EVALUATION OF ANTIDEPRESSANT ACTIVITY OF AMLODIPINE IN MICE

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In partial fulfillment of the award of the degree of

MASTER OF PHARMACY IN

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Submitted by

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APRIL 2020

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"EVALUATION OF ANTIDEPRESSANT ACTIVITY OF AMLODIPINE IN MICE"** submitted by the student bearing **[REG.No: 261825212]** to **"The Tamil Nadu Dr. M.G.R. Medical University"**, Chennai, in partial fulfillment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on……..…….

Internal Examiner **External Examiner**

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DECLARATION

"EVALUATION OF ANTIDEPRESSANT ACTIVITY OF AMLODIPINE IN MICE", submitted to "**The Tamil Nadu Dr. M.G.R Medical University"**, Chennai, for the partial fulfillment of the degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work has been carried out by me during the academic year 2019-2020, under the guidance and supervision of **Dr. C. KALAIYARASI, M.Pharm., Ph.D., Associate Professor, Department of Pharmacology,** J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

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INDEX

ABBREVIATIONS

Abbreviations	Expansion
ANOVA	Analysis of variance
VTA	Ventral tegmental area
PTSD	Post-traumatic stress disorder
CSF	Cerebrospinal fluid
PET	Positron emission tomography
OCD	Obsessive-Compulsive Disorder
PPD	Post-partum depression
COPD	Chronic obstructive pulmonary disease
HVA	Homovanillic acid
CRP	C-reactive protein
TNF-α.	Tumor necrosis factor alpha
HPA	Hypothalamic-pituitary- adrenal axis
SSRI	Selective serotonin reuptake inhibitors
SNRI	Serotonin norepinephrine reuptake inhibitor
MAO-I	Mono amino oxidase inhibitors
GABA	Gamma amino butyric acid
BDNF	Brain derived neurotropic factor
MAPK	Mitogen-activated protein kinase
CREB	Cyclic AMP response element Binding protein
AVP	Arginine vasopressin

1. INTRODUCTION

Depression is a heterogeneous disorder that affects physical health and mental health. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, in particular, norepinephrine**,** serotonin and dopamine[1]. This monoamine hypothesis is also supported by the fact that the known antidepressants like monoamine oxidase inhibitors, tricyclic antidepressants and selective serotonin reuptake inhibitors have been known to potentiate monoamine function.[2]

Depression is reported to place the second most common chronic condition in clinical practice.[3] In addition, depression is the most common of the affective disorders ranging from a very mild condition bordering on normality, to severe depression accompanied by hallucinations and delusions[4][5].

Major depression represents a significant public health problem worldwide. The high prevalence of suicide in depressed patients (up to 15%), coupled with complications arising from stress and its effects on the cardiovascular system, have suggested that it will be the second leading cause of death by the year 2020 and studies show depression as a contributory factor to fatal coronary disease.[6]

Types of depression

Depression is a heterogeneous disorder often confused with single clinical mental illness. Moreover, there exist diverse forms of depression that can either be mild or extremely severe conditions like psychotic depression in which the patients show symptoms such as hallucinations and delusions. Diagnosis of this disorder is more complex as the co-occurrence of many other mental conditions such as anxiety disorders, including panic agoraphobia syndrome, severe phobias, generalized anxiety disorder, social anxiety disorder, post-traumatic stress disorder (PTSD) and obsessive-compulsive disorder (OCD). This co-morbidity is commonly seen in

elderly patients and is also associated with severity of somatic symptoms [9]. The different types of depression are reviewed below.

Major Depressive Disorder(MDD):

Major depressive disorder (MDD) is believed to be a serious condition that can affect patients' ability to sleep, work, eat, and go about their lives.(18) This most complex disease is poorly under stood and is thought to be caused by both genetic and environmental factors. Its development is said to be associated with numerous neurotransmitter abnormalities, including serotonin, norepinephrine, and dopamine, among others(19).

Patients with this type of depressive disorder often show dysphoric mood and anhedonia along with physical changes such as weight loss or gain, increased or decreased appetite, alteration in sleep pattern and sustained fatigue. Disturbances in cognitive and executive functions are also exhibited by lack of concentration and coherent thinking as well as morbid preoccupation by thoughts of death and suicide. Majority of these symptoms normally are present nearly every day and result in significant distress and impaired social life and occupational performance (DSM-V) [8,10].

Dysthymic disorder:

Dysthymic disorder is often referred as persistent depressive disorder. Patients affected by dysthymic disorder display depressed mood or sadness that persists for the majority of the duration of the day for a minimum of two years in adults and one year in children and adolescents. Most of the patients do not meet the full criteria for MDD as there is interruption by short periods of remission. On the other hand, there are instances where patients meet full criteria in which they are diagnosed with MDD [11,12].

Melancholic depression:

The person suffering from melancholia lack the ability to experience pleasure. In addition, psychomotor retardation and early morning worsening of mood is also apparent in this subset of patients. This type of depression is seen more often in the elderly, in patients with more severe forms of depression and psychotic depression [11].

Seasonal affective disorder (SAD):

It is a type of depression described as recurring annually during rain fall or early winter. This 'winter blues' or SAD is characterized by decreased mood, feelings of guilt and worthlessness and increased irritability. Moreover, patients show a significant increase in appetite and craving for foods high in carbohydrates which result in weight gain [8,13].

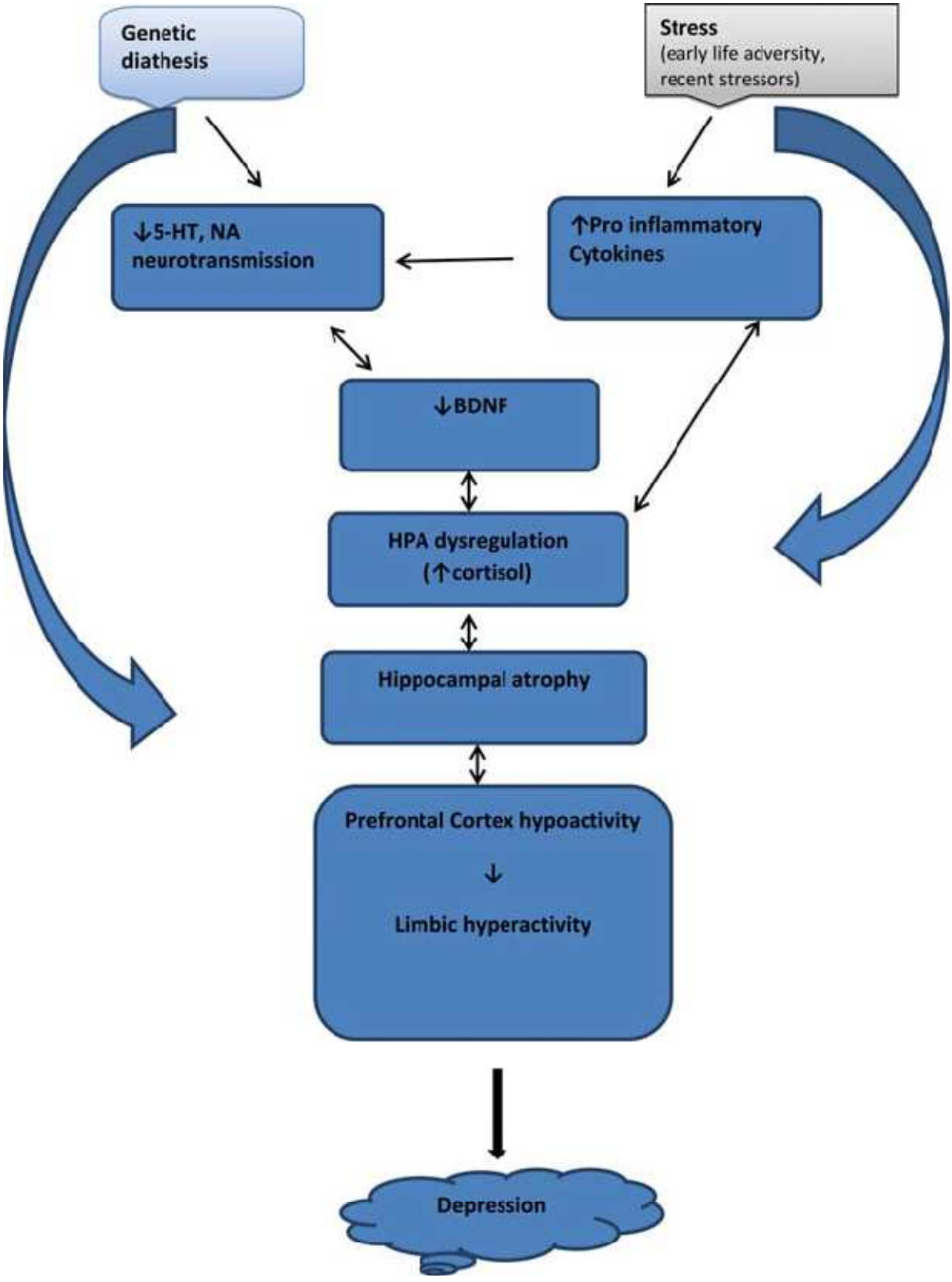
Post-partum depression (PPD):

Post-partum depression is a heterogeneous group of depressive symptoms that affects mothers. These symptoms may surface before or after giving birth [10]. Half of the "postpartum" episodes believed begin before the time of delivery. Therefore, are referred to collectively as "peri-partum" episodes. According to DSM-V [8] mood swings and anxiety symptoms during pregnancy, as well as the "baby blues" increase the risk for a postpartum major depressive episode.

