Effect Of Cilnidipine on Depression Paradigm in Male Swiss Mice - An Experimental Study

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LIST OF ABBREVIATIONS

ACE: Angiotensin converting enzyme

ACTH: Adrenocorticotropic Hormone

ANOVA: One way analysis of variance

ARB: Angiotensin II receptor blocker

5-HT: Serotonin

BDNF: Brain derived neurotrophic factor.

cAMP: Cyclic adenosine monophosphate

Ca^{2+}: Calcium

CA: Catecholamines

CCB: Calcium channel Blockers

CLD: Cilnidipine

CNS: Central Nervous System

CMD: Common Mental disorder

CCSEA: Committee for Control and Supervision of Experiment on Animals

CRF: Cortisol Releasing Factor

CRH: Corticotropin Releasing Hormone

CRP: C-Reactive Protein

DALY: Disability-Adjusted Life Years

DG: Dentate gyrus

DHPs: Dihydropyridines

DSM V: Diagnostic and Statistical Manual of Mental disorders

FSH: Follicular Stimulating Hormone

FST: Forced Swim Test

GABA: γ-amino butyric Acid

GR: Glucocorticoid Receptors

HPA: Hypothalamic-Pituitary-Adrenal axis

IAEC: Institutional Animal Ethics Committee

ICD: International Classification of Diseases

MAO: Monoamine oxidase

MDD: Major Depressive Disorder

Na SSA: Noradrenergic and specific Serotonergic antidepressant

NA: Noradrenaline

NE: Norepinephrine

NCD: Non – communicable disease

NGF: Nerve Growth Factor

NK-1: Neurokinin-1

NMDA: N-Methyl D-Aspartate

NPY: Neuropeptide Y

NRI: Norepinephrine reuptake inhibitors

NT: Neurotrophins

MR: Mineralocorticoid Receptor

PTSD: Post Traumatic Stress Disorder

RIMA: Reversible inhibitors of MAO A

ROS: Reactive Oxygen Species

SAD: Seasonal Affective Disorder

SD: Standard deviation

SSRI: Selective Serotonin Reuptake Inhibitor

SNRI: Serotonin Norepinephrine reuptake inhibitor

TCA: Tricyclic antidepressant

TNFα: Tumour Necrosis Factor α

Trk: Tyrosine Kinase

TST: Tail Suspension Test

VIP: Vasoactive Intestinal Polypeptide

WHO: World Health Organisation

YLD: Years lived with disability

ABSTRACT

Introduction

Depression is a commonly occurring ailment that frequently coexists with cardiovascular problems, impacting approximately 16-23% of individuals. Emerging genetic, ex vivo, and clinical trial evidence suggest that Calcium channel blockers (CCB) have a positive effect on mood and cognitive performance. The hypothesis posits that calcium channel blockers (CCB) alter the release of central neurotransmitters, particularly noradrenaline (NA) and 5-hydroxytryptamine (5-HT) contribute to the occurrence of depression. Nevertheless, the therapy now accessible has limitations in terms of effectiveness and the accompanying negative consequences.

Study Objectives:

1. To evaluate the effects of Cilnidipine on depression paradigm in Male Swiss Mice using the Forced Swim Test, Tail Suspension Test and Locomotor Activity Test.
2. To compare the effects of Cilnidipine and Fluoxetine on depression paradigm in Male Swiss Mice using the Forced Swim Test, Tail Suspension Test and Locomotor Activity Test.

Methods:

Animals categorised into four groups, each consisting of six individuals (n = 6 per group). The participants received the test drug doses of Cilnidipine at 5mg/kg and 10mg/kg, as well as Fluoxetine at 10mg/kg, for a duration of 21 days. The positive effects of antidepressants were evaluated using the Forced Swim Test (FST), Tail Suspension Test (TST), and locomotor activity was measured using an Actophotometer on Day 1, 14, and 21. The duration of immobility was measured for 6 minutes in the Forced Swim Test (FST) and for 5 minutes in the Tail Suspension Test (TST). The count was obtained using an Actophotometer.

Results:

When compared to the normal control, administering Cilnidipine at a dosage of 10mg/kg results in a decrease in depressed symptoms. There was no significant reduction in locomotor activity observed with either the administration of Cilnidipine at a dosage of 5mg/kg or 10mg/kg.

Conclusion:

The present investigation exhibited a significant antidepressant impact of the Cilnidipine dosage of 10mg/kg. Further investigation is required to authenticate the presented findings.

Keywords:

Depressive disorder, Elevated blood pressure, Dihydropyridines, Forced swim test, Tail suspension test, Neurotransmission, and Corticosterone.

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INTRODUCTION

Depressive disorder is a prominent and highly widespread mental disorder worldwide. It is characterised by persistent hopelessness, a lack of enjoyment or interest, low energy, inadequate food and sleep, and even suicidal thoughts, which affect day-to-day activities and psychosocial functioning. [1]

Since 2008, depression has been ranked by the World Health Organisation (WHO) as the third most common cause of disease burden. Globally, depression has a major detrimental economic impact. By 2030, it will probably be in the leading rank.[2,3] Depression was the sixth most prevalent cause of years lived with disability (YLDs), accounting for 34.1 million of all YLDs, according to the 2016 Report on the Worldwide Impact of illnesses, injuries, and associated risks.[4]

In 2016, the prevalence of depression in India was 2.7% for existing and 5.2% for lifetime depression. This indicates that almost 1 in 40 and 1 in 20 people suffer from depression, respectively. Depression is more common in women, those aged 40 to 49, and those who reside within urban areas. Equally high rates were reported amongst the elderly (3.5%).[5]

Several non-communicable diseases (NCDs), such as elevated blood pressure, heart attacks, strokes, memory loss, seizures, and endocrine conditions including hyperglycaemia and hypothyroidism, are closely associated with common mental disorders (CMDs), including depression.[6] Recently, it has been found that the chronic nature of these medical conditions is an additional cause of depressed symptoms in the patient.

Treatment of such comorbidities often necessitates the concurrent use of multiple drugs, which leads to a drug-interaction or drug-disease relationship which can improve or worsen the primary disorder or comorbidity in the latter case, thus contributing to a significantly higher healthcare burden.[5]

The aetiology of depression remains uncertain; however it is commonly acknowledged that a multifaceted interplay of biological, psychological, and social variables contributes to the development of this disorder. Depression is a complex medical condition involving a variety of neurotransmitters, chemicals are commonly considered as messengers that transport signals between brain cells or neurons. The "monoamine hypothesis," which states that an imbalance in monoamine neurotransmitters, namely serotonin (5-HT) or norepinephrine (NA), is the basis for conventional depression treatment.[7] Other neurotransmitters that have been linked to mood and emotion regulation include glutamate and gamma-aminobutyric acid (GABA), which could be a different explanation for depressive symptoms.[7]

Study to determine how calcium channel blockers (CCBs) affect central neurotransmitter release such gamma-aminobutyric acid and noradrenaline has been conducted.[8,9] According to some theories, CCBs may enhance intracellular calcium release through NA and GABA-A receptors, both of which are involved in the pathophysiology of depression. This might result in an antidepressant effect.[9] Moreover, brain penetrant CCBs have been associated to a lower incidence of neuropsychiatric diseases, including depression.[10]

Currently, available therapy modalities for depression, which include selective serotonin receptor inhibitors (SSRIs), selective noradrenaline receptor blockers (SNRIs), and other older classes of drugs, are lacking to provide a complete recovery in most of the patients, and many patients remain resistant to treatment with these medications. Furthermore, antidepressants elicit major side effects such as drowsiness, hypotension, anticholinergic symptoms, and, less commonly, cardiac arrhythmias. Additionally, a significant latent period of 8-12 weeks is required to generate adequate therapeutic response. Unfortunately, only 30-40% of patients achieve remission with a single treatment, and most of these patients are switched to another medication or augmented with another drug.[11] As a result, there is a significant demand for additional treatment choices in depressive illnesses. Numerous effects of calcium channel blockers (CCBs) have been reported, including effects on inflammation, oxidative stress, endothelial function, and central neurotransmitter release.[8,12] These pleiotropic effects may contribute to the potential benefits associated with CCBs in many illnesses, including neuropsychiatric disorders and cardiovascular health. [12,13]

An investigation was conducted into the effect of CCBs, specifically nifedipine and verapamil, on the antidepressant properties of imipramine and alprazolam. These CCBs are frequently used to treat illnesses that have been related to depression.[9]

Furthermore, the effects of combining CCBs with selective serotonin reuptake inhibitors (SSRIs) were examined in a cohort study carried out in three locations. The results indicated that the simultaneous use of selective serotonin reuptake inhibitors (SSRIs) and calcium channel blockers (CCBs) might potentially amplify both depressive symptoms and cognitive performance in elderly adults with hypertension.[14]

Several research investigated the prevalence of depression among hypertensive individuals. An extensive investigation and meta-analysis found that the estimated occurrence of depression in patients with hypertension was 21.3%.[15] Depression was associated in another research project to uncontrolled blood pressure in hypertension patients.[16] These findings emphasise the significance of treating patients with mental health issues with hypertension, as well as the potential impact of depression on blood pressure regulation. The coexistence of hypertension and depression is a major concern, and better therapies for depression in hypertensive individuals are required.[15,17] The association between antihypertensive medication and depression was studied via a network meta-analysis and systematic review. Calcium channel blockers, one of the four major antihypertensive groups, were found to be positively associated with symptoms of depression.[18] However, the significance of CCBs in depression remains unknown, and more extensive clinical and animal studies are required to validate their effects These findings suggest that calcium channel blockers should be studied for their antidepressant effects. Dihydropyridines (DHPs) are lipophilic and the most effective calcium channel blockers; among them, cilnidipine has a pleiotropic effect with cerebroselective action. Considering that into account, the objective of this research is to assess cilnidipine's impact on a mouse depression model.

OBJECTIVES

PRIMARY OBJECTIVE

To evaluate the effects of Cilnidipine on the depression paradigm in Male Swiss Mice using the Forced Swim Test, Tail Suspension Test and Locomotor Activity Test.

SECONDARY OBJECTIVE

To compare the effects of Cilnidipine and Fluoxetine on the depression paradigm in Male Swiss Mice using the Forced Swim Test, Tail Suspension Test and Locomotor Activity Test.

REVIEW OF LITERATURE

The mental well-being of an individual is a crucial component of their total health. Throughout history, humanity has been plagued by mental diseases. Mental disorders are defined by deviations in cognition, emotion, mood, or the most complex parts of behaviour, such as social interactions or future activity planning.[19] A psychiatric condition, also known as a mental disorder, refers to a psychological pattern or abnormality that can be observed in a person's behaviour. It is typically characterised by distress or incapacity and is not regarded a normal aspect of development within a person's culture.[20]

Major depressive disorder (MDD) moved up from the 15th position in 1990 to the 11th position in the rankings of diseases causing the largest DALYs (Disability-Adjusted Life Years) Based on the "Global burden of disease" report - 2010" survey, an increase of roughly 37%.[21] Based on a research undertaken by the World Health Organisation (WHO), more than one-third of individuals in most countries have issues at some point in their lives that fulfil the criteria for diagnosing one or more of the prevalent mental disorders. According to the same study, there are three main categories of illnesses: anxiety, mood disorders, and drug addiction disorders. They play a major part in estimating the general prevalence of mental illnesses.[22]

Of the Disability-Adjusted Life Years (DALYs), mental and behavioural illnesses made up 7.4%. Among this broad category, five distinct diseases were responsible for more than 15 million DALYs each. The main contributory causes were schizophrenia (0.6%), drug abuse disorders (0.8%), anxiety disorders (1.1%), and major depressive disorder (2.5%).[21] By 2030, estimates indicate that if present demographic and epidemiological transition patterns continue, depression will account for 5.7% of the total burden of disease. This would make depression the second most significant cause of DALYs, ranking only below ischemic heart disease.[23] Regarding public health, depression ranks as the third most significant factor, contributing to

4.3% of the worldwide burden of illness as assessed by disability-adjusted life years. If the current patterns persist, it will emerge as the primary source of disease burden by the year 2030.[24] Depression affects people from every group worldwide and is one of the main factors that significantly contributes to the overall impact of illness and health issues worldwide. Currently, it is approximated that over 350 million individuals are impacted by depression. In the 17-nation World Mental Health Survey, about 1 in 20 people said they had had a depressive episode in the previous year.[25] Overall, psychiatric problems play a significant role in causing illness and have a substantial economic impact. It is estimated that mental disorders will account for over one-third of the anticipated US$ 47 trillion cost associated with all non-communicable diseases.[26] Hypertension and depression are commonly observed to occur together, indicating a comorbid relationship between the two disorders.

Hypertension is a constituent of metabolic illnesses prevalent in India. In September 2023, the occurrence of Hypertension in India stands at 18.3%, which is marginally below the worldwide average of 31%. Based on a study conducted in 2022, it was shown that 41% of individuals in India who suffer from hypertension also experience depression. Both depression and hypertension raise the risk of cardiovascular disease and are often related. Depression may make uncontrolled hypertension more likely, while in elderly people, hypertension can cause depressed symptoms. Insufficient dopamine levels in critical areas of the brain can lead to elevated blood pressure and/or initiate depressive symptoms.[27] Moreover, persons with hypertension may be more susceptible to depression due to the cerebrovascular and ischemic alterations in the brain caused by high blood pressure.[28]

The term "depression" is commonly employed to refer to any conditions of lowered mood that do not possess clinical importance. Depression is a condition characterised by a diminished mood and a lack of desire to engage in activities, which can impact a person's cognition, emotions, actions, and overall sense of happiness. A person's capacity to function is severely hampered by physical and mental changes that go along with a continuous sense of melancholy or agitation that characterise depression.[29] The hallmark of MDD, a mental illness, is a persistent and depressing mood together with a low sense of self-worth and a lack of interest or pleasure in frequently enjoyable activities. Depression is a highly expensive neurological disorder globally, both in terms of the immense pain and suffering it causes, as well as the economic costs associated with lost productivity and the risk of mortality due to suicide. There is evidence of a rising occurrence rate and an earlier age at which it is happening.[30]

Depression can appear either as a solitary occurrence or as repeated bouts. The duration of the course may be extended up to 2 years or more in individuals with the single-episode form.[20] While most patients with major depressive disorder have a positive prognosis for recovering from an acute episode of depression, three out of four people with MDD will experience recurring episodes throughout their lifetime. These episodes may vary in intensity and leave behind lasting symptoms.[31]

The DSM V provides diagnostic criteria for Major Depressive Disorder (MDD).[32]

A. Five symptoms from the following list must be present continuously for a minimum of two weeks in order to meet the diagnostic criteria; these symptoms must also show a discernible departure from the person's typical functioning: One of the signs, at minimum, is a depressed mood or a lack of enjoyment.

1. Constant, overwhelming, and pervasive emotions of melancholy, emptiness, and hopelessness that are subjectively reported or seen by others—such as sobbing. (Note: Children and teens may exhibit irritable mood.)
2. 2. The individual experiences a notable decrease in interest or enjoyment in practically all activities during the vast portion of the day, practically every day.

3. Rather than being based just on subjective feelings of restlessness or slowing down, psychomotor agitation or retardation happens practically daily and may be noticed by others.
4. Observable reduction in body weight without intentional modifications to one's diet or substantial weight gain (e.g., a change exceeding 5% of total body weight within a month) or consistent decrease or rise in hunger. (Note: The absence of expected weight increase in paediatric patients should be taken into account.)
5. Having either everyday hypersomnia or insomnia.
6. Persistent sense of insignificance or excessive, inappropriate remorse (perhaps delusional) on a daily basis (not limited to self-blame or guilt related to illness).
7. Everyday incidence of lower cognitive function or trouble making judgements, as stated by the individual or as seen by others.
8. Having daily exhaustion or energy loss.
9. Constant reflection about death (beyond simple dread of dying). Recurrent thoughts of wanting to terminate one's own life without having a particular plan, or of having attempted suicide before.

B. The symptoms cause obvious distress or hinder communal, job-related, or additional significant areas of activity.

C. The episode is not explainable by a drug's physiological effects or by another medical problem.

Criteria A-C indicate the presence of a significant depressive episode.

The monoamine hypothesis suggested that depression was attributed to a deficiency in the functioning or quantity of monoamine neurotransmitters in certain regions of the brain. Recent research has provided mounting evidence that, alongside the lack of monoamines, both endocrine and neurotrophic factors play significant roles in the development of depression.

