bottles were recorded and difference in the respective initial and final weights was calculated [(He et al., 2020; Tianzhu et al., 2014)]. Sucrose preference (%) = $\frac{\text{sucrose intake (g)}}{[\text{sucrose} + \text{water}]} \times 100\%$

4.3.2.2 Forced swim test

A plastic cylinder with 25 cm diameter and 50 cm height was used that was filled with water at the level of 30 cm with maintaining the temperature 24.2 °C. After CUMS, animals swim for 20 minutes in the swim tank, dry under a heat lamp for 10 minutes, and then return to their cages. This test was conducted for recording the total duration of immobility (floating of mouse without any movement). Recording was done during the last 4 minutes of the test [Abdul et al., 2016; Wang et al., 2021)

4.3.2.3 Actophotometer test

Monitoring of locomotor activity of rats was performed by Actophotometer apparatus. In this test, animals were placed in apparatus individually and basal activity score was recorded over the period of 5 minutes. The results of decreased activity score indicated the CNS depression (Gupta et al., 2015).

4.3.2.4 Open field test

Open field testing in a Plexiglas box (75 cm x 75 cm x 42 cm) was used to assess the locomotor activity. To view animal activity, the box top remained open. Animals were kept free for 5 minutes in the center on the last day of CUMS. A three-minute session counts were crossings and rearing after two minutes of acclimatization. The OFT apparatus was also cleaned after every trial [(Abdul et al., 2016; Femandes et al., 2019)

4.3.2.5 Hyponeophagia test

Hyponeophagia was explored to the animal models of depression which was induced using the unanticipated mild stress. All food from the cage hopper was removed at the night before testing and provided regular feed of 1g/rat, about a third of their usual intake. The Hyponeophagia test apparatus was installed and arranged a row of small cages to hold the rats from group home cage before and during the eating. Then, a minute-long eating latency of the rat was measured in the test apparatus (Eltokhi et al., 2021)

4.3.2.6 Clonidine-induced aggression test

A single high dose of clonidine (50 mg/kg, i.p.) was injected into rat for induction of aggressive response as per the standard protocol of anti-depressant activity (Maj et al., 1982)

4.3.3 *In-vivo* pharmacokinetic study

Freshly prepared solutions of Fluoxetine, Galangin, Allicin, Galangin + Allicin, Fluoxetine + Galangin, Fluoxetine + Allicin were administered by oral route. On the last day of these dosing, all the animals in the treatment groups were separated into subgroups (n=3) based on sample collection time points: 0 min (pre-dose), 10 min, 20 min, 40 min, 1 hour, 2 hours, 6 hours, 8hours, 24 hours (post-dose).

At each time point, 0.03 ml of blood sample were collected from Lateral tail vein in heparinized saline solution. The plasma was collected from blood sample using centrifuge at 6000 rpm for 6 min. All the pharmacokinetic parameters including plasma peak concentrations (C_{max}), concentration peak times (t_{max}), the concentration-time curve's area under the curve AUC (0-t), AUC (0-∞) were determined for the blood plasma levels at different time intervals [(Vyas & Galani, 2010; Al-Asmari, Ullah, Al Masoudi, & Ahmad, 2017; Xia et al., 2016)].

4.3.3.1 Brain tissue preparation

Rat brains were meticulously dissected on ice-cold plates. Then the rat brain hippocampus was removed. Icy water was used to rinse the brain's tissues. water and homogenized for examination.

4.3.3.2 Chromatographic state

An RP-C18 analytical column with a mobile phase containing methanol, water, and phosphoric acid (60, 38, 2, and v/v/v, respectively) were used in the chromatographic analysis, which was performed at room temperature. The sample was isocratically eluted at a rate of 1 ml each minute. Each sample run utilized a volume of 20 µl, which was injected into the HPLC apparatus. The UV detector operating at a wavelength of 254 nm was used to monitor the chromatogram.

4.3.3.3 Selection of variables

Table 4.4: HPLC separation variable selection

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 μm
Bonded Phase	Octadecylsilane (C18)
Mobile Phase	
60% Methanol	60
38% water	38
2% Phosphoric acid	40
Flow rate	1.2 ml/min
Temperature	Room Temperature
Sample Size	20 μl
Measurement wavelength	254 nm
Retention time Fluoxetine	13.87 \pm 0.5 min
Retention time Allicin	9.98 \pm 0.5 min
Retention time Galangin	38.88 \pm 0.5 min

4.3.3.4 Standard stock solution preparation

The amount of 1mg of Allicin, Galangin, and Fluoxetine were accurately weighed and moved to separate 10 ml volumetric flasks. The volume was stepped up to the mark using HPLC grade methanol to generate a 100 ppm stock solution, which was subsequently diluted to make a 5 ppm working stock of standard as marker. The working stock was injected into the HPLC system to obtain the standard peak.

4.3.3.5 Analysis of analyte in plasma

Sample preparation

Plasma was obtained by centrifuging 0.03 ml of blood from each time point at 6000 rpm for 6 min. About 100 μl of plasma is taken in clean sterilized 1.5 ml microfuge separately and dilute with 900 μl HPLC grade methanol. The resulting solution was passed through 0.45 μm dissociable nylon syringe filter of using and sonicated for 10

min before injecting into the HPLC machine. This preparation is further diluted as per the requirement to get the peaks in readable range.

HPLC analysis

The reverse phase C18 column was equilibrated using mobile phase combination of methanol, water, and phosphoric acid in ration of 60: 38: 2, isostatically. The eluent was measured at 254nm while the filtered and degassed mobile phase flowed at 1.2 ml/min. A 20 µl fixed loop was used to inject the sample for 55 min. Finally, Chromatography Data System Software N2000 reported marker detection (Science Technology, Hangzhou, China).

4.3.4 Evaluation of antidepressant activity through neurochemical estimation

The institution animal ethical committee approved (1429/PO/a/11/CPCSEA) all pre-clinical study protocols. The following treatments were given as per IAEC guidelines in the different groups of animals.

- Control Group – Healthy control rats + 5 % DMSO solvents
- CUMS Group – CUMS + 5 % DMSO solvents
- Flu Standard Group – CUMS + Fluoxetine (10 mg/kg, oral)
- Gal Test Group – CUMS + Galangin (9 mg/kg, oral)
- All Test Group – CUMS + Allicin (10 mg/kg, oral)
- Gal+ All Co-Ad Test Group – Galangin (9 mg/kg, oral) + Allicin (10 mg/kg, oral)
- Gal Co-Ad Test Group – CUMS + Galangin (9 mg/kg, oral) + Fluoxetine (10mg/kg, oral)
- All Co-Ad Test Group – CUMS + Allicin (10 mg/kg, oral) + Fluoxetine (10mg/kg, oral) All

Fluoxetine as a standard, drug of choice for the treatment of depression (Selective serotonin reuptake inhibitor). Any new depression therapy is based on it.

Drug dose calculation

Allicin (Rumaseuw, 2022)

Animals	Dosage group (mg/kg)	Number of deaths	% death
5	5	0	0
5	50	0	0
5	300	0	0
5	2000	0	0
5	Negative control	0	0

Galangin (Aloud, 2017)

Animals	Dosage group (mg/kg)	Number of deaths	% death
5	4	0	0
5	40	0	0
5	80	0	0
5	160	0	0
5	320	0	0

4.3.5 Estimation of 5-HT, dopamine, and BDNF using ELISA kits**4.3.5.1 Estimation of brain dopamine**

Dissection of rat brain was done while keeping it in ice cold plates. The brain hippocampus was removed to obtain the supernatant, the brain tissues were washed in an ice-cold environment which was used for biochemical investigation of brain monoamines and brain BDNF levels using the ELISA technique.

- To analyze brain BDNF levels, extracts from rat brain samples were exposed to an ELISA test using a sandwich ELISA kit from Elabsciences, and the process was carried out according to the kit's protocol. The experimental results for BDNF level analysis in the brain are given in table 1 and visually in figure 1.

- The Micro ELISA plate that came with this kit was pre-coated with a BDNF-specific antibody.
- When the standard or sample had been put to the wells of the micro-ELISA plate, the antibody was applied. Each microplate well was then filled with an Avidin Horseradish peroxidase (HRP) Conjugate and a biotinylated detection antibody specific for BDNF. The combination was then incubated for 60 minutes at 37 degrees Celsius. Only the wells containing BDNF, biotinylated detection antibody, and Avidin-HRP conjugate were blue before the substrate solution was added. A stop solution was added, which stops the activity of the enzyme substrate and causes a change in color to yellow.

BDNF levels are adversely correlated with optical density. A pre-coated Micro ELISA plate is included in this kit. The Micro ELISA plate involved in this set has been pre-coated with an antibody specific for monoamines like serotonin and dopamine.

4.3.5.2 Estimation of brain serotonin and dopamine level

The levels of serotonin and dopamine were evaluated as biochemical markers for the assessment of monoamine levels in the brain. The supernatant of the homogenate produced from rat brain samples was exposed to an ELISA test using a competitive ELISA kit from LSBbio™, and the process was carried out according to the kit's instructions.

- A pre-coated micro ELISA plate is included in this kit. This kit has a micro ELISA plate that has been pre-coated with an antibody specific to monoamines, such as serotonin or dopamine. standard or sample was put to the wells of the micro ELISA Plate together with the particular antibody [Incubate at 37 degrees Celsius for 90 minutes].
- As soon as the liquid is removed, mix it with a biotinylated detection antibody that is specific for serotonin or dopamine (HRP). Each microplate well should contain conjugate. Incubate for 60 minutes at 37 degrees Celsius. Only the wells containing human serotonin or dopamine, biotinylated detection antibody, and Avidin-HRP conjugate became blue once the substrate solution was added. Following that, each well receives a TMB substrate solution

(Tetramethylbenzidine). The material became yellow as a consequence of the stop solution's addition, which stopped the enzyme substrate process.

- Serotonin or dopamine levels are correlated with optical density (Monoamine).

4.3.6 Statistical Analysis

The finding results of the experiment were expressed through statistics and reported as mean \pm S.E.M. (Standard error mean). All data were analyzed by One-way ANOVA (Analysis of Variance) followed by Bonferroni test.

5. RESULTS & DISCUSSION

The research work has been completed as per discussed methodology and all the findings as results were summarized along with the discussion.

5.2 Identification and characterization of procured drug

5.2.1 Physical evaluation

It relates to the examination of a drug's taste, appearance, odor, and texture, among other sensory characteristics.

Table 5.1: Sensory character list

S.N.	Sensory characters	Result
1.	Appearance	White to Off-White
2.	Odor	Odorless
3.	Texture	Crystalline

5.2.2 Solubility

To measure the medication's solubility, a little amount of the drug (approximately 1-2 mg) was placed in a test tube with 5 ml of solvents (water, methanol, ethanol, 0.1N HCl, and 0.1N NaOH) and violently shaking the tube for a length of time. The drug's solubility in several solvents at room temperature was then ascertained.

Table 5.2: Solubility of Fluoxetine

S.N.	Solvent	Solubility
1.	Water	Soluble
2.	Ethanol	Freely soluble
3.	Methanol	Freely soluble
4.	0.1N HCl	sparingly soluble
5.	0.1N NaOH	sparingly soluble

5.2.3 Melting point

This is one of the factors used to assess medicine purity. The melting points of pure substances are extremely precise and consistent. As the medications comprise a combination of substances, their melting point ranges are specified.

Table 5.3: Melting point of Fluoxetine

Standard Melting Point	Noticed Melting Point
178-180° C	178° C

5.2.4 UV Spectroscopy

Using a double-beam UV spectrophotometer, the λ max of Fluoxetine was measured by scanning the wavelength of drug solution.

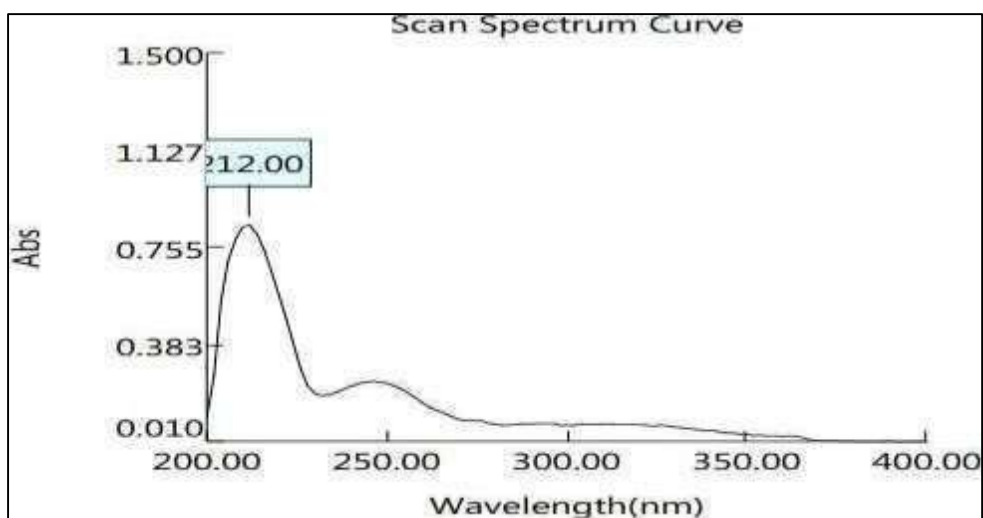


Fig.5.1: Determination of max of Fluoxetine

5.2.5 FTIR spectroscopy

An infra-red spectrum of compound was recorded crucially that provides adequate information about its structure. This approach produces a spectrum with several absorption bands, from which a plethora of evidence about the arrangement of an organic chemical may be extracted. FTIR Spectroscopy was used to identify Fluoxetine relative to the reference spectrum. Fluoxetine was available in the form of a powder that was either crystalline white or virtually white. According to the results of the IR spectrum, it was identified.

