COMBINATION OF LAVENDER OIL AND MYRISTICIN IN TREATMENT OF DEPRESSION IN RATS EXPOSED TO LIPOPOLYSACCHARIDE REPEATED CHALLENGE FOLLOWED BY CHRONIC UNPREDICTABLE MILD STRESS

A Dissertation submitted to
THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY IN
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Submitted by
Ms. Arya P Anil
REGISTRATION No.261725101

Under the guidance of
DR. K. Asok Kumar, M. Pharm., Ph.D.,
.Professor & Head
Department of Pharmacology

COLLEGE OF PHARMACY
SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES
COIMBATORE - 641 044

May 2019

CERTIFICATE

This is to certify that the M. Pharm., dissertation entitled "**Combination of lavander oil and myristicin in treatment of depression in rats exposed to lipopolysaccharide repeated challenge followed by chronic unpredictable mild stress**" being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of Master of Pharmacy programme in Pharmacology, carried to Register No. **261725101** in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision and guidance to my full satisfaction.

Dr.K. Asok Kumar, M. Pharm., Ph. D.,
Professor&Head,
Department of Pharmacology,
College of Pharmacy, SRIPMS,
Coimbatore-44.

Place: Coimbatore

Date:

CERTIFICATE

This is to certify that **Register No. 261725106** Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, worked on the synthesis of *Myristicin* from nutmeg in Pharmaceutical Chemistry, Post Graduate Laboratory which is a part of her dissertation work entitled **"Combination of lavander oil and myristicin in treatment of depression in rats exposed to lipopolysaccharide repeated challenge followed by chronic unpredictable mild stress"** being submitted to the Tamil Nadu Dr. M.G. R. Medical University, Chennai under my direct supervision and to my fullest satisfaction.

Prof. M. FRANCIS SALESHIER, M. Pharm.,
Professor & Head,
Department of Pharmaceutical Chemisty,
College of Pharmacy, SRIPMS,
Coimbatore-44.

Place: Coimbatore

Date:

CERTIFICATE

This is to certify that the M. Pharm., dissertation entitled **"Combination of lavander oil and myristicin in treatment of depression in rats exposed to lipopolysaccharide repeated challenge followed by chronic unpredictable mild stress"** being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of **Master of Pharmacy** programme in Pharmacology, carried out by **Register No. 261725101** in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under the direct supervision and guidance of **Dr. K. Asok Kumar, M.Pharm., (Ph.D).,**Professor and Head, Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore.

Dr. T. K. Ravi, M. Pharm., Ph.D., FAGE.,
Principal,
College of Pharmacy, SRIPMS,
Coimbatore-44.

Place: Coimbatore

Date:

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LIST OF ABBREVIATIONS

Abs	-	Absorbance
ADME	-	Absorption, Distribution, Metabolism and Excretion
ALP	-	Alkaline phosphatase
ALT	-	Alanine transaminase
ANOVA	-	Analysis of Variance
ACTH	-	Adrenocorticotropic hormone
ADHD	-	Attention deficit hyperactivity disorder
AST	-	Aspartate transaminase
b.w	-	Body weight
BBB	-	Blood brain barrier
CBT	-	Cognitive behaviour therapy
Cm	-	Centimetres
CNS	-	Central nervous system
COMT	-	Catechol-O-methyltransferases
CPCSEA	-	Committee for the Purpose of Control and Supervision of Experiments on Animals
CRF	-	Corticotropin releasing factor
CRH	-	Corticotropin releasing hormones
CUMS	-	Chronic unpredictable mild stress
COX	-	Cyclooxygenase
DALYs	-	Disability-Adjusted Life Year

DA	-	Dopamine
DIPFC	-	Dorsal portions of the middle and superior Frontal gyri
DLPFC	-	Dorsolateral prefrontal cortex
dpf	-	Docking parameter file
E.coli	-	Escherichia coli
EDTA	-	Ethylenediaminetetraacetic acid
EPM	-	Elevated plus maze
EL	-	Escape latency
FST	-	Forced swim test
FAD	-	Flavin adenine dinucleotide
gpf	-	Grid parameter file
h	-	Hour
HPA	-	Hypothalamic pituitary adrenal
HPT	-	Hypothalamic pituitary thyroid
3-HK	-	3-hydroxykunurenine
5-HIAA	-	5- hydroxyindoleacetic acid
IL-1β	-	Interleukin 1 beta
IL-6	-	Interleukin 6
INFɣ	-	Interferon-gamma
IDO	-	Indoleamine 2,3 dioxygenase
IUPAC	-	International Union of Pure and Applied Chemistry
LDH	-	Lactate dehydrogenase
LC	-	Locus coeruleus

LPS	-	Lipopolysaccharide
LOS	-	Lipooligosaccharides
MDD	-	Major depressive disorder
MAO	-	Monoamine oxidases
MAOI	-	Monoamine oxidase inhibitors
MFC	-	Middle frontal cortex
Mg/dl	-	milligram per deciliter
Mg/kg	-	milligram/kilogram
MWM	-	Morris water maze
NADH	-	Nicotinamide Adenine Dinucleotide
NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate
NE	-	Norepinephrine
NMDA	-	N-methyl D-aspartate
NMHS	-	National Mental Health Survey
NCRB	-	National Crime Record Bureau
OECD	-	Organization for Economic Co-operation and Development
OCD	-	Obsessive compulsive disorder
Pdb	-	Protein data bank
PFC	-	Prefrontal cortex
PLP	-	Proteolipid protein
QUIN	-	Quinolic acid
RCSB	-	Research Collaboratory for Structural Bioinformatics
SAD	-	Seasonal affective disorder
Sec	-	Seconds

SSRI - Selective serotonin reuptake inhibitors

SNRIs - Serotonin and noradrenaline reuptake inhibitors

SEM - Standard Error of Mean

SGOT - Serum Glutamic Oxaloacetic transaminase

SGPT - Serum Glutamic Pyruvic transaminase

SMILES - Simplified Molecular Input Line Entry System

TST - Tail suspension test

TC - Total Cholesterol

TCA - Tricyclic antidepressant

TM - Traditional medicine

TPH2 - Tryptophan hydroxylase 2

TNFα - Tumor necrosis factor alpha

TRP - Tryptophan

TDO - Tryptophan 2,3-dioxygenase

vmPFC - Ventral portion of the medial prefrontal cortex

WHO - World health organization

YLDs - Year linked with disability

CONTENTS

INTRODUCTION

Among all the diseases and disorders, the most complex and disturbing all age groups are the psychological and neurological disorders, psychiatric disorders have provied to be a major health problem in the recent years, These disorders not only influence the individuals health and mental status but also its social,socioeconomic and family life[1].

The 'global burden of disease' statistics indicates that four out of ten most common diseases in world wide are psychiatric orgin. The various mental disorders, depression, anxiety disorder and adjustment disorders, affect about 10% of population in general. The presence of mental disease is frightfully huge. Mental disorders are all pervading, found in people of all region of the world irrespective of race, gender, age, culture and socio-economic status and are essentially effectively treatable so much so that people with disorders of mind can lead long productive life along with the main stream of the society[2].

There are reports that about 17% of people world wide suffer from depression and about 1 million people commit suicide each year because of depression. An estimated 350 million people of all age suffer from depression (WHO Global report 2016) and major depression disorder (MDD) has become the second most prevalent cause of illness induced disability world wide, Statistical studies conducted by the World Health Organization have shown that psychiatric disorder in adult have a prevalence of about 10% and it has also been found that one in four families has at least one member suffering from a mental or behavioural disorder at one point of time, Such emotions may manifest at certain point of life without any preceivable stressful situations. Sadness is ofcourse a normal human emotion and one that is generally unavoidable in the face of stressful and uncomfortable life situations, However, normal sadness waxes and wanes overtime and generally lasts for short duration and people are usually able to come to terms with such situations [3].

In India, the average national deficit of psychiatrists has been estimated to be 77% approaching over 90% in several states. There is a need to mobilize more resources for dealing with depressive disorders in Indian population. Therefore it is pertinent that the psysicians are sensitized and trained for recognition and treatment of depressive disorder, if current trends continue, it will become the leading cause of disease burden by the year 2030. At an individual level depression affects the mental and emotional wellbeing lower the overall quality of life and may increase the risk of other medical illnesses. It adversily affects

the job and familial functioning. At a societal level, it leads to loss of productivity and economic burden[4].

Health is a positive state of wellbeing; mental health is a sense of wellbeing, an individual experience. People who can carry out their roles in society and whose behaviour is appropriate and adaptive are viewed as mentally healthy. Mental health is determined by hereditary, environmental opportunities, good working conditions, fair support system effective communication autonomy and independence of an individual. Maladaptive response to stressors form internal and external environment. There are many psychiatric disorders among them depression is the common cold of psychiatric disorder among the depression is the common cold of psychiatric disorder[5].

The world is moving fast and so hectic; human beings do not have time to relax, feeling of ill, sadness and disappointment are part of human life and are experienced by everyone in the world. These feelings are associated with academic failures, problems with in relationship, financial problems, failure in love, and loss of loved one which may leads to changes in normal sleep, appetite and disinterest in daily activities. Many of the people get success with their strong coping mechanism while some people do not know the correct way to resolve their problems. People get depression when this happens on a continuous basis. WHO (2018) concludes 350,000,000 (5% of world population) people globally are affected by some form of depression. Adolescents who have a depressive disorder by the age of 18 are 11% and 70% by which women are more likely than men to experience depression in their life time. 14% of women from a 2018 postpartum depression study had the disorder four to six weeks after giving birth 30% of college students reported feeling depressed[6].

Depression is the common cold of psychiatric disorder, many persons get affected by depresssion either directly or indirectly. Depression is a state of low mood and a version to activity that can affect a person's thoughts, behaviour, feelings and total sense of well being. Depressed people fel sad, anxious, empty, hopeless, worried, helpless, worthless, guilty, alone, irritable, hurt or restless and they don't show interest on daily activities[7].

Depression present with depressed mood, loss of interest or pleasure, decreased energy, feeling of guilt or low self worth, disturbed sleep or appetite, and poor concentration. Moreover, depression, depression often comes with symptoms of anxiety. Depression drives the client to feel hopeless and helpless this leads to suicide. Almost 1 million lives are lost every year due to suicide, which translates to 3000 suicides everyday[8].

A large majority of patients with depression present to physicians with complaints of medically unexplained somatic symptoms or marked depression. Depression is classified under mood disorder these include major depressive disorder (MDD) where the person has at least two weeks of depressed mood, loss of interest in pleasure from all activities. Dysthymia is a state of chronic depressed mood symptoms that doesnot meet the severity of major depression, bipolar disorder consists of one or more episodes of elevated mood or one or more episodes of depressive mood[9].Seasonal affective disorder (SAD) is a type of depression that is related to changes in seasons – SAD begins and ends at about the same time every year: symptoms start in the fall and continue into the winter months; sapping energy and making one feel moody. SAD causes depression in the spring or early summer[10].

Epidemiology of depression

According to the WHO (2014), depression is projected to become the second leading contributor to the global burden of disease by 2020. Depression is recognized as the risk factor of coronary artery disease (CAD)[11].

Recently conducted world mental health surveys indicate that major depression is experienced by 10-15% people in their lifetime and about 5% suffer from major depression. If current trend continues, it will become the leading cause of disease burden by the year 2030[11].