Although depression has a significant influence on most people, our understanding of its pathogenesis remains unclear. There are other factors contributing to this disparity. First and foremost, the task of observing degenerative changes in the brain is considerably more challenging compared to other organs. The methods used to document abnormal brain circuit function involve either post-mortem investigations or brain scans techniques. Neuroimaging techniques depend on identifying alterations in neuronal activity through indirect markers of activation. Furthermore, most depression disorders are of idiopathic origin. The specific genes responsible for causing depression in mice have not yet been discovered.[33] Several theories have been suggested to explain depression:

1. The Monoamine Theory: The monoamine theory was initially introduced by Joseph JI. Schildkraut in 1965.[34] According to this idea, there is a deficit in the quantity or function of monoamines such as serotonin, norepinephrine, and dopamine in certain regions of the cortex and limbic system.[35] The evidence supporting this theory is further discussed. Patients undergoing treatment with iproniazid, a monoamine oxidase inhibitor, exhibited a euphoric mood, as reported. In contrast, reserpine, a substance that is recognised for its ability to deplete monoamines, induced depression in certain patients.[35] Genetic studies provide additional support for the monoamine hypothesis. Individuals with a homozygous genotype for the short allele of the polymorphic promoter region of the serotonin transporter gene have a higher vulnerability to suffering severe depression and participating in suicide conduct when exposed to stress.[35] However, the most crucial evidence stems from the fact that the effectiveness of all existing antidepressants is attributed to their capacity to augment the presence of these monoamines at the synapses in different brain areas.[31,34,36]

These medications can either reduce the absorption of monoamines by neurons (such as SSRIs, which block the reuptake of serotonin, thereby increasing its availability in the synapse) or prevent the breakdown of monoamines by inhibiting monoamine oxidase.[31] While monoamine-based medications possess potent antidepressant properties, their antidepressant effects are also influenced by changes in central monoamine function, it is crucial to acknowledge that depression is not solely caused by a deficiency in these central monoamines.[29] Monoamine oxidase inhibitors and selective serotonin reuptake inhibitors (SSRIs) induce an immediate increase in the transmission of monoamine neurotransmitters, but it takes many weeks of treatment for their mood-enhancing effects to become apparent. In contrast, when monoamines are experimentally depleted, non-medicated depressed people may experience a little decrease in mood. However, these treatments have no effect on the mood of healthy individuals.[35] To overcome the limitations of the monoamine hypothesis, researchers have developed other theories of depression.

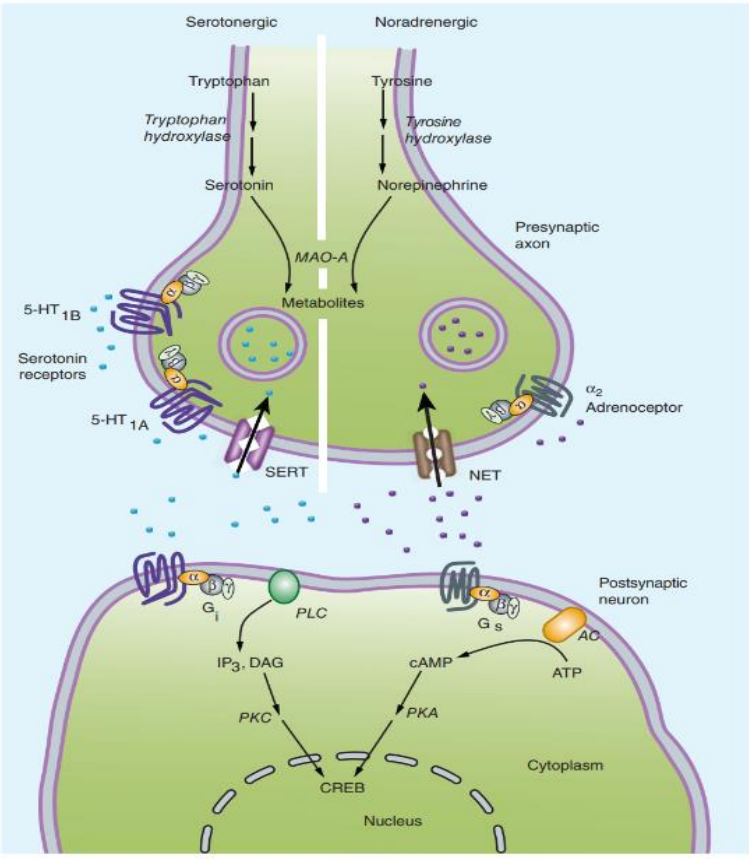


FIGURE 1: Monoamine hypothesis of depression[34]

2.Neurotrophic hypothesis:

The neurotrophic hypothesis posits that neurotrophins, a collection of released peptides, exert a significant impact on the growth and activity of the central nervous system (CNS). Neurotrophins govern the viability of neurons and manage the development of neurites by their interaction with transmembrane glycoprotein receptors, which belong to the trk family of receptor tyrosine kinases. Neurotrophins have a significant impact on the ability of neurons to change and adapt, known as neuronal plasticity.[31] Neurotrophins are classified into many families, and the factor derived from the brain neurotrophic factor (BDNF), belonging to the nerve growth family (NGF), is intensively studied in its connection to depression.[11,31] Research indicates that depression is linked to the decline of neurotrophic support, and that successful antidepressant treatments enhance neurogenesis and synaptic connection in certain regions of the brain, such as the hippocampus. The survival and proliferation of neurons are believed to be influenced by BDNF via its activation of tyrosine kinase receptor B in glial cells and neurons. A decline in brain-derived neurotrophic factor (BDNF) is caused by pain and stress, which causes the hippocampus, cingulate gyrus, and medial frontal cortex to undergo structural modifications and shrink in size.[31] The reduction in volume in the brain regions listed above has also been observed in postmortem examinations of people with depression.[11,31] Even neuroimaging investigations of individuals with depression indicate a decrease in the volume of the hippocampus. The extent of the decrease has been directly correlated with the duration of the sickness.[11] In addition, the direct administration of BDNF into the hippocampus, midbrain, and prefrontal cortex of animals has demonstrated an antidepressant effect. Human studies provide evidence in support of the neurotrophic hypothesis of depression. Research has shown that people diagnosed with depression had lower levels of brain-derived neurotrophic factor (BDNF) in both their cerebrospinal fluid and serum.[31]

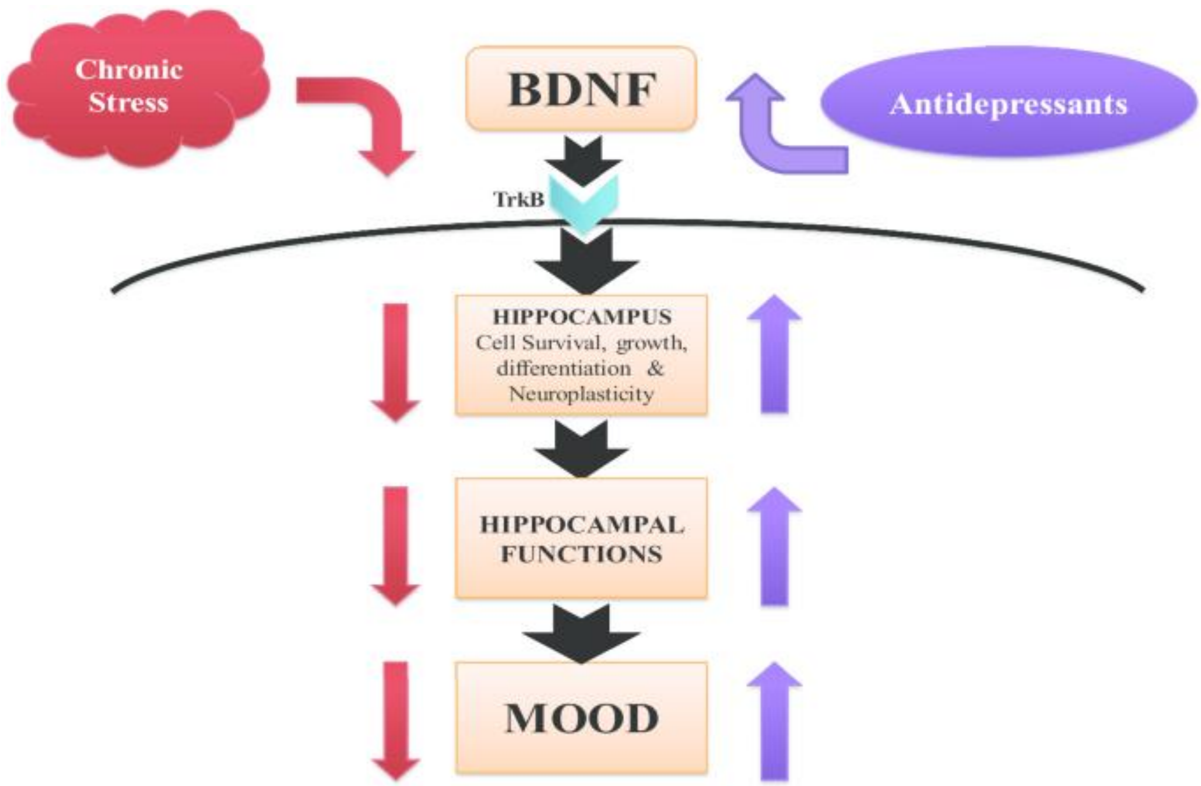


FIGURE 2: Neurotrophic hypothesis of major Depression[37]

3. Cytokines-induced depression via neuroimmune pathways

The neuroimmune mechanisms underlying the development of depression induced by cytokines. Cytokines and other immune factors have been discovered to have a crucial impact on regulating both early brain development and adult neural plasticity. Supporting evidence indicates that pro-inflammatory cytokines (IL-1B, TNF-a, and IL-6), along with immune mediators (CPM, nitric oxide, IL-6), acute phase response protein, and glucocorticoids, are involved in the pathogenesis of depression and its associated symptoms (e.g., anorexia, sleep disturbance, fatigue, and cognitive impairment).[38]

Recent preclinical investigations suggest that inhibiting the signalling of pro-inflammatory cytokines can lead to antidepressant benefits. Mice that have specific gene deletions for IL-6 or the TNF-a receptors exhibit behavioural characteristics that resemble those of antidepressant effects.[39] An IL-1β receptor antagonist delivered centrally effectively counteracted the behavioural and anti-neurogenic consequences of prolonged stress. Approximately 30% of patients receiving recombinant interferons experience depression as an adverse consequence of the medication.[29]

4. The role of neuroendocrine factors in depression:

Depression often correlates with several hormonal imbalances. Major depressive illness is commonly associated with abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis. In addition, MDD is associated to elevated cortisol and corticotropin-releasing hormone levels.[11,31] Both externally administered glucocorticoids and naturally occurring increases in cortisol levels are linked to mood symptoms and cognitive impairments that resemble those observed in Major Depressive Disorder (MDD).[11] Elevated levels of glucocorticoids may impair neurogenesis in the subgranular zone of the hippocampus and lead to atrophy in certain regions of the hippocampus. This could potentially contribute to the observed reductions in hippocampus volume that are associated with depression.[40]

Thyroid dysfunction has also been observed in individuals with depression. Approximately 25% of individuals diagnosed with depression exhibit an atypical thyroid function. These symptoms include a decrease in the body's reaction to thyrotropin-releasing hormone and an increase in the levels of thyroxin in the bloodstream during periods of depression.[40] Depressive symptoms frequently accompany clinical hypothyroidism, although they can be alleviated by supplementing with thyroid hormones. Thyroid hormones are frequently utilised alongside conventional antidepressants to enhance the therapeutic outcomes of the latter.[11,41] Sex steroids are also involved in the development of depression. Oestrogen insufficiency states, occurring during the postpartum and postmenopausal periods, are believed to have a significant impact on the development of depression in certain women.[11,41] Similarly, males who experience a significant lack of testosterone may also exhibit symptoms of depression. Hormone replacement therapy in individuals with hypogonadism may lead to enhanced mood and alleviation of depressive symptoms.[42]

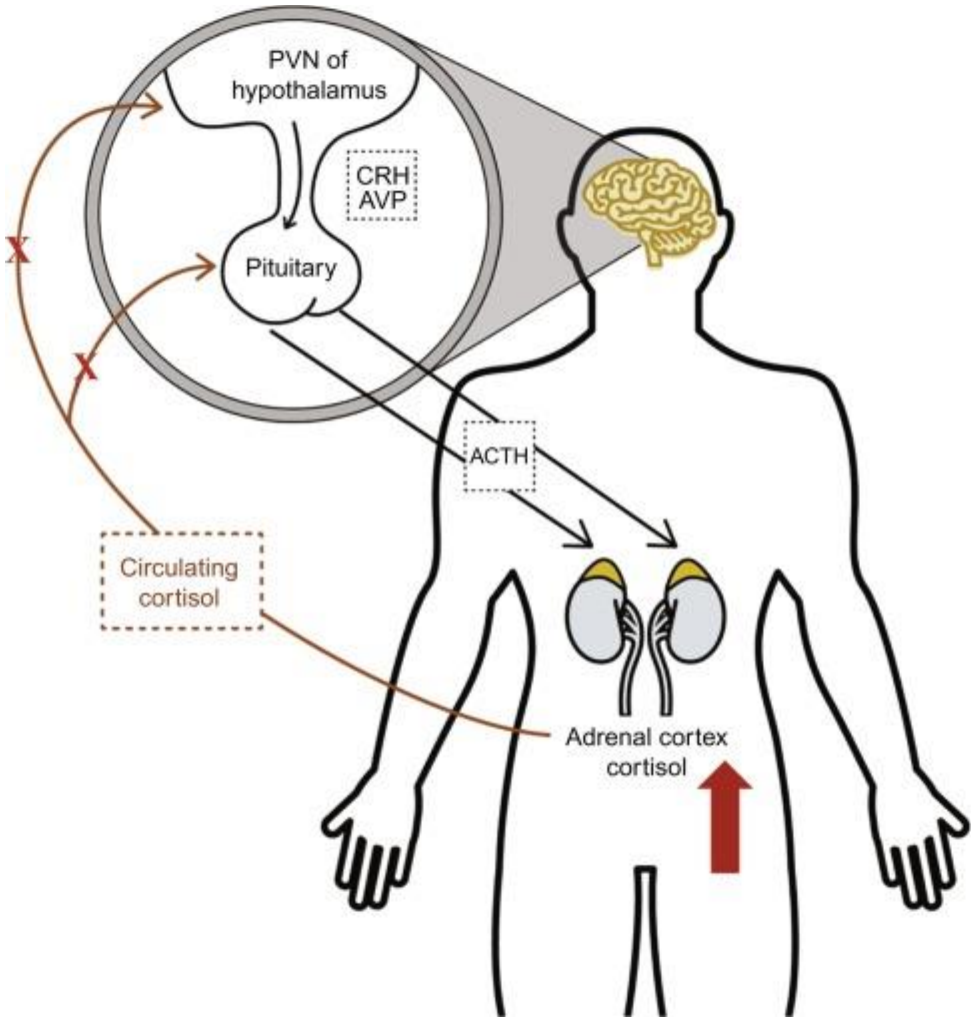


FIGURE 3: Neuroendocrine factors in depression[43]

5. The Epigenetic theory

The epigenetic theory elucidates the mechanism by which certain variables can impact gene expression and cellular function without modifying the fundamental DNA sequence. Epigenetic alterations provide a means by which environmental events can alter gene activity without any changes in the DNA sequence. These alterations may help elucidate the conflicting findings in genetic association studies pertaining to depression. Within the realm of depression research, epigenetic investigations have mostly concentrated on two key chromatin-modifying mechanisms.[33,44] Cytosine DNA methylation is a crucial factor in determining how maternal behaviour influences the emotional processing of adults.[45] In contrast to the progeny of rodents whose mothers exhibit high rates of maternal behaviours such as licking and grooming, those whose offspring have mothers with low rates of these behaviours demonstrate increased anxiety and decreased expression of glucocorticoid receptors in the hippocampus. An increase in methylation can explain why the number of cortisol receptors went down. There is a process in the promoter area of the glucocorticoid receptor gene that stops the gene from being expressed.[33,44] Another mechanism is histone acetylation, which is linked to the activation of transcription and the relaxation of chromatin structure. This process appears to be a crucial target for the action of antidepressants.[39]