Psychotic depression:

Psychotic depression is a type of depressive disorder which is very severe and accompanied by psychotic symptoms [15,16]. It is commonly seen as a combination of psychosis and depression that is not discriminated into either of the two. Symptoms include psychotic features such as hallucinations or delusions. Other than its severity, psychotic depression is often associated with prolonged course, poor response to available drugs and higher relapse rate [13,17].

Fig.1 Neurobiology of depression



Animal and human research has recognized a number of abnormalities, fit together to create a picture of a psychobiological model of the pathophysiology of depression. The main observations, which interact closely with each other, are decreased monoamine (serotonin and noradrenaline) neurotransmission, low BDNF concentrations, raised cytokines, dysregulation of the HPA axis, cortical and subcortical functional and structural brain changes and susceptibility/protective gene variations (see Fig)

The structural changes in the brain in particular the hippocampus and PFC are reported to be due to abnormalities in neuroplasticity rather than neurodegeneration. On the other hand, it remains to be confirmed whether these changes are indeed always reversible, particularly in the PFC and also whether or not they predate the onset of depression. The dysregulation of the HPA axis is responsible to a great extent for these abnormalities. Raised levels of circulating cortisol activate brain receptors stimulating gene transcription and protein synthesis. Although this may have a beneficial effect in the short term, enabling the brain to cope with smaller amounts of stress, persistent hypercortisolaemia in chronic stress can affect voltage-gated ion channels allowing increased calcium entry into the activated neurons and causing neuronal damage. Glucocorticoid-induced damage in the hippocampus may occur directly, via activation of the glutamate systems or via BDNF reduction. CRH also has direct toxic effects on hippocampal neurons. Stress is associated with reduced BDNF concentrations which further impair neuronal survival. The decrease in BDNF concentrations may be due to the reduction in hippocampal neuronal tissue, as well as a direct effect of hypercortisolaemia; decreased activity in monoaminergic neurotransmission or other noxious factors may also be responsible. The resulting impaired hippocampal function fails to adequately regulate (inhibit) the HPA axis, therefore sustaining 'toxic' hypercortisolaemia. The rise in cytokines levels may also contribute to the sustained HPA activation and abnormal IRSs may have a secondary or even a primary role in the dysregulation of the HPA axis.

This vicious cycle of events may be triggered off in susceptible individuals by stress (external: psychological or internal: physical) and sustained activation of the HPA axis. Pre-existing (functional or structural) abnormalities related to hereditary diathesis (susceptibility minus protective genotypes), near the beginning life difficulty or other causes may act as vulnerability factors. The strength of recent life stress required to trigger a depressive state may depend on the

degree of that susceptibility. It might be speculated the higher the genetic loading and other vulnerability factors, the lower the amount of life stress required to bring about a depressive episode and vice versa.

Despite the major role stress and the HPA axis come into view to play in the pathogenesis of depression, given the multiple systems involved (neuroanatomical, neurochemical and immunological), insults other than the effects of stress hormones, cortisol and CRH, need to be also considered. be hippocampal and prefrontal cortex volume changes in depression completely attributable to stress and could they be present in the absence of stress and HPA abnormalities at any time? The decreased hippocampal volume of healthy subjects with a family history of depression suggests some of these changes may be genetically determined.[20]Cushing's syndrome is associated with a reduced hippocampal volume and cognitive dysfunction[23] and sometimes but not always with depression. Only 50% of depressed patients have measurable abnormalities in the HPA axis.[24] In several depressed subjects, there are no particular stressors either in the recent or remote past to explain the onset of a first depressive episode and in such cases possibly there is either very strong genetic susceptibility possibly with multiple vulnerability genotypes present in the same individual or other unidentified mechanisms may be operating. It is possible that in such cases whatever the cause of hippocampal dysfunction, this may in turn cause secondary activation of the HPA axis (in the absence of any stressors) through reduced inhibition of the hypothalamus. Further research is needed to examine these questions.

Experimental lesioning studies, clinical observations of patients with degenerative disorders of the basal ganglia and neuroimaging structural and functional studies in depressed subjects implicate the limbic-cortico-striato-pallido-thalamic circuits related to the medial and orbital PFC networks in depression. Abnormalities which get in the way with the finely balanced interaction/communication within the neurocircuit and in particular a decrease in the inhibitory control of the limbic structures by the PFC is associated with emotional processing, cognitive performance, behavioural and other signs of depression as well as abnormalities in neurotransmitter activity, neuroendocrine function and pain modulation. Price and Drevets[21] propose that impaired function within the circuits involving the medial prefrontal network and related limbic structures can account for these disturbances mood disorders. However, this does not explain what causes the impaired function in the first instance, in

particular in the absence of any vascular, degenerative or other obvious abnormalities. The recently described subcortical white matter structural abnormalities merit further study to clarify their nature and possible consequence in disrupting the connections among the neurocircuit brain structures and in particular decreasing PFC activity. Although in the old age onset depression these can be attributed to vascular changes, it is unlikely that vascular abnormalities can explain the white matter changes in the young.

Based on the evidence that after the first few episodes of depression, the influence of life stressors in further relapses diminishes, it has been suggested that a kindling process may be responsible for the seemingly spontaneous subsequent episodes.(22) It could also be speculated that perhaps at some point in the reduction of neuronal tissue a 'critical mass' is reached which cannot be sustained (in order to maintain a healthy homeostasis within the circuit brain structures) without on-going medication. This may explain the presence of low hippocampal volumes shown in medication-free remitted patients, although in some cases (those with a family history) the structural abnormality may be pre-existing (prior to the onset of depression). The persistence of such changes may result in a fragile system, with higher risk of relapse and therefore serious consideration needs to be given to long-term treatment in recurrent depression. Longitudinal studies examining the status of structural brain changes in relation to the longitudinal clinical course of depression over several years may help clarify these issues.

Pathophysiology of depression

Even though there are several studies attempting to shed light on the pathophysiology of depression, it still remains indefinable. This is in fact the major reason for the slow paced drug development against this disease. There are varied theories on the pathogenesis of depression most based on measurement of indirect markers, post-mortem studies and neuro-imaging techniques. For decades, depression pharmacotherapy and a resultant explanation for the fundamental pathology, focused on the brain monoamine neurotransmitters level following the serendipitous discovery of imipramine and iproniazid as antidepressants [25,26].

Neural circuitry of depression:

A variety of structural and functional studies report abnormalities in the areas of the brain that are accountable for the regulation of mood, prize response and executive functions. Post-mortem and neuro-imaging studies have reported morphological changes indicated by reductions in grey-matter volume and glial thickness in the prefrontal cortex and the hippocampus, regions that have received the most attention in animal research on depression. The decline in hippocampal function, which is supposed to have an inhibitory effect on the hypothalamic-pituitary- adrenal (HPA) axis, could potentially be responsible for the hypercortisolemia seen in depression [26,27]. The mesolimbic dopamine system that consists of the nucleus accumbens (NAc) and the ventral tegmental area (VTA) also are believed to play a role in the pathogenesis of depression. These brain regions mediate the reward response to pleasant stimuli such as food, sex and even drugs. For that reason, a peculiar lack of pleasure in depressed patients can possibly be explained as a dysfunction in this brain reward circuit [27]. Other studies have also shown a decrease in Locus coeruleus (LC) neuron density in some depressed and suicide inhabitants compared with controls [28].

Stress response circuits:

Chronic stress and hyperactivity of the HPA axis (causing chronic hypercortisolemia) have been hypothesized to play a famous role in the incidence of depression and still in recurrence after complete remission. Structural brain abnormalities have been documented in patients with elevated levels of corticosteroids. One of the brain structures affected is the amygdala, area of the brain involved in mainly regulating emotional reactivity and to some degree stress response [29,30]. An additional brain region shown to decrease in size with chronic administration of corticosteroids is the hippocampus, area of the brain that is supposed to exert an inhibitory signal to the HPA axis [31].

There is still a lack of complete understanding on how behavioral stress causes depression. However, chronic stress has been shown to alter the expression of genes regulating antioxidant systems, such as superoxide dismutases (SODs), catalase, glutathione peroxidase, glutathione reductase and NADPH oxidase. Furthermore, animal studies uncovered that

treatment with glucocorticoids cause elevation in the level reactive oxygen species (ROS) both *in vitro* and in the brains of animals, while also down-regulating various antioxidant enzyme and inducing depression-like behavior [32,33].

Genetic vulnerability and environmental interaction:

There is now a compelling argument that in order for depression to surface there needs to be a complex gene-environmental interaction that alters an person response to stressful life situations. No single gene polymorphism seems responsible for causing depression, it has been suggested that genetic factors make certain individuals susceptible to depression by increasing their vulnerability to stressful environmental factors [34].

A hereditary polymorphism that has been perhaps a center of awareness for years is the allelic variation in the promoter region of the gene encoding the serotonin transporter (5- HTT). The promoter region of 5- HTT gene (5-HTTLPR) contains a functional polymorphism resultant in a long (L)/short(S) variant in the promoter region upstream of the transcription starting site. The short allele of 5-HTT has a low-activity and has been shown to set carriers at a greater risk of upward depression in response to stressful life events. This allele has also been related with not as good as outcomes after antidepressant pharmacological and non-pharmacological treatments [35,36]. The rate-limiting enzyme in serotonin biosynthesis, tryptophan hydroxylase (TPH), is encoded by two distinct genes *Tphl* and *Tph2* and has been proposed to play a role in pathogenesis of depressive disorders and suicide. Single nucleotide polymorphisms (SNPs) on *Tph2* gene have been linked with increased incidence of MDD and completed suicide attempts. Also, *Tph l* gene, which is dominantly expressed in the pineal gland, is thought to influence suicidal risk by disrupting the synthesis of melatonin a hormone responsible for regulation of circadian rhythm consequential in an increase in in the depths of despair risk [37,38].