According to global burden of disease (2010), the prevalence of unipolar depressive episode is estimated to be 1.9% for men and 3.9% for women. It estimates the burden of depression will increase to 5.7% of the total burden of disease and it would be the second leading cause of the disability. India has among the highest rate of depression in the world[12]. For every 1 Lakh Indians between 15 and 29 years old, 36 commit suicide annually- the highest rate among the youth in the world. Statistics related with depression in India; out of every 10 Indian professional surveyed across the metropolitan cities, 4 survives from general anxiety disorder or depression. In the first list of all top diseases, depression (42%) ranks at the top. Followed by obesity (23%), high blood pressure (9%) and diabetes (8%)[13]. The top 3 cities where professional were most prone to depression were Delhi, Bangalore and Mumbai, in that order. U.S adults (16 Million 6.9%) had at least one major depressive episode in the year 2012. The average age of depression among Indians is at the age of 31

years (NIMHANS 2009). In India, 9% of people are affected by depression. Major depressive disorder episode is high among Indians (35.9%).(Thiruvananthapuram 2011)NCRB states that 135,455 people committed suicide in the country last year. Tamil Nadu tops the list with 16,927 suicides (National Crime Record Bureau record 2013)[14].

A recent large population based study from South India, which screened more than 24000 subjects in Chennai, reported overall prevalence of depression to be 15.1% (2009). Study conducted in Chennai with 309 subjects of working and non-working women concludes that working women have 2.9% of depression. and non-working women have 2.3% depression. Globally, depression is ranked as the single largest contributor to non-fatal health loss accounting for 7.5% of global years linked with disability (YLDs) and 2.0% of global disability adjusted life years (DALYs) in 2015. According to Global Health estimates 2015, depressive disorders accounted for nearly one third of the total DALYs caused by mental and substance use disorder[15].

The average age of onset for major depression is 24 years as per recent epidemiology research through. It can begin at any time throughout the life span. One of the consistent findings across almost all research studies is that women are twice as likely to have depression compared to males[16].

Depression is much more likely among people who are unmarried, widowed, divorced or separated or without closer inter personal relationships. Those residing in nuclear families and urban areas are possibly at a higher risk. Elderly age and presence of medical disorders pose an even higher risk of depression[16].

Aetiology of depression

Current understanding of depression is based on a biopsycho social framework, with an interplay of biological as well as factors. Studies suggest that depressive disorders are heritable to some extent, with 1.5-3 time increase in risk among those with a family history of depression in first degree relatives. Those with a high familial risk tend to have an early age of onset and a recurrent illness. Few Indian studies have also found genetic factors to play a role in depression and its treatment response. Many studies have implicated norepinephrine and serotonin as possible neurotransmitters involved in aetiology of depression. The alteration in the neuro endocrine system, sleep physiology and immune system has been found in depressed patients[17].

Stressful life events are one of common precedents in the first episode of depression and may leave a person more vulnerable to develop subsequent episodes. Common life events associated with depression are loss of a parent in childhood, loss of a spouse and unemployment. Being a caregiver for a chronically disabled person in family may also increase the risk of depressive symptoms. Researches have suggested the role of psychological factor. Indian studies have suggested the role of poverty, marginalized and other socio economic adversities in increasing the likelihood of depression[17].

Factors associated with occurrence of depression

Biochemical factors

Depression is associated with deficiency of neurotransmitter in a certain region of the brain and includes dopamine, norepinephrine and 5- hydroxy tryptamine. Antidepressants increase the level of these neurotransmitters in the brain[18].

Endocrine factor

Over activity of HPA axis is associated with the affective disorder[18].Cushing's syndrome and hypothroidism is connected with HPA and HPT axis dysfunction[19].

Genetic factor

Serotonine transporter gene is a risk factor for depression. Many common disorder which are also influenced by genes include high blood pressure and diabetes. Cystic fibrosis and Huntigton's disease may be caused due to the single defective gene. The gene responsible for depression, 3p25-26 chromosome isolated by British research team appears to be present in many families with recurrent depression[20].

Environmental factor

Environmental factors responsible for depression are as water, air, synthetic chemicals, food additives and food pollution, hormones, pesticides, drugs and industrial by products are bombarding our bodies at an extreme rate, other sources include stress electrical pollution, natural disasters, noise pollution and other catastrophic environmental events. Some events include the death of a loved one, divorce, job loss, financial problems and disabling illness or injury sometimes called as social and relational causes of depression[21].

Co-morbid medical illness

Recent research has convincingly proved that depression is often present with several medical illnesses. It has been shown that heart disease predisposes to depression while presence of depression significantly increases the probability of heart disease. Similarly diabetes and high blood pressure are associated with depression, stress and anxiety worsens both the condition. Depression following child birth (postpartum blue) is a well documented entity. It has also been shown that depression often follows diagnosis and treatment of cancer and the presence of depression significantly interferes in body's defense mechanism (immunity) which is essential for recovery. Other diseases like viral hepatitis and hypothyroidism often predispose to depression[22].

Cause of depression

Depression does not have angle cause. Several factors can lead to depression. Some people carry genes that increase their risk of depression. But not all people with depression have these genes and not all people with these gene have depression. Environment, surroundings and life experiences can lead to stress[23].

Economic stress : It is the stress related to factors demanding constant adjustment to meet ends. This leads to enormous stress of uncertainty and insecurity. More over with expansion of family, financial needs increases while resources remaining limited. This is a major source of stress in economically backward countries[24].

Occupational stress:

With industrialization and consumerism work related stress is on the risk.[25].

Family stress:

With breakdown of joint family structure, more and more families are becoming nuclear. This has led to stress, increasing breakdown of marital relationship, death of a near and dear ones and living away from family for the first time, feeling alone or isolated, experiencing conflicts in relationships causes enormous stress leading to depression[26].

Signs and symptoms of depression

The symptoms of depression is divided into two categories - psychological symptoms and somatic symptoms[27]. The Psychological symptoms are depressed mood or sadness, anxious,helpless, restless, loss of interest in activities you used to enjoy, loss of energy or

pleasure, problems in concentrating, remembering information or making decision,thoughts of suicide or suicide attempts, perceptual abnormalities and hallucinations or delusions.

The Somatic symptoms are problems in falling asleep, staying asleep or sleeping too much, loss of appetite or eating too much, muscle fatigue, aches, pains, headaches, cramps or digestive problems, psychomotor retardation,constipation,menstrual problems, loss of sex drive and agitation

Types of depression

The most common depressive disorders are

Bipolar mood disorder:

Person experiences episodes of mania and in most cases episodes of depression. Bipolar depression consists of two phases either mania or hypomania, asosociated with strong sign of obsessive - compulsive disorder, panic disorder or social anxiety disorder.

Unipolar mood disorder:

Person experiences only episodes of depression does not have alternating episides and not associated with obsessive – compulsive disorder, panic disorder ror social anxiety disorder.

Major depressive disorder:

The symptoms of major depression are disabling and interfere with everyday activities such as studying, eating and sleeping. People with disorder may have only one episode of major depression in their lifetime. But more often depression comes back repeatedly[28].

Dysthymic disorder:

Dysthymia is mild, chronic depression. The symptoms of dysthymia last for a long time- 2 years or more. Dysthymia is less severe than major depression, but it can still interfere with everyday activities. People with dysthymia may also experience one or more episodes of major depression during their lifetimes[28].

Minor depression:

Symptoms of minor depression are similar to major depression and dysthymia. But they are less severe and/or are usually shorter term without treatment, however people with minor depression are at high risk for developing major depressive disorder[29].

Other type of depression include:

Psychotic depression:

Severe depression accompanied by some form of psychosis, such as hallucinations and delusions[30].

Seasonal affective disorder:

Depression that begins during the winter months and lifts during spring and summer[30]

Endogenous depression:

Endogenous depression is a kind of depression in which there is no apparent external factor causing depression[30].

Neurotic depression:

A kind of depression with mild to moderate and last for a long duration with predominant physical symptoms[30] [31][32].

Cause of depressive disorder

Remission:

Remission is a period in which the individual is asymptomatic, does not meet the syndromal criteria for MDD and has no more than minimal symptom[33]

Recurrence:

A manifestation of a new episode of MDD that occurs during recovery is known as recurrence[34].

BRAIN

The human brain is the central organ of the nervous system, and with the spinal cord makesup the central nervous system, each hemisphere is conventionally divided into four

lobes the frontal, temporal, parietal and occipital lobes[29]. The frontal lobes is associated with executive functions, including self control, planning, and reasoning while, the occipital lobe is dedicated to vision, within each lobe, cortical areas are associated with specific functions, such as sensory, motor and association regions. Although the left and right hemisphere are broadly similar in shape and function. Some functions are associated with one side, such as language in the left and visual spatial ability in the right[35].

Frontal lobe:- Motor control, concentration, planning, problem solving, speech, smell

Parietal lobe:- Touch and pressure, Taste

Temporal lobe:- Hearing, facial recognition

Occipital lobe:- Vision

Cerebellum:- Co- ordination

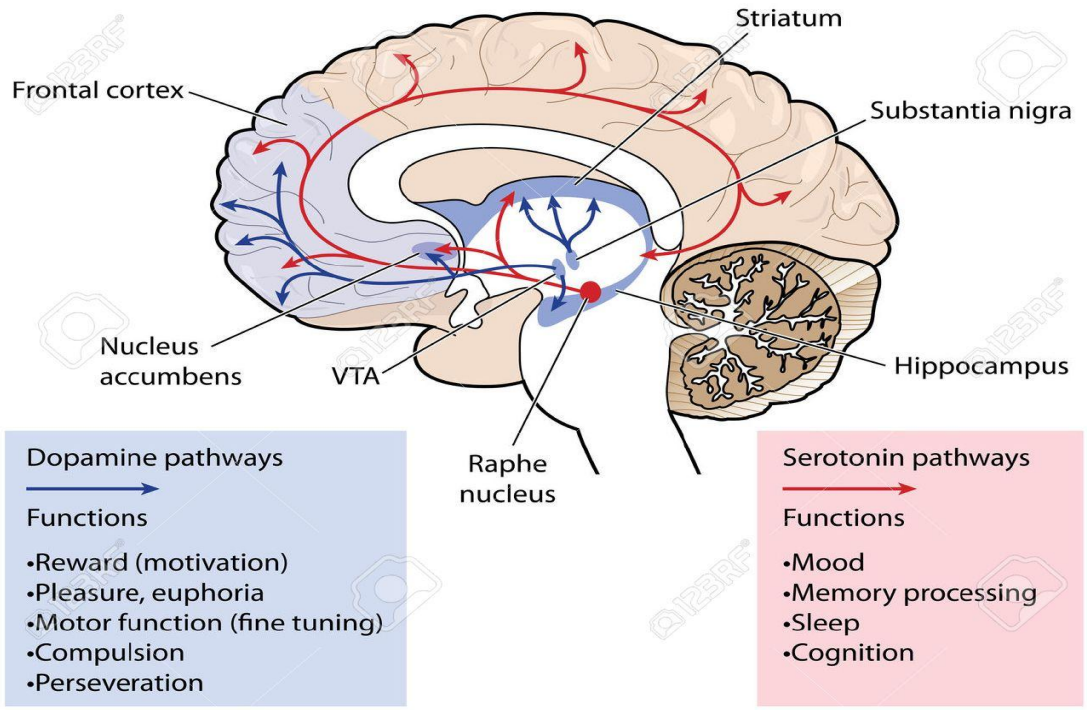

Fig 1: Parts of brain that control different body functions

Areas of the brain affected by depression

Depression is associated with the many brain regions like the hippocampus, basal ganglia and amygdala and cortical brain regions. Abnormalities in the structure and function of these region in patient exhibit distinct pathological changes in the brain and have been found to be associated with depression[36].