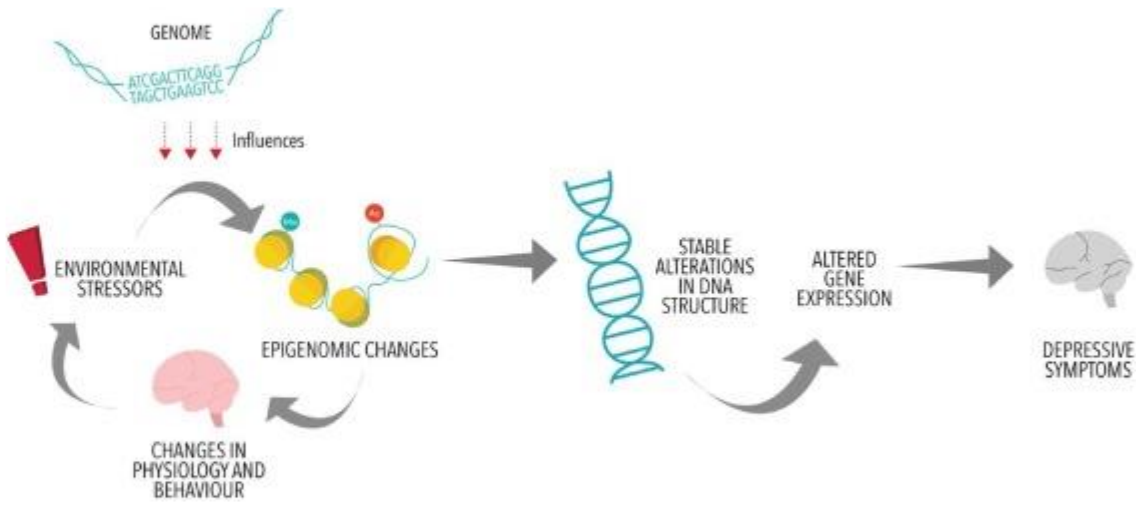


FIGURE 4: Epigenetic theory of depression[46]

Convergence of the various theories of depression

The several pathophysiologic explanations of depression mentioned earlier are not mutually exclusive. There is substantial evidence indicating convergence among the monoamine, neuroendocrine, and neurotrophic systems. HPA and steroid disorders can potentially inhibit the transcription of the BDNF gene. The hippocampus contains many glucocorticoid receptors. The cortisol hormone can bind to glucocorticoid receptors in the hippocampus during prolonged stress, including serious depression. This substance inhibits the production of neurotrophic factors derived from the brain (BDNF), leading to a decrease in volume in regions of the brain that are sensitive to stress, such as the hippocampus. Antidepressants, by the persistent stimulation of monoamine receptors, seem to counteract the effects of stress and lead to an upregulation of BDNF production. Furthermore, the stimulation of monoamine receptors seems to decrease the activity of the HPA axis and potentially restore normal functioning of the HPA system.[11,40]

Pharmacotherapy of depression

The treatment of depression involves various categories of medications. The various groupings are displayed in Table 1.

TABLE 1: Pharmacotherapy of Depression[11,31,47,48]

Classification of drugs	Mechanism of action	Adverse effect
I. Monoamine oxidase inhibitor (MAOI)		
1. Nonselective irreversible monoamine oxidase inhibitors. • Isocarboxazid • Phenelzine • Tranylcypromine	Inhibit deamination of NE, 5-HT and DA resulting in increased levels of NE, 5-HT and dopamine.	• Agitation, hallucination, mania, peripheral neuropathy. • Hypertensive crisis with tyramine rich food.
2. Selective monoamine oxidase B (MAO-B) inhibitor • Selegiline	Acts non-selectively at high doses and exerts antidepressant actions.	• Postural hypotension, nausea, confusion, psychosis. • Is converted into amphetamine may cause insomnia.
3. Reversible inhibitors of MAO-A (RIMA's) • Moclobemide • Clorgyline	Reversibly inhibit MAO-A selectively. Shorter duration of action.	• Nausea, dizziness, headache, insomnia, rarely liver damage. • No hypertensive crisis on ingestion of food containing tyramine.

II. TRICYCLIC ANTIDEPRESSANT (TCA)		
1. NE +5HT reuptake inhibitors • Amitriptyline • Imipramine • Trimipramine • Doxepine • Dothiepin • Clomipramine	Prevent the reuptake of biogenic amines NE and 5-HT into their respective neurons, hence increasing their effectiveness.	• Due to blockade of α1 adrenergic receptors → orthostatic hypotension, dizziness. • Inhibition of muscarinic cholinergic receptors lead to xerostomia, visual impairment, difficulty in urination, and reduced bowel movements. Blockade of H_1 receptors causes sedation, weight gain. • Sodium channels in the brain and heart are additionally inhibited, potentially resulting in cardiac arrhythmias and seizures.
2. Predominantly NE reuptake inhibitors • Protriptyline • Maprotiline • Amoxapine	There may be lesser or no blockade of dopamine reuptake. They differ in their selectivity and potency for different amines.	
III. SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRI's)		
• Fluoxetine • Fluvoxamine	Selectively inhibit the reuptake of 5-HT and	• Actions on 5-HT_{2A}, $5HT_{2C}$ receptors in the

• Paroxetine • Sertraline • Citalopram	increases its levels in the synapses.	limbic cortex nervousness, anorexia, agitation, panic attacks. • Actions on 5-HT_{2A} receptors in the basal ganglia → akathisia, psychomotor retardation, mild Parkinsonism, dystonic movements. • Actions on 5-HT_{2A} receptors in the brainstem - myoclonus and insomnia. • Actions on 5-HT_{2A} receptors in the spinal cord sexual dysfunction. • Action on 5-HT_3 receptors in brainstem or hypothalamus nausea and vomiting.

		• Action on 5-HT3 receptors in gastrointestinal tract ⟶ cramps and diarrhoea.
IV. ATYPICAL ANTIDEPRESSANTS		
1. Norepinephrine reuptake inhibitors (NRI) • Reboxetine • Tomoxetine	Selectively inhibit reuptake of norepinephrine (NE). Increase in the NE levels in the pathway from locus ceruleus to frontal cortex is responsible for antidepressant action. It improves fatigue, apathy and psychomotor retardation by increasing NE levels in the pathway from locus ceruleus to limbic cortex.	• Stimulation of $\beta_1$receptors in cerebellum and peripheral nervous system - tremors. • Stimulation of NE receptors in ▪ limbic system - agitation ▪ brain stem cardiovascular centres - increases the blood pressure. ▪ heart alteration of heart rate. • Stimulation of sympathetic nervous system can cause

		reduction in the parasympathetic tone and can cause dry mouth, constipation, urinary retention.
2. Norepinephrine and dopamine reuptake inhibitor (NDRI) Bupropion	Inhibit reuptake of NE and dopamine.	• Long term effects unknown • Can cause agitation, dry mouth, nausea, reinforcement and abuse.
3. Dual serotonin and norepinephrine reuptake inhibitor (SNRI) • Venlafaxine • Duloxetine	Inhibits the reuptake of NE and 5-HT selectively. No interaction with cholinergic, histamine and adrenergic receptors.	Hypertension, impotence, anxiety nausea, sweating.
4. Noradrenergic and specific serotonergic antidepressant (Na SSA) • Mirtazapine	Antagonizes α_2 action and increases NA, 5-HT release. No action on monoamine transporter. Has antagonistic action at 5-HT_{2A}, 5-HT_{2c}, 5-HT_3 (no nausea, vomiting, sexual	Sedation, weight gain.

	dysfunction) and histamine (H_1) receptors.	
5. Dual 5-HT-receptor antagonist serotonin reuptake inhibitor (SARIs) • Trazodone • Nefazodone	By inhibiting the transporter, it increases 5-HT level at synapses Antagonizes 5-HT_{2A} (no sexual dysfunction, anxiety, insomnia) Inhibits histamine (H_1) receptors.	Sedation, priapism (lesser with nefazodone).

A. Screening methods for antidepressant drugs:

I: In vitro tests:[45,49]

1. Suppression of ^{3}H-norepinephrine absorption in synaptosomes of the rat brain.
2. Suppression of ^{3}H-dopamine absorption in synaptosomes from the striatum of rats.
3. Suppression of ^{3}H-dopamine absorption.
4. Interacting with monoamine transporters.
5. Monoamine oxidase inhibition
6. Inhibition of serotonin uptake counteracts the harmful effects of p-chloramphetamine.

II: In vivo tests[45,49]

There are two approaches to screen antidepressant drugs. Behavioural changes can be studied by subjecting the animals physically to a particular stressful situation without using drugs. Behavioural changes can also be induced by means of drugs to evaluate antidepressants.

A. Drug induced behavioural models:

1. Inhibition of reserpine (2.5-5.0 mg/kg, sc) induced ptosis, hypothermia and catalepsy.
2. Potentiation of amphetamine (10 mg/kg, ip) induced hypothermia, locomotor activity and stereotypy.
3. Inhibition of apomorphine (16 mg/kg, ip) induced hypothermia, stereotypy, climbing behaviour.
4. Potentiation of tryptamine (60 mg/kg, ip) induced convulsions.
5. Potentiation of yohimbine (25 mg/kg, sc) induced lethality.
6. Potentiation of 5-hydroxytryptophan (50 mg/kg, ip) induced head twitch responses or head shakes.
7. Potentiation of l-dopa (25 mg/kg, ip) induced behavior like increased ambulation, salivation, jumping, and irritability.

B. **Behavioural models to screen antidepressant drugs:** They are given in Table 2 below.[45,49]

TABLE 2: Behavioural models to screen antidepressant drugs

Sl.no	Experimental models	End point	Comments
1.	Muricidal behavior in rats	Decrease in muricidal behavior	Response also shown by neuroleptics at doses inducing motor deficits.
2.	Forced swim test	Reduction in immobility	Simple test. Rats are more suitable than mice. Classical TCAs respond

			significantly. Antidepressants acting through 5-HT are inactive. False positives by opiates and antihistamines.
3.	Tail suspension test	Decrease in duration of immobility	Easy to perform. Several strains are resistant. Reliable and rapid method for all antidepressants. MAOIs are inactive.
4.	Learned helplessness in rat	Decrease in the frequency of unsuccessful attempts for escape	Excellent predictive validity. Commonly employed to assess the efficacy of antidepressants, explore their mechanisms of action, and examine the neurobiology underlying mental illnesses. Time consuming.
5.	Isolation induced hypo activity	Abolish isolation induced behaviors	Response seen with newer antidepressants like mianserin, trazodone and SSRIs.
6.	Separation models	Decrease in despair	TCAs selectively reduce depression.
7.	Chronic unpredictable stress	Restoration of normal behavior and physiological functions	Validity is not superior to learned helplessness model. Chronicity of the models. Difficulty in setting up.

			Clinically effective antidepressant have failed in this model.
8.	Incentive disengagement	Improved locomotor activity even on removal of incentive	Good predictive validity. Not popular because of procedural problems.
9.	Disturbed circadian rhythm	Readjustment of behaviours.	TCAs have shown response.

III. Genetic models of depression[49]

There are many strains of rats selectively bred for a particular behavioural trait. Examples of such strains are:

- Flinders sensitive line of rats which are supersensitive for cholinergic agents.
- Congenital learned helpless rats - analgesia, cognitive deficits and hypo responsivity of the HPA axis.
- Wistar-Kyoto rats selectively bred for hyper responsiveness to antidepressants.

Another approach to genetic animal models of depression is to use genetically altered mice. Various candidate genes involved in the pathology of depression are knocked out and the effect on behaviour is studied. Examples are:

- ✓ BDNF knockout mice
- ✓ Substance P knockout mice
- ✓ Mice with inactivated dopamine transporter gene
- ✓ Dopamine D_5 receptor null mutant
- ✓ DARPP-32 knockout mice

Histopathological Changes in Depression

The hippocampus is a brain region that is frequently examined in depression research. It is noteworthy that the hippocampus has a significant presence of glucocorticoid receptors and glutamate, which play a role in regulating the HPA axis. This increases the susceptibility of the hippocampus to stress and depression. Stress and other adverse stimuli can cause modifications in the brain's capacity to undergo changes and adjust (plasticity) in the hippocampus. Stress can trigger the hypothalamic-pituitary-adrenal axis is stimulated, resulting in increased corticosteroid secretion and diminished neurogenesis in the hippocampus.[50]

Hippocampal Volumetric Changes in Depression

There have been numerous reports indicating a substantial decrease in the occurrence of hippocampal depression among patients. The volumetric changes may arise due to alterations in volume in patients with Depression.[51] Neurodegenerative reactions to elevated glucocorticoid levels in depression can lead to alterations in volume.[52] The decrease in size of the prefrontal cortex and hippocampus can also be attributed to the disturbance and degeneration of neurons and glial cells in individuals with depression.[53,54]

Hippocampal Neurogenesis

There are variations in the impact of antidepressant therapies on the growth of new neurons in the adult hippocampus across different species. In rats, the majority of antidepressant treatments utilised in humans, such as electroconvulsive shock and medication, have been found to promote the growth of new neurons in the hippocampus.[55–58]

Hippocampal Apoptosis in Depression

Various studies have demonstrated that sadness and stress can induce apoptosis in the hippocampus of rodents, non-human animals, and humans. Nevertheless, hippocampus apoptosis can also manifest in mice who are not experiencing depression. Animal models and human research have demonstrated that chronic depression has a more prolonged impact on inducing apoptosis in the hippocampus compared to acute depression. The pro-apoptotic effects induced by acute depression may cease entirely within 24 hours of recovery, while the harmful implications associated with chronic depression may take up to three weeks to fully resolve. Nevertheless, the exact time at which melancholy and stress begin to influence the course of apoptosis remains unknown.[59] Furthermore, fluoxetine, a selective reuptake inhibitor of serotonin (SSRI), regulates the ability of the hippocampus to change and adapt by reducing the increase in a specific neural cell adhesion protein called synaptosomal polysialic, which is produced by depression. Additionally, fluoxetine triggers a protective response against cell death in the hippocampus.[60]

HPA Axis in Major Depression

Anxiety and depression have been linked to the activation of the hypothalamic pituitary adrenal (HPA) axis, which is triggered by stress. The axis comprises neural circuits that involve the brain, pituitary, and adrenal glands, and is responsible for regulating the production of glucocorticoids through both forward and feedback inhibition loops. Cortisol, which is secreted by the adrenal glands, demonstrates a diminished affinity for glucocorticoid receptors (GRs) and an increased affinity for mineralocorticoid receptors (MRs) in the brain. GR exhibits a broad distribution across the entirety of the macaque brain, whereas MR demonstrates a predominant localization in the hippocampus.[61] Furthermore, the regulatory domains of numerous genes in the brain contain glucocorticoid sensitive elements. Cortisol primarily exerts its effects through the mineralocorticoid receptor (MR) in the hippocampus. However, feedback actions at the pituitary level and in activated brain regions such as the amygdala are mediated by the glucocorticoid receptor (GR).[62,63] An imbalance in the functioning of MR and/or GR within the hypothalamic-pituitary-adrenocortical system has been proposed as a potential cause of major depression. A significant proportion, ranging from 40% to 60% of persons diagnosed with depression exhibit hypercortisolemia[64] or Additional disturbances of the hypothalamic-pituitary-adrenal (HPA) system, such as a flattened circadian rhythm [65], or an earlier[66] or higher lowest point.[67]

The unfavourable correlation between calcium consumption and depression can be elucidated by various potentially interconnected pathways. Calcium (Ca) plays a crucial function in regulating the HPA system, that is widely acknowledged as the primary mechanism for responding to stress in the body.[68] Corticotropin-releasing hormone (CRH) activates the hypothalamus, prompting it to secrete adrenocorticotropic hormone (ACTH), which controls the synthesis of adrenocorticosteroid. CRH regulates the hypothalamic-pituitary-adrenal (HPA) system.[69,70] Thus, any imbalance in the interaction between the CRH and HPA systems might disrupt the regulation of stress hormones, such as cortisol, leading to an impact on depressive symptoms.[71]

Furthermore, the entry of calcium ions from outside the cell is a crucial element in numerous neural functions.[72] The adjustment of extracellular Ca^{2+} concentrations may have a role in the regulation of emotions, possibly by directly influencing the stabilisation of the plasma membrane. In addition, methyl-D-aspartate can potentially impact brain plasticity.[73] Ca/calmodulin-dependent protein kinase II has been found to facilitate the development of metabotropic glutamate receptors in group I of the rat hippocampus, resulting in long-lasting depression.[74,75] Ca ions activate the enzyme tryptophan hydroxylase in the pathways that produce 5-HT. Moreover, the level of Ca ions in the cytosol plays a vital role in connecting stimuli and reactions in many tissues. Any disturbances in the regulation of calcium ions can have a substantial impact on cellular function, therefore influencing mood.[76,77]

It is evident that the rise in corticosteroid levels, such as those occurring after a short-lived stressor, cause molecular and cellular alterations in the brain, namely in the hippocampus formation. These consequences ultimately lead to behavioural adaptation. Extended exposure to stress can result in maladaptation and may even serve as a risk factor for disorders such as major depression in persons with a hereditary predisposition. A sequence of tests was conducted to analyse alterations in brain function during a period of three weeks of unpredictable stress. Following unpredictable stress, the inhibitory input to neurons responsible for regulating the hypothalamus-pituitary-adrenal (HPA) axis was reduced, perhaps causing dysregulation of the axis and excessive exposure of the brain to glucocorticoids. Furthermore, there was a rise in the transmission of glutamate in the dentate gyrus (DG), maybe due to the modulation of receptor subunits at the transcriptional level. This could potentially increase susceptibility to cell death when combined with heightened calcium channel expression. Neurogenesis and apoptosis in the dentate region had a reduction. Both the dentate and CA1 areas exhibited decreased synaptic plasticity. Together, these impacts can lead to impairments in the process of memory formation. In the end, there was a noticeable reduction in the response to serotonin in the CA1 region, which could contribute to the emergence of depression symptoms in vulnerable individuals. These outcomes offer prospective targets for innovative treatment options for brain illnesses related to stress.