A functional polymorphism, producing a valine to methionine substitution at the codon 66 (Val66Met) in the pro-BDNF region, has been identified in the BDNF gene, exhibiting a detrimental effect on intracellular trafficking and activity-dependent secretion and influencing hippocampal function, episodic memory and brain morphology. Healthy individuals with the BDNF Met variant display a low touching stability and smaller hippocampus volume. Studies

also suggest a multifaceted interaction exists between the polymorphisms in genes indoctrination BDNF and 5- HTT to bring about a depressed phenotype [26,35].

The biogenic monoamine theory:

The monoamine hypothesis of depression came into the picture after the unexpected discovery of the first antidepressant drugs that were otherwise developed for other medical conditions. These clinical observations have contributed greatly to the considerate of the pathophysiological changes that take place in the brains of depressed individuals. The drugs were projected to increase the amount of monoamine neurotransmitters in the brain either by blocking a monoamine degrading enzyme monoamine oxidase inhibitor (MAOI) or by blocking the reuptake of the neurotransmitters into the presynaptic neuron [39].

The Serotonin hypothesis:

Serotonin is a monoamine neurotransmitter with a broad range distribution throughout the central nervous system. It is involved in physiologic activities such as pain sensation, appetite regulation, aggression and mood. Dysfunction in serotonergic system has been implicated in mood and anxiety disorders. The foundation for this hypothesis is the detail that the first antidepressant drugs worked by reviving the diminished monoamine activity in the brain. Along with afterward SSRIs alone were found to be sufficient to treat symptoms of depression effectively. This detail additional strengthened the involvement of 5-HT in the pathogenesis of the disease [40,41].

Subset of depressed patients have been reported to have a lowered level of 5-hydroxyindoleacetic acid (5-HIAA) a metabolite of 5-HT in the cerebrospinal fluid (CSF), which has been related to aggressive behavior and augmented suicidal intent and impulsivity. The plasma level of the amino acid forerunner (tryptophan) of 5- HT decreased and depressive symptoms can be induced in patients who are susceptible to depression by depleting this amino acid. Moreover, positron emission tomography (PET) imaging studies have reported a decrease in density of 5-HT1A receptor subtype on depressed patients in different regions of the brain. There is also a decreased availability of 5-HTT in midbrain and

brainstem regions. But this serotonergic dysfunction associated in depression is debated whether it is an etiologic factor or increases susceptibility [42,43].

The catecholamine hypothesis:

The catecholamine hypothesis of depression emerged in the 1960s after the surveillance that reserpine; an antihypertensive drug depletes central and peripheral amine storage in the nervous system, induced depression.However, there are no consistent findings on the alteration in the levels of NE metabolites in the CSF of depressed individuals. In subsequent years, the "supersensitivity hypothesis" was proposed which links depression to supersensitive presynaptic α2-R which is also supported by an increased density of these receptor types in post mortem studies, leading to an impaired NE activity [28,39].

Additionally, some symptoms of depression including anhedonia and psychomotor retardation are better explained by a derangement in the brain DA systems. These systems include the substantia nigra –basal ganglia motor system and the reward circuitry involving the NAc and VTA. There is a diminished DA activity in the NAc specifically which corresponds to the inability to experience pleasure which is one of the hallmarks of depression. The concentration of the dopamine metabolite homovanillic acid (HVA) in CSF is reported to be lower in depressed patients as well [44-46].

Inflammation and depression:

The claim that depression is an inflammatory disorder is gaining popularity nowadays. This is supported by the fact that many pro-inflammatory marker levels are reported to be elevated in depressed patients. Examples of these markers are C-reactive protein (CRP), interleukin (IL)-6, IL-1 and tumor necrosis factor alpha (TNF-α). In fact depressive like behaviors can be induced in the laboratory by administration of (IFN)-α, a powerful inflammatory cytokine, that has also been shown to produce depression like symptoms in patients taking it for the treatment of hepatitis C [47,48].

An increase in reactive oxygen and nitrogen species generation and damage by oxidative and nitrosative stress (ONS), including lipid peroxidation, damage to deoxyribonucleic acid (DNA) and proteins is also seen. Even though a complete understanding of the mechanisms involved remains obscure, an increase in pro- inflammatory cytokines results in a lack of neuronal plasticity and eventual neurodegeneration. Also pro-inflammatory cytokines can interfere with the activity of growth factors which results in reduced neurogenesis as the immune changes can damage glial cells and neurons [49,50].

Neurothrophic hypothesis:

Significant atrophy of certain prefrontal cortex areas and hippocampus experiential in depression as well as decreased levels of nerve growth factors (NGF) such as BDNF has led to the neurotrophic hypothesis. BDNF is an important molecular regulator of neuronal growth and plasticity. It increases survival of neurons, stimulates the growth of dendrites and increases the spine density and also concerned in maturation of excitatory synapses, processes that are important in learning and version process which seems to be deficient in depression [35,51,52].

The expression of BDNF is supposed to be halted by chronic stress and normal level of this growth factor is attained after a successful treatment with antidepressants. This is reliable with the fact that antidepressants take at least 2-3 weeks to elicit their actions, possibly through causing a longer lasting neuroadaptive changes in the brain rather than a simple increase in the level of neurotransmitters. This neuroadaptive change includes the process of neurogenesis, a phenomenon recently exposed to also occur in specific areas such as the subventricular and subgranular zones of the dentate gyrus giving rise to neurons in the hippocampus. This process includes cell division, migration and differentiation medicated by NGFs [51-53]. Vascular endothelial growth factor (VEGF) is another NGF that promotes proliferation of neuronal cells in some brain regions like the hippocampus. It achieves this by activating intracellular signaling cascades that involves mitogen-activated protein kinase (MAPK) pathway. This signaling pathway has also been postulated to underlie the late antidepressant response of currently available drugs. This is achieved through the activity of gene transcription inducer cyclic AMP

response element Binding protein (CREB) which is activated by MAPK resulting in stabilization of synaptic plasticity [56,57].

Neuropeptides and depression:

There is rising proof that this neuropeptides are involved in the modulation of stress- related behaviors and mood by acting on neurokinin type-1 receptors (NK- 1). Substance P (SP) is one of these neuropeptides known for its extensive spread distribution in the brain and its co-localization with 5-HT and NE neurons [58]. Elevated CSF SP concentrations have been reported in depressed patients and patients with PTSD after exposure to a stressful stimulus. Furthermore, central administration of SP has been shown to induce stress response. This is supported by the antidepressant activity of NK-1 antagonists [59,60].

Hormones and depression

i.Thyroid hormones:

Thyroid hormones (TH) imbalances are concerned in the pathophysiology of neurodegenerative and psychiatric conditions. These hormones are very essential for brain development, maturation and have been shown to encourage neurogenesis, in particular, in the hippocampus [61]. Hypothyroidism has been linked to depressive -like behavior in that it impaired hippocampal neurogenesis which resolved with hormone replacement.

Animal studies also revealed that thyroid hormone causes an increase in seretonergic neurotransmission which supports the fact that TH supplementation has been beneficial in management of refractory cases of depression [62,63].

ii.Estrogen involvement:

Increased female vulnerability to depression mostly overlaps periods of low estrogen levels in the menstrual cycle, postpartum and after the onset of menopause. Animal studies point out

mood ornamental actions of estrogen as well as synergy with monoaminergic drugs. Estrogen enhances mood by increasing the rate of degradation of MAO and intraneuronal 5- HT transport, causing an overall increase in 5-HT availability in the synapse. In addition serotonergic neurotransmission, estrogen also is supposed to have modulatory effect on hippocampal neurogenesis, BDNF signaling,and HPA axis function [64,65].

iii.Vasopressin and depression*:*

Arginine vasopressin (AVP) is a hypothalamic hormone that influences some input symptoms relevant to major depressive disorder. Its stage is reported to be elevated in patients suffering from this mental disorder [66]. AVP has been linked to play a role in the guideline of stress response, one of the prominent features of depression, in that it synergizes with CRF at the level of the pituitary to influence the release of ACTH [67]. Elevated AVP concentrations were also associated with psychomotor retardation in patients with major depressive disorder [68].

Implications of the circadian rhythm in depression:

Melatonin, a hormone secreted by the pineal gland, in a circadian fashion, regulates the rhythm of various biological parameters like body temperature, cortisol secretion, and sleep cycles by acting on receptors in the suprachiasmatic nucleus (SCN) of the hypothalamus [69]. Delayed circadian rhythm in patients with depression has been linked to diminished level of melatonergic signaling in the brain. Patients may manifest with delayed onset of sleep, difficulty in maintaining sleep and early morning awakening. This has given way to the discovery of new antidepressant agent, agomelatin, which acts on melatonin and serotonin receptors on the SCN. Disruption of circadian rhythm has also been planned to build persons susceptible to depression [70-72].

NEUROTRANSMITTERS IMPLICATED IN DEPRESSION

Depression has been connected to harms or imbalances in the brain, specifically with the neurotransmitters serotonin, norepinephrine, and dopamine. It is very difficult to actually measure the level of neurotransmitters in a person's brain and their activity.[73]

The neurotransmitter serotonin is concerned in scheming many vital bodily functions, including sleep, aggression, eating, sexual behavior, and mood. Serotonin is shaped by serotonergic neurons. Recent research suggests that a decrease in the making of serotonin by these neurons can cause depression in some people, and more specifically, a mood state that can cause a few people to feel suicidal.[74]

Another line of research has investigated linkages between stress, depression, and norepinephrine. Norepinephrine helps our bodies to recognize and respond to stressful situations. Researchers suggest that people who are vulnerable to depression may have a norepinephrinergic system that doesn't handle the effects of stress very efficiently.[75]

The neurotransmitter dopamine is also linked to depression. Dopamine plays an important role in controlling our force to look for out rewards, as well as our aptitude to obtain a sense of pleasure. Low dopamine levels may, in part, explain why people with depression don't get the same sense of pleasure out of activities or people that they did before becoming depressed.