The most widely accepted division of prefrontal cortex, based on anatomical connectivity and functional specialization is betweeen the dorsolateral and ventromedial sectors. The ventromedial prefrontal cortex (vmPFC) includes the ventral portion of the medial prefrontal cortex (below the level of the corpus callosum) and medical portion of the orbital surface (approximately the medial one – third of the orbital frontal cortex in each hemisphere)[32]. Target of vmPFC projections include the hypothalamus are periaqueductal gray, which mediate the visceral autonomic acitivity associated with emotion, and the ventral striatum, which signals reward and motivational value[37].

By contrast, the dorsolateral prefrontal cortex (dIPFC) which includes portions of the middle and superior frontal gyri on the lateral surface of the frontal lobes, receives input from specific sensory cortices and has dense interconnection with promotor areas, the frontal eye fields, and lateral parietal cortex[38]

The distinct patterns of connectivity in these two regions of PFC suggest disparate functionally.Indeed dIPFC has primarily been associated with “cognitive” or “ executive” function, whereas vmPFC is largely a scribed “emotional” or “affective” fuctions[39].

Major depressive disorder patients exhibited a cortical thinning of middle frontal cortex (MFC), the brain region needed for maintaining the normal social behaviour. MDD patients have shown reduced gray matter volume in the regions of the prefrontal circuits that included dorsolateral and dorsomedial prefrontal cortices, lateral and medial orbitofrontal cortices[40].

The total gray matter volume is inversely correlated with depression severity and suicide attempt and suicide attempters showed reduced gray matter volume in several brain regions including prefrontal cortex. Possible reduction of gray matter in critical cortical area in suicidal patients causes serious congnitive deprivation in them for performing planned goal directed behaviour coupling this inability with depressive symptoms, they feel more helplessness to attempt suicide. Amygdala (Ag) is a part of the neural circuitry involved in emotional component of experiences. It has been suggested that frontal cortex modulates the

amygdala activities and mediates congnitive emotion regulation. In MDD patients deviated activity of the Ag has been reported which is found to be associated with the hypoactivation of dorsolateral prefrontal cortex (DLPFC). This increased activity of Ag is supposed to arise from the hypoactivity of DLPFC which make it less efficient in exerting regulatory influence over Ag. Hippocampus (H) is a brain region which is very important for learning, memory acquisition, motivation and emotion[41].

Amygdala

The amygdala part of the limbic system, are two almond shape masses of neurons on either side of thalamus linked to emotions like anger, sorrow, fear, pleasure and sexual arousal. A charged memory is responsible for activation of the amygdala. Functional neuroimaging studies have supported the hypothesis that the amygdala is abnormally hyperactive in depressed patients. Amygdala regulates neuroendocrine response and cortical arousal as well as emotional learning and memory. It also stimulates the hypothalamus CRF-containing neurons to result in the release of corticotrophin[42,43].

Hippocampus

The hippocampus is one of the divisions of the limbic system and consists of two horns that curved back from the amygdala and has an important role in the translation of short term memory to long term memory recollection and responds to stess hormone in the blood. Neuroimaging has been used to map changes in the brain structure of depressed patients related to their symptoms[44].

Prefrontal cortex

The prefrontal cortex is the component of the frontal lobe. It plays an important role in modulating the activity of limbic region and basal ganglia and has cortical and subcortical interconnection. It regulates, emotion, mood, memory, motivation and decision making[45]

The prefrontal cortex (PFC) processess and modulates the physiological, neuroendocrine and behavioral responses (via the amygdala) and is also involved in fear and anxiety related conditional responses. Neuroimaging study state that frontal cortex volume reduction ranges from 7% - 48% in the subgenual prefrontal cortex in major depression[46].

Thalamus

The thalamus is involved in the transmission of sensory information and regulation of learning, speech, behavioral reactions, movement and thinking. The problems in thalamus leads to bipolar depression[47].

Pathways in the brain

The distributions of a noradrenergic and serotonergic neuron are similar in pons and medulla. The cell bodies of this neurons extensively send the axon to different part of the brain and spinal cord. The most prominent cluster is the locus coeruleus (LC) located in the pons. Other noradrenergic neurons lie close to the LC in the pons and medulla and widely project to the hypothalamus, hippocampus, prefrontal cortex and other part of the fore brain as well as to the cerebellum and spinal cord. The locus coeruleus is involved in the descending control of pain pathways. The regulation of the stressful environmental response correlates with the involvement of the norepinephrine system in the pathogenesis of depressive and anxiety disorder[48]

A small cluster of epinephrine neurons which release epinephrine rather than norepinephrine lies more ventrally in the brain stem, projecting mainly to the pons, medulla and hypothalamus, rather little is known about them but they are believed to be important in cardiovascular control[49].

Serotonergic pathway:

The cell bodies of 5-HT neurons are localized in the midbrain, while their terminals innervate all brain regions (except some areas of the cerebellum), Tryptophan hydroxylase 2 (TPH2) is the rate-limiting and the only specific enzyme of 5-HT synthesis and metabolism in the brain (with TPH1 playing a similar role in the periphery). An irreversible TPH1/TPH2 inhibitor, p-chlorophenylalanine, and TPH2 gene knockout dramatically reduce 5-HT concentration in the brain. Synthesized 5-HT is stored in synaptic vesicles, transported to presynaptic terminals, and released in the synaptic cleft. The 5-HT secretion is regulated by the feedback mechanism including presynaptic 5-HT1A and 5-HT1B autoreceptors on the cell body of 5-HT neuron. The secreted 5-HT interacts with 14 types of currently known 5-HT receptors with four different mechanisms of signal transduction. Released 5-HT is removed from the synaptic cleft by the plasma membrane 5-HT transporter (5-HTT), which takes it into the presynaptic 5-HT neurons, where the neurotransmitter can either storage in the vesicles or be oxidized to 5-hydroxyindoleacetic acid (5-HIAA) by the monoamine

oxidase A (MAOA). Therefore, 5-HTT, TPH2, presynaptic 5-HT1A receptors, 5- HTT and MAOA regulate 5-HT concentration and 5-HT signaling in the brain. Moreover, 5-HTT and MAOA and are the molecular targets for the majority of antidepressant drugs. [50]

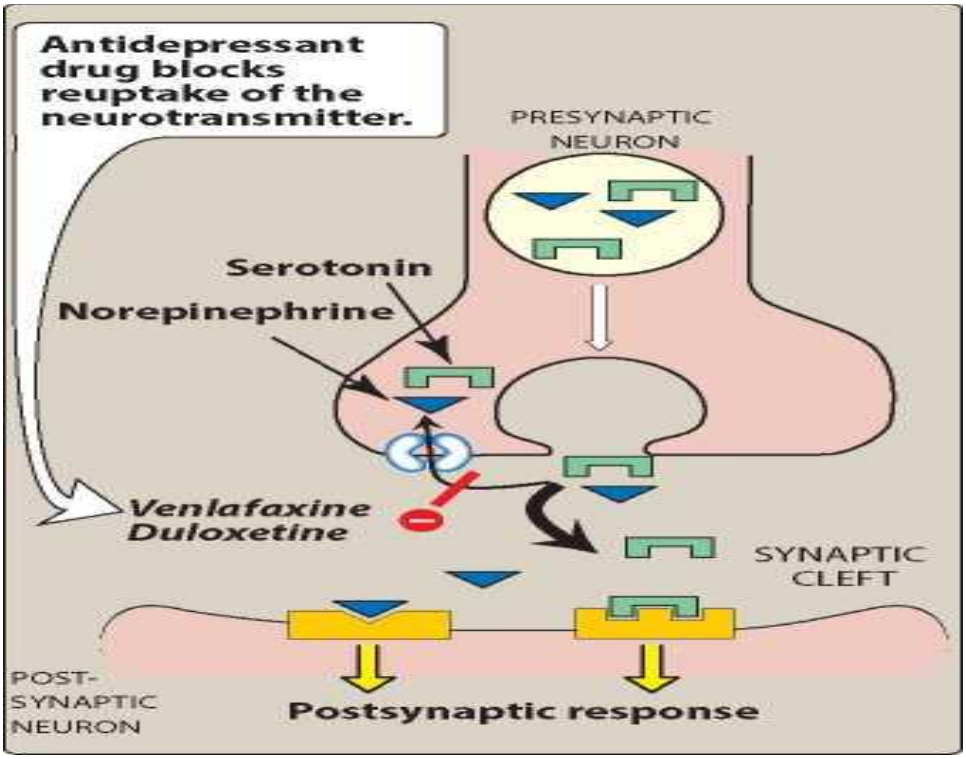

Fig 2: Serotonergic pathway

Dopaminergic pathway in the CNS

Four major dopaminergic pathways have been identified in the mammalian brain; the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular systems that originate from the A9 (nigrostriatal), A10 (mesocorticolimbic pathway), and A8 (tuberoinfundibular) groups of dopamine-containing cells. Dopaminergic signaling has also been suggested for a host of other brain disorders, such as bipolar disorder, major depression Once released from presynaptic terminals, dopamine activates members of a family of G protein-coupled dopamine receptors named D_1 to D_5. Targeting these receptors using specific agonists and antagonists has provided an opportunity to significantly influence dopaminergic transmission and dopamine-dependent functions by enhancing or blocking the actions of dopamine. The D_1 and D_2 classes of dopamine receptors presynaptically localized autoreceptors generally provide an important negative feedback mechanism that adjusts neuronal firing rate, synthesis, and release of the neurotransmitter in response to changes in extracellular neurotransmitter levels. Activation of presynaptic D_2-class autoreceptors generally causes a

decrease in dopamine release that result in decreased locomotor activity. D_2 dopamine receptors seem to be the predominant type of autoreceptors that are involved in the presynaptic regulation of the firing rate, synthesis of dopamine and release of dopamine. D_2 dopamine receptors are likely to play a critical role in the psychotic reactions observed in schizophrenia and bipolar disorder. Other functions are mediated in part by various dopamine receptor subtypes in the brain, such as affect, attention, impulse control, decision making, motor learning, sleep, reproductive behaviors, and the regulation of food intake.

The distribution of dopaminergic neuron is similar to that serotonergic pathway. The antidepressant act by down regulation or desensitization of presynaptic auto receptors. This increased release of neurotransmitter in the synaptic cleft which desensitize or 'down regulate' the post synaptic receptors thus lift the depression in the central nervous system, dopamine containing circuit regulates concentration, psychomotor speed, motivation and the ability to experience pleasure. Depression leads to impairment of these functions. Dopamine present most abundantly in the corpus straitum is concerned with the movement coordination[53].