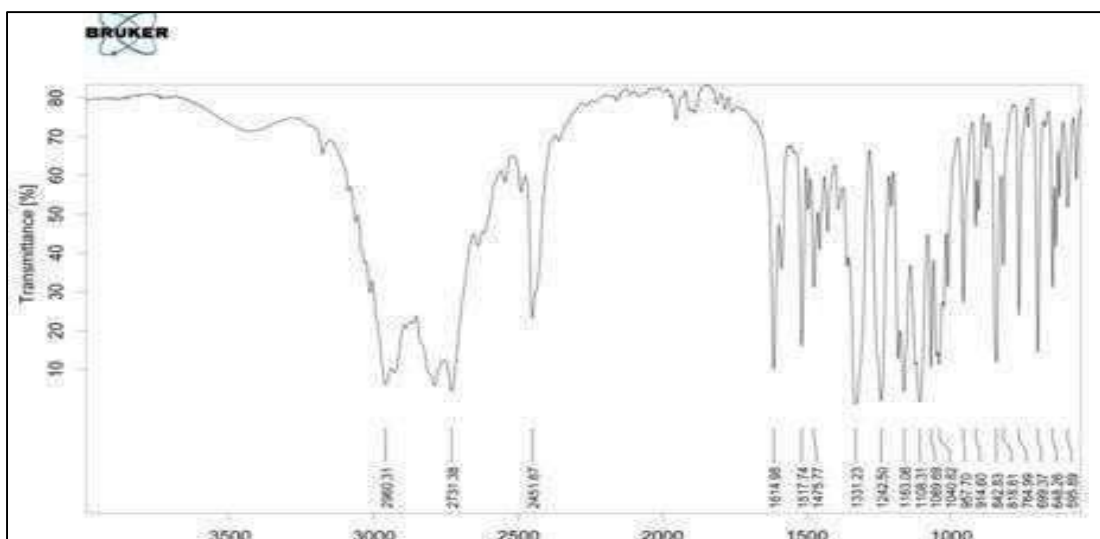


Fig. 5.2: Reported FT-IR spectrum of Fluoxetine (Ampati, S, 2016)

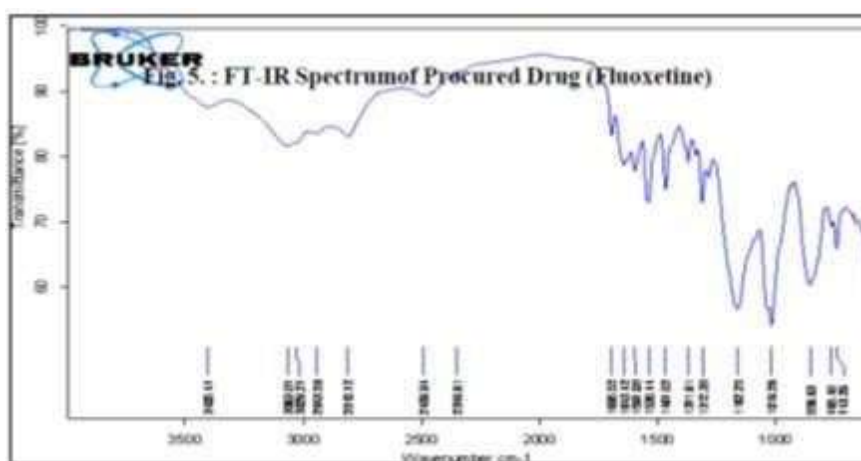


Fig. 5.3: FT-IR Spectrum of procured Fluoxetine

5.3 Development of MDD induced model

According to protocol, rats were observed for six weeks (42 days) under the chronic unexpected mild stress (CUMS). The procedure included stressors, including sound, cage swaying, overnight illumination, food and drink deprivation, dense housing, filthy cage, and depressive-like behavior. All these behavioral symptoms confirmed the induction of depression successfully.

5.4 Assessment of depression level in animal models using different parameters

5.4.1 Sucrose preference test

Based on procedure, observed result of sucrose preference test were as follows: In sucrose preference test, data was analyzed via One-way Analysis of Variance (Bonferroni t-test) and was found considerable difference between treatments. In a group wise comparison, percentage of sucrose preferences was decrease in CUMS group (A2) in comparison to that of control group (A1). Treatment with (A3) Flu (10 mg/kg); (A4) Gal (10mg/kg); (A5) Alli(9 mg/kg) does not shown a satisfactorily increase, however co-treatment group such as Gal + Flu demonstrated increase in percentage of sucrose preference test that substantially have greater efficacy.

Table 5.4: Sucrose preference test of rats

S.N.	Groups	Sucrose Solution	Water	% Sucrose preference (Mean \pm SEM)
1.	Control	30 ml	20 ml	60 \pm 1.865
2.	CUMS group	28 ml	25 ml	52.8 \pm 0.699*
3.	CUMS + Flu	10 ml	20 ml	33.3 \pm 1.051###
4.	CUMS + Gal	8 ml	14 ml	36.3 \pm 1.403###
5.	CUMS + Alli	12 ml	28 ml	30 \pm 0.930###
6.	CUMS + [Gal + Alli]	17 ml	30 ml	36.1 \pm 1.466 #,@,@,\$\$\$
7.	CUMS + [Flu + Gal]	36 ml	30 ml	54.5 \pm 3.418#, $\Delta\Delta\Delta$, @@@
8.	CUMS + [Flu + Alli]	28 ml	30 ml	48.2 \pm 1.853###, $\Delta\Delta\Delta$, \$\$\$

*P<0.05 vs. Control; ###P<0.001, #P<0.05 vs. CUMS group; @@@ P<0.001, @@P<0.01 vs. CUMS + Gal; \$\$\$P<0.001 vs. CUMS + Alli; $\Delta\Delta\Delta$ P<0.001 vs. CUMS + Flu.

There was a statistically significant difference (P< 0.05), all pairwise multiple comparison procedure (Bonferroni t-test) specified in Table 5.4.

5.4.2 Forced swim test

In the Forced swim test, result data was evaluated via one-way ANOVA through Bonferroni t-test. The immobility time of rats was found to be increased in CUMS group. However, treatment with Gal + Flu and Alli + Flu showed significant decrease in immobility time i.e., 68 sec. and 70 sec. respectively. Other group treatment did not show much efficacy.

Table 5.5: Forced swim test of rats

S.N.	Groups	Total time (sec.) (Mean \pm SEM)
1.	Control	81 \pm 0.24
2.	CUMS group	93.3 \pm 1.59 ^{***}
3.	CUMS + Flu	72 \pm 1.72 ^{###, ,}
4.	CUMS + Gal	91.3 \pm 3.07 [#]
5.	CUMS + Alli	90 \pm 0.98 [#]
6.	CUMS + [Gal + Alli]	87.6 \pm 0.24 ^{#, @, \$}
7.	CUMS + [Flu + Gal]	68 \pm 1.35 ^{###, Δ, @@@}
8.	CUMS + [Flu + Alli]	70 \pm 0.12 ^{###, Δ, \$\$\$}

*P<0.05 vs. Control; ^{###}P<0.001, [#]P<0.05 vs. CUMS group; ^{@@@} P<0.001, ^{@@}P<0.01 vs. CUMS + Gal; ^{\$\$\$}P<0.001 vs. CUMS + Alli; ^{ΔΔΔ}P<0.001 vs. CUMS + Flu.

There was a statistically significant difference (P< 0.05), all pairwise multiple comparison procedure (Bonferroni t-test) specified in Table 5.5.

5.3.3. Actophotometer test

In Actophotometer test, result data was evaluated via one-way ANOVA through Bonferroni t-test. Actophotometer test results show the baseline activity. Reduced activity indicates a CNS depression at some level. When compared to the control treated group, the mean value of the A2 treated group (CUMS group) exhibited a substantial decrease in basal activity that continued to decline after 30 minutes ("P<0.05"). Co-treated populations, such as A6 (GAL+ALLI) and A7 (GAL + FLU). A7 and A8 treatment groups were shown to have a substantial rise in basal activity score, although GAL + ALLI treated groups showed little to no increase in

comparison to the group, with mean values of 280, 277, and 273 ("P<0.05"), respectively. Their antidepressant impact was seen by the rise in baseline activity in the co-treated group (data shown in table 5.6). In group-wise comparisons locomotion was significantly higher ("P<0.05") in co-treatment groups A6 (GAL + ALLI), A7 (GAL + FLU), and A8 (ALLI + FLU) compared to single treated group in the open field test that was used to measure behavioral evaluations.

Table 5.6: Actophotometer test for rats

	Mean Score in 5 minutes			
	Control (Mean±SEM)	CUMS group (Mean ±SEM)	CUMS + Flu (Mean±SEM)	CUMS + Gal (Mean±SEM)
Basal	280±0.12	275±1.25 ^{***}	276±0.36 [#]	265±0.24 ^{###}
30 Min	275±0.61	270±0.12 ^{***}	275±0.24 ^{###}	200±0.24 ^{###}
60 Min	279±1.72	273±0.24 ^{***}	271±0.12 [#]	175±0.61 ^{###}

	Mean Score in 5 minutes			
	CUMS + Alli (Mean±SEM)	CUMS + [Gal + Alli] (Mean ±SEM)	CUMS + [Flu + Gal] (Mean ±SEM)	CUMS + [Flu+ Alli] (Mean ±SEM)
Basal	271±0.12 ^{###}	280±0.36 ^{###, @@@, \$\$\$}	277±0.49 ^{#, Δ, @@@}	273±0.36 ^{#, \$, ΔΔΔ}
30 Min	175±0.61 ^{###}	145±0.12 ^{###, @@@, \$\$\$}	235±0.36 ^{###, ΔΔΔ, @@@}	201±0.49 ^{###, ΔΔΔ, \$\$\$}
60 Min	98±0.24 ^{###}	109±0.86 ^{###, @@@, \$\$\$}	187±0.24 ^{###, ΔΔΔ, @@@}	176±0.12 ^{###, ΔΔΔ, \$\$\$}

^{***}P<0.001 vs. Control; ^{###}P<0.001, [#]P<0.05 vs. CUMS group; ^{@@@}P<0.001 vs. CUMS + Gal; ^{\$\$\$}P<0.001 vs. CUMS + Alli; ^ΔP<0.05, ^{ΔΔΔ}P<0.001 vs. CUMS + Flu.

There was a statistically significant difference (P<0.05), all pairwise multiple comparison procedure (Bonferroni t-test) specified in Table 5.6.

5.3.4. Open field test

Findings of open field test mentioned below helped for the assessment of behavioral evaluations. In the Open field test, data was analyzed via one-way ANOVA through Bonferroni t-test. OFT was done to evaluate locomotor activity. Followed by chronic stress locomotor activity in outer square (P<0.001), central square (P<0.001) and

rearing ($P < 0.001$) which was enhanced by co-treatment of Gal + Alli (A6) and Gal + Flu (A7).

Table 5.7: Open field test for rats

	Control (Mean \pm SEM)	CUMS group (Mean \pm SEM)	CUMS + Flu (Mean \pm SEM)	CUMS + Gal (Mean \pm SEM)
Outer square	32 \pm 0.24	27 \pm 0.73 ^{***}	34 \pm 0.364 ^{###}	89 \pm 0.491 ^{###}
Central square	1 \pm 0.12	3 \pm 0.12 [*]	12 \pm 0.24 ^{###}	8 \pm 0.24 ^{###}
Rearing	4 \pm 0.12	3 \pm 0.12 [*]	5 \pm 0.12 ^{###}	17 \pm 0.49 ^{###}

	CUMS + Alli (Mean \pm SEM)	CUMS + [Gal + Alli] (Mean \pm SEM)	CUMS + [Flu + Gal] (Mean \pm SEM)	CUMS + [Flu + Alli] (Mean \pm SEM)
Outer square	101 \pm 0.612 ^{###}	98 \pm 0.24 ^{###, @@@, \$}	93 \pm 0.24 ^{###, $\Delta\Delta\Delta$, @@@}	94 \pm 0.24 ^{###, $\Delta\Delta\Delta$, \$\$\$}
Central square	17 \pm 0.491 ^{###}	14 \pm 0.24 ^{###, @@@, \$\$\$}	13 \pm 0.733 ^{###, Δ, \$\$\$}	19 \pm 0.24 ^{###, $\Delta\Delta\Delta$, \$}
Rearing	21 \pm 0.24 ^{###}	12 \pm 0.12 ^{###, @@@, \$\$\$}	11 \pm 0.24 ^{###, $\Delta\Delta\Delta$, @@@}	9 \pm 0.12 ^{###, $\Delta\Delta\Delta$, \$\$\$}

* $P < 0.05$, ** $P < 0.001$ vs. Control; ### $P < 0.001$ vs. CUMS group; @@@ $P < 0.001$ vs. CUMS + Gal; \$ $P < 0.05$, \$\$\$ $P < 0.001$ vs. CUMS + Alli; Δ $P < 0.05$, $\Delta\Delta\Delta$ $P < 0.001$ vs. CUMS + Flu.

There was a statistically significant difference ($P < 0.05$), all pairwise multiple comparison procedure (Bonferroni t-test) specified in Table 5.7.

5.3.5. Hyponeophagia test

Animals' drinking times were analyzed using this method. As per methodology, the results were reported as below in Table 5.8. In Hyponeophagia test, reduction in feeding was measured via One-way Analysis of variance (ANOVA) through Bonferroni t-test. Increases in latency during group-wise comparisons indicate CNS

depression, which was shown to be significantly reduced ("P<0.05") in the co-treatment group A7 (GAL+FLU). The difference in mean value between the co-treated group and the A7 treated group, which was discovered to be a little higher, indicates their lower efficacy.

Table 5.8: Hyponeophagia test for rats

S. N.	Groups	Latency to drink (Time in sec.)		
		Test 1 (Mean± SEM)	Test 2 (Mean ± SEM)	Test 3 (Mean ± SEM)
1.	Control	80±2.42	118±5.36	146±6.52
2.	CUMS group	56±1.79 ^{***}	87±3.06 ^{***}	123±6.46 [*]
3.	CUMS + Flu	76±2.13 ^{###}	92±1.44 [#]	110±5.94 [#]
4.	CUMS + Gal	44±2.42 [#]	78±2.59 [#]	112±6.5 [#]
5.	CUMS + Alli	49±1.79 [#]	69±0.75 [#]	106±5.8 [#]
6.	CUMS + [Gal + Alli]	56±1.44 ^{#, @, \$}	105±4 ^{#, @@@, \$\$\$}	102±4.96 ^{#, @, \$}
7.	CUMS + [Flu + Gal]	64±3.52 ^{#, Δ, @@@}	111±5.13 ^{#, Δ, @@@}	99±3.23 ^{#, Δ, @}
8.	CUMS + [Flu + Alli]	59±2.94 ^{#, Δ, \$}	99±2.59 ^{#, Δ, \$\$\$}	104±4.85 ^{#, Δ, \$}

^{###}P<0.001 vs. CUMS Group; [@] P<0.05, ^{@@@} P<0.001 vs. CUMS + Gal; ^{\$}P<0.05, ^{\$\$\$}P<0.001 vs. CUMS + Alli; ^ΔP<0.05 vs. CUMS + Flu.

There was a statistically significant difference (P< 0.05), all pairwise multiple comparison procedure (Bonferroni t-test) specified in Table 5.8.

5.3.6. Clonidine induced aggression

In Clonidine induced aggression test, data was analyzed via One-way ANOVA through Bonferroni t-test. Treatment includes clonidine administration cause attack within 7 minutes which was significantly increased by co-treatment with Alli+Flu (A8) followed by Gal+Flu (A7) (P<0.05).