In addition, new studies are viewing that other neurotransmitters such as acetylcholine, glutamate, and Gamma-aminobutyric acid (GABA) can also play a role in depressive disorders.[76]

Nor ephinephrine:

A number of lines of evidence suggest that Nor-epinephrine is a neurotransmitter of major importance in the pathophysiology and treatment of depressive disorders.[77]

Nor-epinephrine projections from the locus coeruleus innervate the limbic system, which is concerned in the regulation of emotions. Numerous differences have been found in elements of the NE system in postmortem brains from depressed patients and healthy controls. Genetic studies show that mice with genetically engineered functional enhancement of the NE system are

protected from stress-induced depression-like behaviors. Experimental depletion of NE in the brain results in a return of depressive symptoms after successful treatment with NE antidepressant drugs. Therapeutic agents which specifically increase NE activity are effective antidepressants.

In similar with the serotonergic system, the noradrenergic system has been a precious target for antidepressants. Norepinephrine is found throughout the brain and its functions include acting as a general regulator of mood and responses to stimuli such as stress [78]. As with the serotonergic system, the noradrenergic system consists of a complex circuitry with many connections to other neurological systems. Depression seems to be associated with a hypofunction of the noradrenergic system [78], and some antidepressants act by increasing the synaptic availability of norepinephrine [79]. α2-adrenergic and β-adrenergic receptors present in the frontal and prefrontal cortex appear to be closely associated with depression. Studies have demonstrated a downregulation of α2-adrenergic receptors in depression [79]. The antidepressants mirtazapine and robexetine arbitrate at least some of their effects through α2-autoreceptors [79]. Norepinephrine transporters, which are responsible for norepinephrine reuptake in the synapse, are also a target of antidepressants. Administration of the norepinephrine reuptake inhibitor (NRI), desipramine, to rats shows that a decrease in norepinephrine transporter function is a result of decrease in transporter binding sites and not in gene expression [80]. The study of the interconnectedness between the serotonergic and norepinephrine systems has proven fruitful since serotonin/norepinephrine reuptake inhibitors (SNRIs) exhibit higher efficacy than SSRIs or NRIs alone [81]. Studies with knockout mice that are not capable to create norepinephrine and epinephrine show that these mice are unfeeling not only to the NRIs, desipramine and reboxetine, the monoamine oxidase inhibitor (MAOI) pargyline and the norepinephrine/dopamine reuptake inhibitor bupropion, but also to the SSRIs fluoxetine, sertraline and paroxetine [82]. These results demonstrate the important role norepinephrine plays in antidepressant therapy.

Serotonin:

The serotonergic system has long been implicated in the pathogenesis of anxiety and depression. Some of the most compelling evidence involves the mitigation of depression caused by serotonin selective reuptake inhibitors (SSRIs), which increase the availability of serotonin at

the synapse [83]. Tryptophan depletion studies also confirmed the relationship between serotonin and anxiety and depression [84]. Numerous studies have been conducted in an effort to uncover how antidepressants operate on the serotonergic system to alleviate mood disorders.

Serotonin and its receptors are among the major targets for depression therapeutic drugs such as tricyclics, selective serotonin re-uptake inhibitors (SSRIs) and serotonin and noradrenaline re-uptake inhibitors (SNRIs) [85]. Serotonin as of the dorsal raphe (DR) located in the periaqueductal grey area and other raphe nucleus innervates many brain areas involved in depression such as the amygdala, the NAc and the PFC [87]. Elevated levels of serotonin contribute to the antidepressant effect [88]. By binding to serotonin receptors, serotonin activates a series of signaling pathways including cAMP-PKA-CREB pathway to generate antidepressant effects [86]. The X-ray structure of 5-HT3 receptor in complex with stabilizing nanobodies [88] may help us understand the antidepressant effects of drugs at the molecular level and reasonably design drugs targeted at this receptors with less side effects. These structures of transmitters receptors involved in the pathology of depression will help us to study the antidepressant molecular mechanism and to design more rational chemical drugs with specific target sites.

Dopamine:

The neurotransmitter dopamine may also contribute to symptoms. Dopamine influences, among other functions, a person's energy levels, attention, rewards, and movement, which may lead to anxiety symptoms if imbalanced. Nor epinephrine is also related to anxiety as it involves the fight-or-flight response or how a person reacts to stress.

Dopamine neurons in ventral tegmental area (VTA) determine vulnerability versus pliability to social defeat stress, while vulnerable phenotype will apparent depressive behaviors [89]. Induction of phasic rather than tonic firing by optogenetic methods in VTA dopamine neurons (projecting to NAc rather than to mPFC) of mice which practiced a social defeat stress beneath the threshold, caused a rapid vulnerable phenotype evidenced by increased social avoidance and reduced sucrose preference [90]. Optogenetic induction of VTA phasic firing also transformed resilient mice that underwent repeated social defeat stress previously into a vulnerable phenotype. Optogenetic suppression of the VTA-NAc dopamine projections generated resilience while suppression of VTA-mPFC dopamine projection induced vulnerability [90]. These

projectionspecific and fire-pattern-specific findings improve our understanding regarding roles of VTA dopamine neurons in susceptibility to stress and in pathology of depression [90].

Gamma Amino Butyric Acid (GABA):

Gamma amino butyric acid (GABA)-ergic system has been supposed to play a role in psychiatric disorders, including anxiety and major depression. A clear link between GABA(A) receptors and anxiety has long been recognized. However, in spite of the GABA system being the prominent inhibitory neurotransmitter in the brain role in depression has been less well validated.

Preclinical and clinical data recommend that a modification in $GABA_B$ receptor expression and function may give to the symptoms of major depression and the response to antidepressant.

The GABA system is another system grave to our understanding of anxiety and depression. The neurotransmitter GABA acts on ionotropic GABAA and GABAC receptors as well as metabotropic GABAB receptors [91] [92] projected the involvement of GABAergic dysfunction in mood disorders based on studies with the mood stabilizer, valproate, which effectively treats bipolar patients. Since valproate causes an enhancement in the concentration of GABA in the brain, the authors postulated that the pathophysiology of mood disorders involved GABAergic deficiency [92,93]. Data obtained on the levels of GABA in plasma and cerebrospinal fluid are conflicting, with some studies reporting decreases and others no change [92]. However, neuroimaging studies suggest the involvement of reduced GABAergic neurotransmission in major depressive disorder [94]. In addition, benzodiazepenes, anxiolytics used in the treatment of depression, directly enhance GABA function by interacting allosterically with the α2 and α5 subunits of the GABA A receptor [92]. Furthermore, the selective GABA reuptake inhibitor, tiagabine, which targets the GABA transporter GAT-1, has been effectively used in the treatment of anxiety-related behaviors in mouse and human studies [95]. Additional evidence for the involvement of the GABAergic system in anxiety and depression comes from GABAB(1) subunit knockout mice which exhibit antidepressant-like activity in the forced swim test [96]. An interesting observation is the reduction in the number of GABA neurons detected in the orbitofrontal cortex of postmortem cases of major depression [97]. The mechanisms involved in

this occurrence are not known, although it could be owing to a stress-mediated decrease in brain derived neurotropic factor (BNDF), a molecule involved in neurogenesis [98]. One more possibility involves a reduction in functional genes such as bcl-2, or decreased neurogenesis of GABA neurons if they undergo substantial turnover [99]

Acetylcholine:

Acetylcholine plays a major role in learning and memory processes. Acetylcholine levels can be modulated by stress in several brain regions. Acetylcholinesterase present in the CNS catalyzes the hydrolysis of acetylcholine to choline. [100]

Acetylcholine is released in the synaptic cleft where it activates both presynaptic and postsynaptic cholinergic receptors namely nicotine and muscarinic leading to an increase of cholinergic transmission which results in cognitive impairment. [101] Cholinergic input to hippocampus is enhancing in response to anxiogenic and stressful stimuli. [102]

Rodent studies confirm that increasing ACh levels by treating with physostigmine acutely can induce anxiety- and depression-like behaviors, whereas chronic treatment with the serotonergic antidepressant fluoxetine increases levels and activity of AChE, particularly in the hippocampus[103].

Local administration of physostigmine or knockdown of AChE in the hippocampus is sufficient to increase anxiety- and depression-like behaviors that can be reversed by administration of fluoxetine, suggesting that they are consistent with symptoms of depression [103].