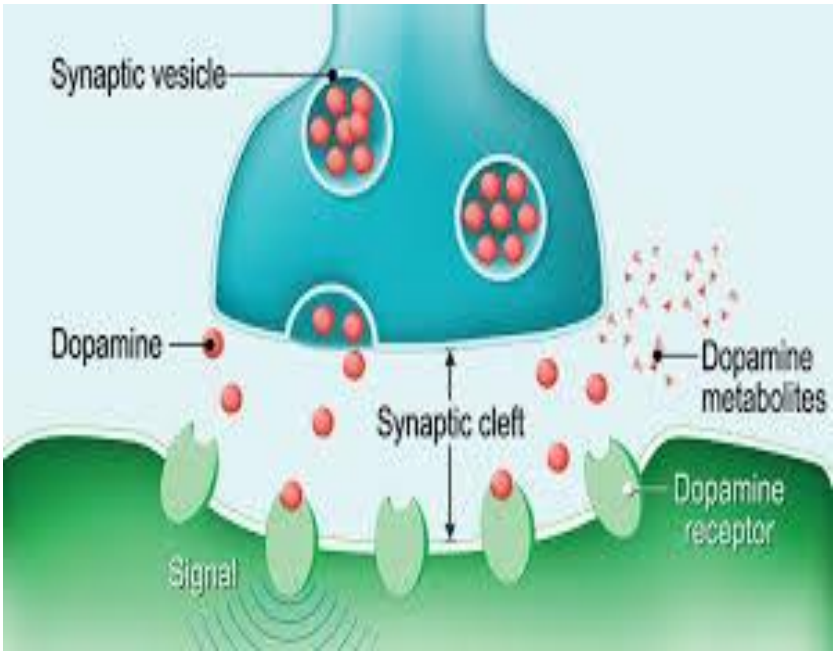

Fig:3 Dopaminergic pathway

HPA axis pathway

The stress response itself occurs primarily within the Hypothalamic-Pituitary-Adrenal Axis (HPA axis), involving the hypothalamus, pituitary gland, and adrenal cortex. Ultimately,

the adrenal cortex releases glucocorticoids, which have effects throughout the body on metabolism and immune function, amongst other things. Glucocorticoids also inhibit earlier steps of the stress response within the hypothalamus and pituitary gland, preventing the system from becoming overactive.The major activating input comes from the amygdala, a part of the brain important for the processing of emotions, particularly negative emotions. Thus when someone experiences negative emotions, the amygdala becomes more active, which can trigger the stress response in the HPA axis. The released glucocorticoids then trigger a positive feedback loop with the amygdala and negative feedback loops with the hippocampus and prefrontal cortex. If the balance of excitation from the amygdala and inhibition from the hippocampus and prefrontal cortex favours HPA activation (amygdala excitation > hippocampus, prefrontal cortex inhibition), positive feedback occurs and chronic stress and depression can result. Chronic stress produces excess levels of glucocorticoids, which can lead to the death of neurons, particularly in the hippocampus. This may be relevant to the mode of action of the most commonly prescribed antidepressant drugs, SSRIs (selective serotonin reuptake inhibitors)[54].

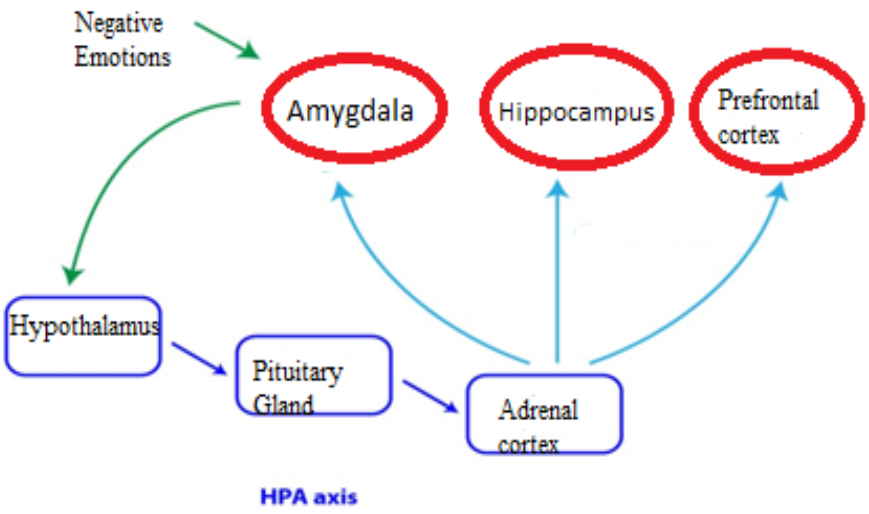

Fig 4: HPA axis and its interactions

Monoamines

The monoaminergic systems (eg.Serotonin, norepinephine, dopamine) play an important role in the control of cognition, motor function, endocrine secretion,

chronobiologic rhythms and appetite. Most of the antidepressant drugs used in clinical practices acts by increasing the concentration of monoamine in the synaptic cleft by neuronal reuptke inhibition or by blocking alpha2 auto and heteroreceptors[55].

Synthesis, release and metabolism of monoamines

Catacholamine are derived from the aminoacid, thyrosine, which derived from dietary sources as well as sysnthesis from phenylalanine. Catacholamines are water-soluble and are 50% bound to plasma protein in circulation. Included among catecholamine are epinephrine (adrenaline) norepinephrine (noradrenaline) and dopamine. Release of the hormones epinephine and norepinephine from the adrenal medulla of the adrenal glands is part of the fight-or-flight response[55].

Tyrosine is created from, phenylalanine by hydroxylation by the enzyme phenylalanine hydroxylation by the enzyme phenylalanine hydroxylase. Tyrosine is also ingested directly from dietary protein. Catecholamine secreting cells use several reactions to convert tyrosine serially to L-DOPA and then to dopamine. Depending on the cell type, dopamine may be further converted to norepinephrine or even further converted to epinephrine production and degradation[55][56].

Location

Catacholamines are produced mainly by the chromaffin cells of the adrenal medulla and the postganglionic fibers of the sympathetic nervous system. Dopamine which act as a neurotransmitter in the central nervous system is largely produced in neuronal cell bodies in two areas of the brainstem the ventral tegmental area and the substantia nigra, the latter of which contains neuromelanin-pigmented neurons.The similarly neuromelanin- pigmented cell bodies of the locus coeruleus procedure norepinephrine. Epinephrine is produced in small group of neurons in the human brain which express its synthesizing enzyme, phenyl ethanolamine N- methyl transferase, these neurons project from a nucleus that is adjacent to the area postrema and from a nucleus in the dorsal region of solitary tract[56].

Biosynthesis

Dopamine is the first catecholamine synthesized from DOPA, in turn, norepinephrine and epinephrine are derived from futher metabolic modification of dopamine.The enzyme

dopamine β hydroxylase requires copper as a cofactor and DOPA decarboxylase requires myelin proteolipid protein (PLP)[56]

Degradation

Catacholamine have a half – life of a few minutes when circulating in the blood. They can be degraded either by methylation by catachol– o- methyl transferases (COMT) or by monoamino oxidase (MAO)

Catabolism of catecholamines is mediated via two main enzyme catechol-o-methyl transferase (COMT) which is present in the synaptic cleft and cytosol of the cell and monoamine oxidase (MAO) which is in the mitochondrial membrane. Both enzyme require cofactors. COMT uses Mg^{2+} as a cofactor while monoamine oxidase (MAO) use flavin adenine dinucleotide (FAD). The first step of the catabolic process is mediated by either MAO or COMT which depends on the tissue and location of catecholamine[56].

The next catabolic steps in the pathway involve alcohol dehydrogenase, aldehyde dehydrogenase and aldehyde reductase. The end product of epinephrine and norepinephrine is VMA (vanillylmandelic acid) which is excreted in the urine[57]

The indoleamine serotonin (5-HT) is a transmitter that is synthesized within the nerve endings from the amino acid tryptophan.In chromaffin cells and neuron conversion of tryptophan to 5-hydroxytryptophan take place in the presence of enzyme tryptophan hydroxylase. 5-hydroxytryptophan is decarboxylated to serotonin by decarboxylase[58].

Anti- depressants

Antidepressants are medications that can help relieve symptoms of depression, social anxiety disorder, anxiety disorders, seasonal affective disorder, and dysthymia, or mild chronic depression, as well as other conditions Antidepressants were first developed in the 1950s. Their use has become progressively more common in the last 20 years. According to the Centers for Disease Control and Prevention (CDC), the percentage of people aged 12 years and over using antidepressant in the United States rose from 7.7 percent in 1999-2002 to 12.7 percent in 2011-2014. Around twice as many females use antidepressants as males[59][60].

Types

Antidepressants can be divided into five main types:

Serotonin and noradrenaline reuptake inhibitors (SNRIs)

Used to treat major depression, mood disorders, and possibly but less commonly attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), anxiety disorders, menopausal symptoms, fibromyalgia, and chronic neuropathic pain. SNRIs raise levels of serotonin and norepinephrine, two neurotransmitters in the brain that play a key role in stabilizing mood. Examples include duloxetine (Cymbalta), venlafaxine (Effexor) and desvenlafaxine (Pristiq)[62].

Selective serotonin reuptake inhibitors (SSRIs)

The most commonly prescribed antidepressants. They are effective in treating depression, and they have fewer side effects than the other antidepressants. SSRIs block the reuptake, or absorption, of serotonin in the brain. This makes it easier for the brain cells to receive and send messages, resulting in better and more stable moods. They are called "selective" because they mainly seem to affect serotonin, and not the other neurotransmitters. Examples include:- citalopram (Celexa), escitalopram (Lexapro), fluoxetine (Prozac, Sarafem), fluvoxamine (Luvox), paroxetine (Paxil) and sertraline (Zoloft)[63].

Common side effects of SSRIs and SNRIs:

The adverse effects observed were hypoglycemia, nausea, rashes, dry mouth, constipation or diarrhea, weight loss, sweating, tremor, sedation, sexual dysfunction, insomnia, headache, dizziness, anxiety and agitation[63]

Tricyclic antidepressants (TCAs)

Tricyclic antidepressants (TCAs) are so named because there are three rings in the chemical structure of these medications. They are used to treat depression, fibromyalgia, some types of anxiety, and they can help control chronic pain. Examples include amitriptyline (Elavil), amoxapine- clomipramine (Anafranil), desipramine (Norpramin), doxepin (Sinequan), imipramine (Tofranil), nortriptyline (Pamelor), protriptyline (Vivactil) and trimipramine (Surmontil). The adverse effects produced are seizures, insomnia, anxiety, arrhythmia, hypertension, rash, nausea and vomiting, abdominal cramps, weight loss, constipation, urinary retention, increased pressure on the eye and sexual dysfunction[62][63].

Monoamine oxidase inhibitors (MAOIs)

This type of antidepressant was commonly prescribed before the introduction of SSRIs and SNRIs. It inhibits the action of monoamine oxidase, a brain enzyme. Monoamine oxidase helps break down neurotransmitters, such as serotonin. If less serotonin is broken down, there will be more circulating serotonin this leads to more stabilized moods and less anxiety. Examples of MAOIs include phenelzine (Nardil), tranylcypromine (Parnate), isocarboxazid (Marplan) and selegiline (EMSAM, Eldepryl)[63].

Side effects of MAOIs are blurred vision, seizures, edema, weight loss or weight gain, sexual dysfunction, diarrhea, nausea, and constipation, anxiety, insomnia and drowsiness, headache, dizziness, arrhythmia, fainting and hypertension.

Noradrenaline and specific serotoninergic antidepressants (NASSAs)

These are used to treat anxiety disorders, some personality disorders, and depression. Examples include Mianserin (Tolvon) and Mirtazapine (Remeron, Avanza, Zispin). Possible side effects include constipation, dry mouth, weight gain, drowsiness and sedation, blurred vision and dizziness[63].

Alternative and complementary treatments for depression

Alternative and complementary treatments for depression may include vitamin and herbal supplements, acupuncture, and relaxation techniques, such as mindfulness meditation and yoga,[64]

Traditional medicine

World Health Organization (WHO) offers the collective term traditional medicine (TM) to refer systems of medicine such as traditional Chinese, indian ayurveda, arabic unani, and verious system of indigenous medicine. TM is also referred as complementary or alternative or non- conventional medicine herbal medicine is the most commonly used form of complementary and alternative medicine therapies herbs used to treat depression, anxiety or insomnia. Herbal medicine is commonly employed to cure mental disorders by various mechanisms of action in different systems. Mental disorders such as depression, anxiety, and insomnia are frequently found together in a single patient and they share some neurological basis, the mechanisms of curing drugs for these diseases might be intertwined with each other. For most of the synthetic antidepressants have severe defects such as adverse reactions,

high drug price and easy recurrence exist. Many people are gradually turning towards herbal medicine in order to find out the multi-target antidepressants with a low level of toxicity. This resulted in the discovery of various antidepressant drugs using herbal formulations Some herbal medicines play antidepressant role *via* sensitization of serotonin receptors or inhibiting monoamine oxidases. As a herb, ginseng has been frequently used for centuries in traditional Chinese medicine to improve mood and keep healthy in the western world. Several herbs have been proven to have a beneficial effect on depression and its symptoms of anxiety, sleeplessness, and inability to concentrate [64].