Table 5.9: Clonidine induced aggressive behaviors for rats

Groups indicator	Control (Mean±SEM)	CUMS group (Mean±SEM)	CUMS + Flu (Mean±SEM)	CUMS +Gal (Mean±SEM)
Number of attacks	2±0.12	3±0.24*	15±0.49 ^{###}	34±0.73 ^{###}
Attack latency (Min)	23±0.73	7.4±0.36 ^{***}	9.2±0.23 [#]	12±0.36 ^{###}

Groups indicator	CUMS + Alli (Mean±SEM)	CUMS + [Gal + Alli] (Mean±SEM)	CUMS + [Flu + Gal] (Mean±SEM)	CUMS + [Flu + Alli] (Mean ±SEM)
Number of attacks	38±0.61 ^{###}	32±0.36 ^{###, @, \$\$\$}	18±0.24 ^{###, Δ, @@@}	24±0.36 ^{###, ΔΔΔ, \$\$\$}
Attack latency (Min)	9±0.24 [#]	11±0.12 ^{###, @, \$}	14±0.49 ^{###, ΔΔΔ, @}	17±0.36 ^{###, ΔΔΔ, \$\$\$}

*P<0.05, ***P<0.001 vs. Control; #P<0.05, ###P<0.001 vs. CUMS Group; @ P<0.05 @@@ P<0.001 vs. CUMS + Gal; \$P<0.05, \$\$\$P<0.001 vs. CUMS + Alli; ΔP<0.05, ΔΔΔ P<0.001 vs. CUMS + Flu.

There was a statistically significant difference (P< 0.05), all pairwise multiple comparison procedure (Bonferroni t-test) specified in Table 5.9.



Fig. 5.4: Experimental images of rat

5.4. *In-vivo* pharmacokinetic study

The HPLC chromatograms of marker compounds were obtained at optimized chromatographic conditions which have been reported in Fig. 5.5.

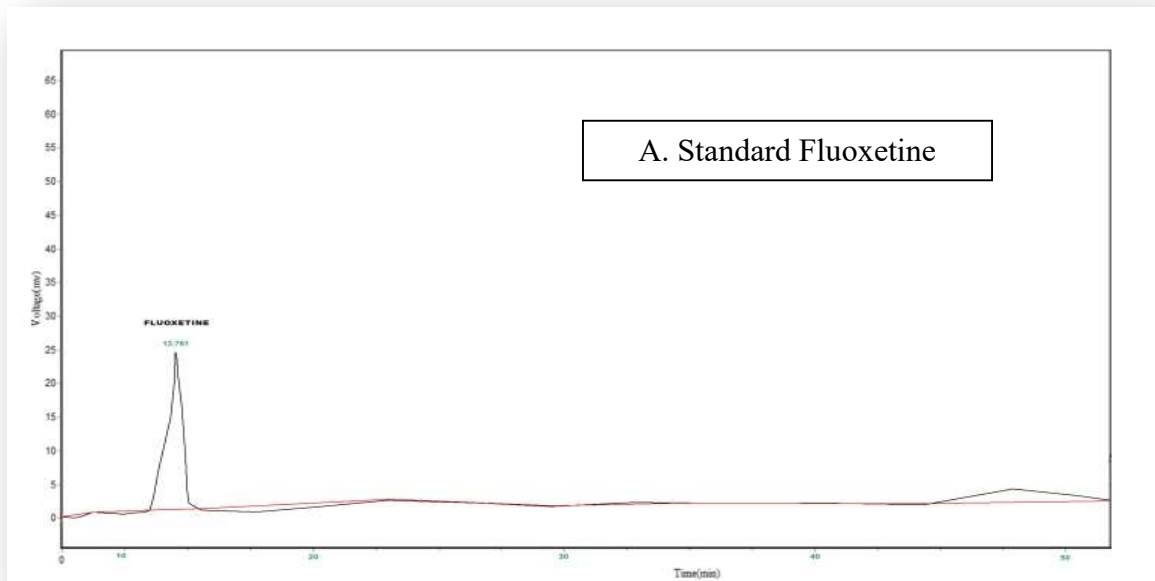


Fig. 5.5: (a) Chromatogram of Fluoxetine standard marker with retention time at 13.87 ± 0.5 min

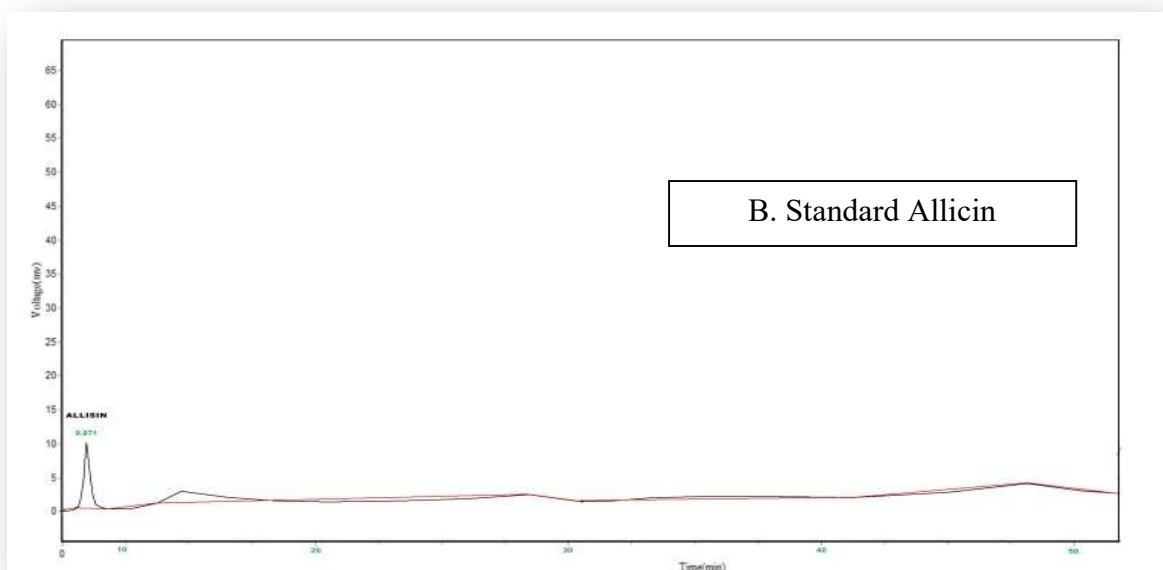


Fig. 5.5: (b) Chromatogram of Allicin standard maker with retention time at 9.98 ± 0.5 min; and

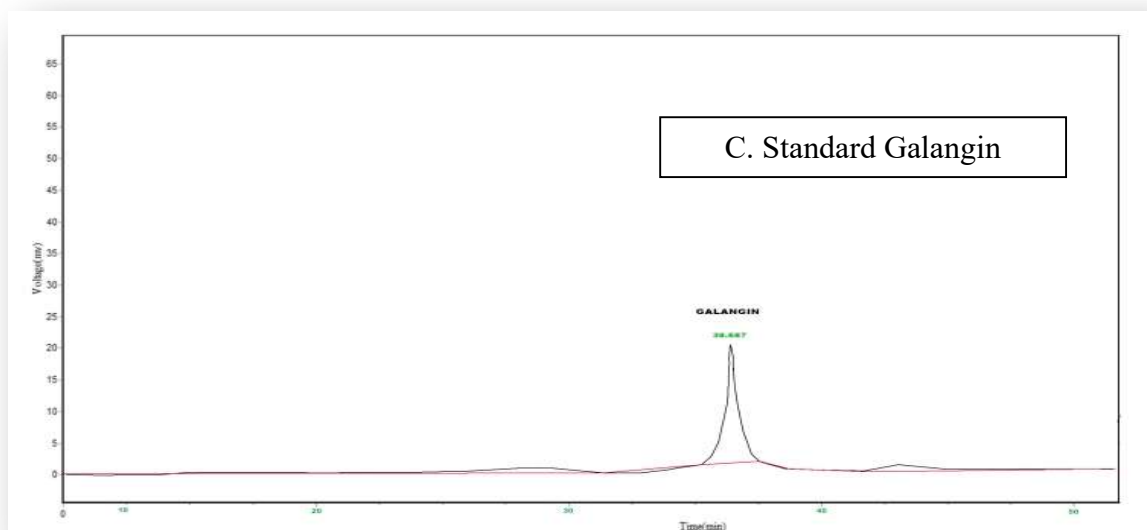


Fig. 5.5: (c) Chromatogram of Galangin standard marker with retention time at 38.88 ± 0.5 min

5.4.1. HPLC estimation of Fluoxetine

Fluoxetine was used as a standard anti-depressant drug for the estimation using the High- Performance liquid chromatography (HPLC) technique. Pharmacokinetic parameters including plasma peak concentrations (C_{max}), concentration peak times (t_{max}), the concentration-time curve area under the curve AUC (0-t), AUC (0- ∞) were determined by plasma levels at different time intervals.

Table 5.10: HPLC detection for Fluoxetine in plasma Sample

S.N.	Time	Peak concentration	Concentration peak time	Area under concentration time curve
1.	10 Minutes	-	-	-
2.	20 Minutes	-	-	-
3.	40 Minutes	-	-	-
4.	1 hr	-	-	-
5.	2 hrs	-	-	-
6.	6 hrs	1.78	13.782	3812
7.	8 hrs	0.684	13.691	1793
8.	24 hrs	-	-	-

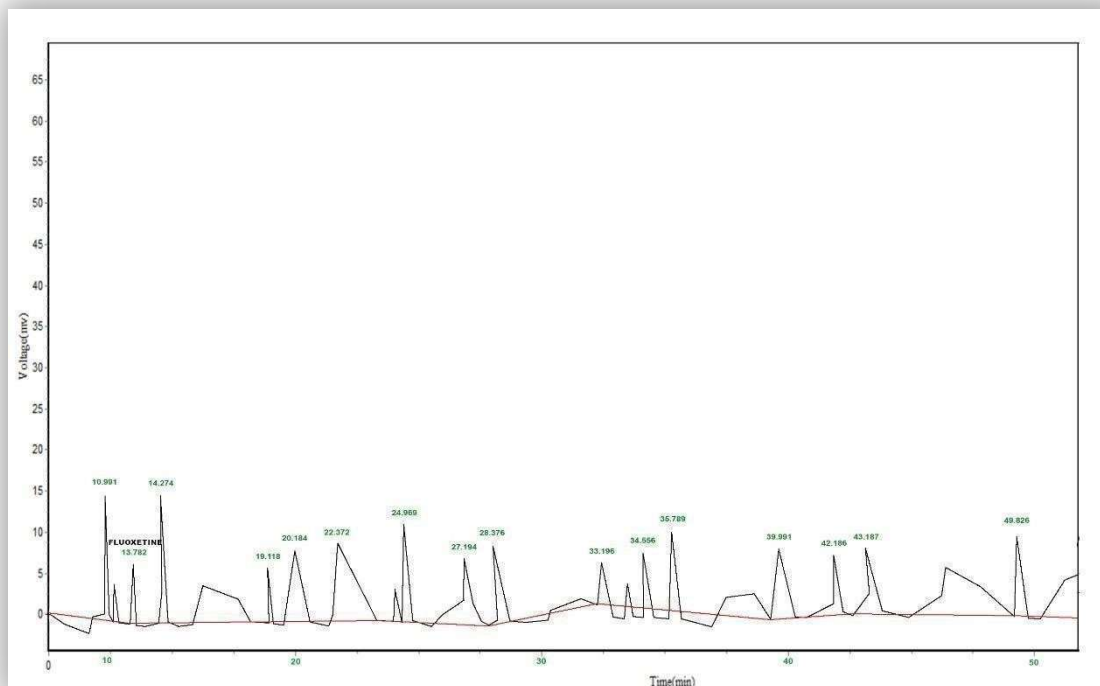


Fig. 5.6: Chromatogram of plasma sample with retention time at 13.782 ± 0.5 Min for Fluoxetine after 6 hrs.

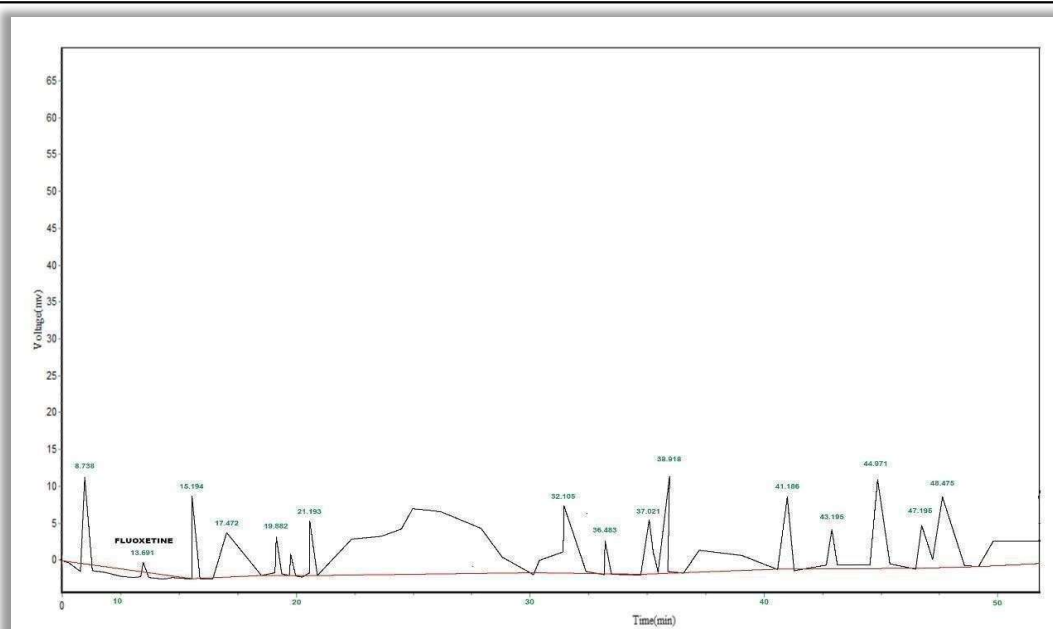


Fig. 5.7: Chromatogram of plasma sample with retention time at 13.691 ± 0.5 min for Fluoxetine after 8 hrs

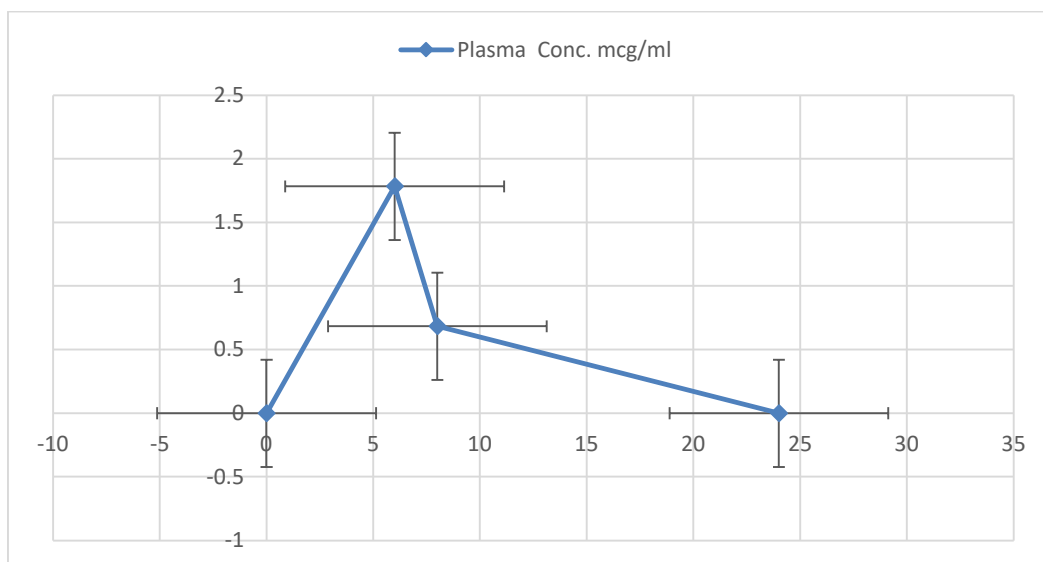


Fig. 5.8: Mean plasma concentration time curve of Fluoxetine

5.4.2. HPLC estimation of Allicin

Analysis of Allicin was evaluated through High Performance liquid chromatography (HPLC) technique.