Glutamate:

The excitatory action of amino acid L-glutamate in the mammalian brain and spinal cord has been known since mid 19th century. [104, 105] Glutamate is the primary excitatory neurotransmitter in the human Central Nervous System (CNS). Glutamate is abundant within the central nervous system and has been shown to play important roles in different brain functions, including neurodevelopment (e.g., differentiation, migration and survival), [106]learning (e.g., long-term potentiation and depression),[107] acute neurodegeneration (e.g., cerebral ischemia, traumatic brain

injury)[109] chronic neurodegeneration (e.g., Huntington's disease, Alzheimer's disease) [109] and, also, the stress response and anxiety disorders. [110]

Glutamate controls the synaptic release by a variety of presynaptic receptors. These include the Group II and Group III glutamate metabotropic receptors and cholinergic (nicotinic and muscarinic) receptors, adenosine (A1), kappa opioid, γ-amino butyric acid (GABA B), cholecystokinin and neuropeptide Y (Y2) receptors. [111,112]

Glutamate primarily show its actions through ligand-gated ion channel (ionotropic) receptors, including the N- methyl-d-aspartate (NMDA), kainate, and -amino- 3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtypes, and G protein-coupled metabo- tropic receptors (mGluR1-8). [113]

An significant role is played by Glutamatergic system in the pathogenesis of anxiety and fear conditioning. Enormous studies suggest that glutamatergic neurotransmission of limbic system plays a pivotal role in the pathogenesis of anxiety disorders. [114-117] Severe stress exposure in a straight line leads to glutamate excitotoxicity, which can cause neuronal damage and/or death. By decreasing the level of endogenously released glutamate, the anxiolysis could be induced. NMDA receptor would be activated by diminished glutamate release but to less extent and CNS excitation would remain at a stable stage. Such an effect can be achieved by switching on the regulatory machinery of presynaptic glutamate release. [118] Monosodium glutamate (MSG) [$C_5H_8NO_4NaH_2O$] a sodium salt of naturally occurring (non-essential) L- form of glutamic acid, is one of the flavor enhancers, which is primarily used as an ingredient in various food products. [119] Glutamate Consumption has been linked to obesity and metabolic syndrome independent of physical activity and calorie intake. [120] Major depressive disorder (MDD) is severely disabling, and current treatments have limited efficacy. The glutamate N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine was recently repurposed as a rapidly acting antidepressant, catalysing the vigorous investigation of glutamate-signalling modulators as novel therapeutic agents for depressive disorders.

Cholecystokinin:

Cholecystokinin, a neuropeptide, was like 5-HT, discovered originally in the digestive tract and found in the CNS later. [121,122] CCK-immunoreactive fibers and CCK (2) receptors are most abundantly present in anatomical locations like periaqueductal gray (PAG), which mediate anxiety. [123] The CCK2 receptor regulates the fear-related behaviours in humans and animals [124] CCK-4 injection triggers the panic attacks in patients with a history of panic disorder. [125,126]

Adenosine:

Adenosine results due to hydrolysis of 5-adenosine monophosphate and is transformed to inosine, which is then stored as adenosine triphosphate. [127] possible therapeutic target for the anergia component of depression is adenosine receptors. Adenosine is a neuromodulator in the central nervous system (CNS) that plays an important role in the regulation of synaptic transmission and neuronal excitability

Corticotrophin releasing factor:

Corticotrophin Releasing Factor Evidence for the important role played by CRF in depression increased over the past decade. Acting as a neurotransmitter in the central nucleus of amygdala and bed nucleus of stria terminalis, concentrations of CRF in those areas, as well as the number of CRF-secreting neurons in the hypothalamus and locus coeruleus, were found elevated in patients with depression [128,129,130]. CRF overexpression in transgenic mice induced depressive behaviors and histonic signatures such as hypercortisolemia, anorexia, weight loss and decreased libido, which could also be achieved by infusion of CRF into CNS 129 CRF exerts its physiological function by binding to its G-protein coupled receptors, CRF1 and CRF2, to activate the downstream cAMP signaling [129,131]. CRF1 receptors are highly expressed in the pituitary and the limbic areas where they modulate the activity of HPA axis . Selective deletion of CRF1 receptors in limbic areas leads to antidepressant-like behaviors in mice subjected to stress while antagonists of CRF1 receptors could attenuate a series of depressive behaviors

generated by withdrawal of drugs of abuse, but inconsistent results still exist [128,129]. A major frustration about developing antagonists of CRF1 receptors as antidepressants is the pharmacokinetic issue as well as hepatotoxicity [128]. The major function of CRF2 receptors might be keeping the HPA axis response, rather than activating the HPA axis when exposed to stress, by functioning as auto-receptors on some neurons in paraventricular nucleus (PVN) [132]. Genetic deletion of CRF2 receptors induces anxiety-like symptoms in mice and antagonists exhibit anxiolytic effects, and some antagonists even exhibit antidepressant effects in chronic mild stress model [128]. With less side effects compared to antagonists of CRF1 receptors, exploring antagonistsof CRF2 receptors is of great interest to treat depression even though there are much more efforts to be made [128,129]

Substance P:

Substance P neurotransmission has been associated with aversion and anxiety behavioral model. Substance P act primarily at the neurokinnin-1 (NK1) receptor. . NK-1 receptor exhibit anxiolytic effects in several models including elevated plus maze and social interaction tests. Disruption of NK-1 receptor results in 5-HT_{1A}-receptor desensitization and anxiolytic behavior and the effect of substance P play a role in anxiogenic behavior. [133]

Neuro Active Steroids (NAS):

A detailed assessment on steroid biosynthesis in the CNS is given elsewhere [134]. Although the majority of studies have focused on the modulatory potential of neuroactive steroids at the GABAA receptor, also other receptors, for example the N-methyl-D-aspartate (NMDA)-gated ion channel or the σ1 receptor, may be a target for neuroactive steroids However, in view of their GABA-enhancing properties, 3α-reduced neuroactive steroids are of particular interest for neuropsychopharma- cological research. Preclinical studies suggested that neuroactive steroids influence the neuro- chemical responses to acute or chronic stress conditions [135,136]and may modulate anxiety- and depression-related behavior. Moreover, it has been suggested that neuroactive steroids may be involved in the therapeutic effects of antidepressant drugs and several clinical studies suggested that changes in neuroactive steroid concentrations might be involved in the pathophys- iology and course of certain psychiatric disorders. In particular, this review will focus on the role of neuroactive steroids in depression and anxiety disorders.

Animal Models for screening anti-depressant activity

The development of preclinical animal models is an significant step on the way to discovering new treatments for diseases. However, establishing animal models of depression presents an obvious difficulty, given that mice and rats cannot display the complex cognitive and emotional traits that characterize major depressive disorder in humans. While laboratory animals can't exhibit depression per sec, they can display some of the core behavioral and physiological traits of depression; these traits are referred to as endophenotypes [137]Some endophenotypes common to both human depression and animal models of depression include

- Anhedonia
- Appetite and sleep disturbances
- Behavioral despair
- Anxiety behaviors

Anhedonia

Anhedonia, defined as the inability to experience pleasure, is evaluated in rodents using the sucrose-preference test and the intracranial self-stimulation test. The sucrose-preference test measures the tendency of an animal to consume a sucrose solution over regular water when provided with access to both [138]Healthy animals will drink a greater volume of sucrose, while those exhibiting anhedonia will show a reduced preference for the sugar. The intracranial self-stimulation test evaluates the motivation of rodents to engage in an activity (pressing a lever or running on a wheel) that is paired with a rewarding electrical stimulus delivered through electrodes implanted in the brain[139].

Appetite and sleep dysregulation

Appetite and sleep disturbances are another common component of major depressive disorder. People with depression tend to sleep and eat less than usual, although individuals with atypical depression display an increase in sleep and in food consumption. Changes in appetite can be easily assessed in experimental animals by quantifying food intake and body weight, while sleep

abnormalities can be measured using electroencephalographic recordings. Like depressed patients, some animal models of depression display altered sleep patterns[140].

Behavioral despair

Behavioral despair refers to the tendency of an individual to view their circumstances as immutable and to cease trying to control the outcome of the situation. Behavioral despair can be screened for in animals using the tail suspension test and the forced swim test [141].

Forced Swimming Test (FST):

This test was a modification of the method of Porsolt et al. [3] Briefly, animals were forced to swim individually, for 15 min, in a glass beaker (11 cm dia, 15 cm ht) containing fresh water upto a height of 6 cm, at a temperature of. 22±1°C. This constituted the "pre-test" session. Twenty four hours later, the animals were administered either a drug (test group) or distilled water (control group) and each animal was once again forced to swim in a similar environment for a period of 6 min in a "test-session". The animal's attempts to get out of the beaker were interspersed with periods of immobility signifying "behavioural despair". The total duration of the immobility during the last 4 min of the 6 min test was recorded.

Tail Suspension Test (TST):

This test was based on the method described by Steru et al .A mouse was suspended by a plastic string 75 cm long, about 20 cm above a table top with adhesive tape fixed approximately 1 cm from the tip of the tail. The mouse made vigorous attempts at regaining an upright posture and later lapsed into a motionless frozen state (immobility phase) interspersed with bursts of agitation. The duration of immobility was recorded for a period of 6 min with the various drugs administered prior to testing. Baseline immobility was measured using distilled water treated animals.

Evaluating Animal Models of Depression:

A variety of methods can be used to recapitulate many of the core symptoms of major depressive disorder in animals. These animal models can be evaluated based on their face validity, construct validity, and predictive validity[144]. Face validity refers to how well the animal model mimics aspects of the human disorder and can be assessed by testing for common endophenotypes. Construct validity evaluates how well the model shares a similar set of causes with the human disorder, while predictive validity assesses whether the treatments that are effective for depression in humans can also reverse the depression-like symptoms in the animals.

Stress-based depression models:

The learned helplessness model is based on the observation that following repeated exposure to an uncontrollable, inescapable stressor, such as an electrical shock, animals will subsequently fail to try to escape the shocks when presented with an escape route[145]. Animals exposed to this procedure can also develop symptoms associated with depression, such as weight loss and sleep alterations. This model is considered an acute stress model, as some of its effects can be produced within one day.

Other models rely on longer-term stressors. The chronic unpredictable stress model involves the random, intermittent administration of stressors that change periodically, preventing the animal from adapting to any particular stressor[144]. Some examples of these stressors include predator odor exposure, overnight light exposure, wet cage bedding, and physical restraint. Several weeks of chronic unpredictable stress produce depression-like symptoms in the animals. Another long-term stress model is the social defeat model, in which a male rodent is introduced to the home cage of older, larger aggressor animal. Repeated physical subordination causes stress-susceptible animals to develop a depression-like syndrome over the course of several weeks.