CHEMICAL INDUCED DEPRESSION

Lipopolysaccharide

Lipopolysaccharide (LPS) also known are lipoglycons and endotoxine, lipooligosaccharides (LOS) are glycolipids found in the outer membrane of some types of gram-negative bacteria, such as Neisseria Spp and Haemophilns Spp. LOS plays a central role in maintaining the integrity and functionality of the outer membrane of the gram-negative cell envelope. Lipooligosaccharides plays an important role in the pathogenesis of certain bacterial infections because they are capable of acting as immunostimulator and immunomodulators[65]

Mechanism of action

Peripheral administration lipopolysaccharide (LPS) results in production of pro-inflammatory cytokines, including IL-1β, IL-6, IFNɣ and TNF-α. There peripheral inflammation signals may be transmitted to the brain through humoral and neural pathway, leading to neuroinflammation. Some of the pro-inflammatory cytokine that induce sickness behaviour also enhance activity of enzyme indoleamine 2,3 dioxygenase (IDO). Activation of IDO result in decreased tryptophan (TRP) leves and increased production of kynurenine (KYN) and other tryptophan derived metabolites. Thus inflammation associated disorder of serotonergic and glutamatergic neurotransmission ultimately induces depression like behavior[66][67].

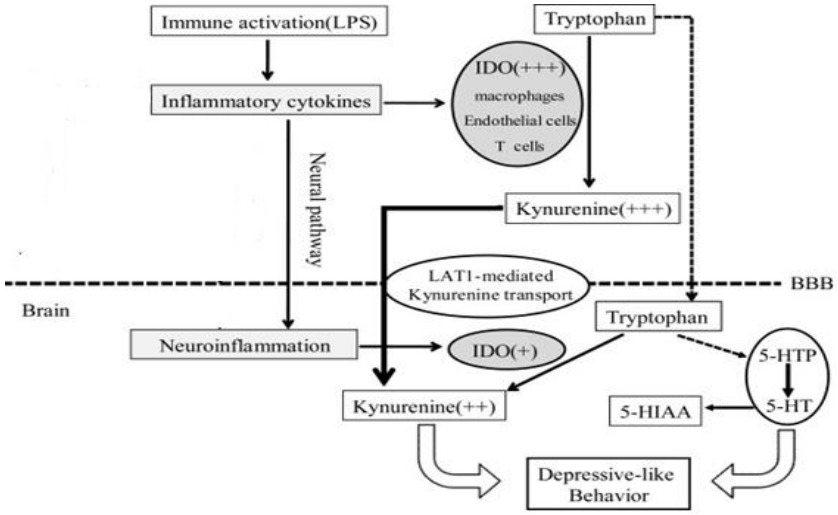

Fig 5: Mechanism of lipopolysaccharide induce depression

Indoleamine 2,3 dioxygenase

Neuroinflammation is defined as the activation of an immune response in the central nervous system (CNS). During neuroinflammation, microglia, the most important resident immunecells, become activated and as a consequence their morphology starts to change and secretion of pro-inflammatory cytokines, such as interferon ɣ (IFN ɣ), tumor necrosis factor α (TNF α) or interleukin 6 is initiated. Chronic inflammatory changes have been shown to play a key role in the pathogenesis of neurodegenerative diseases[68].

Mechanism of action

During peripheral infection, either acute or chronic the immune system is activated by macrophage stimulation and pro-inflammatory cytokines are released which also act on the brain causing sickness behavior, sickness behavior shares similar feature with major depression. Both are characterized by malaise, weakness, loss of appetite, lethargy or decreased interest in the surroundings, chronic activation of the immune system in the brain can lead to the development of depression the overall idea being that the overexpression of proinflammation agents is associated with increased activity of the ubiquitous intracellular enzyme indoleamine 2,3 dioxygenase (IDO) which catalyze tryptophan catabolism through the kynurenine pathway. The depletion of tryptophan in brain cells reduces the production of brain serotonin (5-HT). The degradation of tryptophan along the kynurenine pathway also generate neurotoxins, like quinolic acid (QUIN) or 3-hydroxykynurenine (3-HK), which leads to apoptosis in neurons, which can add to local excitotoxic neuronal overstimulation

next to modulating serotonergic neurotransmission.Cytokine induced IDO mediated tryptophan depletion and QUIN mediated neurotoxicity hypothesized to be involved in the pathophysiology of mood disorder like major depression[68][69].

Tryptophan 2,3- dioxygenase

Tryptophan 2, 3-dioxygenase (TDO) a heme containing enzyme found in mammalian liver is responsible for tryptophan (Trp) catabolism. TDO is cytosolic heme dioxygenase that catalyzes the oxidative cleavage of the C2-C3 bond of the indole ring of L-tryptophan (Trp). This reaction is first and rate-limiting step of the kynurenine pathway of tryptophan catabolism, which eventually leads to the formation of nicotinamide dinucleotide (NAD+), a process regarded as the primary biological function of TDO. Trp availability to the brain also play important role in central 5-HTsynthesis because the rate limiting enzyme of the serotonin–biosynthetic pathway, Trp hydroxylase, is unsaturated with its Trp substrate, brain Trp concentration is the most important single metabolic determinant of the rate of serotonin synthesis. Consequently, peripheral factors influencing circulating Trp availability to the brain play important roles in central serotonin synthesis. These include, at the primary level of control, activity of inhibition of TDO decreases catabolism of endogenous Trp in the body,elevate plasma Trp and thereby increase uptake by the brain. It will inturn increase the saturation of Trp hydroxylase, which remain unsaturated with its substrate under normal physiological conditions thus serotonin availability to the brain increases. A pharmacokinetically, inhibitor of TDO should also produce liver TDO compared to the transient effect of Trp administration, leading to greater antidepressant efficacy[70].

CHRONIC UNPREDICTABLE MILD STRESS

Stress is an important precipitant factor in depression, and the changes in various body systems that occurred in depression are similar to those observed in response to stress. Chronic unpredictable mild stress (CUMS), the most promising rodent model for depression, is widely used for preclinical testing and screening of antidepressants. In the CUMS paradigm, animals are subjected to a variety of mild stressors presented intermittently for prolonged periods of time, which mimic chronic stressful life events and result in a behavioral deficit anhedonia, a core symptom of human depression[71-73].

CUMS paradigm have been long used to model depression, and consists in the continuous exposure of animals to stressful situations, usually for at least 4 weeks, including

some stressors that involve water and /or food deprivation. In contrast, CUMS was originally used to study mechanisms underlying the stress-response and involves the intermittent exposure to a daily stressful stimulus, lasting at least 4 weeks, being one of the main advantages of this protocol the absence of stressors that interfere with water and/or food deprivation, which might better mimic everyday life stress. CUMS battery consisted of exposure to a variety of mild unpredictable stressors: water deprivation (24hr); hot air stream (10 min), cage tilt (45º) for 7hrs, soiled cage (1 h) inverted light cycle (24hr), physical restraint (2hrs) and tail pinch (1hr), food deprivation for 24 h, sawdust empty (24h), glare flash (3h), loud noise (3h) and empty bottle (24h).[74][75][76].

***Insilico* docking studies**

Docking

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Preferred orientation helps to predict the strength of association of or binding affinity between two molecules. The association with biological molecules such as protein, nucleic acids, carbohydrates and lipids play an important role in signal transduction i.e agonism or antagonism. So docking is a useful tool for predicting both the strength and type of signal produced [76][77].

Molecular docking may be defined as a computational ligand-target docking approach was used to analyze structural complexes of the target with ligand in order to understand the structural basis of this protein target specificity. Initially, protein-ligand attraction was investigated for hydrophobic/hydrophilic properties of these complexes docking were carried out by Autodock 4.2 options based on scoring functions. The energy interaction of ligand and protein was evaluated using atomic affinity potentials computed on a grid[78].

Various software used for docking studies are:

- AutoDock 4.2, Gold, Vega, Glide, Flexidock, Flex, Fred, Hint etc

Autodock 4.2

Autodock is a suit of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Autodock uses *Monte Carlo* method and simulated annealing in combination with genetic algorithm for building the possible conformations. The genetic algorithm is used for global optimizations. Autodock word in Linux platform. Cygwin is used as a user friendly interface. The local search method is energy minimization and Amber " force field" model helps in the evaluation of binding positions compatible with several scoring functions based on the free energy. The atomic affinity grids can be visualized. This is helpful to guide organic synthetic chemists to design better binders[78-80].

Application of Autodock

- Lead identification
- Lead optimization
- Structural elucidation by X-ray crystallography
- Virtual screening analysis
- Structure based drug desing
- Combinatorial library design[80]

DRUG PROFILE:- Myristicin

Chemical name	3-methoxy 4,5-methylenedioxyallybenzene
Formula	$C_{11}H_{12}O_3$
IUPAC	6-Allyl-4-methoxy-1,3-benzodioxole
Source	Myristica fragrans (Nutmeg)
Botanical name	*Myristica fragrans*
Family	*Myristicaceae*
Genus	*Myristica Gronov*
Species	Myristica fragrans Houtt
English	Nutmeg
Tamil	Jaadhikaai
Malayalam	Jaadhika

Fig 6: *Myristica fragrans* whole plant

Ethano medical information:

Myristica fragrans is known as "nutmeg", its extracts and essential oil are important in drug development with numerous pharmacological activities, *M. fragrans* has been used in traditional medicines as a carminative, stimulant, narcotic, emmenagogue and abortifacient nut meg is traditionally used as hepatoprotective, antibacterial, anti- inflammatory, antioxidant, cytotoxic and psychoactive. Nutmeg is also prescribed for the treatment of many diseases, such as rheumatism, muscle spasm, decreased appetite and diarrhea. Myristicin has recently been shown to have antioxidant, anticonvulsant, analgesic, anti-inflammatory, antidiabetic, antibacterial and antifungal activities and neuropharmacological properties[81,82]

Phytochemical Properties

M. fragrans seed consist of Sabinene (41.7), α-pinene (9.4%), β-pinene (7.3%), terpine-4-ol (5.8%), limonene (3.7%), safrole (1.4%) and myristicin (2.7%). Myristicin is isolated from trymyristicin it is a alkenylbenzene derivative[82].

Habitat

The genus *Myristica* comprises 72 tropical species occurring from Asia. It is an evergreen tree indigenous to the Moluccas, or Spice Islands, and exotic to Grenada, India, Mauritius, Singapore, South Africa, Sri Lanka, and United States of America. Indonesia and Grenada dominate world production and exports of both nutmegs and mace with a world market share

of 75 and 20%, respectively. Other producers include India, Malaysia, Papua New Guinea, Sri Lanka, and St. Vincent[82,83].

Traditional uses:-

Stomach cramps, rheumatism, nervousness, vomiting, whooping cough, and The nutmeg seed and mace were used as a stimulant, digestive, aphrodisiac, and post childbirth tonic nutmeg as a remedy for overeating, distended stomach, appetite stimulant, malaria, and madness, nutmeg butter, consisting of nutmeg oil and vegetable fat, was used as soap and as an outward application in cases of rheumatism and sprains. Nutmeg oil can also be rubbed on the temples to relieve headaches and that a drop of the oil in a cup of tea can be helpful against indigestion and vomiting and also used as psychoactive properties[82][83].