Table 5.11: HPLC detection for Allicin in plasma sample

S.N.	Time	Peak concentration	Concentration peak time	Area under concentration time curve
1.	10 Minutes	-	-	-
2.	20 Minutes	-	-	-
3.	40 Minutes	-	-	-
4.	1 hr	-	-	-
5.	2 hrs	-	-	-
6.	6 hrs	2.801	9.891	4013
7.	8 hrs	1.682	9.419	2981
8.	24 hrs	-	-	-

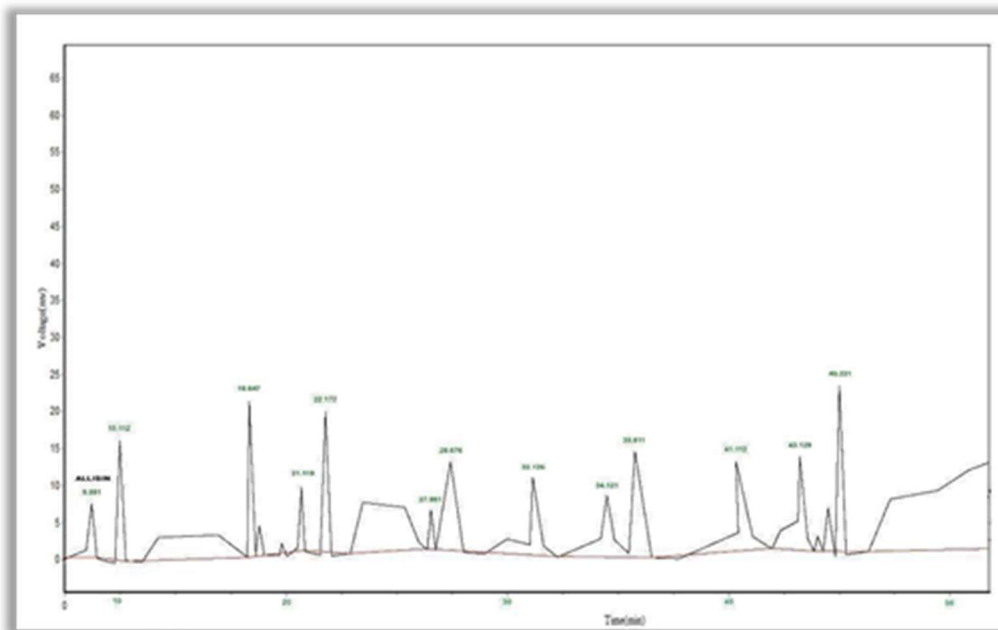


Fig. 5.9: Chromatogram of plasma sample with retention time at 9.891 ± 0.5 Min for Allicin after 6 hrs

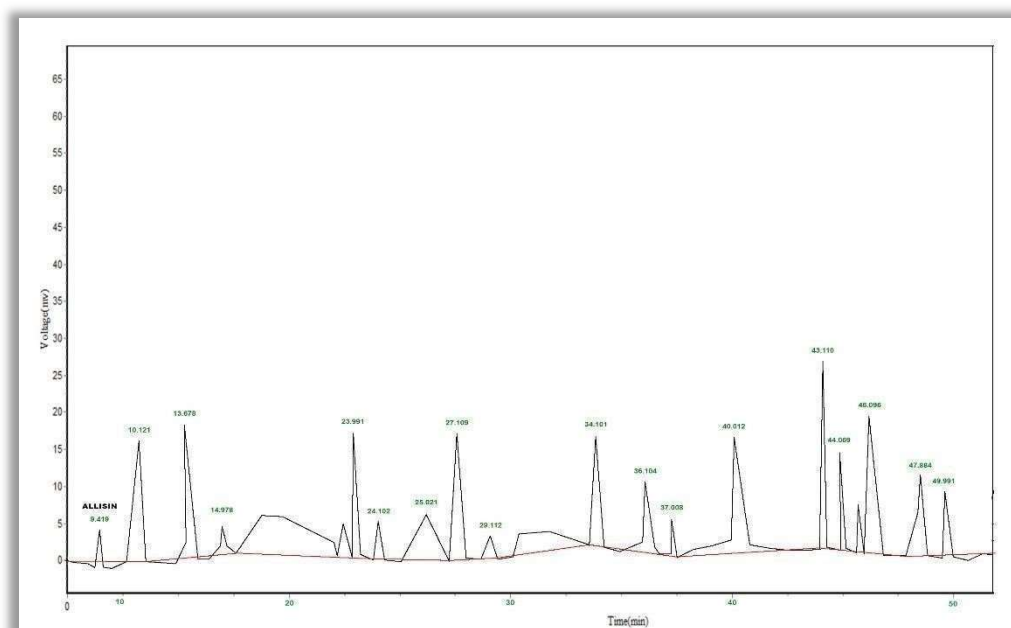


Fig. 5.10: Chromatogram of plasma sample with retention time at 9.419 ± 0.5 min for Allicin after 8 hrs

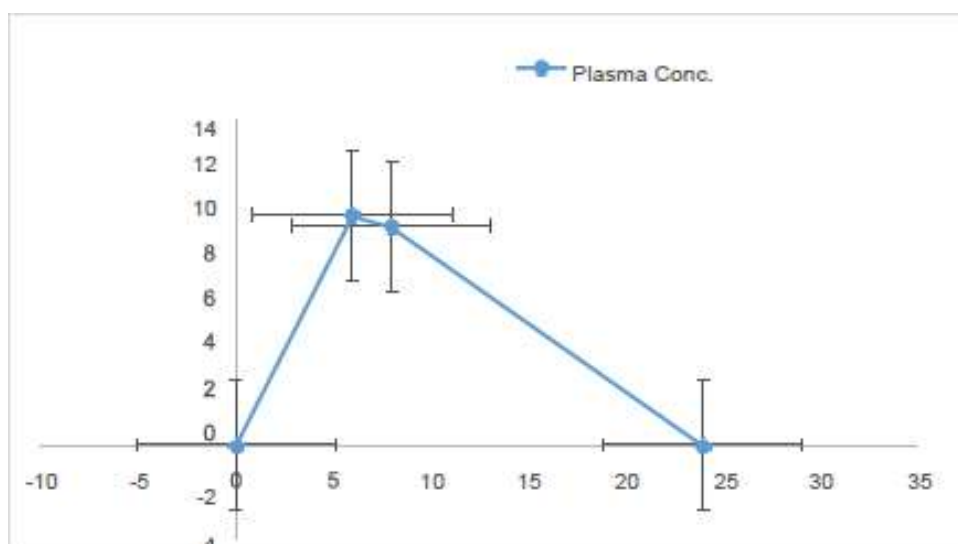


Fig. 5.11: Mean plasma concentration time curve of Allicin

5.4.3. HPLC estimation of Galangin

Analysis of Galangin as evaluated through High Performance liquid chromatography (HPLC) technique.

Table 5.12: HPLC detection for Galangin in plasma sample

S.N.	Time	Peak concentration	Concentration peak time	Area under concentration time curve
1.	10 Minutes	-	-	-
2.	20 Minutes	-	-	-
3.	40 Minutes	-	-	-
4.	1 hr	-	-	-
5.	2 hrs	-	-	-
6.	6 hrs	3.718	38.846	3892
7.	8 hrs	1.013	38.651	2913
8.	24 hrs	0.493	38.663	1030

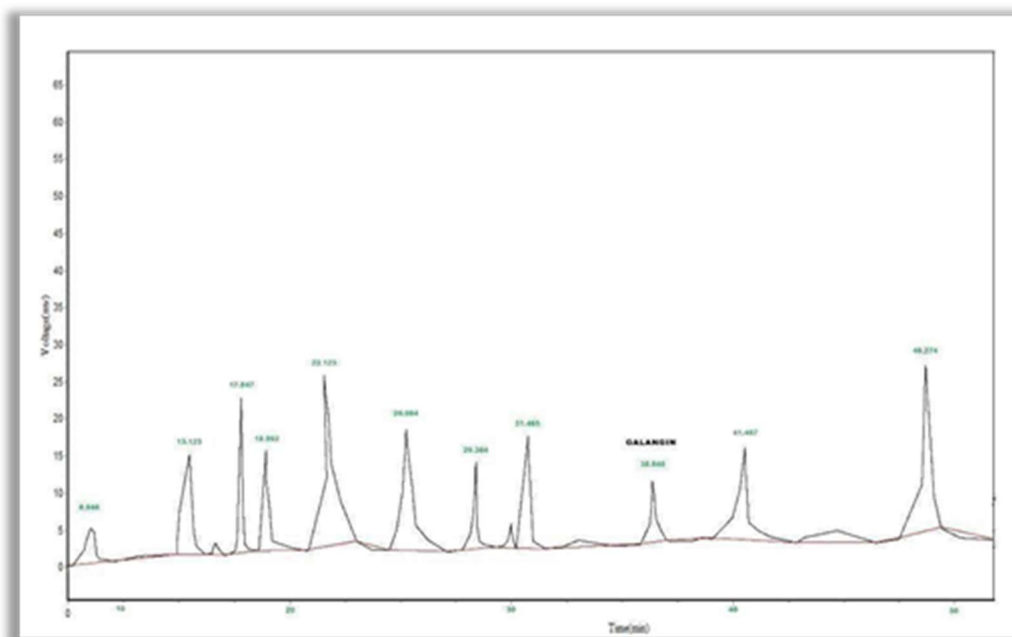


Fig. 5.12: Chromatogram of plasma sample with retention time at 38.846 ± 0.5 min for Galangin after 6 hrs

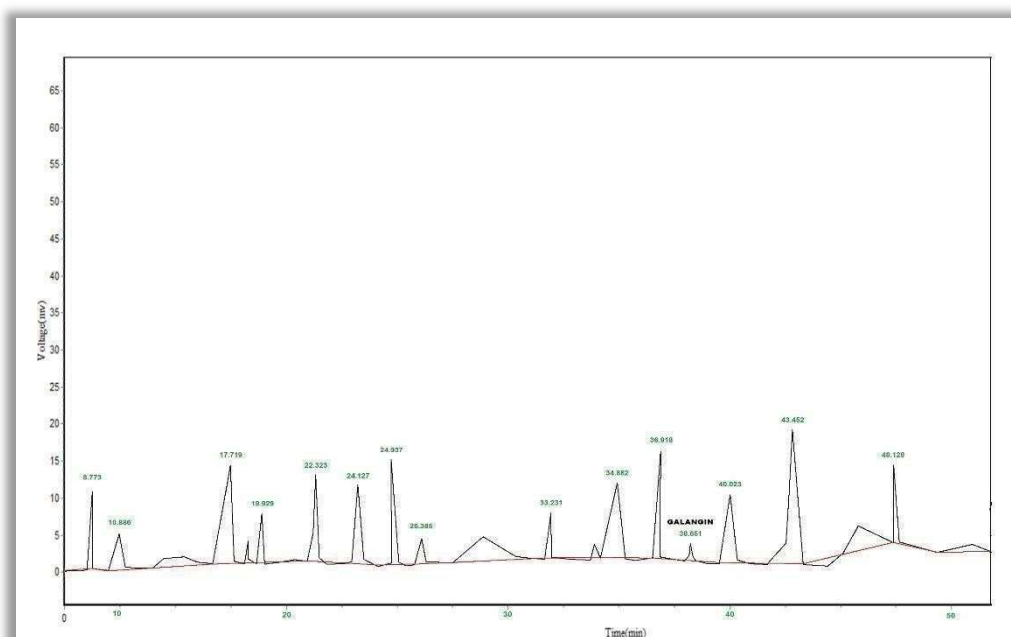


Fig. 5.13: Chromatogram of plasma sample with retention time at 38.651 ± 0.5 min for Galangin after 8 hrs

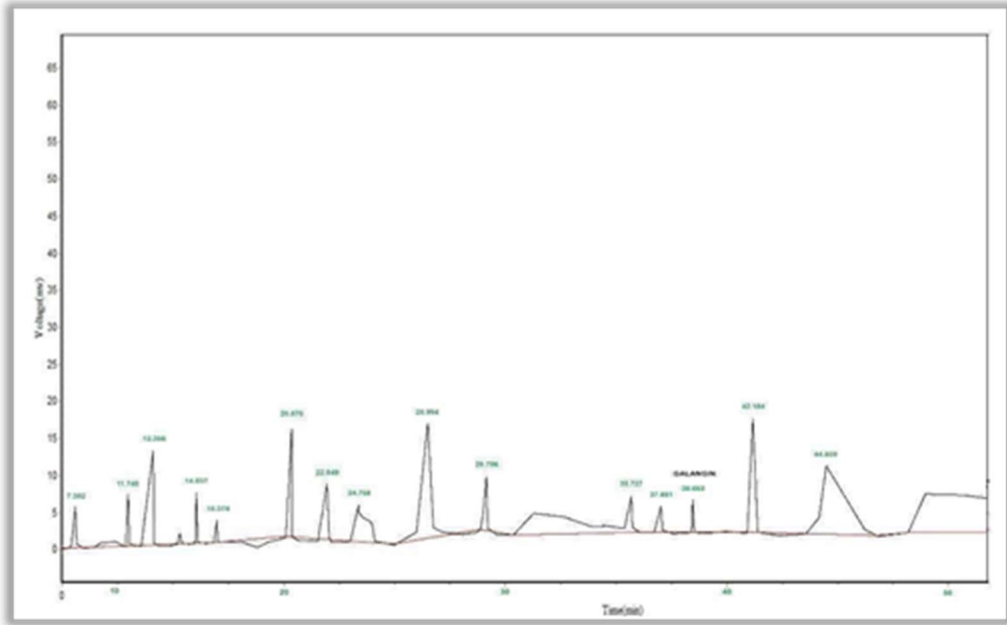


Fig. 5.14: Chromatogram of plasma sample with retention time at 36.663 ± 0.5 min for Galangin after 24 hrs