Transient activation of the HPA axis during a short-term stressful event is crucial for maximum performance. However, prolonged and excessive activation of the axis can be detrimental and eventually result in a pathological condition. In humans, it is widely acknowledged that individuals with a genetic predisposition may experience either heightened or reduced activity in the HPA axis. This might contribute as an additional factor of risk for advancement of several disorders, including major depression. Various research provide evidence for the latter. It is widely recognised that certain patients with significant depression have abnormalities in the function of the HPA axis. This is particularly obvious through higher levels of cortisol at rest, resistance to the suppressive effects of dexamethasone, or excessive responses in the combined dexamethasone-CRH challenge test.[78,79] These anomalies are partially restored when the clinical symptoms are addressed with medication; the restoration of the axis is actually inversely related to the likelihood of relapse. Moreover, studies on individuals with a heightened susceptibility to major depression have uncovered Hypothalamic-pituitary-adrenal (HPA) axis dysfunctions prior to the manifestation of clinical symptoms.[80] This indicates that these abnormalities not only correlate with the condition but also make individuals more susceptible to developing it. If this is the case, it might be anticipated that a medication specifically targeting the disruption caused by anomalies in the HPA axis will promptly alleviate the clinical symptoms.

While the exact mechanisms through which calcium channel blockers impact depression are not well comprehended, it has been proposed that these blockers may regulate the excessive functioning of the HPA axis, which is recognised for exerting a significant impact on the development of despair.[81] Consistently high levels of cortisol, caused by a disruption in the HPA axis, have been proven to negatively affect cognitive function in individuals with depression.[82] A study based on observations found that the combination of selective serotonin reuptake inhibitors (SSRIs) with calcium channel blockers led to improved relief of depression symptoms in older adults receiving treatment for hypertension. The concurrent use of SSRIs and calcium channel blockers may be effective in alleviating depressive symptoms, according to these findings.[83]

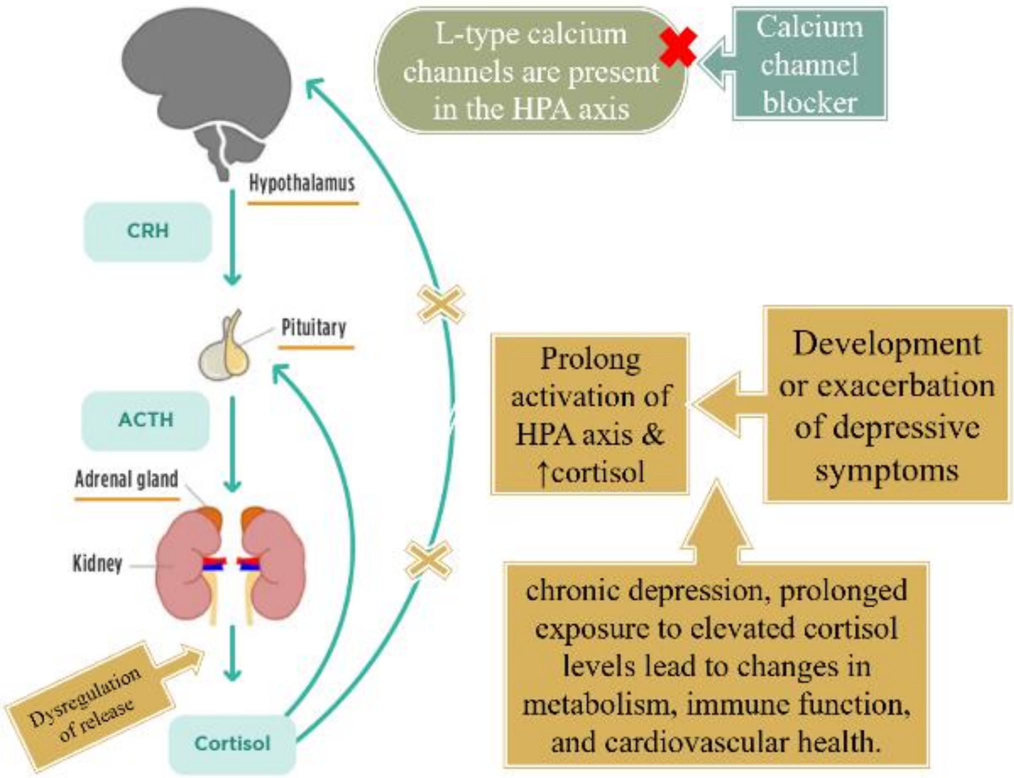


FIGURE 5: HPA Axis in Depression [68,69]

Experimental drug used in this study:

Various antihypertensive drugs, such as calcium channel blockers (CCBs), diuretics, angiotensin II type 1 receptor blockers (ARB), angiotensin converting enzyme (ACE) inhibitors, alpha-blockers, and beta-blockers, are commonly prescribed to treat cardiovascular complications. These medications can be used alone or in combination.[84,85]

Calcium channel blockers (CCBs), which can be divided into two subclasses known as dihydropyridines and non-dihydropyridines, have long been a fundamental part of hypertension treatment. Dihydropyridine (DHP) calcium channel blockers (CCBs) are generally more effective at dilating blood vessels compared to non-dihydropyridine (non-DHP) agents. On the other hand, non-DHP agents have a stronger impact on reducing the force of heart contractions. Both subclasses possess comparable efficacy in reducing blood pressure.

Non-dihydropyridine (non-DHP) calcium channel blockers show promise with diabetic nephropathy and chronic kidney disease as treatment components.

DHPs, introduced in the 1960s, are widely used medications for the therapy for cardiac disorders.

Currently, there are four successive iterations of dihydropyridines that are available.[86]

Generation	Properties
First-generation • Nicardipine • Nifedipine	Rapid onset and brief duration of vasodilator action
Second generation • Benidipine • Efonidipine	Delayed-release and immediate-release formulations
Third generation • Amlodipine • Azelnidipine	Pharmacokinetics of these drugs are more stable, they have lower selectivity for the heart, and they are well tolerated by patients with cardiac failure.
Fourth generation • Lercanidipine • Lacidipine • Cilnidipine	High lipid soluble Indicated for use in cases of ischemia and highly beneficial in heart failure(congestive).

Dihydropyridine are classified into three generations based on their duration of action. Long-acting calcium channel blockers have been discovered to elicit reduced reported activation of the sympathetic nervous system to provide more favourable effects compared to short-acting medicines. Calcium channel blockers (CCBs) suppressed the activity of L-type channels in humans. Inhibition of inward calcium flux leads to relaxation of vascular smooth muscle cells, resulting in vasodilation and a decrease in blood pressure (BP). The CCBs are classified into two categories: dihydropyridines and non-dihydropyridines. These categories have been used in therapeutic settings to enhance blood pressure. While all CCBs operate using the same mechanism of action, their therapeutic effects vary.[87,88]

Cilnidipine (CLD) is a calcium antagonist belonging to the dihydropyridine class. The development of CLD was a collaborative effort by Fuji Viscera Pharmaceutical Company and Ajinomoto, Japan. It received approval in 1995. Unlike most calcium antagonists, CLD could target both the N-type calcium channel found in sympathetic nerve endings and the L-type calcium channel. CLD has been authorised in Japan, China, India, Korea, and several European nations.[89]

Physiochemical properties

The chemical structure depicted in Figure 1 is unveiled by the CLD. The chemical substance discussed is 1,4-Dihyro2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic. The chemical is denoted as 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester. CLD is a pale-yellow crystalline powder with a chemical formula of C27H28N2O7 and a molecular weight of 492.5 grammes per mole. The compound exhibits high solubility in acetonitrile, moderate solubility in methanol and ethanol (99.5), and very low solubility in water. Its melting point ranges from 107 to 112 ◦C.[90]

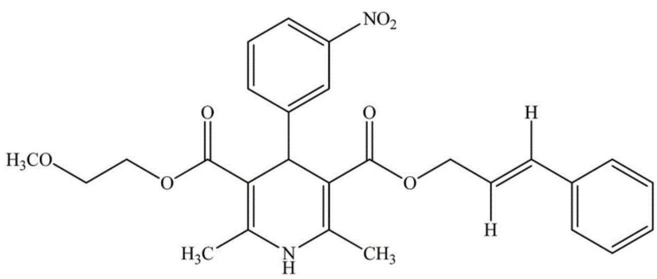


Figure 6: Chemical structure of cilnidipine[90]

Pharmacological profile

Absorption: Oral absorption of CCBs is efficient. However, numerous substances have limited potential to be absorbed into the bloodstream due to their metabolism in the liver, which is mostly controlled by the enzyme CYP3A4.

Distribution: Calcium channel blockers (CCBs) have a high degree of binding to proteins (98%) and achieve their maximum concentration shortly after 2 hours. They also have large volumes of distribution. The suggested dosage for adults is 5–10 mg taken once day, with a maximum daily dosage of 20 mg. The medication has a half-life of 20.4 minutes.[91]

Metabolism: Calcium channel blockers (CCBs) undergo significant hepatic metabolism, specifically through the process of dehydrogenation by liver microsomes. When the liver enzymes responsible for breaking down drugs are overwhelmed by repeated dosages or an overdose, they become saturated and are less effective at reducing the initial effects of the drug. This leads to increased absorption of the active drug. The half-life of many CCBs is increased by modified release formulations and saturation of metabolism of these medicines. The possibility of drug-drug interactions arises from the fact that CCBs are metabolised by CYP3A4, an enzyme that is also responsible for metabolising numerous other foreign substances.

Excretion: CCB is excreted 20% through urine and 80% through faeces.

Mechanism of action

Calcium channel blockers are effective drugs that suppress the Long lasting type voltage-gated calcium channels promote the widening of blood arteries. They are frequently employed as a primary or supplementary treatment for hypertension. There are four parts that make up the voltage-gated calcium channel. They are α1, α2-ε, and γ. The α1 subunit is the primary component of calcium channels and forms the structure that facilitates the movement of ions through them. According to reports, there are a total of 10 distinct α1 subunits, each with its own unique distribution and ion conductance values for its channels. The distinct constituents of L-, N-, T-, P-, Q-, and R-type calcium channels determine their individual channel properties.The numbers 92 and 93. The primary focus of the CCB is in the examination of L-type calcium channels, with particular emphasis on discerning the distinctions among various channel types. Calcium channel blockers (CCBs) are categorised into three classes according to their chemical composition.

Subgroups	**Drugs**
Benzothiazepines	Diltiazem Clenazem
Phenylalkylamines	Verapamil Gallopamil
Dihydropyridines	Nifedipine, Nicardipine, Felodipine, Amlodipine, Aranidipine, Azelnidipine, Cilnidipine, Efonidipine, Manidipine and Nilvadipine

Calcium channel blockers (CCBs) attaching to the α1 subunit decreases the flow of calcium, hence lowering cell excitation.

Significant research has been conducted on cilnidipine throughout its preclinical and clinical development stages. Clinical study and animal studies have shown that cilnidipine has beneficial effects in protecting the kidneys, brain, and heart. Cilnidipine has been observed to demonstrate pleiotropic effects, alongside its core role of inhibiting Cav2.2 -type Ca2+ channels. Hence, the suppression of Cav2.2 -type Ca2+ channels could offer a novel approach to address cardiovascular disorders.

Cilnidipine is a novel calcium channel blocker (CCB) that effectively blocks both Long lasting and Cav2.2 -type calcium channels. Because nerves and the brain both have Cav2.2-type calcium, cilnidipine is likely to have a unique effect on nerve activity, mostly by slowing down the sympathetic nervous system.

Takahara A et al. demonstrated that cilnidipine efficiently inhibits Cav2.2 type channels in IMR-32 human neuroblastoma cells.[94] Cilnidipine demonstrates better efficacy in blocking Cav2.2 -type Ca2+ channels compared to other Ca^{2+} channel blockers. Nap A et al (2004) conducted in vitro experiments that showed cilnidipine effectively suppresses the release of norepinephrine from sympathetic nerve terminals.[95] The study conducted on a rabbit model of heart attack showed that cilnidipine successfully reduced the levels of norepinephrine in the gap between heart cells during both the period of reduced blood flow and the period of restored blood flow. Administration of cilnidipine led to a decrease in the extent of myocardial infarction and the ventricular premature beats frequency, demonstrating the cardioprotective effects of cilnidipine.[96] Furthermore, in animal experiments conducted within a living organism, cilnidipine has demonstrated antianginal properties in a model of angina caused by vasopressin. It also helps with treating abnormal cardiac repolarization in a dog model of long QT syndrome.[84,97]

Nagahama S et al. (2007) and Iimura O et al. (1993) conducted clinical studies demonstrating the antihypertensive effects of cilnidipine in individuals with elevated blood pressure and significant hypertension. [98,99]

Clinical trials have proven the effectiveness of cilnidipine in Managing mornings high blood pressure and white-coat hypertension, both of which are linked to the activation of sympathetic nerves.[100,101] After completing a comprehensive analysis of the multiple risk factor intervention trial (MRFIT), a notable and steady association was discovered between blood pressure levels and the occurrence of end-stage renal disease.[102]

A study conducted by Takashi Masuda, Misao N. Ogura, Tatsumi Moriya, et al (2011) showed that Long lasting and Cav2.2 -type calcium channel blockers have People with high blood pressure and type II diabetes mellitus have been shown to benefit from changes in their glucose and cholesterol metabolism as well as their kidney function.[103]

According to research done by Fan et al (2011), cilnidipine functions as a Ca2+ channel antagonist, leading to the relaxation of human arteries. Additionally, it enhances the synthesis of nitric oxide by improving the functionality of nitric oxide synthase in the internal thoracic artery of the human body.[104]

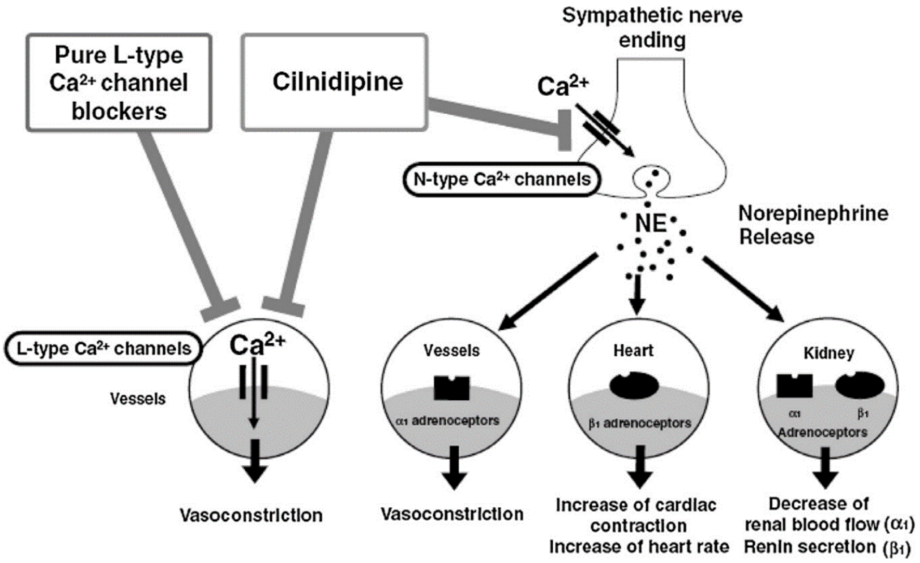


FIGURE 7: Diagram illustrating the Cilnidipine's effect on L & N type calcium channels.[105]

Cilnidipine is a suitable alternative antihypertensive drug for those who experience oedema as a result of using amlodipine.[105]

Cilnidipine is a highly promoting calcium channel blocker of the fourth generation, known for its acceptable pharmacological profile. Its mechanism of action involves the inhibition of L-type and Cav2.2-type calcium channels, resulting in its dual effect. Inhibiting Cav2.2 Ca2+ channels effectively disrupt the neurohumoral control of the cardiovascular system, that has the renin angiotensin aldosterone system and the sympathetic nerve system.