While a number of other models for depression exist, including genetic models, olfactory bulbectomy, and early maternal deprivation, stress-induced depression models are some of the most widely used. They exhibit strong face validity, sharing many phenotypic outcomes with depression. They share a common etiology with depression, as the disorder often develops following periods of extreme or chronic stress. Further, these stress-induced syndromes are at least partially reversible by the administration of antidepressant medications. These and other

animal models of depression will continue to be important tools in the search for new and more effective depression treatments.

2.LITERATURE REVIEW

Hasegawa Y(2014)[11] investigated the significant beneficial effects of the combination therapy using amlodipine and irbesartan against stroke onset in hypertensive rats. Although calcium channel blockers, angiotensin II receptor blockers, and combination therapy are effective for hypertensive patients, the significant differences among them against stroke onset are undetermined. Amlodipine and the combination therapy significantly reduced BP compared with the vehicle. Although the rates of stroke-related signs and mortality were high in the vehicle group, the rats in the treatment groups were mostly healthy until 35 days. After all drugs were discontinued, stroke onset was frequently seen in the monotherapy groups until 42 days, but no signs were observed in the combination therapy group. Although there were no significant differences in CBF or brain edema, the combination therapy reduced blood-brain barrier disruption, white matter injury, and reactive astrocytes compared with irbesartan, and the combination also inhibited left ventricular hypertrophy and preserved brain-derived neurotrophic factor (BDNF) expression on cerebral vessels compared to the monotherapies. These data suggest that the combination therapy had a persistent preventive effect on stroke onset in hypertensive rats, and the effects might be associated with BDNF preservation on cerebral vessels.

Leenen et al, 2016[11] found once-daily dihydropyridines exert both indirect sympatho-excitatory and direct central sympatho-inhibitory effects. Age may affect this balance by influencing blood pressure (BP) or renin responses.

We evaluated BP, sympathetic and cardiac responses after the first dose and after 8 weeks of treatment with placebo, amlodipine or felodipine extended release (ER) in 29 young versus 37 older hypertensive patients, using a double-blind, parallel group design.

In the young group, neither dihydropyridine dose decreased BP after the first dose and both caused decreases by after chronic treatment. In the older group, felodipine ER decreased BP rapidly and amlodipine more gradually, and after chronic treatment, systolic BP decreased .

Felodipine ER increased the heart rate after the first dose in both age groups and caused persistent increases in the cardiac index . and the ejection fraction only in the older group. Amlodipine did not affect cardiac function in the young, and with chronic dosing decreased the heart rate by and the cardiac index by a metre in the older group. In the young hypertensive patients, both dihydropyridines increased plasma norepinephrine (NE) after chronic dosing, with little effect after the first dose. In contrast, in the older group felodipine ER increased plasma NE after the first dose but not with chronic dosing, whereas amlodipine had no effect after the first dose, and after chronic dosing tended to decrease plasma NE .We conclude that age is a major determinant not only of the BP but also of the cardiac and sympathetic responses to once-daily dihydropyridines

Lee Y J(2011)[122] examined the neuroprotective effects of the long-acting third-generation dihydropyridine Ca(2+) antagonists, amlodipine besylate (AB) and amlodipine camsylate (AC), on neuronal cell death induced by oxidative stress. Cell viability and levels of free radicals and intracellular signaling proteins were measured after treating primary cultures of cortical neurons with AB, AC, and/or hydrogen peroxide under various conditions. Cell viability was not affected by concentrations of AB or AC but decreased at higher concentrations. Following its exposure, the viability of cortical neurons decreased in a concentration-dependent manner; however, treatment with AB or AC restored the viability of Hydrogen peroxide -injured cortical neurons. Treatment with Hydrogen peroxide increased the level of free radicals in cortical neurons, and pre-treatment with AB or AC counteracted this in a dose-dependent manner. Similarly, treatment with AB or AC reduced the declines . phosphorylated Akt, phosphorylated GSK-3β, heat-shock transcription factor-1, and Bcl-2 induced by H(2) O(2) , as well as the increases in cyclooxygenase-2, cytosolic cytochrome c, cleaved caspase 9, and cleaved caspase 3. Our results indicate that AB and AC exert similar neuroprotective effects by reducing oxidative stress, enhancing survival signals, and inhibiting death signals.

Leenen H(2011)[12] reviewed highlights new findings in two areas. First, in animal studies, direct central administration of dihydropyridines such as nifedipine or amlodipine lowers sympathetic nerve activity and thereby blood pressure. Peripheral administration of nifedipine or amlodipine at low rates appears to result in gradual accumulation of drug in the central nervous system, and also causes lowering of sympathetic nerve activity and thereby lowering of blood pressure (rather than by arterial vasodilation). Second, in hypertensive humans treated with long-acting dihydropyridines and presumably little activation of the arterial baroreflex, some studies have demonstrated lowering of sympathetic activity (as assessed by plasma norepinephrine), but others reported increases (as assessed by plasma norepinephrine or microneurography). This sympathoexcitatory response may be due to activation of the renin-angiotensin system, particularly at higher doses. It is generally assumed that the arterial vasodilation induced by inhibition of Ca(2+) influx into vascular smooth muscle cells represents the main mechanism for the hypotensive effect of dihydropyridine calcium channel blockers. Increases in sympathetic tone have been related to activation of the arterial baroreflex by rapid lowering of blood pressure.

Toma L (2011)[12] evaluated the potential of amlodipine (Am) to restore endothelial dysfunction induced by irreversibly glycated low density lipoproteins (AGE-LDL), an in vitro model mimicking the diabetic condition. Amlodipine, alone or in combination with other drugs, was successfully used to treat hypertension.Human endothelial cells (HEC) from EA line were incubated with AGE-LDL in the presence/absence of Am and the oxidative and inflammatory status of the cells was evaluated . The cellular NADPH activity, 4-hydroxynonenal (4-HNE) and 3-nitrotyrosine levels in the culture medium and the adhesion of human monocytes to HEC were measured by chemiluminescence, UHPLC, Western Blot and spectrofluorimetric techniques. The gene expression of NADPH subunits (, NOX4), eNOS and inflammatory molecules (MCP-1, VCAM-1) were determined by Real Time PCR. Results showed that in HEC incubated with AGE-LDL, Am led to: (i) decrease of the oxidative stress: by reducing , NOX4, iNOS expression, NADPH oxidase activity, 4-HNE and 3-nitrotyrosine levels; (ii) decrease of the inflammatory stress: by the reduction of MCP-1 and VCAM-1 expression, as well as of the number of monocytes adhered to HEC; (iii) inhibition of ROS-sensitive signalling pathways: by

decreasing phosphorylation . In conclusion, the reported data demonstrate that amlodipine may improve endothelial dysfunction in diabetes through anti-oxidant and anti-inflammatory mechanisms.

Hirooka Y (2006)[123] examined whether treatment with amlodipine reduced oxidative stress in the brains of stroke-prone spontaneously hypertensive rats (SHRSP). Amlodipine is a dihydropyridine calcium channel blocker that is widely used for the treatment of hypertensive patients and has an antioxidant effect on vessels in vitro.The animals received amlodipine, nicardipine or hydralazine for 30 days in their drinking water. Levels of thiobarbituric acid-reactive substances (TBARS) in the brain (cortex, cerebellum, hypothalamus, and brainstem) were measured before and after each treatment. Systolic blood pressure decreased to similar levels in the amlodipine-, nicardipine-, and hydralazine-treated groups. Urinary norepinephrine excretion was significantly reduced in SHRSP after treatment with amlodipine, but not with nicardipine or hydralazine. Levels of TBARS in the cortex, cerebellum, hypothalamus, and brainstem were significantly higher in SHRSP than in Wistar-Kyoto rats (WKY), and were reduced in amlodipine-treated, but not in nicardipine- or hydralazine-treated, SHRSP. Electron spin resonance spectroscopy revealed increased levels of reactive oxygen species in the brains of SHRSP, which were reduced by treatment with amlodipine. Intracisternal infusion of amlodipine also reduced systolic blood pressure, urinary norepinephrine excretion, and the levels of TBARS in the brain. These results suggested that oxidative stress in the brain was enhanced in SHRSP compared with WKY rats. In addition, antihypertensive treatment with amlodipine reduced oxidative stress in all areas of the brain examined and decreased blood pressure without a reflex increase in sympathetic nerve activity in SHRSP.

Huang BS(2007) [11] examined whether central actions contribute to the hypotensive effects of peripherally administered amlodipine, a lipophilic dihydropyridine with slow onset and long duration of action. After 5 to 6 weeks of high or regular salt intake, changes in renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP), and heart rate (HR) were recorded at rest and in response to intravenous (iv) and intracerebroventricular (icv) injection, and prolonged iv infusion of amlodipine, in conscious spontaneously hypertensive rats (SHR). Iv injection of

amlodipine decreased MAP but increased RSNA and HR in a dose-related manner. In contrast, icv injection of amlodipine caused parallel decreases in MAP, RSNA, and HR. Iv infusion of amlodipine for 3 hours followed by for 2 hours also decreased in parallel RSNA, MAP, and HR. Maximal decreases in RSNA, MAP, and HR in response to icv injection and iv infusion were significantly larger in SHR on H-Na versus R-Na. All responses lasted at least 1 hour following iv and icv injection, or after the termination of iv infusion of amlodipine. These data suggest that in SHR during prolonged iv infusion, amlodipine appears to cross the blood-brain barrier, block brain l-type Ca2+ channels, and decrease sympathetic outflow and thereby BP. Central actions may prevail during iv infusion of amlodipine at low rates, and the decrease in BP is associated with sympathoinhibition. High salt intake markedly enhances its sympathoinhibitory action, likely through central mechanisms.