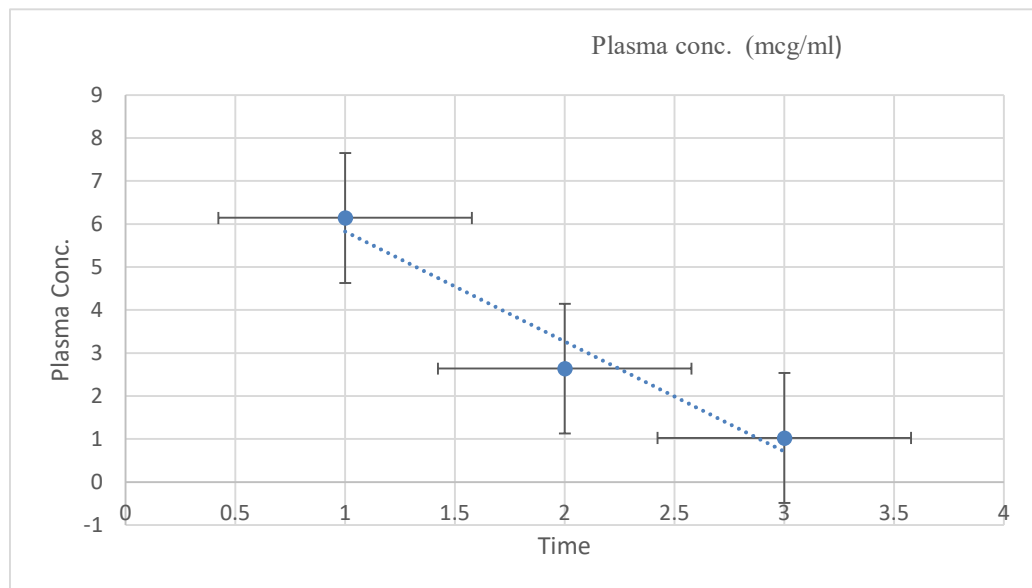


Fig. 5.15: Mean plasma concentration time curve of Galangin

5.4.4. HPLC estimation of Allicin + Galangin

Analysis of pharmacokinetic parameters was evaluated based on various combination of Allicin and Galangin using HPLC technique. Basis on the methodology described earlier, the following findings were observed.

Table 5.13: HPLC detection for Allicin + Galangin in plasma sample

S.N.	Time	Peak concentration		Concentration peak time		Area under concentration time curve	
		Allicin	Galangin	Allicin	Galangin	Allicin	Galangin
1.	10 Min	-	-	-	-	-	-
2.	20 Min	-	-	-	-	-	-
3.	40 Min	-	-	-	-	-	-
4.	1 Hr	-	-	-	-	-	-
5.	2 Hrs	-	-	-	-	-	-
6.	6 Hrs	1.184	1.869	9.732	38.663	2948	3104
7.	8 Hrs	0.783	0.938	9.573	38.659	2149	1185
8.	24 Hrs	0.682	0.783	9.645	38.633	817	791

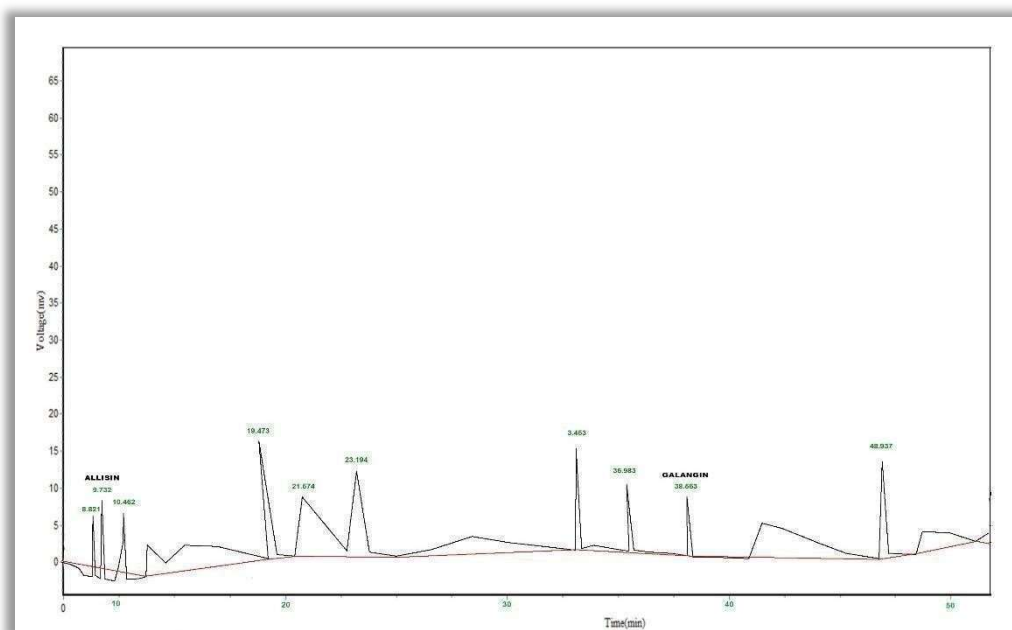


Fig. 5.16: Chromatogram of plasma sample of groups dosed with Galangin and Allicin after 6 hrs

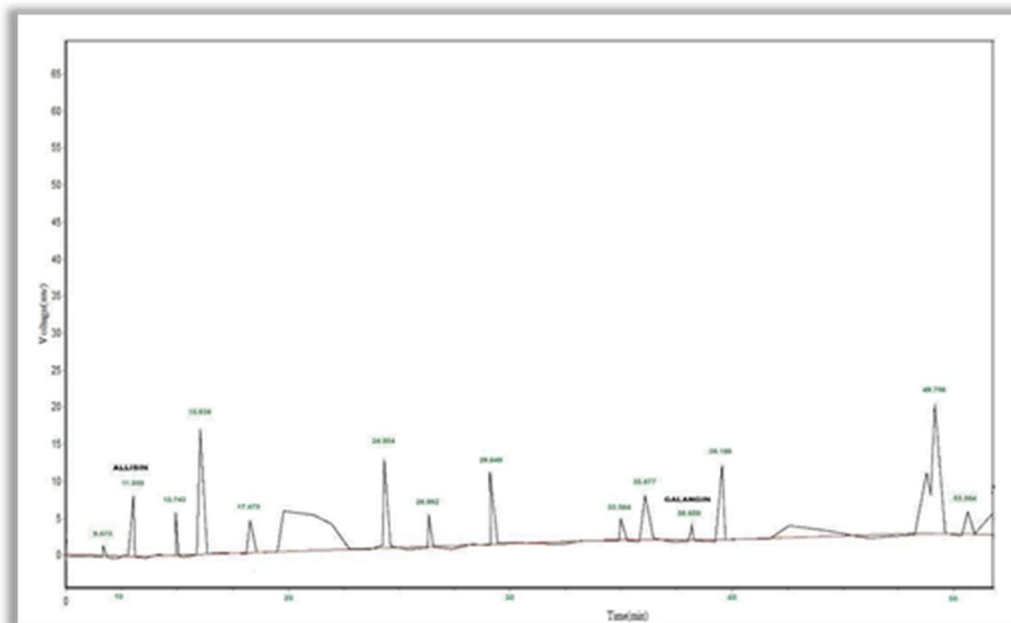


Fig. 5.17: Chromatogram of plasma sample of groups dosed with Galangin and Allicin after 8 hrs

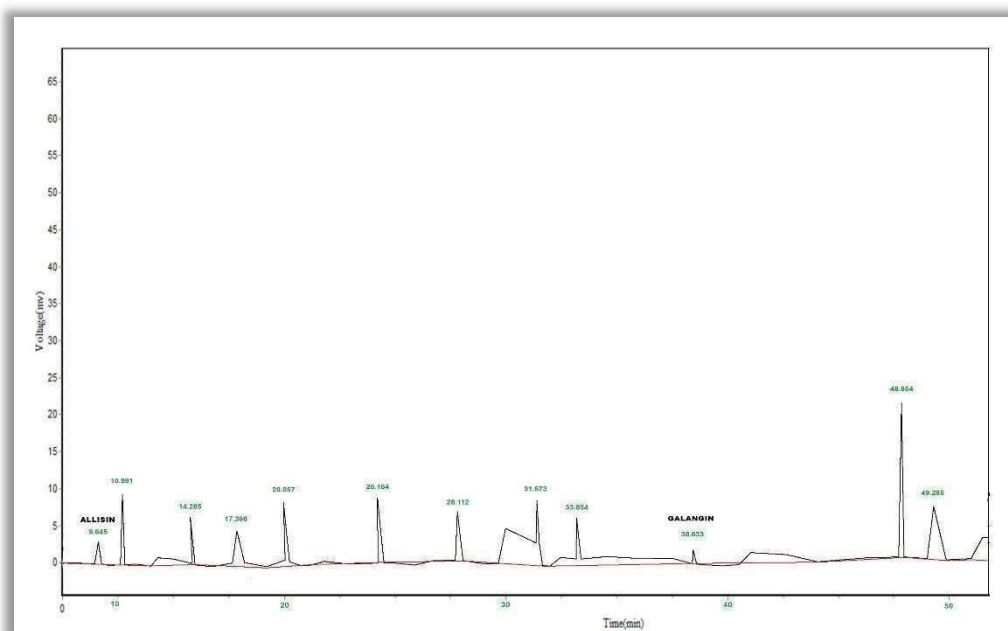


Fig.5.18: Chromatogram shows a sample of plasma groups after 24 hours after a dosage of Galangin and Allicin

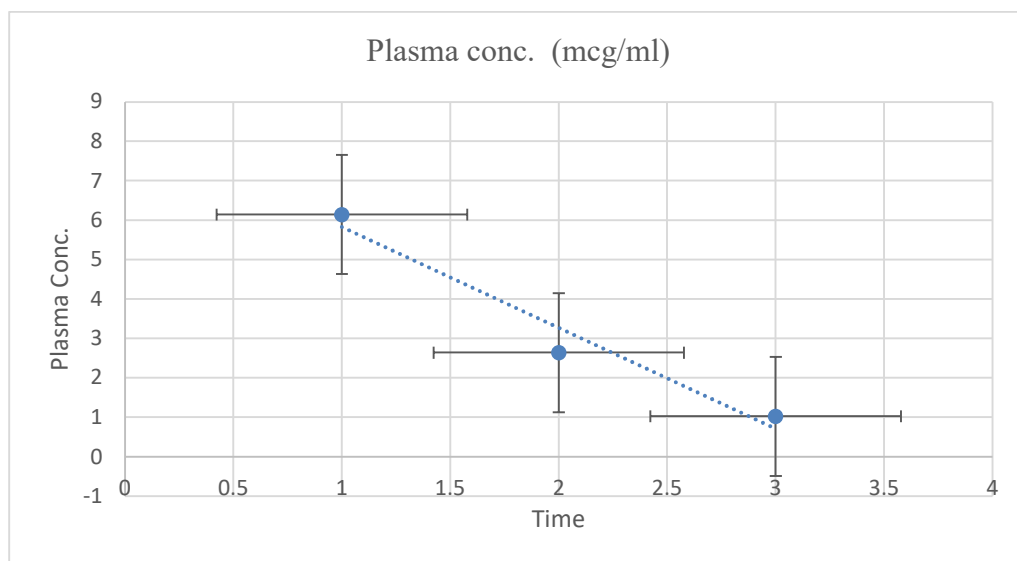


Fig. 5.19: Mean plasma concentration time curve of Galangin + Allicin

5.4.5. HPLC estimation of Fluoxetine + Galangin

Analysis of pharmacokinetic parameters was evaluated based on different combination of Fluoxetine and Galangin using HPLC technique. Basis on the methodology described earlier, the following findings were observed.