Adverse effect:[106]

1. Flushing
2. Headache
3. weight gain.
4. dizziness
5. decrease in pulse rate
6. fatigue and asthenia
7. ankle oedema
8. palpitation
9. GI disturbances
10. Increased micturition frequency
11. Lethargy

Drug Interaction:

Cilnidipine undergoes predominant metabolism via the cytochrome P450 enzyme CYP3A4, with a minor contribution from CYP2C19. [30]

Cilnidipine exhibits interactions with various medications, including quinidine (an antiarrhythmic agent), phenytoin (an antiepileptic medicine), rifampicin (an anti-TB treatment), erythromycin (an antibiotic), amlodipine (another blood pressure-lowering medication), and antipsychotic drugs.

Dose:

The oral dose of Cilnidipine for adults is 5-10 mg, to be administered once day. The dose can be adjusted to a maximum of 20 mg.

Precaution:

Hypotension, inadequate cardiac reserve, cardiac failure. Abrupt discontinuation can worsen angina symptoms. Discontinue treatment in patients who encounter ischemia discomfort after receiving the medication. Pregnancy, breastfeeding.

Indications:

FDA-approved indications include the management of hypertension for end organ protection, coronary artery disease, and chronic stable angina.

Off-Label Uses - Migraine prophylaxis, Raynaud phenomenon, hypertrophic cardiomyopathy, pulmonary hypertension, anal fissures, and high-altitude pulmonary oedema.

METHODOLOGY

The present study was conducted over 1.5 years from September 2022 to March 2024.

Study design:

This was an experimental study involving male Swiss albino mice. The sample size was 24. Animals were categorised into four categories, with each group comprising six animals.

Ethical committee approval:

The study obtained permission from the Institutional Animal Ethics Committee (IAEC) on June 25, 2022, under Resolution No. 17/3 (Annexure-I). The research was done according to the guidelines issued by the Committee for Control and Supervision of Experiments with Animals (CCSEA), which is based in New Delhi.

Experimental Animals:

We acquired adult, robust male Swiss albino mice with an average weight of 20-25 gms from the central animal facility of J.N. Medical College, KAHER, Belagavi (CPCSEA Reg No.627/PO/Re/S/02/CPCSEA)

The animals were raised in polypropylene cages that had stainless steel top grills and facilities for food and water. The mice were provided unrestricted availability of both water and food in the form of pellets. The enclosures were equipped with bedding composed of paddy husk. Prior to the trial, the animals underwent a 7-day period of acclimatisation to a 12:12 hour light-dark cycle.

Study drugs and their dosages: Table No. 1

Standard antidepressant Fluoxetine (Flunil-10), and Cilnidipine (Cilnimine-5, Cilnipox 10) were purchased from the local pharmacy.

The table developed by Paget and Barens was utilised to translate clinical dosages of these medications into doses equal to those in mice.[107]

TABLE 4 Drugs and dosages used in the study

GROUP	TREATMENT	DOSE
A	Normal saline	0.2ml
B	Fluoxetine	10mg/kg(0.2ml)
C	Cilnidipine	5mg/kg(0.1ml)
D	Cilnidipine	10mg/kg(0.2ml)

Distilled water was used to dissolve every drug.

Each drug solution was made right before usage, and the drug was injected intraperitoneally into each group i.e. control, standard and test groups.

TEST FOR ANTIDEPRESSANT ACTIVITY

Male Swiss albino mice were subjected to the Tail Suspension Test (TST) and the Forced Swim Test (FST) in order to evaluate the antidepressant effects. In the afternoon, from 2:00 pm to 5:00 pm, behavioural research was conducted under low illumination.

1. **FORCED SWIM TEST**

Proposed by Porsolt et al., in 1981.

Rodents are confined to swim in restricted spaces (cylinders) from which they are unable to flee, and after a brief period of intense activity, they quickly cease swimming. Being immobile could be taken as evidence that they had given up hope and concluded that there was no chance of escape. Thus, immobility eventually came to be known as "behavioural despair."

Several laboratories have since demonstrated that a wide spectrum of clinically efficacious antidepressant medications lowers this immobility.[107]

In this study, mice in different groups were administered the respective drugs for 21 days and the experiment was conducted on 1st, 14th and 21st day.[108,109]

The animals were placed within a cylindrical glass container with a height of 25 cm and a diameter of 10 cm.[110]

PRINCIPLE

This test depends on the behavioural despair paradigm. An undesirable circumstance will cause a normal animal to fluctuate between two different behaviours: agitation and immobility. These are usually grouped into two categories: searching conduct (characterised by strong motor activity and energy consumption) and waiting behaviour (distinguished by immobility and energy storage). It's often known as the "searching and waiting" approach. The balance of these kinds of behaviours is altered by antidepressant drugs, promoting searching.[45,111]

If mice are made to swim in a restricted area, they eventually cease swimming and only make moves to stay above the water (Hinge position) (FIGURE 8a). The mouse exhibits a unique and recognizable immobility behaviour that suggests a state of despair when it realizes it is impossible to escape and gives up on the conditions of study. It is believed that these behaviours are identical to clinical depression. Hence, medications that decrease this immobility would have an antidepressant effect.[112–114]

PROCEDURE

Forced swim test was carried out on 1st, 14th and 21st days.

On the first day, thirty minutes following a single test or vehicle doses, each animal was allocated six minutes to swim within the cylinder. The intense activity (escape activity) observed in the first two minutes of the session was eliminated from the analysis.[107,115] During the final four minutes of the session, the duration of immobility was noted[107,110]. An animal that remained still in the water, apart from the essential leg motions required to keep its head above the surface, was deemed immobile. The mice were removed, dried for 15 minutes (FIGURE 8b), then placed back in their cages and provided with food and drink.[110]

On the 14th and 21st day, thirty minutes following drug administration mice were subjected to the same test and the immobility duration was recorded.

The mean immobility of all groups was calculated for the 1st, 14th and 21st day for analysis.

NOTE: After six 6-minute tests, the water in the cylinder was replaced for every animal.

2. TAIL SUSPENSION TEST

Devised by steru et al. in 1987.

Some researchers propose that the lack of movement observed in mice when faced with unavoidable stressors could be a manifestation of behavioural despair, which may be analogous to depressive disorders in humans. Antidepressants that are clinically effective enhance the mice's ability to move after they have made vigorous but failed attempts to escape while being suspended by their tails. The Tail suspension test is likely more efficient in assessing depressive-like behaviour and the effects of antidepressants in mice.[116]

The structure consists of two metallic rods that are positioned 35 cm apart. These rods are connected by a horizontal rod, which serves as a support for a nylon thread that hangs from the centre. In order to enable the animal to hang on its tail, a hook was fastened to the loose end of the thread and a sticky tape was positioned around 1 centimetre from the end of the tail. The thread length was adjusted to provide 58 cm from the ground to the hook. (FIGURE 9)

In this study, mice in different groups were administered the respective drugs for 21 days and the experiment was done on the 1st, 14th and 21st day.[108,109]

PRINCIPLE

When exposed to an inescapable, undesirable environment, a normal animal alternates between agitation and immobility. These behaviours can be categorised as waiting, which involves immobility and energy conservation, and searching, which involves high motor activity and energy expenditure. The searching-waiting strategy is the term for the decision-making sequences that occur between these types of actions. Antidepressant medications shift the balance of these behaviours in favour of searching.[117]

PROCEDURE

The tail suspension test was conducted on the 1st, 14th, and 21st days.

On the first day, 30 minutes after administering a single dosage of the test substance or vehicle, a mouse is attached to a hook using adhesive tape, positioned 1cm from the end of its tail, for a duration of 5 minutes. Mouse should be 15 cms away from the nearest object. When mice hang motionless and still for at least a minute, they considered immobile. Immobility time in seconds is recorded. The mice were removed and returned them to their cages and provided with food and water.[107,118,119]

On the 14th and 21st day, 30 minutes after the drug was given, mice underwent the Tail Suspension Test and the length of time they remained motionless was measured for a period of 5 minutes.

The mean immobility of all groups was calculated for 1st, 14th and 21st day for analysis.

TEST FOR LOCOMOTOR ACTIVITY

ACTOPHOTOMETER

PRINCIPLE

Devised by Dews P.B in 1953

A state of alertness is indicated by the locomotor activity. Locomotor activity was assessed with an Actophotometer (INCO Ambala, India). It consists of a 40 cm enclosed circular arena with four infrared beam cells mounted on the circular wall (2 cm above the floor). The light beam landing on the photocell in an Actophotometer is broken up when an animal moves, and a count is digitally recorded. As a result, the total counts reflect the animal's movements within the Actophotometer chamber.[120,121] (FIGURE 10)

PROCEDURE

After administering a single dosage of test/vehicle, mice from various groups were individually placed in the Actophotometer on the first day. The number of movements made by the mice was then observed and recorded for a duration of 10 minutes before returning them to their cages. The same experiments were repeated on the 14th and 21st day, half an hour after drug administration.

The mean of all groups was calculated for the 1st, 14th and 21st day for analysis.

TEST FOR ANTIDEPRESSANT ACTIVITY

FORCED SWIM TEST

Figure 8a

Figure 8b

TAIL SUSPENSION TEST

Figure 9

TEST FOR LOCOMOTOR ACTIVITY

ACTOPHOTOMETER

Figure 10

STATISTICAL ANALYSIS

The data was examined using analysis of variance (ANOVA), and each experiment's outcome was represented as the mean ± SD.

The post-hoc analysis was performed using Graph Pad Prism software, version 8.0.1.

The Bonferroni's multiple comparison test was used to compare the experimental groups with the control group.

Observed changes are considered statistically significant if their p-value is less than 0.05.

RESULTS

In the present study the drug Cilnidipine for its possible antidepressant effect was investigated using depression models in animals.

ANTIDEPRESSANT ACTIVITY

The effectiveness of several Dihydropyridines in treating depression was evaluated by performing the forced swim test on male Swiss albino mice. Fluoxetine served as the reference antidepressant medication for the purpose of comparison.

Forced Swim Test in male Swiss albino mice.

Duration of Immobility on Day 1

The duration of immobility, measured in seconds, was documented for different groups throughout a 6-minute period. The average duration of immobility in the control group was 182.4±4.1, while in the Fluoxetine group it was 176±3.3. For the Cilnidipine group, the duration of immobility was 180.2±1.8 for a dose of 5mg/kg and 183.7±7.6 for a dose of 10mg/kg.

The duration of immobility was considerably reduced in the Fluoxetine group ($p < 0.05$) compared to the control group. There were no significant differences in outcomes between the control group and the groups who received Cilnidipine at dosages of 5mg and 10mg, based on statistical analysis.

Duration of Immobility on Day 14

The time of immobility, measured in seconds, was noted for various groups throughout a 6-minute period. The average duration of immobility in the control group was 183.4±4.1, while in the Fluoxetine group it was 175.7±1.6. For the Cilnidipine group, the duration of immobility was 179.8±3 for the 5mg/kg dose and 177.3±2.3 for the 10mg/kg dose.

In compared to the control group, the groups given Fluoxetine ($p<0.01$) and Cilnidipine (10 mg) showed a significant reduction in immobility duration ($p < 0.05$). A statistically significant decrease ($p < 0.05$) was seen in the group treated with Cilnidipine 10mg compared to the group treated with Fluoxetine. When compared to the placebo group, there was no statistically significant effect of 5 mg of cilnidipine.

Duration of Immobility on Day 21

Duration of immobility, measured in seconds, was recorded for various groups throughout a 6-minute time frame. The average duration of immobility in the control group was 184.1±4, whereas in the Fluoxetine group it was 171.1±1.9. For the Cilnidipine 5mg/kg dose, the duration of immobility was 177.3±1.6, and for the Cilnidipine 10mg/kg dose, it was 173.02±1.5.

The duration of immobility was considerably reduced in the Fluoxetine ($p<0.001$), Cilnidipine 5mg group ($p<0.05$), and Cilnidipine 10mg group ($p<0.01$) compared to the control group. Both the group receiving Cilnidipine 5mg ($p < 0.05$) and the group receiving Cilnidipine 10mg ($p < 0.01$) exhibited a statistically significant decrease in comparison to the group receiving Fluoxetine.

Duration of immobility was significantly decreased in Fluoxetine on day1, 7 and 21, Cilnidipine 5mg/kg group shows significant decreased only on 21st day when compared to that of Control and Fluoxetine group. Whereas Cilnidipine 10mg/kg shows significant decrease from 14th day onwards. On day 21st when compared with the standard drug Fluoxetine, Cilnidipine 10mg/kg group showed similar results. (Table 5, Graph 1-4)

Forced Swim Test - Duration of Immobility

TABLE 5

Effect of various drugs on duration of immobility in Forced swim test.

Day of study	**Duration of immobility in seconds**			
	Control	**Fluoxetine 10mg/kg**	**Cilnidipine 5mg/kg**	**Cilnidipine 10mg/kg**
1	184.4±4.1	176±3.3 *	180.2±1.8	183.7±7.6
14	183.4±4.1	175.7±1.6 **	179.8±3	177.3±2.3 *#
21	182.1±4	171.1±1.9 ***	177.3±1.6 *#	173.02±1.5 **,##

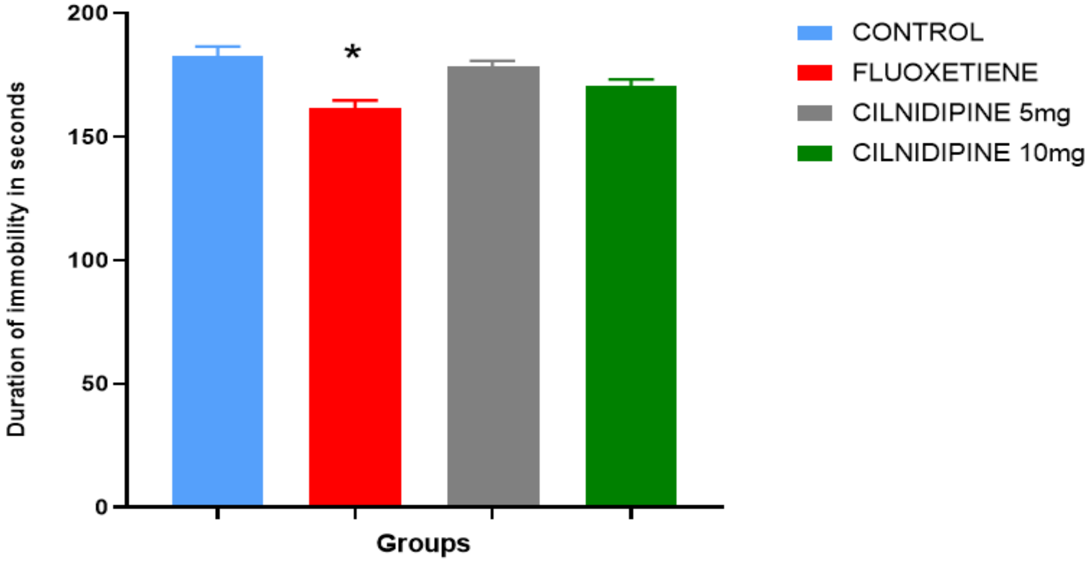
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *p<0.05, **p<0.01, and ***p<0.001in comparison to control group.
4. #p<0.05 and ##p<0.01 in comparison to standard group.

DAY 1

GRAPH 1

Effect of various drugs on duration of immobility in forced swim test.