Lix(2017)[121] hypothesized that hypotensors could alleviate the decline of GR gene expression and ameliorate age-induced learning and memory deficits in a D-gal-induced ageing mice model. Glucocorticoid receptors (GRs) exert actions on the hippocampus that are important for memory formation. There are correlations between vascular dysfunctions and GR-related gene expression. Both vascular dysfunction and GR gene expression decline occur during the ageing process. Therefore, hypotensors, which have effects on improving vascular dysfunction, may be able to ameliorate GR gene expression decline in ageing mice and improve ageing-mediated memory deficits. In line with our hypothesis, we found that chronic D-gal treatment decreased GR and DCX expression in the hippocampus, leading to learning and memory deficits. Amlodipine (AM) and puerarin (PU) treatment improved GR gene expression decline in the hippocampus and ameliorated the learning and memory deficits of D-gal-treated mice. These changes correlated with enhanced DCX expression and brain-derived neurotrophic factor (BDNF) expression in the hippocampus. Furthermore, PU treatment conveyed better effects than AM treatment, but combination therapy did not enhance the effects on improving GR expression. However, we did not find evidence of these changes in non-D-gal-treated mice that lacked GR gene expression decline. These results suggest that AM and PU could improve D-gal-induced behavioural deficits in correlation with GR gene expression.

Luszczki (2008)[11] assessed the effect of 3 calcium channel antagonists (amlodipine, diltiazem, and verapamil) on the anticonvulsant action of topiramate (a new generation antiepileptic drug) in the mouse maximal electroshock seizure (MES) model. Amlodipine significantly enhanced the anticonvulsant activity of topiramate in the MES test in mice, reducing its ED50 value. Similarly, diltiazem markedly potentiated the antiseizure action of topiramate against MES, lowering its ED50 value .. In contrast, lower doses of amlodipine and diltiazem and all doses of verapamil had no significant impact on the antiseizure action of topiramate. Pharmacokinetic verification of the interaction of topiramate with amlodipine and diltiazem revealed that neither amlodipine nor diltiazem affected total brain topiramate concentration in experimental animals, and thus, the observed interactions were concluded to be pharmacodynamic in nature. The favorable combinations of topiramate with amlodipine or diltiazem deserve more attention from a clinical viewpoint because the enhanced antiseizure action of topiramate was not associated with any pharmacokinetic changes in total brain topiramate concentration.

Luszczki(2008)[11] assessed the effect of three calcium channel antagonists (amlodipine, diltiazem and verapamil) on the anticonvulsant action of lamotrigine (a second generation antiepileptic drug) against maximal electroshock-induced seizures in mice. Results indicated that all three calcium channel antagonists when administered alone [amlodipine , diltiazem and verapamil , did not significantly affect the threshold for maximal electroconvulsions in mice. However, amlodipine at a non-protective dose of , significantly enhanced the anticonvulsant activity of lamotrigine in the maximal electroshock-induced seizure test in mice by reducing its ED(50) value . In contrast, amlodipine at lower doses , diltiazem and verapamil had no significant impact on the antiseizure action of lamotrigine in the maximal electroshock-induced seizure test in mice. In conclusion, one can ascertain that the favorable combination of lamotrigine with amlodipine deserves more attention from a clinical viewpoint because of the enhanced antiseizure action of lamotrigine.

KishiT(2012)[11] found orally administered atorvastatin reduces sympathetic nervous system (SNS) activation via an anti-oxidant in the rostral ventrolateral medulla (RVLM) of hypertensive rats, whereas amlodipine did not. Furthermore, several previous reports have suggested that atorvastatin or amlodipine improves cognitive dysfunction during hypertension. The aim of the present study was to determine whether a combination of atorvastatin and amlodipine causes sympathoinhibition via reduction of oxidative stress in the RVLM and improves cognitive dysfunction of hypertensive rats.

Stroke-prone spontaneously hypertensive rats (SHRSPs), as a hypertensive model with sympathoexcitation, were divided into 4 groups; a combination of atorvastatin and amlodipine-treated (COM), atorvastatin-treated (ATR), amlodipine-treated (AML), hydralazine-treated (HYD), and vehicle-treated SHRSPs (VEH). After treatment for 28 days, the mean blood pressure did not change in ATR rats, and was reduced to the similar levels in COM, AML, and HYD rats. However, SNS activation and oxidative stress in the RVLM were significantly lower only in COM than in ATR, AML, HYD, and VEH rats. Cognitive performance and manganese-superoxide dismutase activity in the hippocampus were significantly higher, and oxidative stress in the hippocampus was significantly lower in COM than in VEH, AML, and HYD rats to a greater extent than in ATR rats.

A combination of atorvastatin and amlodipine causes sympathoinhibition via an anti-oxidant in the RVLM and improves cognitive dysfunction via an anti-oxidant in the hippocampus in hypertensive rats, independent of the blood pressure-lowering effect.

Cohen et al(1997), Assessment of the antidepressant-like effects of L-type voltage-dependent channel modulators in FST,TST .However, amlodipine was reported to have no antidepressant activity.

3. AIM AND OBJECTIVE

Drug repositioning (also known as drug repurposing or drug reprofiling) is referred to as the process of redeveloping a compound for use in a different disease.

Drug Repositioning has many advantages over traditional drug discovery approaches in that it can significantly reduce the cost and developmental time as many compounds have demonstrated safety in humans. Drug repositioning removes the need of phase 1 clinical trials.

Amlodipine has long been used as antihypertensive and its safety in humans has been established. It has been reported to act through blocking L type calcium channels. The role of L type calcium channel in behavioral depression has been studied extensively and well documented [146]. The antidepressant activity of a drug candidate can be confirmed only in animal models like mice as they better mimic certain human metabolism, behavior and they are easy to handle.

Therefore based on the extensive literature survey, the objective of present work focused on probable anti-depressant activity of amlodipine in mice models.

The objectives are to evaluate the antidepressant activity of amlodipine

a. Forced swim test model
b. Tail suspension model.

Evaluate the effect of amlodipine on locomotor activity using actophotometer.

4. MATERIALS AND METHODS

Animals

Six weeks old male Swiss albino mice , weighing 20 ± 5 gms were used for this study. The animals were group housed (n=6 per cage) in a room with controlled temperature (21-22°C), and in normal light-dark cycle (12 h/12 h).They had free access to food and water *ad libitum*. All the experimental protocols employed in this study were approved by the Institutional Animal Ethical Committee of J.K.K. Nattraja College of Pharmacy and experiments were performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines on the ethical use of animals (JKKN/IAEC/M.Pharm/14/2019 dated 10/4/2019).

Drugs

Table 1. List of chemicals used in the present study

S. No.	Chemicals	Manufacturer
1.	Fluoxetine	Cadila
2.	Amlodipine	Ranbaxy

Drug solutions

Fluoxetine was diluted with normal saline (0.9% NaCl) and administered at a dose of 20 mg/kg i.p. Amlodipine was suspended in normal saline and administered at a dose of 5 mg/kg p.o. and 10 mg/kg, p.o..

Protocol for evaluation of antidepressant activity of amlodipine in FST, TST

The following table shows the grouping pattern animals used for the evaluation of antidepressant activity in FST, TST. Animals were treated with vehicle (control)/

Fluoxetine(standard) or 5/10 mg/kg of amlodipine through intraperitoneal/oral route and 30/60 minutes thereafter individually tested for depressant behaviour in FST,TST.

Table. 2. Grouping of animals for evaluation of antidepressant activity of amlodipine in FST,TST.

S.No	Group	Number of animals	Treatment
1	Group I	6	Control (Vehicle 10 ml/kg)
2	Group II	6	Standard (Fluoxetine 20 mg/kg i.p.)
3	Group III	6	Amlodipine (5 mg/kg, i.p.)
4	Group IV	6	Amlodipine (10 mg/kg, i.p.)

Methods

Forced swim test

The method described by porsolt and co-workers was used. Unlike porsolt's method for mice which consists of direct immersion of animals after injecting drugs,we subjected the animals to "pre-test session"to avoid variations and for maintaining consistency in the immobility time between different groups. Briefly.mouse was forced to swim individually for 15 mins in glass cyclinder(21 12 cm)containing fresh water up to the height of 9 cm at a temperature of 25±2 c.This constituted the "pre test session",twenty four h later ,the animal were treated either with a drug (test group)or vehicle (control group)and each animal was again forced to swim in similar environment for a period 6 minin "test session" and the duration of immobility was recorded. In this experimental paradigm after a phase of vigorous swimming in water,mice show an immobile floating posture.This immobility is postulated to represent a depression like state and is reduced or eliminated by clinically effective antidepressant drugs. Mouse was judged to be immobile it it ceased struggling and remained floating motionless in water making only those movements necessary to keep its head above water. Reduction in the duration of immobility by a drug was considered,as it possesses antidespair or antidepressant like effect.

Each experimental group consists 5 mice and chosen by means of completely randomized method. Lllumination of 100Lux was maintained above the FST model throughtout the experiment.

Tail Suspension Test

Tail suspension test was derived from the porsolt's forced swim test and wasbased on the observation that the mouse suspended by its tail shows alternate period of agitation and immobility similar to that of FST. Briefly, mice were suspended from the edge of the shelves 50 cm above the floor by its tail. The duration of immobility was recorded for 6 min. The reduction in the duration of immobility by a drug was considered as it possess antidepressant activity.

Actophotometer test

The animal locomotor behavior was monitored using actophotometer. Animals were placed in actophotometer individually, and basal activity score was recorded over the period of 5 min. Each animal was treated with respective drug, and activity score was recorded after 30 min and 1 h. Decreased and increased activity score was taken as index of CNS depression and stimulation, respectively.