Table 5.14: HPLC detection for Fluoxetine + Galangin in plasma sample

S.N.	Time	Peak concentration		Concentration Peak time		Area under concentration time curve	
		Fluoxetine	Galangin	Fluoxetine	Galangin	Fluoxetine	Galangin
1.	10 Min	-	-	-	-	-	-
2.	20 Min	-	-	-	-	-	-
3.	40 Min	-	-	-	-	-	-
4.	1 Hrs	-	-	-	-	-	-
5.	2 Hrs	-	-	-	-	-	-
6.	6 Hrs	2.984	3.159	13.524	38.773	3895	3491
7.	8 Hrs	0.894	1.746	13.719	38.601	1194	2372
8.	24 Hrs	-	1.026	-	38.194	-	899

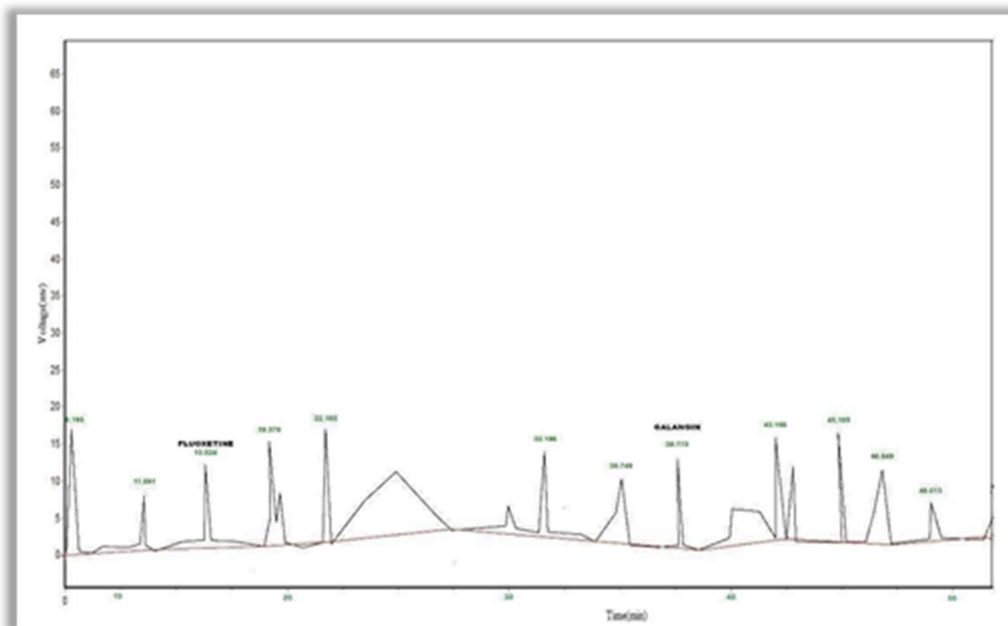


Fig. 5.20: Chromatogram of Fluoxetine and Galangin-dosed plasma samples after 6 hrs

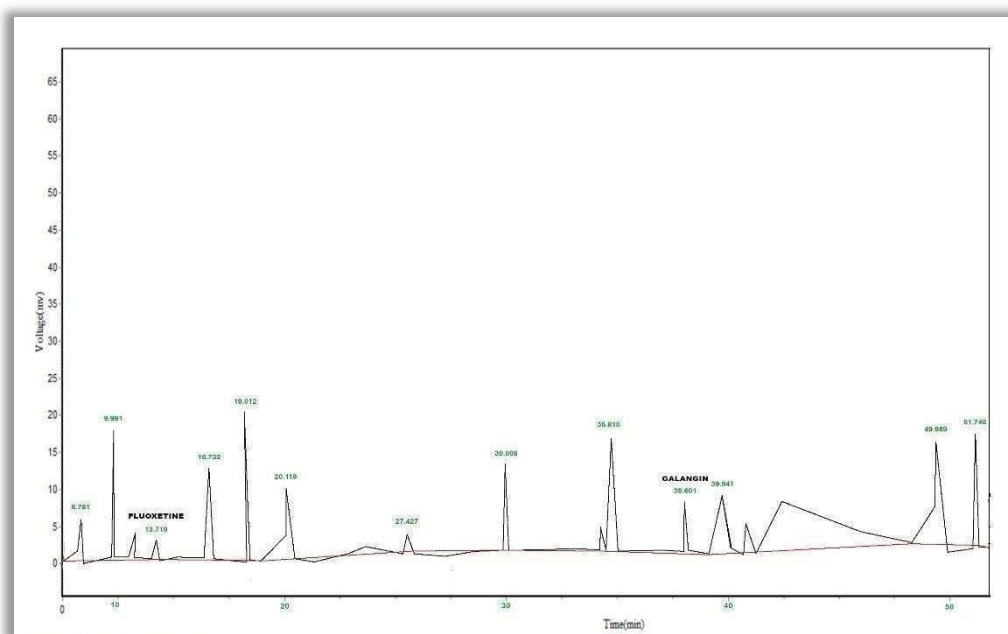


Fig. 5.21: Chromatogram of Fluoxetine and Galangin-dosed plasma samples after 8 hrs

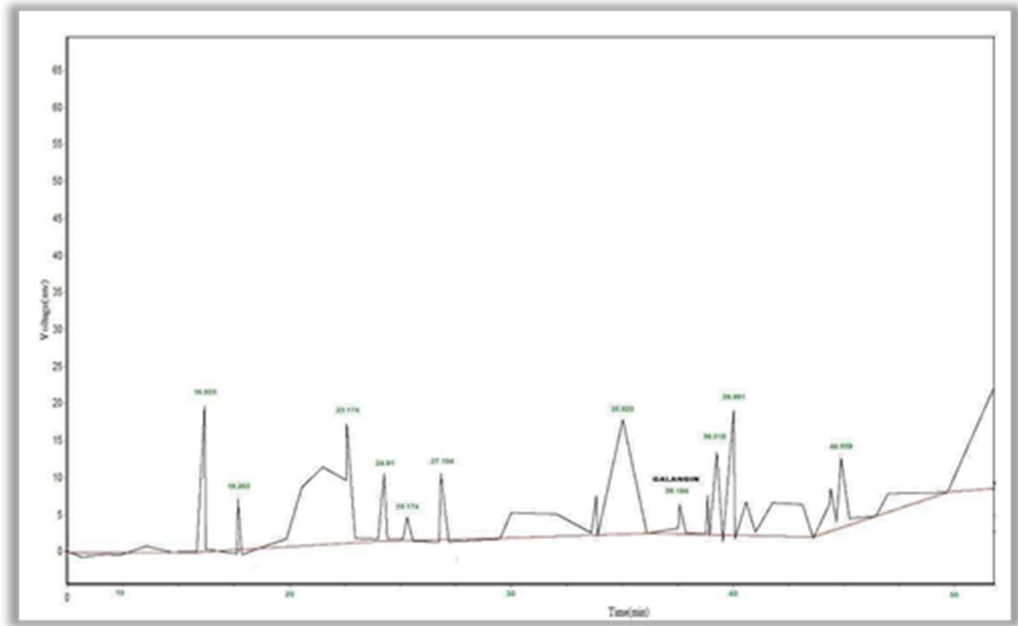


Fig. 5.22: Chromatogram of Fluoxetine and Galangin-dosed plasma samples after 24hrs

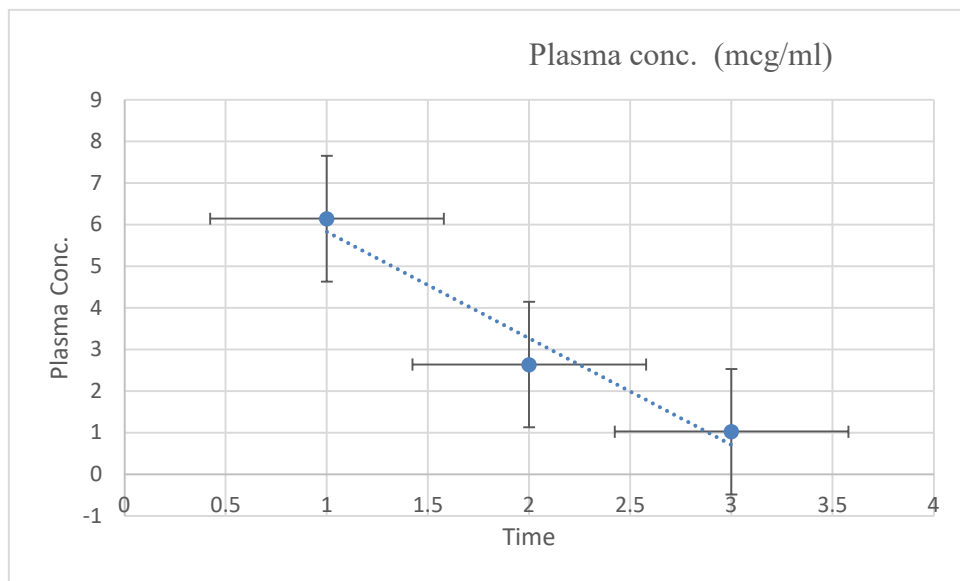


Fig. 5.23: Mean plasma concentration time curve of Fluoxetine + Galangin

5.4.6. HPLC estimation of Fluoxetine +Allicin

Analysis of Pharmacokinetic parameters was evaluated based on combination of Fluoxetine and Galangin using HPLC technique. Based on the methodology described above. The following findings were as follows.

Table 5.15: HPLC detection for Fluoxetine +Allicin in plasma sample

S.N.	Time	Peak concentration		Concentration peak time		Area under concentration time curve	
		Fluoxetine	Allicin	Fluoxetine	Allicin	Fluoxetine	Allicin
1.	10 Min	-	-	-	-	-	-
2.	20 Min	-	-	-	-	-	-
3.	40 Min	-	-	-	-	-	-
4.	1 Hr	-	-	-	-	-	-
5.	2 Hrs	-	-	-	-	-	-
6.	6 Hrs	3.183	0.928	13.658	9.891	3183	912
7.	8 Hrs	2.173	-	13.894	-	1921	-
8.	24 Hrs	-	-	-	-	-	-

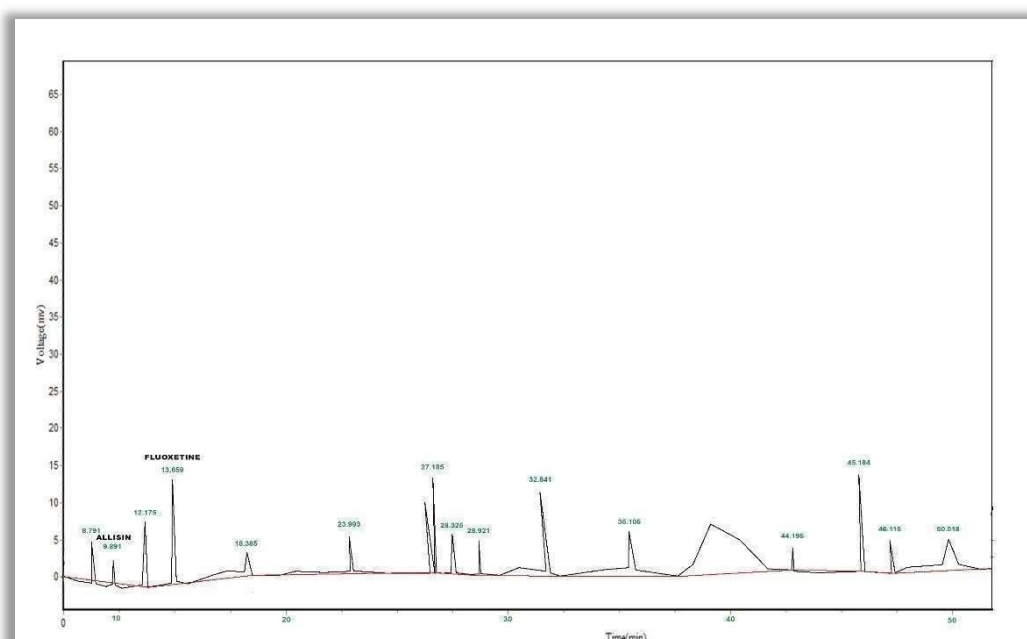


Fig. 5.24: Chromatogram of plasma sample of groups dosed with Fluoxetine and Allicin after 6 hrs.

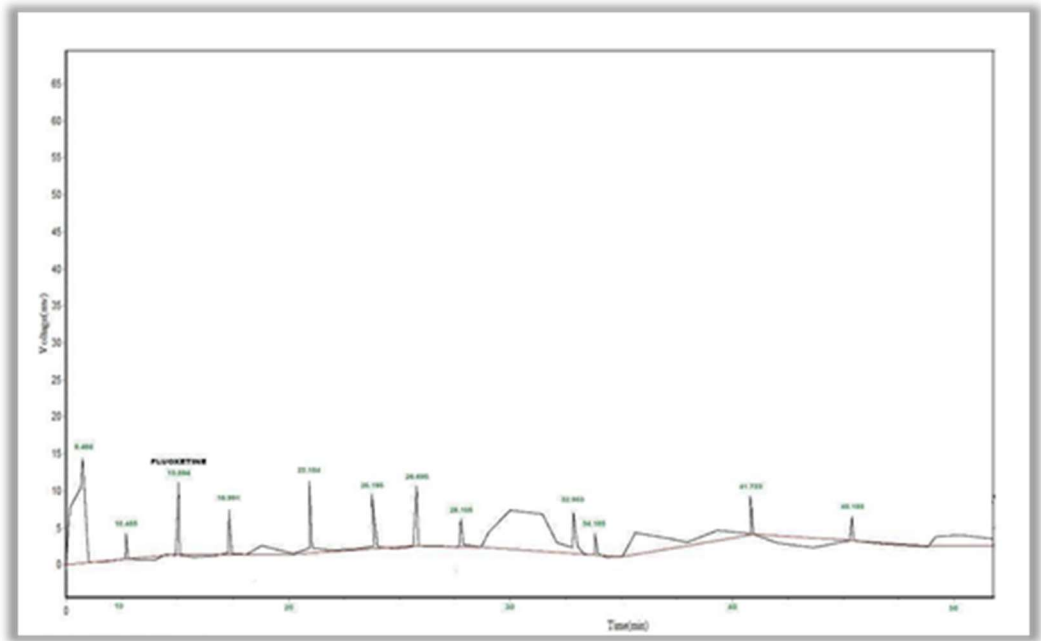


Fig. 5.25: Chromatogram of plasma sample of groups dosed with Fluoxetine and Alicin 8 hrs

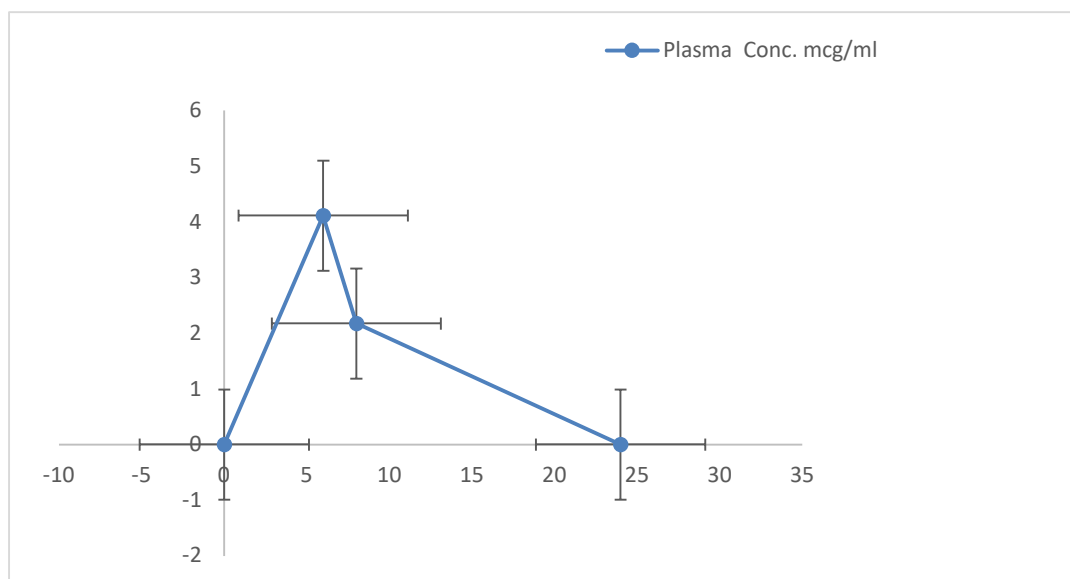


Fig. 5.26: Mean plasma concentration time curve of Fluoxetine + Alicin

5.5. Evaluation of antidepressant activity through neurochemical estimation

5.5.1 Estimation of Brain BDNF level

Estimation of brain neurotrophin and monoamine level was analyzed using ELISA kit. The hippocampus removal from rat brain has been demonstrated in Fig. 5.27.