Reported activities

The reported activities of nutmeg are analgesics[83], anticancer[84], antiinflammatory[85], antimicrobial[85], antioxidant[86],and antidepressant[87].

Table: 2 LAVANDER OIL:

Botanical name	*Lavandula angustifolia*
Synonyms	*Lavandula Officinalis*
Family	Lamiaceae
Genus	Lavandula L.
Species	*Lavandula angustifolia Mill.*
English	Lavander
Tamil	Paneer malar
Malayalam	Lavander

Fig 7: Lavandula angustifolia

Ethanomedical information:

It has been used cosmetically and medicinally throughout world. In modern times, lavender is cultivated around the world and the fragrant oils of its flowers are used in aromatherapy, baked goods, candles, cosmetics, detergents, jellies, massage oils, perfumes, powders, shampoo, soaps, and teaAcne, alopecia and also possess analgesia, angioprotectant, anticolic, anticonvulsant, antidepressant, antiflatulant, antifungal, anti-inflammatory, antimicrobial, antioxidant, antipyretic, antiseptic, anxiety, appetite stimulant, asthma, balenotherapy (functional circulatory disorders), cholagogue, choleretic, chronic bronchitis, and treat diabetes, diuretic, douche, emmenagogue, gas, hangovers, hyptension, infertility, insect repellent, insomnia, lice, migraine, non-tubercular mycobacteria (NTM), parasitic infection, psychosis, rheumatism, Roehmheld's syndrome, rubefacient, toothache, varicose veins and vomiting[88].

Habitat:

Lavender is native to the Mediterranean, the Arabian Peninsula, Russia, Africa Spain, France, Italyand India[88].

Phytochemical properties

Phytochemical studies revealed that the major constituents of *Lavandula angustifolia* essential oil (LEO) are 1,8-cineole, camphor, and endo-borneol. Other components can also

be found in minor quantities, such as α-pinene, camphene,-pinene, β-pinene, p-cymene, limonene, terpinen-4-ol, cryptone so on[88].

Traditional use

Flower used as antiseptic, analgesic, anti rheumatic, anti spasmodic and oil used in treatment of nausea, bee sting, sleep aid, minor burn, eczema The topical use of lavender oil is very common in perfumes, cosmetics, and cleaning products [89,90].

Reported activity

Lavender is reported to possess anticonvulsant [89], anxiolytic [89], antioxidant, anti-inflammatory and wound healing [89]. Antimicrobial agent [90].

REVIEW OF LITERATURE

Xia *et al* ., (2018) examined the effects of lipopolysaccharide (LPS) challenge at different time points on CUMS-induced anxiety- and depression-like behaviors. At 1 day before, 18 or 35 days following the initial of Chronic unpredictable mild stress, mice were intraperitoneally given a single LPS (0.1 mg/kg). Neurobehavioral and biochemical studies were performed at the indicated time points. LPS challenge had different effects on CUMS-induced anxiety- and depression-like behaviors depending on the timing of stimulation. When given 1 day before CUMS, LPS restored brain-derived neurotrophic factor level and reversed anxiety- and depression-like behaviors. When given at 18 days after the initial of CUMS, LPS seemed to promote the immune response and even induce a slight exacerbation of neurobehavioral performance, although the difference did not reach statistical significance. Intriguingly, when given at the end of CUMS, LPS reversed some of the anxiety- and depression-like behavior. study highlights the complex interaction between stress and immune challenge.[91]

Deyama *et al* ., (2018) investigated the antidepressant effects of resolvin E3 (RvE3) in a mouse model of lipopolysaccharide (LPS)-induced depression. It was observed that LPS (0.8 mg/kg, i.p.) significantly increased immobility time on the tail suspension test, and this depression-like behavior was dose-dependently attenuated by intracerebroventricular infusion of RvE3 (10 or 100 ng). No effects of LPS or intracerebroventricular infusion of RvE3 on locomotor activity were observed. These results indicate that RvE3, as well as RvE1 and RvE2, have antidepressant effects.[92]

Arulmozhi ***et al*., (2018)** investigated the antidiabetic and antihyperlipidemic effects of myristica fragrans in animal models by using the hydroalcoholic extract of fruits of Myristica fragrans Houtt. (Myristicaceae) was investigated on chlorpromazine-induced glucose and triglyceride elevations in male Swiss albino mice. After 7 days of oral administration, the extract, at doses of 150 and 450 mg/kg, ameliorated the metabolic abnormalities caused by chlorpromazine as evidenced by significant reduction of glucose and triglyceride (TG) levels (maximal effect of 41% and 53% reduction of glucose and TG, respectively, at 450 mg dose, $P < 0.01$). The standard antidiabetic rosiglitazone at 10 mg significantly ($p < 0.01$) reduced the TG (63%) and glucose (40%) levels in this model, while the standard antidiabetic glimepiride has exhibited 55% and 16% reduction in TG and

glucose, respectively. In rats fed a high-cholesterol diet, *Myristica fragrans* extract significantly reduced theelevated TG (47% reduction at 450 mg, $p < 0.01$) and cholesterol (66.7% reduction at 450 mg, $p < 0.01$), and also exhibited a reduction in hepatic TG secretion aftertyloxapol administration. These data suggest that *Myristica fragrans* extract ameliorates hyperglycemia and abnormal lipid metabolism in animal models.[93]

Adell *et al.*,(2018) examined the signaling pathways responsible for the rapid antidepressant-like effects of a GluN2A-preferring NMDA receptor antagonist the duration of this behavioral effect as well as the molecular readouts involved. The results showed that NVP-AAM077 reduced the immobility in the forced swim test 30 min and 24 h after its administration. However, this effect waned 7 days later. The rapid antidepressant-like response seems to be associated with increases in the GluA1 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, mammalian target of rapamycin (mTOR) signaling, glia markers such as glial fibrillary acidic protein (GFAP) and excitatory amino acid transporter 1 (EAAT1), and a rapid mobilization of intracellular stores of brain-derived Neurotrophic factor (BDNF) in the medial prefrontal cortex[94]

Martins *et al.*, (2018) reviewed and summarized 110 medicinal plants and their phytochemical constituents that have been shown to possess antidepressant activity. It also highlights the various mechanisms of anti-depressant action of some of these plants and their plant parts like roots, stem, leaves, flowers, fruit or whole plant; phytochemical compounds showing anti-depressant activity such flavanoids, steroids, saponins, sugars, lectins, alkaloids, etc.; and various anti-depressant screening models used such as tail suspension test, forced swim test, chronic unpredictable stress test, sucrose preference test, monoamine oxidase inhibition assay, learned helplessness test, open field test, hole board test, etc.[95]

Gill *et al* ., (2017) evaluated the effect of a methanolic extract of *Saraca asoca* bark in rats exposed to chronic unpredictable mild stress (CUMS) daily for 8 weeks using a forced-swim test, an open-field test, and a sucrose-preference test. The effect of the extract on endogenous antioxidant levels in the brain was also assessed using catalase activity, superoxide dismutase activity, reduced glutathione levels, and malondialdehyde levels in the brain. Male *Sprague dawley* rats received 100 mg/kg (oral) of the extract daily 1 h before daily stress exposure for 8 weeks. The extract showed a significant reduction in the

immobility time in the forced-swim test, increased the total number of line crossing, rearing, and grooming in the open-field test, and increased the sucrose consumption as well as the levels of endogenous antioxidants significantly in comparison with the CUMS control group. Therefore, *Saraca asoca* might be a useful agent for the treatment or alleviation of symptoms associated with depression possibly by reducing CUMS-induced oxidative stress and reactive oxygen species in the brain.[97]

Megala *et al* .,(2017)studied anxiolytic- and antidepressant-like effects of a methanolic extract of *Morinda citrifolia Linn*. (noni) fruit (MMC) in well-established mouse models of anxiety and depression. The administration of MMC (1 g/kg, p.o.) and diazepam (1 mg/kg, i.p.) significantly attenuated anxiety-like behaviour in mice by increasing the percentage of time spent and number of entries in the open arms in the elevated plus maze (EPM), and significantly enhanced the exploration in the light box in the light/dark test (LDT). The pre-treatment with flumazenil (6 mg/kg, i.p.) or bicuculline (3 mg/kg, i.p.) or WAY 100635 (1 mg/kg, i.p.) antagonized the anxiolytic-like effect elicited by MMC (1 g/kg, p.o.). These results suggest the possible involvement of benzodiazepine-$GABA_A$ergic and serotonergic mechanisms in the anxiolytic-like effect of noni fruit., The administration of MMC (0.5 and 0.75 g/kg, p.o.) and desipramine (30 mg/kg, i.p.) significantly reduced the duration of immobility in the tail suspension test (TST). Furthermore, pre-treatment of mice with 4-chloro-DL-phenylalanine methyl ester hydrochloride (PCPA; 100 mg/kg, i.p., an inhibitor of serotonin synthesis) for four consecutive days or a single dose of WAY 100635 (1 mg/kg, i.p., $5HT_{1A}$ receptor antagonist) or α-methyl-DL-tyrosine (100 mg/kg, i.p., an inhibitor of noradrenaline synthesis) significantly reversed the anti-immobility effect of MMC (0.5 g/kg, p.o.) in TST by indicating the specific involvement of the serotonergic and noradrenergic systems in the antidepressant-like effect of noni fruit.[98]

Timothy *et al.*, (2017) examined the neurophysiological and behavioural effects of lavender oil in rats with experimentally induced anxiety.The experimental rats were divided into five groups, which respectively received inhalation of saline, 1.25% lavender oil, 2.5% lavender oil, chlordiazepoxide (CDP), and 2.5% lavender oil co- administered with CDP. Anxiety was induced in rats using animal models including elevated plus maze and open field. The levels of serotonin in the pre-frontal cortex and striatumof the rats, the anxiolytic effects of lavender oil and its augmentation effect as to co-administration with CDP were

evaluated. The neurophysiological findings showed that groups receiving lavender oils, CDP, and 2.5% lavender oils co-administered with CDP had significantly higher level of serotonin in the pre-frontal cortex. However, the anxiolytic behavioural effects of lavender oil were found to have mixed results. This study provided preliminary evidence that inhalation of lavender oil paralleled effects of CDP in up-regulating synthesis of serotonin in rat pre-frontal cortex, and the co-administration of CDP with 2.5% lavender oil tended to augment effect of CDP on serotonin in their pre-frontal cortex and striatum.[99]

Agric *et al* ., (2017) studied the hepatoprotective effect of myristicin from nutmeg (*Myristica fragrans*) on lipopolysaccharide/D-galactosamine-induced liver injury. About 21 different spices were fed to rats with liver damage caused by lipopolysaccharide (LPS) plus D-galactosamine (D-GalN). As assessed by plasma aminotranferase activities, nutmeg showed the most potent hepatoprotective activity. Bioassay-guided isolation of the active compound from nutmeg was carried out in mice by a single oral administration of the respective fractions. Myristicin, one of the major essential oils of nutmeg, was found to possess extraordinarily potent hepatoprotective activity. Myristicin markedly suppressed LPS/D-GalN-induced enhancement of serum TNF-R concentrations and hepatic DNA fragmentation in mice. These findings suggest that the hepatoprotective activity of myristicin might be, at least in part, due to the inhibition of TNF-R release from macrophages. However, further studies are needed to elucidate the hepatoprotective mechanism(s) of myristicin.[100]