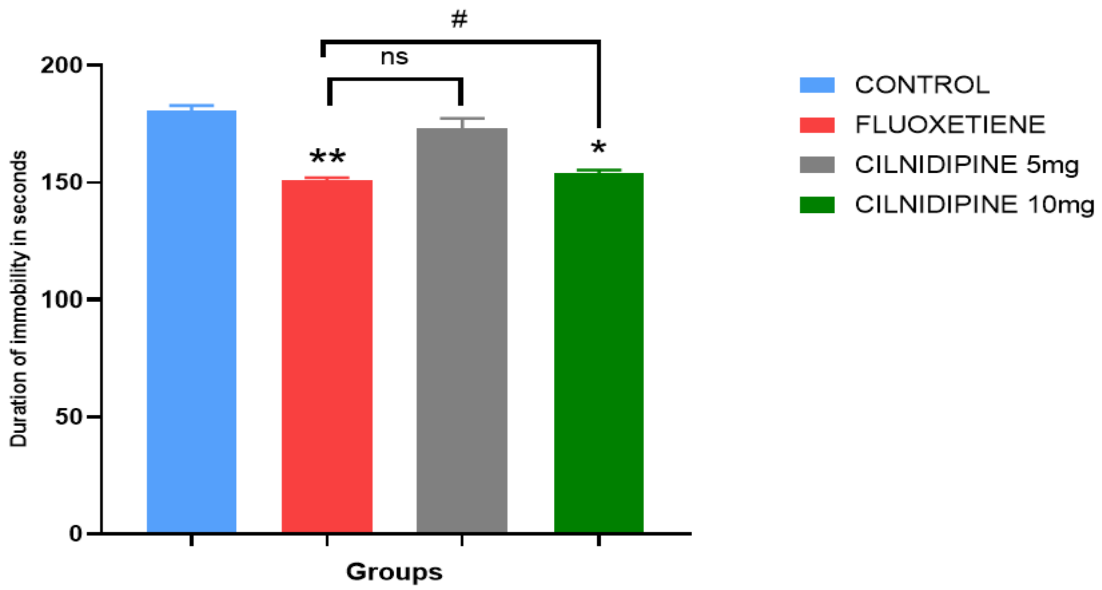
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *$p<0.05$ as compared to control group.

DAY 14

GRAPH 2

Effect of various drugs on duration of immobility in Forced swim test.



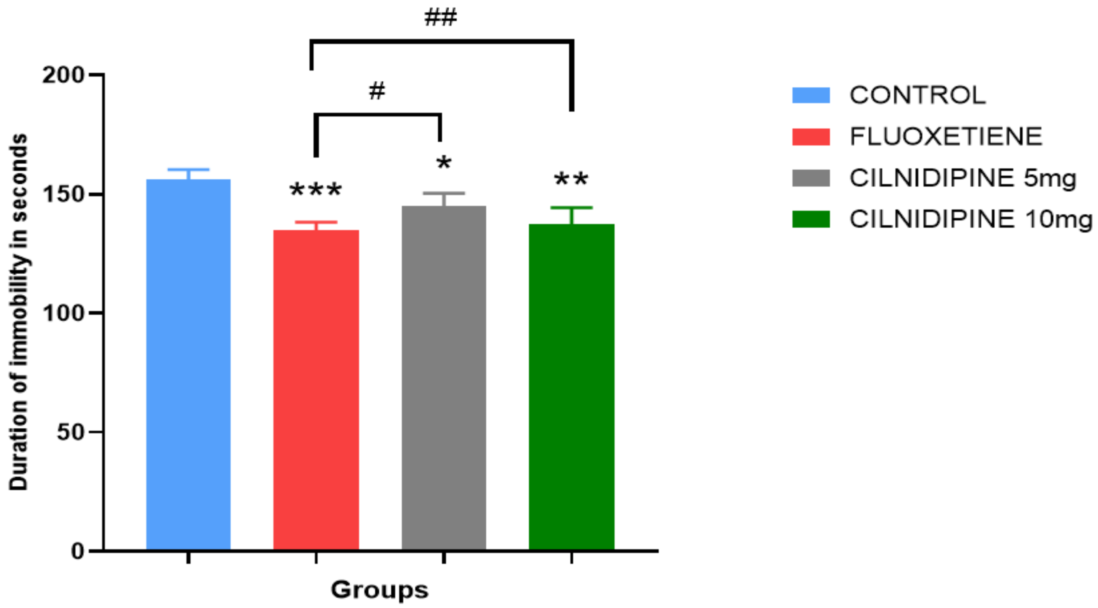
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *p<0.05 as compared to control group.
4. #p<0.05 as compared to standard group
5. ns- nonsignificant

DAY 21

GRAPH 3

Effect of various treatment on duration of immobility (in seconds) in forced swim test.

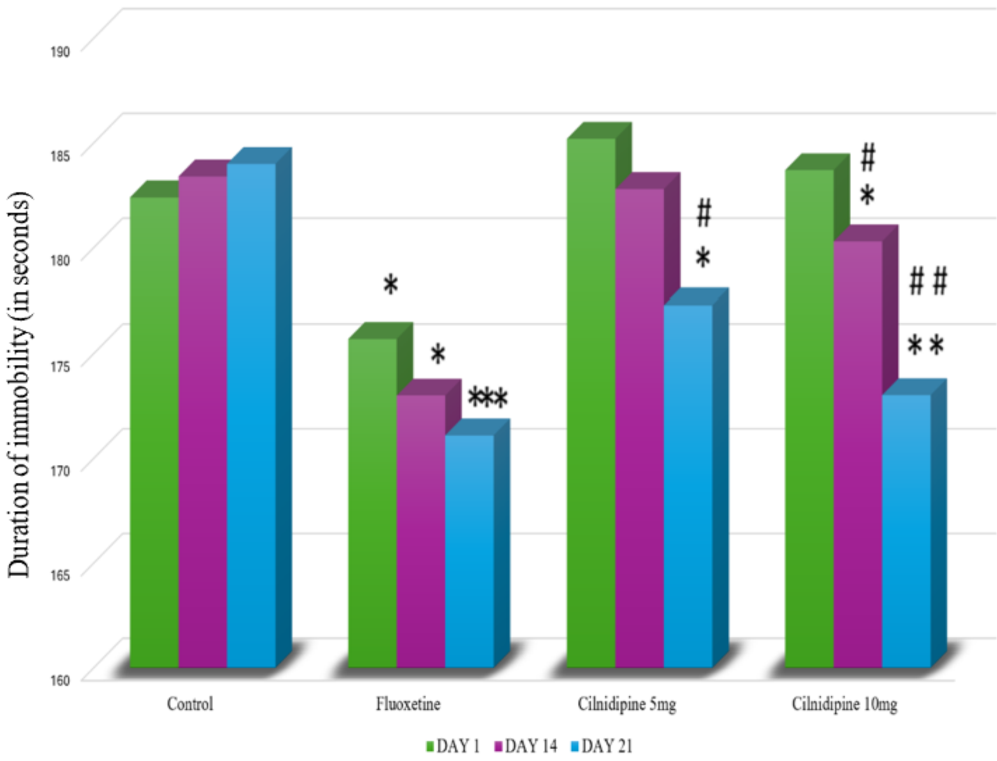


Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3.*p<0.05, **p<0.01as compared to control group.
4. #p<0.05, ##p<0.01as compared to standard group.
5. ns - nonsignificant

GRAPH 4

Overall effect of various drugs on duration of immobility in Forced swim test.



Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3.*p<0.05, **p<0.01, ***p<0.001as compared to control group.
4. #p<0.05, ##p<0.01as compared to standard group.

Tail suspension Test in male Swiss albino mice.

Duration of Immobility on 1st day

The duration of immobility, calculated in seconds, was documented for various groups over a 5-minute period. The average duration of immobility in the control group was 180.4±4.1, whereas in the Fluoxetine group it was 174.9±1.4. For the Cilnidipine 5mg/kg dose, the duration was 179.1±2.1, and for the Cilnidipine 10mg/kg dose, it was 183.2±8.

In the Fluoxetine group, the period of immobility was significantly shorter than in the control group ($p < 0.05$). There was no statistically significant difference between the control group and those given 5 or 10 milligrammes of cilnidipine.

Duration of Immobility on 14th day

Duration of immobility, measured in seconds, was recorded for various groups throughout a 6-minute period. The average duration of immobility in the control group was 183.1±4.4, while in the Fluoxetine group it was 175±2.7. For the Cilnidipine 5mg/kg dose, the duration of immobility was 179.5±1.3, and for the Cilnidipine 10mg/kg dose, it was 177±1.9.

The duration of immobility was considerably reduced in the Fluoxetine group ($p<0.01$) and the Cilnidipine 10mg group ($p < 0.05$), compared to control group. In addition, the Cilnidipine 10mg group ($p < 0.05$) exhibited a statistically significant decrease compared to the Fluoxetine group. When compared to the control group, there were no statistically significant results from administering 5 mg of cilnidipine.

Duration of immobility on the 21st day.

The length of time during which participants remained motionless, measured in seconds, was documented for different groups over a period of 6 minutes. The average duration of immobility in the control group was 181.1±4, while in the Fluoxetine group it was 168.1±1.9.

For the Cilnidipine 5mg/kg dose, the duration was 174.3±1.6, and for the Cilnidipine 10mg/kg dose, it was 170.02±1.5.

In comparison to the control group, the groups given Fluoxetine ($p<0.001$), Cilnidipine (5mg) ($p<0.05$), and Cilnidipine (10mg) ($p<0.01$) had significantly shorter durations of immobility. Both the 5mg and 10mg groups with Cilnidipine showed a statistically significant decrease when compared to the Fluoxetine group ($p < 0.05$ and $p < 0.01$, respectively).

Duration of immobility was significantly decreased in Fluoxetine on day1, 7 and 21, Cilnidipine 5mg/kg group shows significant decreased only on 21st day when compared to that of Control and Fluoxetine group. Whereas Cilnidipine 10mg/kg shows significant decrease from 14th day onwards. On day 21st when compared with the standard drug Fluoxetine, Cilnidipine 10mg/kg group showed similar results. (Table 6, Graph 5-8)

Tail Suspension Test - Duration of Immobility

Table 6

Effect of various drugs on duration of immobility in Tail suspension test.

Day of study	**Duration of immobility in seconds**			
	Control	**Fluoxetine 10mg/kg**	**Cilnidipine 5mg/kg**	**Cilnidipine 10mg/kg**
1	180.4±4.1	174.9±1.4 *	179.1±2.1	183.2±8
14	183.1±4.4	175±2.7 **	178.5±1.3	177±1.9 *#
21	181.1±4	168.1±1.9 ***	174.3±1.6 *#	170.02±1.5 **,##

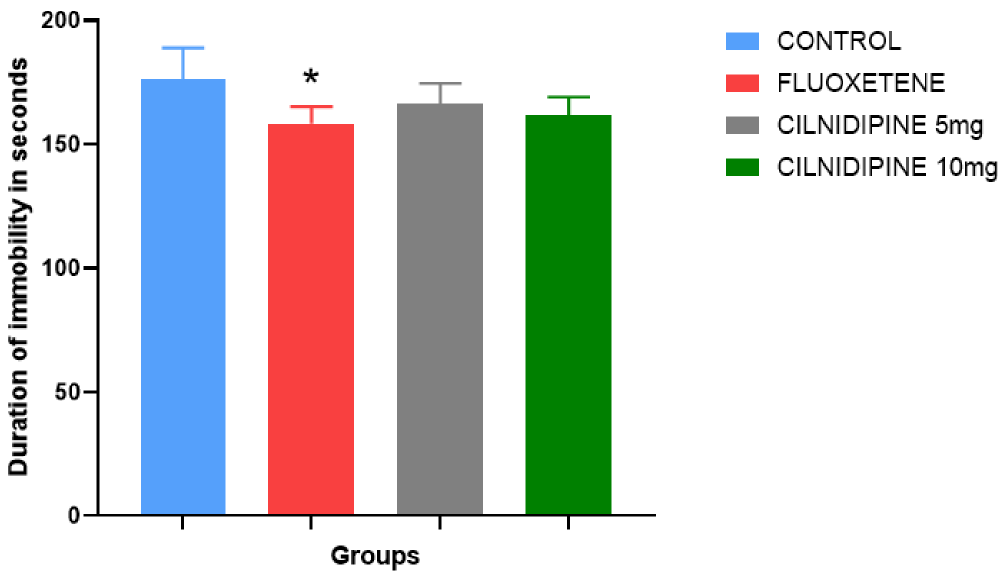
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *p<0.05, **p<0.01, and ***p<0.001in comparison to control group.
4. #p<0.05 and ##p<0.01 in comparison to standard group.

DAY 1

GRAPH 5

Effect of various drugs on duration of immobility in Tail suspension test on Day 1.



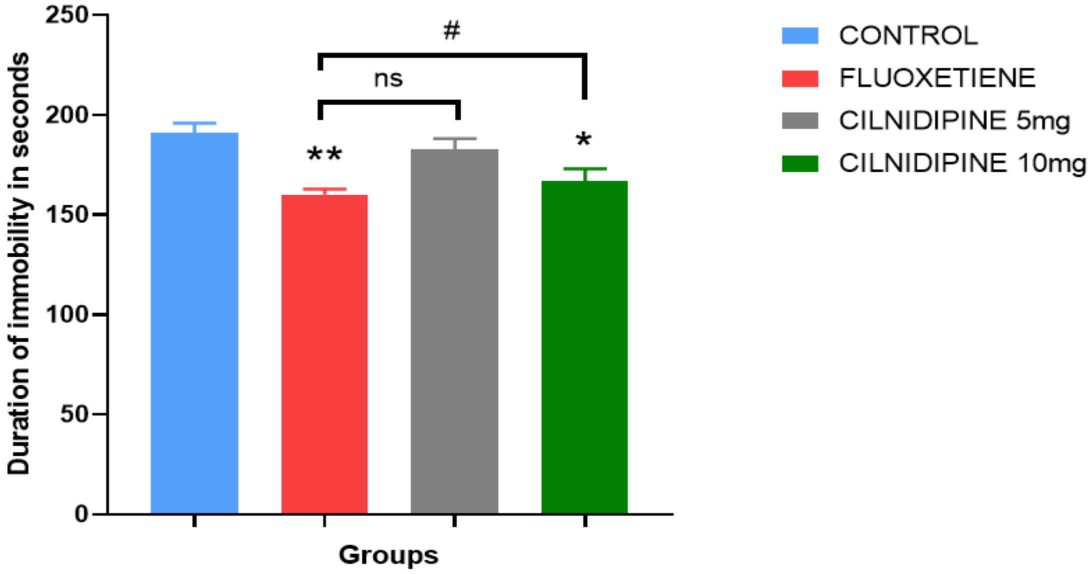
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *$p<0.05$ as compared to control group.

DAY 14

GRAPH 6

Effect of various drugs on duration of immobility in Tail suspension test on Day 14.



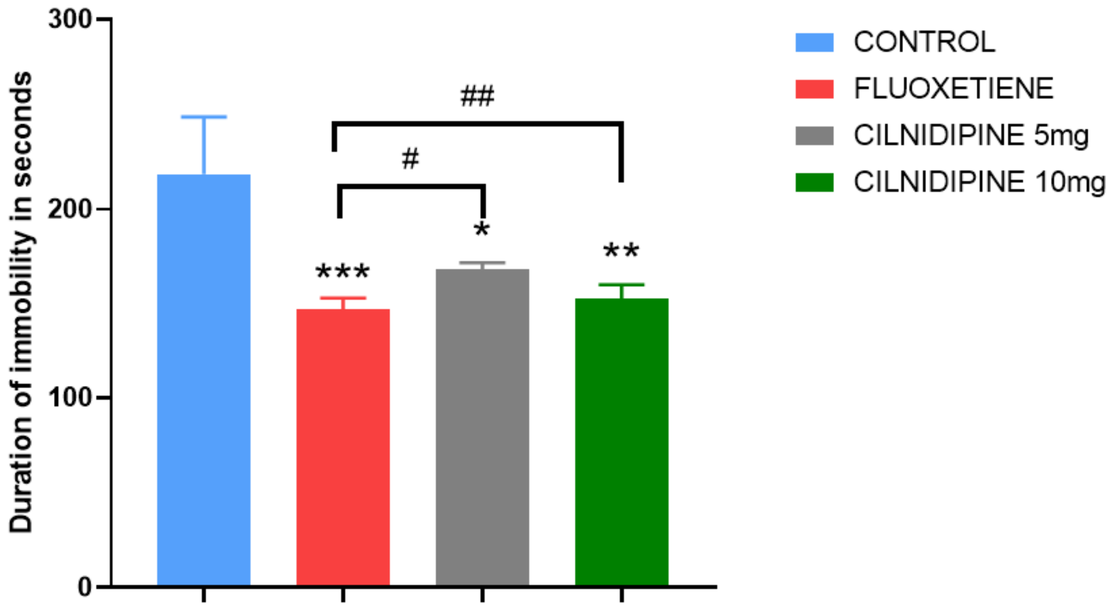
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *p<0.05 as compared to control group.
4. #p<0.05 as compared to standard group
5. ns- nonsignificant

DAY 21

GRAPH 7

Effect of various drugs on duration of immobility in Tail suspension test on Day 21.