Table 5.16: Brain BDNF level analysis of rats

S.N.	Groups	OD at 450 nm	Concentration in pg/ml	SEM
1	Control	0.914	59.9	4.2± 2.425
2	CUMS	0.108	19.2	1.1±0.635***
3	CUMS + Flu	0.149	33.00	3.4±1.963###
4	CUMS + Gal	0.272	12.8	1.1±0.635#
5	CUMS + Alli	0.511	25.6	2.1±1.212#
6	CUMS +[Gal + Alli]	0.884	50.2	4.3±2.483###, @@@, \$\$\$
7	CUMS + [Flu + Gal]	0.382	28.1	2.1±1.212#, Δ, @@@
8	CUMS + [Flu + Alli]	0.794	40.8	3.2±1.848###, Δ, \$\$\$

***P<0.001 vs. Control; #P<0.05, ###P<0.001 vs. CUMS Group; @@@ P<0.001 vs. CUMS + Gal; \$\$\$P<0.001 vs. CUMS + Alli; ΔP<0.05 vs. CUMS + Flu.

There was a statistically significant difference (P< 0.05), all pairwise multiple comparison procedure (Bonferroni t-test) specified in Table 5.16.

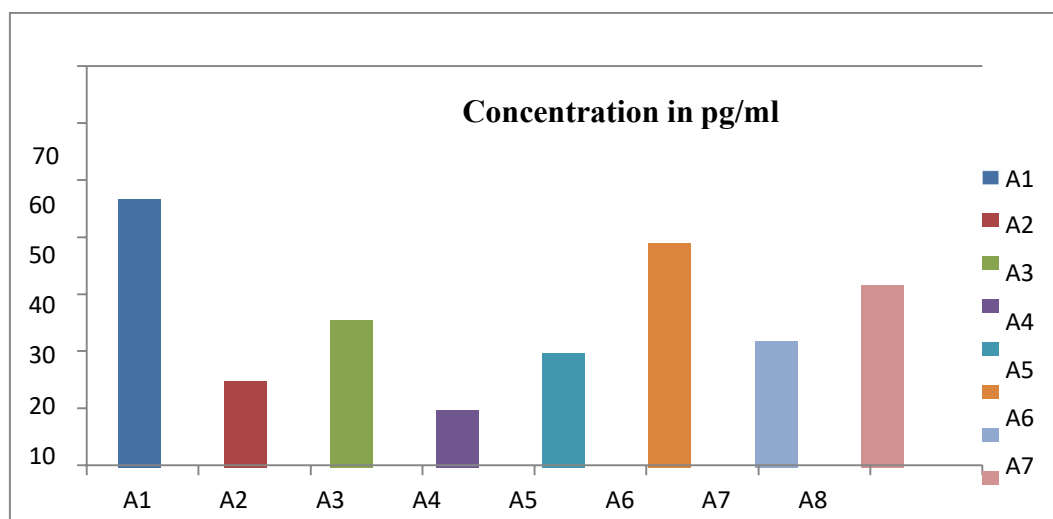


Fig. 5.27: Graphical presentation of the level of brain BDNF assessed in rats under experiment for antidepressant potential of Galangin, Allicin and standard Fluoxetine compared to control

5.5.2 Estimation of brain serotonin level

Table 5.17: Brain serotonin level analysis of rats

S.N.	Groups	OD at 450 nm	Concentration in pg/ml	SEM
1	Control	0.819	5.6	2.4± 1.386
2	CUMS	0.234	0.06	0.01± 0.005 ^{***}
3	CUMS + Flu	0.301	1.4	0.11± 0.063 [#]
4	CUMS + Gal	0.654	4.3	0.4± 0.231 ^{###}
5	CUMS + Alli	0.651	4.2	0.1± 0.05 ^{###}
6	CUMS + [Gal + Alli]	0.954	6.0	0.3± 0.173 ^{###, @, \$}
7	CUMS + [Flu + Gal]	0.784	5.7	0.2± 0.115 ^{###, ΔΔΔ, @}
8	CUMS + [Flu + Alli]	0.732	5.1	0.2± 0.115 ^{###, Δ, \$}

^{***}P<0.001 vs. Control; [#]P<0.05, ^{###}P<0.001 vs. CUMS Group; ^{@@@}P<0.001 vs. CUMS + Gal; ^{\$\$\$}P<0.001 vs. CUMS + Alli; ^ΔP<0.05 vs. CUMS + Flu.

There was a statistically significant difference (P< 0.05), all pairwise multiple comparison procedure (Bonferroni t-test) specified in Table 5.17.

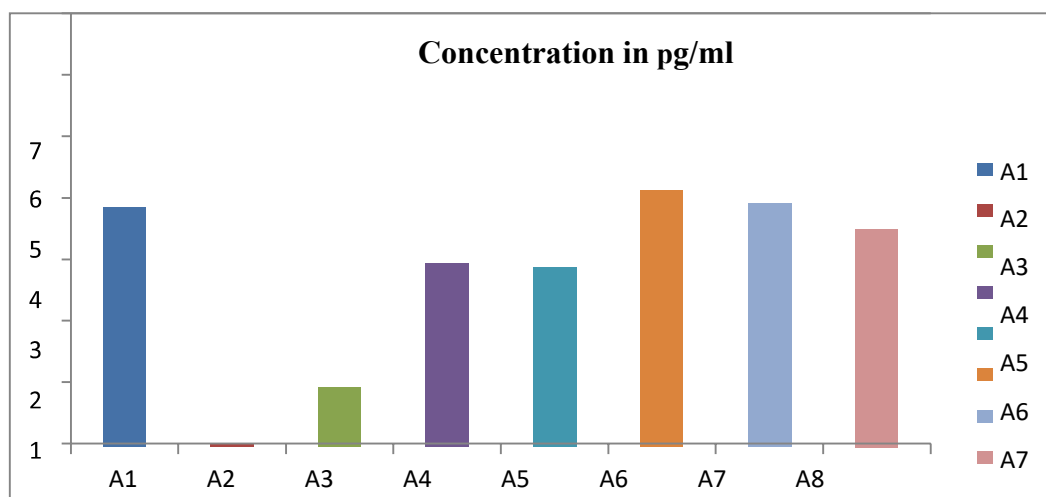


Fig. 5.28: Graphical presentation of the level of brain serotonin assessed in rats under experiment for antidepressant potential of Galangin, Allicin and standard Fluoxetine compared to control

5.5.3 Estimation of brain dopamine level

Table 5.18: Brain dopamine level analysis of rats

S.N.	Groups	OD at 450 nm	Concentration in pg/ml	SEM
1	Control	0.769	56.2	4.3±2.483
2	CUMS	0.120	28.0	1.8±1.039 ^{***}
3	CUMS + Flu	0.653	50.0	4.8±2.771 [#]
4	CUMS + Gal	0.578	47.7	3.7±2.136 [#]
5	CUMS + Alli	0.698	52.5	4.3±2.483 [#]
6	CUMS + [Gal + Alli]	0.932	143	12.3±7.101 ^{###, @@@, \$\$\$}
7	CUMS + [Flu + Gal]	0.835	65.5	5.9±3.406 ^{###, Δ, @}
8	CUMS + [Flu + Alli]	0.781	57.8	4.7±2.714 ^{###, Δ, \$}

^{***}P<0.001 vs. Control; [#]P<0.05, ^{###}P<0.001 vs. CUMS Group; [@] P<0.05, ^{@@@} P<0.001 vs. CUMS + Gal; ^{\$}P<0.05, ^{\$\$\$}P<0.001 vs. CUMS + Alli; ^ΔP<0.05 vs. CUMS + Flu. There was a statistically significant difference (P< 0.05), all Pairwise Multiple Comparison Procedure (Bonferroni t- test); specified Table 5.18.

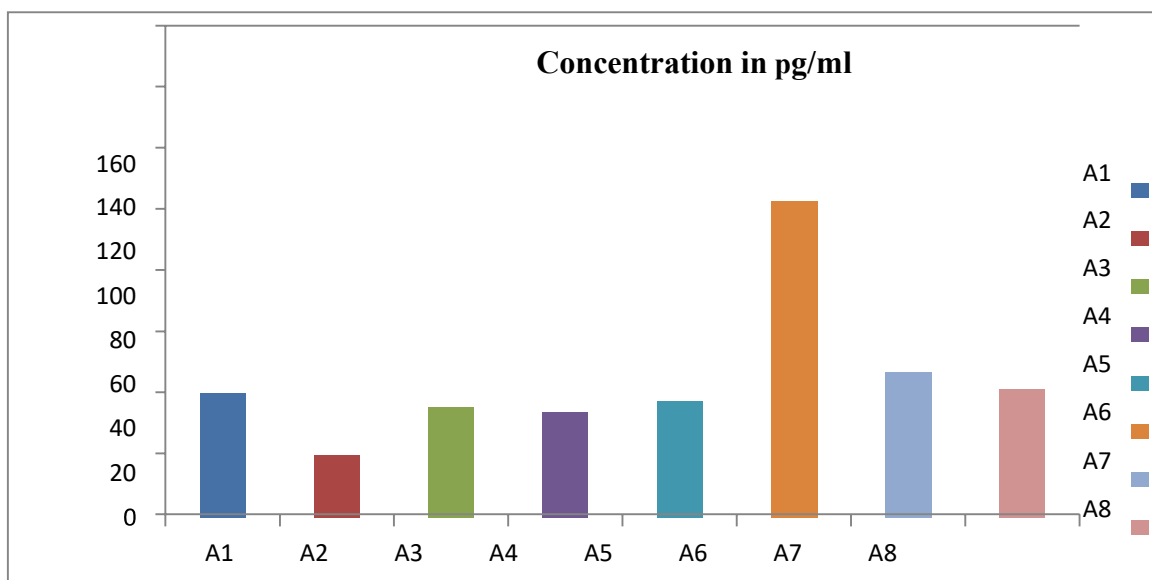


Fig. 5.29: Graphical presentation of the level of brain dopamine assessed in rats under experiment for antidepressant potential of Galangin, Allicin and standard Fluoxetine compared to control.

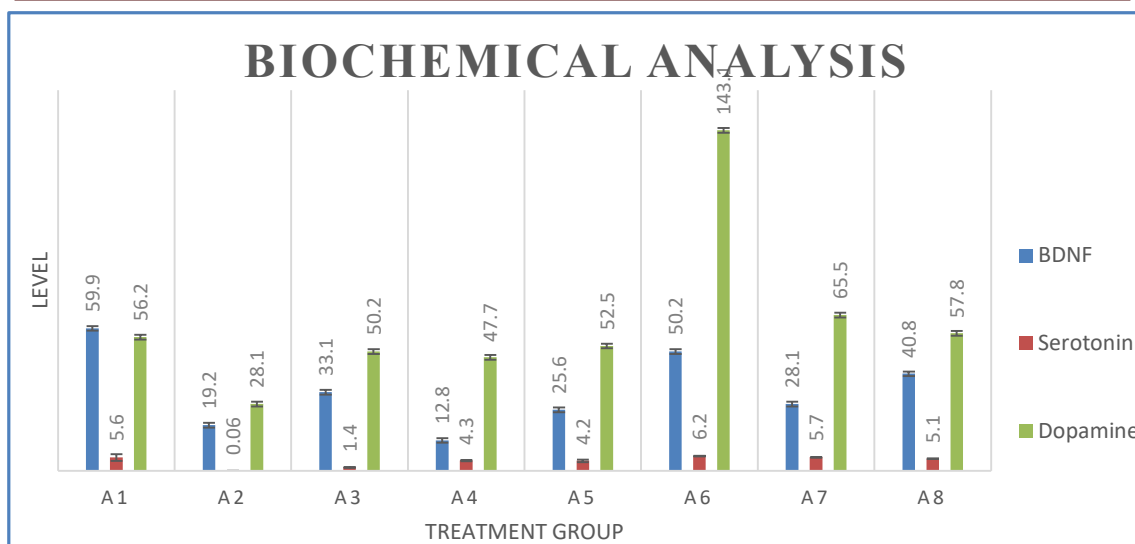


Fig. 5.30. Biochemical Analysis

This figure demonstrated the concentration of BDNF, Serotonin, and Dopamine in plasma sample which indicates the level of anti-depressant activity.

5.6 DISCUSSION

The intent of this investigation intended to evaluate the neuropharmacological effects of co-administration of Galangin and Allicin with Fluoxetine on rats with major depressive disorder caused by chronic unpredictable mild stress. The animal model of depression known as chronic unpredictable mild stress has become popular due to its ability to produce illness in animals through the use of varied, unexpected and unmanageable chronic stress, which is well established risk factor for depression. Furthermore, the CUMS model was linked to the emergence of anhedonia, as well as displays neurological problems such as diminished levels of monoamine and impaired hippocampus neurogenesis (Strekalova et al., 2022; Nollet, 2021). Galangin dose (9mg/kg, b.w.; p.o.) were selected based on research studies (Aloud, 2017). As per previous study, the neuroprotective effect of Galangin in preventing neuronal damage and oxidative insults in rat hippocampus during forebrain ischemia are examined (Mundugaru, 2018). Allicin dose (10mg/kg, b.w., p.o.) were selected based on previous research study (Sani et al., 2023; Rumaseuw, 2022). Allicin has been reported to penetrate blood-brain barrier leading to neuroprotective effects in brain. The anti-depressant like effects of garlic extract is believed to be mostly due to its ability to suppress levels of MAO-A and MAO-B enzymes as well as its interaction with the adrenergic, dopaminergic, serotonergic and GABAergic system (Munshi, Joshi,

& Rane, 2014; Dhingra & Kumar, 2008). Alone as well combination of Galangin and Allicin was tested to explore the potential effect on neuroprotection and overcome the unwanted side effects of marketed anti-depressant drugs on CUMS animal model. Connection between Fluoxetine administration and suicidal thought linked with the dysregulation of serotonin metabolism. As serotonin metabolite 5-Hydroxy indole acetic acid (5-HIAA) was found to be more in depressed patients (Reeves & Ladner, 2010). In this study, CUMS procedure were applied in rats for six weeks (42 days) based on established protocol (Abdul et al., 2016). The Fluoxetine was identified and characterized prior to the CUMS model's induction of depression. The different physical characteristics properties like solubility, melting point, UV spectroscopy, and FTIR spectroscopy were studied for the confirmation of Fluoxetine. It had a white appearance and crystalline structure without any odor. Solubility was another evaluating criterion in addition to physical inspection. Fluoxetine was observed to be soluble in water, ethanol, and methanol. Their melting point, which was another physical characteristic, was estimated to be around 178°C. The procured medicine was characterized and analyzed using UV-Spectroscopy. The main variable utilized to calculate the wavelength of the pharmaceutical solution was max, which produced a result of 212 nm. The application of Fourier Transform Infrared Spectroscopy employed various function groups in their chemical structure.