Maciel *et al*., (2017) examined the depression-like behaviour in the classical model of chronic inflammation induced by Complete Freund's Adjuvant (CFA). Male Swiss mice received an intraplantar (i.pl.) injection of CFA (50 µl/paw) or vehicle. Behavioural and inflammatory responses were measured at different time-points (1 to 4 weeks), and different pharmacological tools were tested. The brain levels of IL-1β and BDNF, or COX-2 expression were also determined. CFA elicited a time-dependent edema formation and mechanical allodynia, which was accompanied by a significant increase in the immobility time in the tail suspension (TST) or forced-swimming (FST) depression tests. Repeated administration of the antidepressants imipramine (10 mg/kg), fluoxetine (20 mg/kg) and bupropion (30 mg/kg) significantly reversed depression-like behaviour induced by CFA. Predictably, the anti-inflammatory drugs dexamethasone (0.5 mg/kg), indomethacin (10

mg/kg) and celecoxib (30 mg/kg) markedly reduced CFA-induced edema. The oral treatment with the analgesic drugs dipyrone (30 and 300 mg/kg) or pregabalin (30 mg/kg) significantly reversed the mechanical allodyinia induced by CFA. Otherwise, either dipyrone or pregabalin (both 30 mg/kg) did not significantly affect the paw edema or the depressive-like behaviour induced by CFA, whereas the oral treatment with dipyrone (300 mg/kg) was able to reduce the immobility time in TST. Noteworthy, CFA-induced edema was reduced by bupropion (30 mg/kg), and depression behaviour was prevented by celecoxib (30 mg/kg). The cotreatment with bupropion and celecoxib (3 mg/kg each) significantly inhibited both inflammation and depression elicited by CFA. The same combined treatment reduced the brain levels of IL-1β, as well as COX-2 immunopositivity, the combination of antidepressant and anti-inflammatory agents bupropion and celecoxib might represent an attractive therapeutic strategy for depression.[101]

Dobosa *et al*., (2016) determined whether neuroinflammation-induced increased indoleamine 2,3-dioxygenase levels in the mammalian brain will lead to depressive-like behavior. Neuroinflammation was initiated in mice by a single intracerebroventricular injection of lipopolysaccharide (LPS). Cerebral inflammation was monitored 1, 2, 3 and 4 days after the injection with small-animal positron emission tomography (PET) using the inflammatory marker [11C]-PK11195. In the presence or absence of systemically applied 1-methyl-tryptophan (1-MT), a competitive IDO-inhibitor, the development of depressive-like behavioral symptoms was assesed parallel with IDO expression and activity. The PK11195 PET signal reached a highly significant peak 3 days after LPS injection, while these animals displayed a significant increase of depressive-like behavior in the forced swim test compared to vehicle-injected animals. These findings were paralleled by a significant increase of IDO in the brainstem, and an increased kynurenine/tryptophan ratio in the serum. Inhibition of IDO by 1-MT in centrally induced neuroinflammation under experimental conditions can prevent the development of depressive-like behavior.[102]

Mori *et al* ., (2016) explored the wound healing potential of lavender oil by acceleration of granulation and wound contraction through induction of TGF-β in a rat model. Topical application of lavender oil promoted collagen synthesis and differentiation of fibroblasts, accompanied by up-regulation of TGF-β. These data suggest that lavender oil has the potential to promote wound healing in the early phase by acceleration of formation of

granulation tissue, tissue remodeling by collagen replacement and wound contraction through up-regulation of TGF-β. The beneficial effect of lavender oil on wound healing may raise the possibility of new approaches as complementary treatment besides conventional therapy.[103]

Jaiswal *et al*., (2016) examined the biological effects *of Myristica fragrans*. The chemical constituents of *M. fragrans* have been investigated for hypolipidaemic and hypocholesterolemic effects, antimicrobial, antidepressant, aphrodisiac, memory enhancing, antioxidant and hepatoprotective properties. Recent studies have revealed strong insecticidal and molluscicidal activities of *M. fragrans*. Despite some laboratory studies on the insecticidal / molluscicidal activity of *M. fragrans*, more field studies are recommended for effective control of pests. It is clearly evident from the literature review that *M. fragrans* deserves more attention by scientific community and public health specialists to explore its full range of benefits in the welfare of the society.[104]

Barua *et al*., (2016) evaluated the effect of hexane extract of *Z. alatum* seeds (ZAHE) on lipopolysaccharide (LPS)induced depression-like behaviour in Swiss albino mice.Mice were treated with ZAHE (100 and 200 mg/kg, p.o.) and imipramine (10 mg/kg injected i.p.) for 14 days. On 14th day of the treatment, depression-like behaviour was induced by LPS(0.83 mg/kg injected i.p.) and after 24 h of LPS administration, it was assessed by measuring behavioural parameters and biochemical estimations. Behavioural tests, including the open field test, forced swimming test, tail suspension test and sucrose preference test revealed that ZAHE (100 and 200mg/kg, p.o.) and imipramine (10 mg/kg injected i.p.) alleviated the depression symptoms of LPS-induced mice. Moreover, ZAHE treatments reversed the LPS-induced alterations in the concentrations of norepinephrine and serotonin (5-HT) and inhibited the expression of brain-derived neurotrophic factor, pro-inflammatory cytokines and oxido-nitrosative stress in the mice. Acute toxicity was calculated to be LD50 >2500mg/kg. This study showed that LPS-induced depression in mice was significantly prevented by ZAHE at both the dosages. ZAHE exhibited an antidepressant activity by altering monoaminergic neurotransmitters in the brain combined with its anti-inflammatory potential. Thus, it could be an effective therapeutic against inflammation-induced depression and other brain disorders.[125]

Umukoro *et al*., (2015) investigated that the antidepressant-like activity of methyl jasmonate involves modulation of monoaminergic pathways in mice the role of monoaminergic systems in the antidepression-like activity of methyl jasmonate. Mice were given i.p. injection of MJ (5, 10 and 20 mg/kg), imipramine (10 mg/kg) and vehicle (10 mL/kg) 30 min before the forced swim test (FST) and tail suspension test (TST) were carried

out. The involvement of monoaminergic systems in the anti-depressant-like effect of MJ (20 mg/ kg) was evaluated using p-chlorophenylalanine (pCPA), metergoline, yohimbine, prazosin, sulpiride and haloperidol in the TST. MJ significantly decrease the duration of immobility in the FST and TST relative to control suggesting antidepressant-like property. Pretreatment with yohimbine (1 mg/kg, i.p., an a2- adrenergic receptor antagonist) or prazosin (62.5 mg/kg, i.p., an a1-adrenoceptor antagonist) attenuated the antidepressant-like activity of MJ. Also, pCPA; an inhibitor of serotonin biosynthesis (100 mg/kg, i.p) or metergoline (4 mg/kg, i.p., 5-HT2 receptor antagonist) reversed the anti-immobility effect of MJ. Sulpiride (50 mg/kg, i.p., a D2 receptor antagonist) or haloperidol (0.2 mg/kg, i.p., a dopamine receptor antagonist) reversed the anti-immobility effect of MJ the results of this study suggest that serotonergic, noradrenergic and dopaminergic systems may play a role in the antidepressant-like activity of MJ.[120]

Elgarf *et al* ., (2014) studied the effect of combined exposure to repeated challenge using low doses of lipopolysaccharide (LPS) and chronic mild stress (CMS) together. This combined exposure is thought to expose the animals to more realistic challenges, testable on different level (behavioral, neurochemical, immunohistochemical and gene expression). The role of glial cells was examined, as well. Additionally, the effects of chronic administration of the tricyclic antidepressant imipramine and the anti-TNF-α pentoxyphylline were investigated.[112]

Al- Shaimma *et al*., (2014) studied the effect of combined exposure to repeated challenge using low doses of lipopolysaccharide (LPS) and chronic mild stress (CMS) together. This combined exposure is thought to expose the animal to more realistic challenges, teastable on different level (behavioral, neurochemical, immunohistochemical and gene expression).The role of glial cells was examined, as well the effect of chronic administration of the tricyclic antidepressant imipramine and the anti TNF-α pentoxyphylline were investigated.[114]

Asgarpanah *et al*., (2012) examined the phytochemistry and pharmacologic properties of *Myristica fragrance* its extracts and essential oil are important in drug development with numerous pharmacological activities and also used as tropical stimulant, narcotic. Nutmeg is also prescribed for the treatment of many diseases, such as rheumatism,

muscle spasm, decreased appetite and diarrhea. *M.fragrance* possess antioxidant, anticonvulsant, analgesic, anti-inflammatory, antidiabetic, antibacterial and antifungal activities. Trimyristicin, myristic acid, myristicin, safrole and elimicin are obtained from nutmeg[115]

Esteves *et al*., (2011) evaluated the effect of *Lavandula angustifolia* Mill. essential oil (LEO) on acute inflammatory response. LEO was analyzed using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR) methods and showed predominance of 1,8-cineole (39.83%), borneol (22.63%), and camphor (22.12%). LEO at concentrations of 0.5, 1, 3, and 10 μg/ml did not present *in vitro* cytotoxicity. LEO did not stimulate the leukocyte chemotaxis *in vitro*.The LEO topical application at concentrations of 0.25, 0.5, and 1mg/ear reduced edema formation, myeloperoxidase (MPO) activity, and nitric oxide (NO) production in croton oil-induced ear edema model. In carrageenan-induced paw edema model, LEO treatment at doses of 75, 100, and 250 mg/kg reduced edema formation, MPO activity, and NO production. In dextran-induced paw edema model, LEO at doses of 75 and 100mg/kg reduced paw edema and MPO activity. In conclusion, LEO presented anti-inflammatory activity, and the mechanism proposed of LEO seems to be, at least in part, involving the participation of prostanoids, NO, proinflammatory cytokines, and histamine.[116]

Browne *et al*., (2010) investigated the differential lipopolysaccharide induced immune alteration in the hippocampus of two mouse strains two strains which differ in stress susceptibility, namely the BALB/c and C57BL/6J mice, would respond differentially to LPS and swim-stress in cytokine profile, corticosterone concentrations and mRNA expression of genes coding for the tryptophan metabolising enzymes, IDO1, IDO2, Tph1 and Tph2. The stress-sensitive BALB/c strain exhibited increased depressive-like behaviour and enhanced corticosterone concentrations in response toLPS.Furthermore, swimstress attenuated the LPS-induced corticosterone response in BALB/c mice only. LPS significantly increased plasma interleukin (IL)-1b and tumour necrosis factor a (TNFa) concentrations to a greater extent in BALB/c mice. The LPS-induced increase in IL-1b mRNA expression was significantly attenuated by swim-stress in the hippocampus of C57BL/6J but not in BALB/c mice. TNFa mRNA expression was significantly increased in BALB/c mice only; this increase was attenuated by swim-stress. Tph1 mRNA expression was upregulated in the brainstem of

C57BL/6J mice post-LPS and following the combination of swimstress and LPS in BALB/c mice. In the hippocampus Tph1 and Tph2 mRNA expression was increased in C57BL/6J but not BALB/c mice in response to LPS challenge and swim-stress. IDO2 but not IDO1 mRNA expression was significantly altered following swim-stress and LPS, particularly in the hippocampus of BALB/c mice. These data indicate altered central mRNA expression of tryptophan metabolising enzymes and immune activation in BALB/c mice compared to the normo-sensitive C57BL/6J strain.[117]