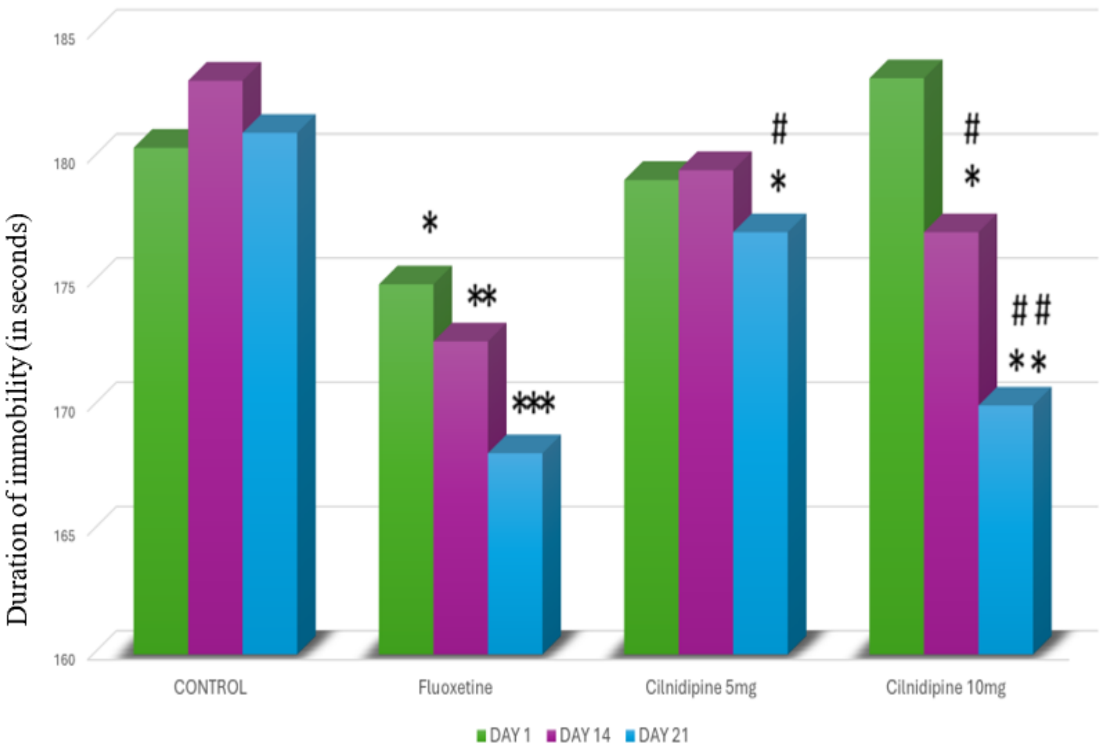


Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3.*p<0.05, **p<0.01as compared to control group.
4. #p<0.05, ##p<0.01as compared to standard group.
5. ns - nonsignificant

GRAPH 8

Overall effect of various drugs on duration of immobility in Tail suspension test.



Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3.*p<0.05, **p<0.01, ***p<0.001as compared to control group.
4. #p<0.05, ##p<0.01as compared to standard group.

Actophotometer in male Swiss albino mice.

Locomotor activity on Day 1

The locomotor activity was assessed for different groups over 10 minutes. The locomotor activity in terms of counts in the control group was 21.83±2.9 while in the Fluoxetine group was 19.66±2.4, Cilnidipine 5mg/kg dose: 22±2.8, Cilnidipine 10mg/kg dose: 21.6±4.6.

Locomotor activity on Day 14

The locomotor activity was assessed for different groups over 10 minutes. The locomotor activity in terms of counts in the control group was 22.6±3.1while in the Fluoxetine group was 20±3.7, Cilnidipine 5mg/kg dose: 23.8±3.7, Cilnidipine 10mg/kg dose: 20±3.7.

Locomotor activity on Day 21

The locomotor activity was assessed for different groups over 10 minutes. The locomotor activity in terms of counts in the control group was 20±3.3 while in the Fluoxetine group was 18.8±3.4, Cilnidipine 5mg/kg dose: 19.8±4.2, Cilnidipine 10mg/kg dose: 20±5.8.

On the 21st day, the Fluoxetine group showed a notable difference in the average locomotor activity compared to control group. On days1,14, and21, the treatment group's mean locomotor activity was not notably distinct from that of the control and standard groups. (Table 7, Graph 9-12)

Actophotometer - Locomotor activity

TABLE 7

Effect of various drugs on Locomotor activity in Actophotometer.

Day of study	Effect on locomotor activity			
	Control	**Fluoxetine**	**Cilnidipine 5mg**	**Cilnidipine 10mg**
1	21.83±2.9	19.66±2.4	22±2.8	21.6±4.6
14	22.6±3.1	20±3.7	23.8±3.7	20±3.7
21	20±3.3	18.8±3.4 *	19.8±4.2	20±5.8

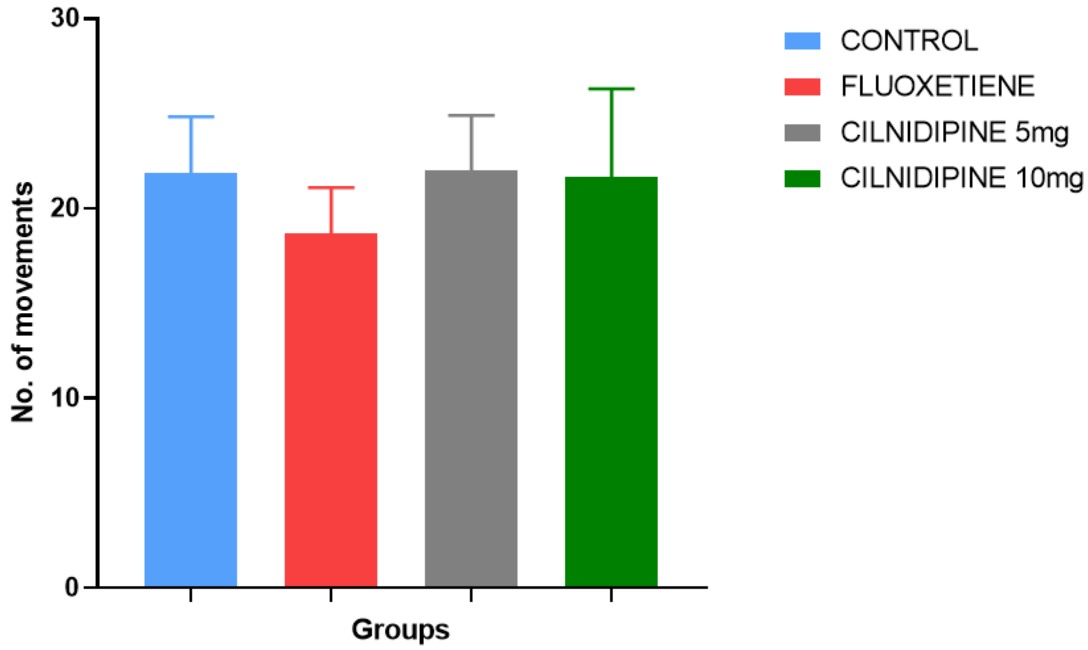
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *$p<0.05$ in comparison to control group.

DAY 1

GRAPH 9

Effect of various drug on locomotor activity in Actophotometer on Day 1



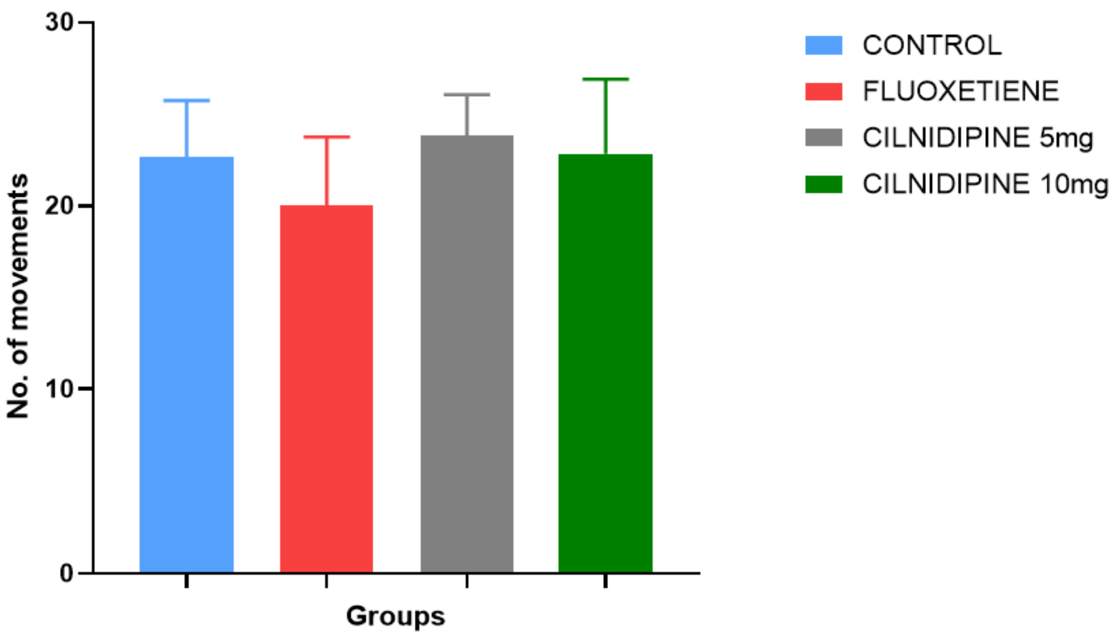
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.

DAY 14

GRAPH 10

Effect of various drugs on locomotor activity in Actophotometer on Day 14



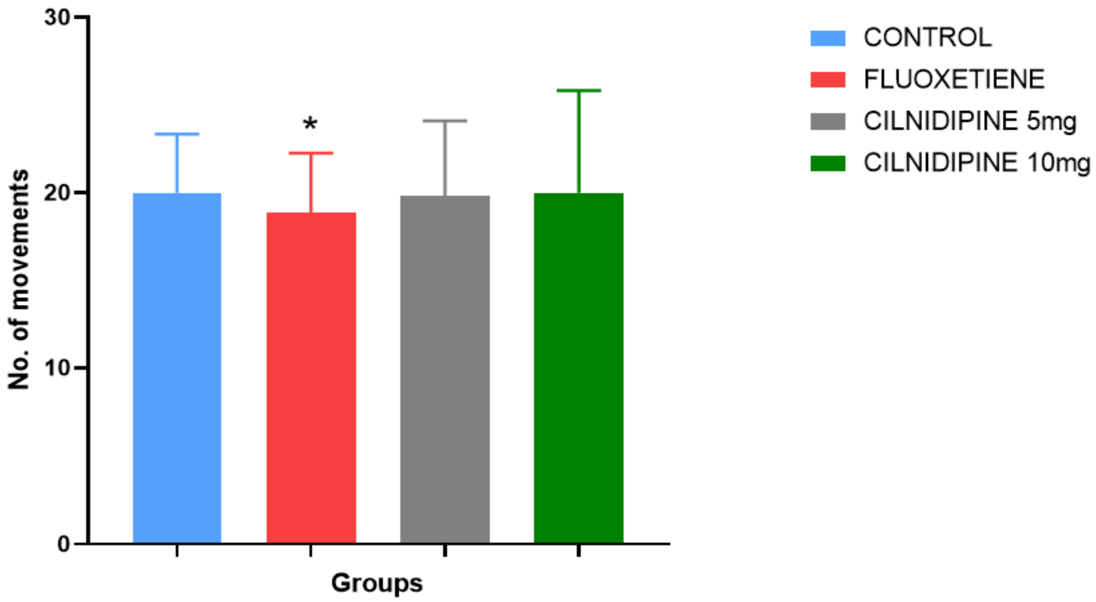
Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.

DAY 21

GRAPH 11

Effect of various Drugs on locomotor activity in Actophotometer on Day 21.

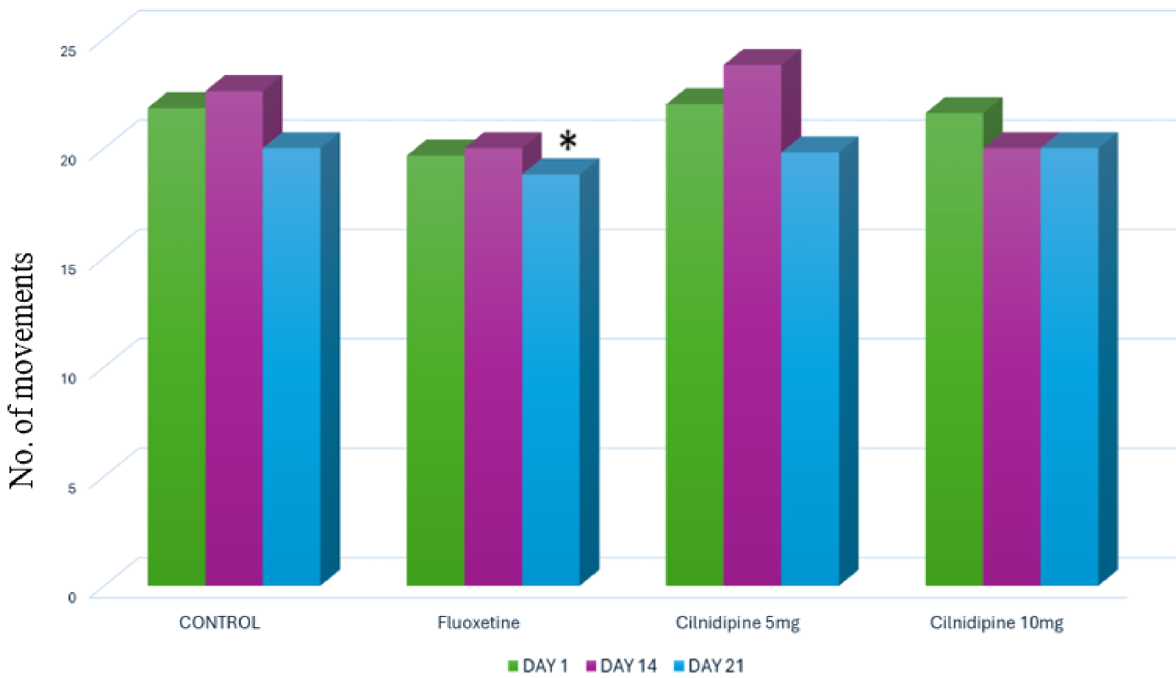


Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *$p<0.05$ in comparison to control group.

GRAPH 12

Overall effect of various Drugs on locomotor activity in Actophotometer



Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *p<0.05 in comparison to control group.

DISCUSSION

This study evaluated the antidepressant effectiveness of Cilnidipine, a dihydropyridine (DHP), in male Swiss albino mice utilising an experimental depression paradigm. The efficacy of the antidepressant was assessed through the utilisation of the Forced Swim Test and the Tail Suspension Test, while the measurement of locomotor activity was conducted using an Actophotometer. The objective of our ongoing research is to investigate the potential antidepressant effects of Cilnidipine, a calcium channel blocker that targets the L/N type calcium channels. Calcium channel blockers like verapamil, nifedipine, and nimodipine have compelling evidence behind their effects. Our current study establishes the antidepressant impact of Cilnidipine, and since it relies on existing literature, it can be used as an alternative or add-on medication in the management of Depression with Hypertension.