The FTIR spectrum was confirmed to be the drug sample as Fluoxetine. In the pharmacological evaluation, the stressors were then employed to further create depression, which was then measured using a variety of criteria, included Sucrose preference test, forced swim test, actophotometer test, open field test, hyponeophagia test (Belovicova, Bogi, Csatlosova, & Dubovicky, 2017) and clonidine-induced aggression test (Nikulina, 1993). The effect of sucrose preference test indicates the percentage of sucrose preference. In a group-by-group comparison, it was discovered that the CUMS group's sucrose preference % had significantly dropped from that of the control group. Fortunately, the effect was significantly increased ("P<0.05") by co-treatment of the common medicine Fluoxetine with flavonoid (Galangin) (GAL + FLU). However, the percentage threshold did not increase with a single intake of GAL, ALLI, or FLU. Other co-treatment groups, such (GAL + ALLI) ("P<0.05") and (ALL + FLU) ("P<0.05"), shown a slight increase, but not to the same extent as the GAL+ FLU group. The results of the sucrose

preference test therefore show that A7 (GAL+FLU) demonstrated substantially greater efficacy (data shown in table 5.4). The forced swim test was the next evaluation parameter used to track the total amount of immobility. The results showed that rats in the CUMS group had a much higher degree of immobility, which indicates that they were depressed. This level of depression declined after the rats were also given the standard medication Fluoxetine (Galangin). A8 treated group (ALLI+FLU) likewise shown a significant decrease ("P<0.05") in immobility length, though much less so than A7 treated group (GAL + FLU). Groups treated with Standard Drugs ("P<0.05"), GAL (—P<0.05||), and ALLI ("P<0.05") did not exhibit a significant decline. These findings imply that groups receiving co- treatment were far more successful than those who received a single medicine (data shown in table 5.5). Actophotometer test results show the baseline activity. Reduced activity indicates a CNS depression at some level. When compared to the control treated group, the mean value of the A2 treated group (CUMS Control) exhibited a substantial decrease in basal activity that continued to decline after 30 minutes ("P<0.05"). Co-treated populations, such as A6 (GAL+ALLI) and A7 (GAL + FLU). A7 and A8 treatment groups were shown to have a substantial rise in basal activity score, although GAL + ALLI treated groups showed little to no increase in comparison to the group, with mean values of 280, 277, and 273 ("P<0.05"), respectively. Their antidepressant impact was seen by the rise in baseline activity in the co- treated group (data shown in table 5.6). In group-wise comparisons, locomotion was significantly higher ("P<0.05") in co-treatment groups A6 (GAL + ALLI), A7 (GAL + FLU), and A8 (ALLI + FLU) compared to single treated group in the open field test that was used to measure behavioral evaluations (data shown in table 5.7).

Next evaluation parameter was hyponeophagia test that measure the minute-long eating latency of rat in the test apparatus. Increases in latency during group-wise comparisons indicate CNS depression, which was shown to be significantly reduced ("P<0.05") in the co-treatment group A7 (GAL+FLU). The difference in mean value between the co-treated group and the A7 treated group, which was discovered to be a little higher, indicates their lower efficacy. The evaluation of depressive disorder also included the use of clonidine-induced aggressiveness. Compared to other parameters, it was insufficient to discern behavior. When aggressiveness was examined across groups, co-treatment groups were shown to have a significantly lower level of

aggression (" $P < 0.05$ ") than single treatment groups (data shown in table 5.8). Following the evaluation, pharmacokinetic research was conducted to calculate the plasma peak concentrations (C_{max}), peak times (t_{max}), and area under the concentration time curve (AUC). HPLC was employed as the detection method in order to measure the drug concentration in a plasma sample at different time points. Their absorption rate is determined by their presence in plasma, so the later the peak time, the slower the absorption. The HPLC method's Fluoxetine peak time of detection was noted using standardization with a different solvent system before the detection process began. Their concentration peaked at that wavelength of peak detection. According to the most recent HPLC examination of Fluoxetine in a plasma sample, the drug was clearly visible after 6 hours after dosing, with a peak concentration of 1.78 g/ml. The concentration quickly dropped to 0.68 g/ml after 8 hours, demonstrating their absorption rate. For the detection of absorbance that demonstrates their efficacy, additional drugs such as Galangin and Allicin were used in addition to Fluoxetine. Allicin was shown to be detectable at maximum level of 2.801 g/ml in 6 hours, and then declined to 1.682 g/ml in 8 hours. Nonetheless, their concentration, which is significantly higher than Fluoxetine, demonstrates their efficacy. Galangin was the next medication used as a natural ingredient for HPLC detection. After 24 hours, Galangin was still present in the plasma sample, and its concentration of 0.493 g/ml showed that it was more efficient than Fluoxetine and Allicin. The peak concentration of GAL+ALLI significantly decreased after 24 hours, showing that the drug's concentration persisted in the plasma sample for that amount of time and may have strong antidepressant effects. Nevertheless, HPLC detection was employed when FLU and GAL were administered concurrently. The concentration of Fluoxetine was substantially lower after 24 hours. Galangin's presence, however, reveals their synergistic effect. At 6 hours, the peak concentration of Allicin began to rapidly fall, Based on the results of the HPLC analysis of the co-administration of Allicin and Fluoxetine. Fluoxetine, however, was detected in a plasma sample after 8 hours and similarly dropped quickly. These findings imply that ALLI + GAL and FLU+GAL co-administration are more effective than ALLI + FLU.

Hippocampal levels of brain derived neurotrophic factor, dopamine, and serotonin were all significantly higher in the A6, A7, and A8 treated groups, respectively (" $P < 0.05$ "). BDNF is an important regulator of early neuronal development and

survival and maintain neural plasticity. Neural plasticity refers to the ability of neurons to adapt and respond to the environment. This includes the production of new cells and controlled demise of health brain cells (Duman et al., 1999). The mechanism underlying depression and the action of antidepressants can be elucidated by considering the concept of an intracellular signal transduction cascade and neural plasticity. Learning, memory, stress and the environment activate distinct brain circuits, which in turn initiates an intracellular signal transduction cascade. This cascade is a fundamental process of neural plasticity (Jeon and Kim, 2016). However, a single dose of the common drug Fluoxetine, Galangin, and Allicin had no favorable impact on the level of neurotrophin and neurotransmitters in the brain. Anhedonia is regarded as a fundamental characteristic of a major depressive disorder, and the dopamine system plays a crucial role in the pleasure-related deficiencies. The regulation of dopaminergic activity involves various brain areas such as hippocampus and amygdala. While both fundamental and Clinical research indicate impairments in the dopaminergic system in depression, it is likely that these impairments are caused by dysregulation of circuits that regulate it (Belujon & Grace, 2017). Report revealed that serotonin plays an important role in progressiveness of depression (Moncrieff et al., 2023) a key regulator in the metabolism of monoamines like serotonin, dopamine, and norepinephrine are the mitochondrial enzymes MAO-A and MAO-B. These flavanoidal medications significantly inhibited the activity of the MAO enzyme by interacting with the dopaminergic and serotonergic systems and raising neurotropic levels such as BDNF. These inhibitors have a favourable impact on depression by lowering serotonin metabolite levels, which in turn lowers suicidal thoughts.

6.1 SUMMARY AND CONCLUSION

Neurological illnesses are the major issues across the world. Among them, major depressive disorder that is the most prevalent mental ailment which nowadays affects every age from young to elder people. In the next ten years, according to the World Health Organization (WHO), depression will be the second leading cause of death globally. According to study, symptoms including sadness, inability to make decisions, lack of energy, libido loss and trouble doing work occur more frequently in females than in males. Emotional changes, a change in thinking, change in behaviors, physical changes were the different identified by scientist. It has been shown that the morbidity of depression and monoaminergic system dysfunction are closely related. It was discovered that severe depression was linked to monoamine reserpine depletion. Additionally, monoamine oxidase inhibitors (MAO), may stop the deterioration of Nor Epinephrine and 5-HT (Serotonin), may alleviate depressed symptoms, indicating that the Nor Epinephrine and 5-HT (Serotonin) systems are crucial in the development of depression. There may be a pathophysiological connection between decreased central and peripheral immune systems and the development of the neurological abnormalities found in MDD, as shown by recent research that lends credence to the neuro immune theory. There are currently many synthetic drugs available to treat depressive symptoms, however some of these drugs have serious adverse effects that have been seen during depression treatment. Some of the negative effects of antidepressant usage include yawning, a reduced libido, decreased arousal, anorexia, dry mouth, headaches, nausea, diarrhea, insomnia, and dry mouth (visible as reduced lubrication in females and difficulty erecting in males), anxiety, nervousness, and yawning are the most common adverse effects reported by adults. Fluoxetine users may experience Suicidal thoughts, agitation, sleeplessness, and anxiety.

The present study was designed to overcome the side effects caused by SSRI drug Fluoxetine . With such objectives, we analyzed the neuroprotective effect of flavanoidal drugs such as Allicin and Galangin alone as well as in combination with Fluoxetine . Connection between Fluoxetine administration and suicidal thought linked with the dysregulation of serotonin metabolism. As serotonin metabolite 5-Hydroxy indole acetic acid (5-HIAA) was found to be more in depressed patients.

Many research have been undertaken in recent years to investigate the antidepressant properties of natural chemical substances, particularly flavonoids, which have diverse effects on the brain. Certain flavonoids have been reported to restore depressed behavior in rodents in animal models, according to a number of preclinical investigations. The proposed underlying mechanisms for anti-depressant activity include increased levels of different neurotransmitter expression, neurotrophic factors, and neurogenesis in the brain. Galangin, is a polyphenol that is primarily derived from several medicinal plants, including *Alpinia officinarum* Hance, *Alnus pendula* Matsum, and *Plantago major* L. It has been used for a very long time in Asian culture as a traditional remedy for diabetes, diarrhea, digestive problems, coughs, and colds. Along with these properties, Galangin also has positive effect on brain and therefore exhibit neuroprotective effect. The aroma and flavour of freshly cut or crushed garlic are attributed to Allicin, a naturally occurring sulphur-containing molecule with a wide range of biological benefits. Previous research indicates the importance of Allicin to maintain antioxidant enzymes in alleviating anxiety and depression. In a rat model of persistent unexpected moderate stress, this research compared the effects of two flavonoids, Galangin and Allicin, with Fluoxetine, a traditional antidepressant drug (CUMS). Critical markers such as BDNF, 5-HT, and dopamine were used to evaluate the efficiency of various drugs (DA). This research examined the effects of Fluoxetine in combination with Galangin and Allicin on rats with mild stress-induced major depressive disorder. The study's initial phase was chronic unpredictable mild stress-induced depression in rats. According to the published methodology, the chronic unexpected mild stress (CUMS) approach treatment that lasted a whole six weeks in rats (42 days). Eight stressors were included in the protocol, including noise, cage tilting, overnight illumination, food and water deprivation, crowded housing, and soiled cage. These stressors were tested with Fluoxetine (10 mg/kg, p.o.), Galangin (10 mg/kg), and Allicin (9 mg/kg). The Sucrose preference Test, Tail suspension Test, Forced swim Test, and Open field Test, Clonidine-induced hyponeophagia assessed depression in all therapy. Pharmacokinetic studies evaluated peak concentration, duration, and concentration time curve area. BDNF, serotonin, and dopamine were biochemically analyzed using ELISA. This study concluded that combination of ALLI + GAL and FLU + GAL are more effective than ALLI + FLU. Peak Plasma Concentration of ALLI + GAL combination was found to be higher and remain in plasma after 24 hrs. that indicate it's higher

bioavailability in comparison with other combination of drug and hippocampal levels of Brain derived neurotropic factor, dopamine, and serotonin were all significantly higher in the A6, A7, and A8 treated groups, respectively (“ $P < 0.05$ ”). However, a single dose of the common drug Fluoxetine, Galangin, and Allicin had no favorable impact on the level of neurotrophin and neurotransmitters in brain. So, it can be suggested to explore the combination effect of flavonoids as an alternate therapy against depression and reduces the side effects of marketed drug.

The synergism effect of Allicin and Galangin was found to be effective against depression and suicidal side effects caused by Fluoxetine. Mitochondrial enzymes (MAO-A and MAO-B) act as important regulator in the metabolism of monoamines such as serotonin, dopamine and norepinephrine. These flavanoidal drugs showed significant anti-depressant activity against MAO enzyme through the interaction with dopaminergic, serotonergic system as well as also increased neurotropic level like BDNF. These inhibitions decrease the level of serotonin metabolite and therefore reduce the suicidal tendency and exert positive effect against depression.

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Date: 15/1/21

Institutional Animal Ethics Committee (IAEC)

(Constituted under Committee for the purpose of control and supervision of experiments on animals)(CPCSEA)

Registration No: 1429/PO/a/11/CPCSEA

CERTIFICATE

This is to certify that the project entitled "**Impact of administration of fluoxetine with galangin and allicin against mild stress induced major depressive disorder in rodents**" submitted by Mr. Ashish Mishra for the dissertation work for Ph.D. has been approved by the IAEC meeting held on 15.01.2021.

Project Ref. – SIRT-P/IAEC/112



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Current Opinion in Environmental Science & Health

Volume 19, February 2021, 100210




Neuroplasticity and environment: A pharmacotherapeutic approach toward preclinical and clinical understanding

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<https://doi.org/10.1016/j.coesh.2020.09.004> 

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Abstract

Emerging research in the field of behavioral neuroscience has demonstrated the fundamental role of the cerebral organization. For brain development, various important functions are required for instance synaptic plasticity and neurogenesis. This property is described in terms of neuroplasticity. Neuroplasticity denotes the extraordinary ability of



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ISSN (Online): 1875-6646

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Research Article

Role of Allicin in Combination with Fluoxetine for the Treatment of Major Depressive Disorder in Rodents: In-Vitro Pharmacokinetic and Pharmacodynamic Study

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(E-pub Ahead of Print)

Published on: 10 July, 2023
Article ID: e160523216966
DOI: 10.2174/1573407219666230516124135
Price: \$95

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Abstract

Background: Depression is one of the most common neurological disorders for which only symptomatic treatment is available with synthetic drugs with serious side effects. Allicin [S- (2-propenyl)-2-propene-1-sulfinothioate] is a bioactive component derived from *Allium sativum* L. (Garlic). It has the property to penetrate the blood-brain barrier, which reveals its neuroprotective property. A synthetic drug named fluoxetine (selective serotonin re-uptake inhibitor) was introduced for the clinical treatment of depression. It is a selective serotonin re-uptake inhibitor that is well absorbed after oral administration.

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