5. RESULTS

Antidepressant activity of amlodipine in FST:

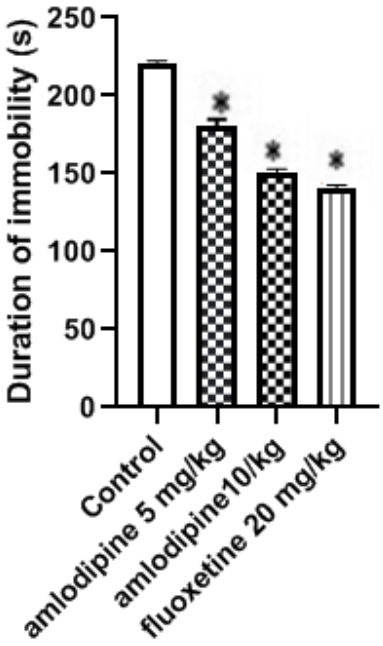
The results illustrated in Figure 2 show the effect of amlodipine on the duration of immobility time in the Forced swim Test model. One‑way ANOVA revealed the significant changes in duration of immobility among the treatment groups { F (3, 20) = 146.7(P<0.0001)}. Post hoc Dunnet's test showed that the treatment of amlodipine and fluoxetine significantly (p<0.0001) reduced the duration of immobility when compared to control

Table 3 : Anti-depressant activity of amlodipine in FST

S.No	Treatment	Duration of immobility Mean±SEM
1.	Control	220±2.49
2.	Fluoxetine 20 mg/kg	141±1.56*
3.	Amlodipine 5mg/kg	180.5±4.27*
4.	Amlodipine 10mg/kg	150.33±2.75*

Mice were injected with normal saline and different doses of amlodipine (i.p),30 mins after administration,the duration of immobility was noted.The data represents± SEM, showed significant result in 5,10 mg/kg of Amlodipine when compared with control,performed ANOVA followed by Dunnett's test. *p<0.0001 compared to control group.

Fig 2.Anti-immobility effect of amlodipine in FST



Anti-Depressant activity of amlodipine in Tail suspension test

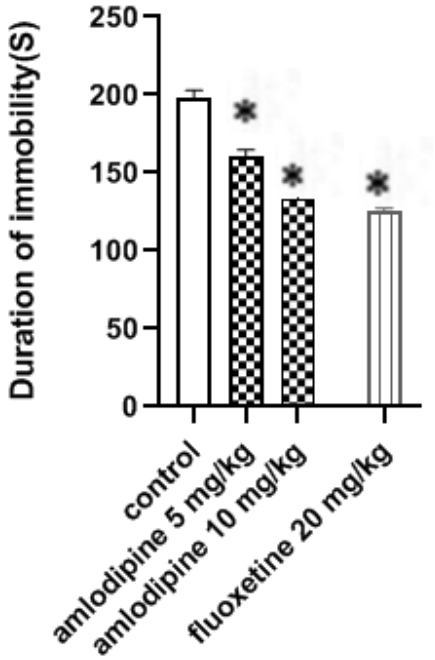
The results illustrated in Figure 3 show the effect of amlodipine on the duration of immobility time in the Tail suspension test. One‑way ANOVA revealed the significant changes in duration of immobility among the treatment groups {F (3, 20) = 85.38, P<0.0001). Post hoc Dunnet's test showed that the treatment of amlodipine and fluoxetine significantly (p<0.0001) reduced the duration of immobility when compared to control

Table 4: Anti-depressant activity of amlodipine in TST

S.No	Treatment	Duration of immobility Mean±SEM
1.	Control	198.5± 4.33
2	Fluoxetine 20 mg/kg	125.33 ± 1.99*
3.	Amlodipine 5mg/kg	160.5±4.27*
4.	Amlodipine 10mg/kg	133.66±3.13*

Mice were injected with normal saline and different doses of amlodipine (i.p),30 mins after administration,the duration of immobility was noted.The data represents± SEM, showed significant result in 5,10 mg/kg of Amlodipine when compared with control, performed ANOVA followed by Dunnett's test. *p<0.0001 compared to control group.

Fig 3.Anti-immobility effect of amlodipine in TST



Effect of amlodipine on Locomotor Activity:

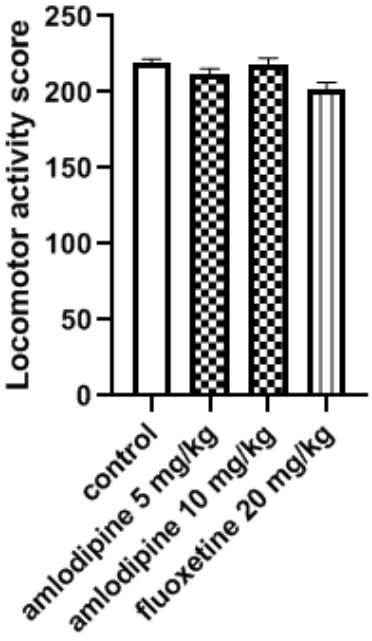
The results of One way ANOVA revealed no significant changes { F (3, 20) = 3.740, P=0.0278} on the treatment of amlodipine (5,10 mg/kg) and fluoxetine (20 mg/kg) on locomotor activity in actophotometer.

Table 5 : Effect of amlodipine administration on Locomotor Activity in Actophotometer using mice.

S.No	**Treatment**	**Duration of immobility Mean±SEM**
1	Control	218.83± 2.90
2.	Fluoxetine 20 mg/kg	202.16± 4.28
3.	Amlodipine 5mg/kg	212± 3.23
4.	Amlodipine 10mg/kg	217.5± 4.90

Mice were injected with normal saline and different doses of amlodipine (i.p),30 mins after administration,the Locomotor activity was observed in actophotometer.The data represents± SEM, showed significant result in 5,10 mg/kg of Amlodipine when compared with control,performed ANOVA followed by Dunnett's test. P>0.05 compared to control group.

Fig 4.Effect of amlodipine on Locomotor activity in actophotometer



6. DISCUSSION

Depression is a heterogeneous disorder that affects physical health and mental health. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, in particular, norepinephrine**,** serotonin and dopamine[1]. This monoamine hypothesis is also supported by the fact that the known antidepressants like monoamine oxidase inhibitors, tricyclic antidepressants and selective serotonin reuptake inhibitors have been known to potentiate monoamine function.[2]

Calcium channel blockers (CCBs) comprise a heterogeneous group of medications that reduce calcium influx and attenuate cellular hyperactivity. Evidence of hyperactive intracellular calcium ion signaling in multiple peripheral cells of patients with various neurological and psychological disorders have been reported. Earlier, a role for LTCCs in regulating depression-related behavior was first realized using LTCC pharmacological agents demonstrating that the DHP LTCC blocker nifedipine has an antidepressant-like effect in rats [146]. This was further expanded in a later study that showed that, in addition to nifedipine, other DHP blockers, including nicardipine, nitrendipine, isradipine, felodipine, and nimodipine but not amlodipine, had a similar antidepressant-like effect [147]. More recently, in support of the pharmacological antidepressant-like effect, the use of genetic mutant mice has revealed that Cav1.2 heterozygous mice exhibit antidepressant-like behavior [149, 150]. Furthermore, focal knockdown of cacna1c (Cav1.2) in the adult PFC was sufficient to induce a similar antidepressant-like effect [152], consistent with antidepressants exerting their effects through cellular changes in the PFC [148]. In the context , the present study evaluated the antidepressant activity of amlodipine in FST and TST.

Behavioral despair refers to the tendency of an individual to view their circumstances as immutable and to cease trying to control the outcome of the situation. Behavioral despair can be screened for in animals using the tail suspension test and the forced swim test [141].

In the present study, acute administration of amlodipine dose dependently reduced the duration of immobility in both FST and TST. FST and TST are considered as effective behavioral despair models for evaluating anti-depressant activity of a drug candidate as several clinically used anti-depressants showed to be effective in this model. The reduction of duration of immobility by the administration of a pharmacological agent is considered as it posses anti-depressant like effect.

The major drawback of this study is that the general psycho stimulant drugs which devoid anti-depressant activity also tend to reduce the duration of immobility. Hence, the present study evaluated the effect of amlodipine on locomotor activity in actophotometer. Administration of amlodipine at the doses used in the present study did not alter the locomotor activity. This clearly indicates that the antidepressant like effect exhibited by amlodipine is not through general psychostimulant effect.

However, further chronic studies are required to confirm the anti-depressant activity as clinical management of depression requires at least three weeks of treatment with anti-depressants but these drugs when administered acutely show antidepressant activity in behavioral despair models.

This study also warrants further studies in the line of receptor binding assays and interaction with various neurochemical analogs may be beneficial in exploring the molecular mechanism for the anti-depressant like effect of amlodipine.

7. SUMMARY AND CONCLUSION

Previous research reports revealed that treatment of amlodipine influences the central nervous system physiology and it has shown to potentiate antiepileptic activity of antiepileptic drugs. L-type calcium channels have been implicated in the pathology of behavioral depression and some L- type calcium channel blockers have been shown to produce anti-depressant activity. Hence,the present study was aimed to evaluate the probable anti depressant activity of amlodipine in valid animal models. The results obtained in this study revealed the anti-depressant activity of amlodipine. However, further chronic studies are required as clinical management of anti depressant requires chronic treatment. In conclusion, amlodipine produces the anti depressant activity in valid animal models similar to that of other L-type calcium channel blockers.

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CERTIFICATE

Name of the student : **Ms . Sathya .A**

This is to certify that the project **"Evaluation of antidepressant activity of amlodipine in mice."** has been approved by the IAEC, meeting held on 10-04-2019

Proposal number : **JKKN/IAEC/M.Pharm/14/ 2019**

Approval date : **10-04-2019**

No of animals sanctioned : **12 Swiss Albino Mice**

10/4/19

Dr R.Sambath kumar

IAEC Chairperson

Dr R.SAMBATH KUMAR
Chair Person
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10/04/19

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