The selected DHPs – Cilnidipine 5mg & 10mg was administered over 21 days. The results suggest that the administration of Cilnidipine 10mg significantly reduces immobility in mouse models of depression, as observed in FST and TST tests. The advantageous impacts of Cilnidipine were further improved on the 7th and 21st day of treatment ($P < 0.05$, < 0.01 respectively). According to the post-hoc test, Cilnidipine 10mg showed the highest level of significance on the 21st day of therapy. Nevertheless, after receiving an equal quantity of therapy, there is no noticeable disparity in locomotor activity between the experimental group and the control group. Cilnidipine had an antidepressant effect that varied in intensity based on the dosage administered. This aligns with the results published in current research.[122]

Despite substantial research, existing theories about major depression do not adequately explain the exact cause and nature of depression. Fluoxetine, a frequently prescribed antidepressant, was used as a control drug in our study. By blocking the reuptake transporter protein in the presynaptic terminal, fluoxetine acts by preventing serotonin from being taken up by presynaptic serotonin neurons. Traboulsie et al. observed that fluoxetine also blocks a variety of neuronal ion channels, despite its primary function being that of a selective serotonin reuptake inhibitor. Fluoxetine concentration-dependently blocked CaV3.1, CaV3.2, and CaV3.3 T channels. Norfluoxetine inhibited CaV3.3 T current better than fluoxetine. Overall, our data indicate that voltage-dependent T channel blockade caused by fluoxetine at clinically relevant levels may contribute to the antidepressant's pharmacological effects.[123]

It has been determined that calcium ions control long-term neuroplastic processes, cytoskeletal remodelling, neuronal excitability, and neurotransmitter production and release. Studies indicate that intracellular Ca2+ is crucial to mood disorders. Serotonin drives platelet intracellular calcium migration in bipolar, melancholy, and depressed patients more often than in healthy people as per the studies conducted by Eckert et al. (1993), Yamawaki et al. (1998), and Tomiyoshi et al.[124–126] Emamghoreishi et al. (1997) found that bipolar affective disorder platelets and lymphoblasts may have greater baseline intracellular calcium levels.[127] Yamawaki et al. (1998) showed that the immediate use of antidepressants in cultured neuronal cells and glioma cells resulted in a decrease in the cellular signalling related to calcium and Ca2+.[124] Mice treated with dihydropyridine channel blockers showed antidepressant-like effects, as noted by Cohen et al. (1997) and Biala (1998). [128,129]

The present study's findings, which are like those of Galeotti N et al.'s study, showed that therapy with pharmaceutical substances that may prevent the release of Ca^{2+} from intracellular reserves reduced the amount of time that mice were immobile and induced a therapeutic response akin to that of the selective antagonists of serotonin reuptake (SSRIs) Fluoxetine, which were employed as standard medications.[130]

When depression first sets in, the hypothalamic-pituitary-adrenal (HPA) axis is profoundly affected. Research has shown that stimulation of the HPA axis is prevalent in individuals with depression, often resulting in elevated levels of cortisol and corticotropin-releasing hormone (CRH).[131] Previous research has shown that the HPA axis possesses L-type calcium channels, and that calcium is necessary for corticosterone release.[132] Studies have also revealed attenuated corticosteroid secretion in the presence of calcium channel blockers.[133]

Consequently, it is plausible that blocking L-type calcium channels on the HPA axis is the reason for reduced corticosterone synthesis, which might be the reason for calcium channel blockers' antidepressant effects.

As a result, current evidence may not support the use of cilnidipine as a primary antidepressant. Further research and clinical studies would be required to determine its efficacy and safety for this specific application. L as well as N-type calcium channel blocker cilnidipine restored behavioural changes similarly in this investigation. N-type calcium channels have been discovered to exist in human adrenocortical cells, and their critical role in the production of corticosterone has been demonstrated.[134] Thus, it's probable that cilnidipine-mediated corticosterone attenuation was significantly aided by blockage of N-type calcium channels.

According to Takahara A's research, the dihydropyridine calcium channel blocker cilnidipine prevents noradrenaline (norepinephrine) from being released from sympathetic nerve terminals, hence preventing sympathetic neurotransmission.[135] There is evidence that noradrenaline (also called norepinephrine) plays an integral role in depression.[136] Depression may be exacerbated by disruptions in noradrenergic neurotransmission in the central nervous system.[136] Antidepressants which enhance noradrenaline activity are beneficial in treating depression. Furthermore, investigations on catecholamine depletion have demonstrated that reducing monoamine catabolism, especially noradrenaline can improve pathological mood states.[137] In contrast to our study findings, the results reported here show a clear disparity in outcome. In addition, some studies suggest that Cilnidipine inhibits noradrenaline release from sympathetic nerve ends without harming the adrenal medulla, and it has been demonstrated to reduce norepinephrine release from sympathetic nerve endings.[135,138,139] The inconsistency between the findings of the two studies underscores the need for further investigation to clarify the factors contributing to the observed disparity and to establish more robust conclusions regarding antidepressant activity of Cilnidipine.

These days, depression models are often assessed based on attaining three essential requirements:

i) Face validity (symptomatic similarity),

ii) Construct (etiological) validity (same causal causes), and

iii) Pharmacological validity (reversal of depressed symptoms with existing antidepressants).[33]

Although each of these standards has fundamental flaws, they are useful as standards for comparing models.[140] Unfortunately there are several issues with modern animal models, from limited predictive value for drug effectiveness in human illness to inadequate validation.[38] While numerous neurochemical and neuroendocrine abnormalities have been linked to depressed individuals and brain imaging research has yielded vital knowledge about the brain circuitry underlying mood, no abnormality has shown to be sufficiently strong or consistent to diagnose depression in humans or support an animal model.[141] Furthermore, highly penetrant genetic variations associated with depression have yet to be found. The following issues show how difficult it is to create and validate depression animal models.

Because the Forced swim test is quick, easy to administer, and needs little equipment, it has been used to study antidepressant effects.[142] The Tail Suspension Test and Forced Swim Test are the most common stressor-induced depression models in rats and mice. In the Porsolt test, also known as the forced swim test, the animal is submerged in a tank of lukewarm water so that neither rats nor mice can escape out. The animal's time to become immobile is then measured. In the Tail suspension test, the rodent hangs passively by the tail, and immobility is assessed in seconds. An interpretation of these tests as a depression model suggests that the amount of time spent immobile reflects a diminished capacity to respond to stress. Furthermore, the observation that administering antidepressants prolongs the period of struggling or swimming would enhance the predictive nature of the model.[143]

An Actophotometer was used to conduct an independent evaluation of locomotor activity to determine whether the therapy of interest has an impact on overall activity levels and to rule out the potential for inaccurate results in the FST.[144] Our analysis indicated that Cilnidipine does not exert a notable impact on the locomotor activity of mice.

Thus, Forced Swim & Tail suspension tests demonstrated that cilnidipine may reduce depression in male Swiss albino mice. Results suggest that Ca^{2+} has a role in regulating the HPA axis in depression, perhaps due to its pleiotropic features. More study is needed to support and clarify the effect of calcium channel blockers like cilnidipine in behavioural disorders such as depression. As a result, there is a significant need to pursue these medications for further evaluation in well-planned clinical trials.

Based on the current findings, it is possible to hypothesise that in therapeutic settings, further research could examine cilnidipine's antidepressant potential, as these appear to be better antihypertensives in hypertensive patients with comorbidities such as depression.

CONCLUSION

It can be concluded based on the study's findings that Cilnidipine, a Dihydropyridine calcium channel blocker commonly used as an antihypertensive, also comprises antidepressant qualities, as shown by a decrease in the immobility time male Swiss albino mice spend immobile on the FST and TST. The drugs showed results similar to that of the standard drug Fluoxetine. However, lower doses of the test drugs (Cilnidipine 5mg) did not provide significant effects. Cilnidipine's antidepressant effect is dosage and treatment duration dependent.

According to our findings, cilnidipine may be a newer target for antidepressant activity, and more research is needed to generalise this impact to patients. It can be used to improve the quality of life of hypertensive individuals suffering from depression when two medications are required.

Funding and Declaration of Interest:

The study was self-funded, and we declare no conflict of interest.

LIMITATION

This study has certain limitations:

1. Significant antidepressant effects were observed with Cilnidipine at clinically utilised dosages. However, the precise mechanism of action of Cilnidipine cannot be explained just by changes in behavioural research. To further comprehend the occurrences, more research is necessary.
2. Further investigation is required to ascertain the antidepressant properties and molecular mechanisms of Cilnidipine prior to recommending its use for treating depression in animal models.
3. Another drawback is that, unlike conventional antidepressants, which become effective only after long-term therapy, the FST model is known to generate false-positive results and respond to acute or subacute pharmacological treatment (Willner, 1990).[145] Therefore, to verify the existence of these effects and investigate the underlying processes, more research utilising long-term therapy and other animal models of depression is required.

We anticipate that this data can shed light on the impact of Cilnidipine on depression and has instructional implications for future pathology research on depression.

SUMMARY

Depression is the most serious hazard to public health worldwide today. It is rapidly worsening and affecting all age groups. It is widespread, particularly in developed nations, and is becoming more common in developing nations. Depression not only causes morbidity, which reduces the quality of life, but it also causes major deaths in the form of suicide and homicide. Depression is linked to and often regarded as an essential comorbid condition in the development of noncommunicable diseases such as hypertension.

The current study examined the effects of dihydropyridines, specifically Cilnidipine. These medications demonstrated antidepressant activity in male Swiss albino mice. These findings are supported by several clinical observations.

Human clinical equivalent mouse doses of the medication demonstrated therapeutic efficacy in all tested parameters. Lower doses had just a marginally compared to the disease control.

Several mechanisms have been proposed to explain Cilnidipine's neuroprotective action, including reduced cortisol levels. These are the same mechanisms that experts have linked to the onset of depression for many years. As a result, Cilnidipine appears to be promising and can be further investigated as a possible antidepressant medicine in humans.

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ANNEXURE I – IAEC APPROVAL CERTIFICATE

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed to be University)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI - 590010, (KARNATAKA).
INSTITUTIONAL ANIMAL ETHICS COMMITTEE.
Phone No. JNMC (0831)- 2444040

Dr.(Mrs)P.P.Patil
Chairperson, IAEC.
Prof & Head Physiology,
J.N.Medical College, Belagavi

Dr.P.A.Patil
Main Nominee - CPCSEA
Prof & Head of Pharmacology,
USM-KLE, IMP, Belagavi

Dr.(Mrs)Rekha Nayaka M.R
Member - Secretary IAEC
Asso Prof of Pharmacology
J.N.Medical College, Belagavi

CPCSEA Reg.No.: 627/PO/Re/S/02/CPCSEA

MEMBERS:

Dr.Banappa Unger
Scientist-D, RMRC,
ICMR, Belagavi.

Shri Sunil.R.Patil.
Non-scientific Social worker,
Nidasosi.

Dr. Sudha Devareddy.
Hon.Veternarian,
Belagavi.

Dr. (Mrs)S.A.Hogade,
Officer Incharge,
Central Animal House,
JNMC, Belagavi.

Dr. (Mrs)S.M.Bhimalli,
Prof of Anatomy.
JNMC,Belagavi

Dr. Vishwanatha Swamy
AHM
Link Nominee CPCSEA.
Dept of Pharmacology &
Toxicology
KLE's Coll Of Pharmacy,
Hubballi

CERTIFICATE

This is to certify that the M.D/ M.D.S/ Ph.D/ Research project Entitled "Effect of Cilnidipine on Depression paradigm in Male Swiss Albino Mice : An experimental study".

Submitted by- BO0121003 PG Pharmacology, JNMC.

Has been approved by the Institutional Animal Ethical Committee

Meeting held on 25-6-22 vide Resolution No. 17/3.

For sanction of 24 Male Swiss Albino Mice

Main Nominee CPCSEA
IAEC-JNMC, Belagavi.
Signature and Name:
CPCSEA-Main Nominee

Member Secretary
IAEC-JNMC, Belagavi.
Signature and Name:
Chairman/Mem.Secretary

ANNEXURE II – CCSEA REGISTRATION AND RENEWAL

No.25/1/99 – AWD (Pt.)
Government of India
Ministry of Statistics & Programme Implementation
(Committee for the Purpose of Control and Supervision of Experiments on Animals)
*****

Shastri Bhavan, New Delhi-110001.
Dated the 19th June 2002.

To

The Prinicipal/Director/Dean
K.L.E. Society's Jawaharlal Nehru Medical College
Nehru Nagar
Belgaum - 590 010
Karnataka

Subject: Registration of Establishments/ Breeders under Rule 5(a) of the "Breeding of and Experiments on Animals (Control and Supervision) Rules 1998".

Sir/Madam,

With reference to your application on the above-mentioned subject, this is to inform that your Establishment is hereby registered for "Research". Your Registration Number is **627/02/a/CPCSEA**. The nominee of CPCSEA on the Institutional Animal Ethics Committee (IAEC) of your Establishment will be intimated in due course.

1. You are requested to quote the above Registration Number in all your future correspondence with the Committee.
2. You are also requested to convene IAEC meeting at the earliest.
3. For further correspondence you are requested to contact Office of CPCSEA at Chennai, at the address given below:

Office of the CPCSEA,
Ministry of Statistics & Programme Implementation
3rd Seaward Road, Valmiki Nagar,
Thiruvanmiyur, Chennai-600 041 (Tamil Nadu)

Yours faithfully,

(R.K.JAIN)
MEMBER SECRETARY (CPCSEA) / DIRECTOR (AW)
Tel. No.3381498

Copy to: - Ms. Prema Veeraraghavan, Expert Consultant (CPCSEA), 3rd Seaward Road, Valmiki Nagar, Thiruvanmiyur, Chennai

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No. 25/373/2010-AWD
Government of India
Ministry of Fisheries, Animal Husbandry and Dairying
Department of Animal Husbandry and Dairying
O/o Committee for the purpose of Control and Supervision of Experiments on Animals
(CPCSEA)
*****

Delhi Milk Scheme Complex,
Shadipur, Delhi – 110008
Date: 19.12.2022

To,

Dr Parwati Patil, Chairperson, IAEC
K.L.E.Society's Jawaharlal Nehru Medical College Nehru Nagar,
Belgaum - 590 010 Karnataka
Email: docparwati@yahoo.co.in
Mobile: 9449019436

Subject: Renewal of Registration and Reconstitution of Institutional Animals Ethics Committee (IAEC)-regarding

Madam,

The registration of Animal House Facility of your establishment with CPCSEA has been **renewed for a period of five years from the date of issue of this letter**.

2. The registration number of Animal House Facility of your establishment is **627/PO/Re/S/02/CPCSEA for Research for Education purpose on small animals.** Henceforth, the registration number may kindly be quoted in all your future correspondence.

3. The CPCSEA has accepted the following members recommended by the establishment.

Name of the IAEC Members	Designation in IAEC
1) Dr.Parwati .P.Patil	Biological Scientist, Chairperson
2) Dr.Netravathi A Kavi	Scientist from different biological discipline, Member Secretary
3) Dr.Veereshkumar S Shirol	Scientist from different biological discipline
4) Dr.Mohan C Singanalli	Veterinarian
5) Dr.Manjula A Vagarali	Scientist Incharge of Animal House Facility

4. CPCSEA hereby nominates the following members to the Institutional Animals Ethics Committee (IAEC) of your establishment:

Details of Nominee(s)	Nominated as
1) Dr. Manish Barvaliya Scientist-E, ICMR-National Institute of Traditional Medicine (NITM) Nehru Nagar, National Highway No. 4 Belagavi590010, Karnataka Contact No :9726901845 Email :drmanishbarvaliya@gmail.com	Main Nominee
2) Dr. Prabhakar Adake Professor of Pharmacology, KAHER's JGMM Medicel College, Kotgonadhunshi,Gabbur cross, Hubballi-580028 Karnataka Contact No :9886554800	Link Nominee

-2-

3) Dr. Shabbir Rafik Pendhari Department of Pharmacology Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli. 416414 Contact No :9766417420 Email :itsshabbir@gmail.com	Scientist from outside the Institute
4) Mr. Atul Ramchandra Chopade Dept of Pharmacology, Rajarambapu College of Pharmacy, Kasegaon, Tal: Walwa, Dist. Sangli – 415404, Maharashtra Contact No :9226346106 Email :chopadearv@gmail.com	Socially Aware Nominee

(Please note that any change in IAEC members can be made only with prior approval of CPCSEA.)

5. The IAEC is valid for a period of five years and is coterminous with renewed period of registration. IAEC is required to be reconstituted at the time of renewal of registration as per CPCSEA guidelines.

6. You are requested to convene the meeting of the re-constituted IAEC within a period of 30 days and upload the same on the website of the CPCSEA.

7. It is stated that only above approved IAEC members shall sign, with date, on the attendance sheet of the IAEC meetings, and decisions will be taken only in meetings where quorum is complete. The quorum for holding IAEC meeting is six (6), and Main Nominee, Scientist from outside the Institute and Socially Aware Nominee must be present in such meetings. Link Nominee can attend in case main nominee conveys his unavailability in writing to the chairman IAEC. However, the Link Nominee should be invited once a year to update him/ her about the activities of the IAEC. Any decision taken in the meetings of IAEC without quorum shall be considered invalid.

8. It is also to inform you that before commencing any research on large animals you are required to send research protocols with due recommendation of IAEC to CPCSEA for further approval (procedure for submission of Research Protocols is available on the website of CPCSEA).

Yours Sincerely,

(Dr. S. K. Dutta)
Member Secretary (CPCSEA)

Copy for necessary action to: Nominees of CPCSEA.

The Main Nominee is requested to ensure that the IAEC meetings are held regularly as stipulated in the SOP of CPCSEA and submit the Annual Inspection Reports of the Animal House Facility regularly on the Website of CPCSEA.