Lewis *et al*., (2009) studied the inhibition mechanisms of human indoleamine 2,3 dioxygenase**.** Human indoleamine 2,3-dioxygenase 1 (hIDO1) and tryptophan dioxygenase (hTDO) catalyze the same dioxygenation reaction of Trp to generate N-formyl kynurenine (NFK). They share high structural similarity, especially in the active site. However, hIDO1 possesses a unique inhibitory substrate binding site (Si) that is absent in hTDO. In addition, in hIDO1, the indoleamine group of the substrate Trp is H-bonded to S167 through a bridging water, while that in hTDO is directly H-bonded to H76. Here we show that Trp binding to the Si site or the mutation of S167 to histidine in hIDO1 retards its turnover activity, and that the inhibited activity can be rescued by an effector, 3-indole ethanol (IDE). Kinetic studies reveal that the inhibited activity introduced by Trp binding to the Si site is a result of retarded recombination of the ferryl moiety with Trp epoxide to form NFK, and that IDE reverses the effect by preventing Trp from binding to the Si site. In contrast, the abolished activity induced by the S167H mutation is primarily a result of ~5000-fold reduction in the O2 binding rate constant, possibly due to the blockage of a ligand delivery tunnel, and that IDE binding to the Si site reverses the effect by reopening the tunnel. The data offer new insights into structure-based design of hIDO1-selective inhibitors.[118]

Griglio *et al*., (2007) determined a multicomponent approach in the discovery of indoleamine 2,3- dioxygenase 1 inhibitors: Synthesis, biological investigation and docking studies. Indoleamine 2,3-dioxygenase plays a crucial role in immune tolerance and has emerged as an attractive target for cancer immunotherapy multicomponent reactions have been employed to assemble a small library of imidazothiazoles that target IDO1. While the p-bromophenyl and the imidazothiazole moieties have been kept fixed, a full SAR study has been performed on the side-chain, leading to the discovery of nine compounds with sub-micromolar IC50 values in the enzyme-based assay. Compound 7d, displaying a a-

acyloxyamide substructure, is the most potent compound, with an IC50 value of 0.20 mM, but a low activity in a cell-based assay. Compound 6o, containing a a-acylaminoamide moiety, shows an IC50 value of 0.81 mM in the IDO1-based assay, a full biocompatibility at 10 mM, together with a modest inhibitory activity in A375 cells. Molecular docking studies show that both 7d and 6o display a unique binding mode in the IDO1 active site, with the side-chain protruding in an additional pocket C, where a crucial hydrogen bond is formed with Lys238.[111]

Samina *et al*., (2006) evaluated the binding of *Hypericum perforatum* (HP) against IDO enzyme using Virtual Docker software acute and chronic effects of HP on IDO enzyme activity. Docking results show that HP fit well in the allosteric site of IDO. Energy scores for HP -158.687 Kcal/mol. Administration of HP (500mg/kg/3ml) shows that serum IDO activity was significantly increased (171%, P<0.01) and (114%, P<0.01) respectively after acute and chronic treatment. Brain IDO activity was decreased by 42%, (P<0.01) after acute and 43% (P<0.01) chronic treatment. It is concluded from the present study that HP is noncompetitive inhibitor of IDO as proofs by docking further its inhibitory effects on brain IDO reveals its anti-inflammatory effect.[119]

Dawood *et al* ., (2003) examined the docking studies of antidepressants against single crystal structure of tryptophan 2, 3-dioxygenase using Virtual Docker software. Tryptophan 2, 3-dioxygenase (TDO) a heme containing enzyme found in mammalian liver is responsible for tryptophan (Trp) catabolism. Trp is an essential amino acid that is degraded in to N-formylkynurenine by the action of TDO.The binding of established antidepressants (ADs) against TDO enzyme using in-silico docking studies for this purpose, Fluoxetine, Paroxetine, Sertraline, Fluvoxamine, Seproxetine, Citalopram, Moclobamide, Hyperforin and Amoxepine were selected. In-silico docking studies were carried out using Virtual Docker software. Docking results show that all ADs fit well in the active site of TDO moreover Hyperforin and Paroxetine exhibited high docking scores of -152.484k cal/mol and -139.706k cal/mol, respectively. It is concluded that Hyperforin and Paroxetine are possible lead molecules because of their high docking scores as compared to other ADs examined. Therefore, these two ADs stand as potent inhibitors of TDO enzyme.[126]

AIM AND OBJECTIVE

Depression has a high lifetime prevalence of 21% and it is among the severe psychiatric disorder. Lower rates of fertility can occur with depression. Major depressive disorder is a chief cause of disability worldwide, by the year 2020. Women are about twice as likely to suffer from a major depressive event as men. In a year, about 1 million lives are lost due to suicide i.e. 3000 suicide deaths every day. This lethal disorder can be treated with antidepressants. Intracellular enzyme indoleamine 2,3 dioxygenase (IDO), which catalyzes tryptophan catabolism through the kynurenine pathway. The depletion of tryptophan in brain cells reduces the production of brain serotonin (5-HT). The degradation of tryptophan along the kynurenine pathway also generates neurotoxins, like quinolinic acid (QUIN), or 3-hydroxykynurenine (3-HK), which leads to apoptosis in neurons, which can add to local excitotoxic neuronal overstimulation next to modulating serotonergic neurotransmission. Cytokine-induced IDO-mediated tryptophan depletion and quinolinic acid mediated neurotoxicity are hypothesized to be involved in the pathophysiology of mood disorders, like major depression.

Tryptophan 2, 3-dioxygenase (TDO) is cytosolic heme dioxygenase that catalyzes the oxidative cleavage of the C2-C3 bond of the indole ring of L-tryptophan (Trp). This reaction is first and rate-limiting step of the kynurenine pathway of tryptophan catabolism, which eventually leads to the formation of nicotinamide dinucleotide (NAD+), a process regarded as the primary biological function of TDO. Trp availability to the brain also play important role in central 5-HT synthesis. The increased cortisol induces TDO leading to decreased brain serotonin (5- hydroxytrptamine) which is also considered as anetiological factor for depression. Systemic increase in LPS is associated with systemic inflammation, resulting in production of cytokines, including IL-1β, IL-6, IFN-γ), and TNF-α. These peripheral inflammation signals may be transmitted to the brain through humoral and neural pathways, produce neuroinflammation leading to depression.

The aim of this study is to carryout for the phytoconstituents present in the lavandar oil **(**α-pinene, D- limolene, eucalyptol, linalyl acetate, camphora, α- terpinol, linalol etc.) and myristicin against indoleamine 2,3 – dioxygenase and tryptophan 2,3-dioxygenase using autodock 4.2

Pharmacological studies have confirmed the use of myristicin from *Myristica fragrans* extracts in different central nervous system (CNS) diseases arachnoidcysts, (Attention deficit/hyperactivity disorder, autism, bipolar disorder, catalepsy, depression,

encephalitis, epilepsy, meningitis, multiple sclerosis, antidiarrheal, antimicrobial, antioxidant, anxiogenic activity. The therapeutic and biological activities of *Lavandula angustifolia* essential oil are anticonvulsant, anxiolytic, antioxidant , anti inflammatory and antimicrobial activities. The objective of the study is to examine the synergistic activity of lavender oil and myristicin in treatment of depression in rats exposed to repeated challenge using lipopolysaccharide (LPS) and chronic unpredictable mild stress (CUMS). This combined exposure is thought to expose the animal to more realistic challenge, testables on different levels (behavioral and neurochemical).

PLAN OF WORK

- Literature review
- Selection of myristicin and active constituent from lavender oil and determination of IDO inhibitory activity
- Determination of bioactivity score by molinspiration
- Molecular docking studies using Autodock 4.2 based on bioactivity score
- Isolation of trimyristin from nutmeg
- Isolation of myristicin from trimyristin
- Spectral analysis of myristicin
- *In vivo* antidepressant activity of myristicin and lavander oil against lipopolysaccharide combined with chronic mild stress induced depression in rat
- Dissection and preparation of tissue homogenate using brain for biochemical estimation
- Histopathology of brain
- Tabulation of result and statistical analysis of data obtained

MATERIALS AND METHODS

In Silico binding interaction studies

Softwares and data bases used

- Accerlys discovery studio viewer 4.0.1
- Molinspiration
- RCSB protein data bank
- Online SMILES translator
- MGL tools-
    - AutoDock 4.2
    - Python 2.7 molecule viewer 1.5.6
    - Vision 1.5.6
    - Cygwin 64
- ChemSketch
- PreADMET

Evaluation of drug likeness properties

Pharmacologically active substituents were characterized by calculating steric, hydrophobic, electronic, and hydrogen bonding properties as well as by the drug-likeness score. The theory of drug-likeness score helps to optimize pharmaceutical and pharmacokinetic properties, for example, chemical stability, solubility, distribution profile and bioavailability. The molecular descriptors have developed as rationally predictive and informative, for example, the Lipinski's Rule-of-Five (Ro5). The better oral absorption of the ligands and drug likeness scores were constructed by getting information about the solubility, diffusion, Log P, molecular weight etc. Molinspiration software was used to evaluate the Lipinski's rule of five.

Lipinski's Ro5 calculations

1) Open the molinspiration home page (http://www.molinspiration.com/).
2) For calculating using molinspiration, it requires JAVA in the computer.
3) Click calculation of molecular properties of drug likeness.
4) Draw the structure of flavonoids in the active window.
5) Click calculate properties and predict bioactivity.
6) Save the properties.

Evaluation of admet properties

The ADMET studies was performed by using the software PreADMET. From this absorption, distribution, metabolism, of the selected ligands was evaluated. The 2D structure was directly introduced into PreADMET to carry out ADMET screening by using ChemSketch software. The data for descriptors are blood brain barrier (BBB), plasma protein binding, aqueous solubility, skin permeability, hepatotoxicity etc. After loading the structure through the function model various descriptors were tabulated [113,118].

***In Silico* docking study on indoleamine 2,3 - dioxygenase and tryptophan 2,3-dioxygenase using autodock 4.2**

Step 1

Ligand file format conversion

- The desire ligand are drawn in ChemSketch.
- Tools→clean structure.
- Tools→ generate→SMILES notation.
- Copied the smile notation and uploaded the smiles in online smile translator-cactus.nci.nih.gov/services/translate.
- By choosing the required file format and save the file as pdb format.

Step II

Protein structure refinement

The enzymesIndoleamine 2,3- dioxygenase , were downloaded from RCSB (Research Co-laboratory for Structural Bioinformatics) Protein Data Bank and the protein was refined before use for docking.

- Opened Accelrys discovery studio viewer.
- File→open→RCSBPDB file.
- View→hierarchy→click water molecules→select all water molecules → delete.
- Selected ligand, which was unnecessary and deleted.
- Saved the molecule in a desired location.

Step III

Docking with autodock 4.2

- Opened the refined protein from the location in pdb format.
- Preparation of target and ligand in AutoDock 4.2

Step IV

Preparation of protein

- AutoDock 4.2 → File → Read molecule → Choose refined enzyme file.

Elimination of water molecule carried out by:

- Select → Select from string → Residue (*HOH*) → Add → Dismiss.
- Edit → Hydrogen → Add → Polar only → Ok.
- Edit → charges → Add kollmann charges → Ok.
- File → save → Write pdb → Browse → Save → Ok.
- Edit → Delete all molecules → Continue.

Step V

Preparation of ligand

- Ligand → input → open.
- Ligand → torsion tree → detect root.
- Ligand → torsion tree → show root expansion.
- Ligand → torsion tree → choose torsions → done.
- Ligand → torsion tree → set number of torsions → dismiss.
- Ligand → torsion tree → hide root expansion.
- Ligand → torsion tree → show/hide root marker.
- Ligand → output → save as pdbqt file.
- Edit → delete → delete all molecules → continue.

Conversion of pdb files of protein in to pdbqt file

- Grid → Macromolecule → Open → Save as pdbqt.

AutoGrid calculation and creating "gpf" file

- Grid → set map types → open ligand.
- Grid → grid box → set 60 points in XYZ.
- File → close saving current.
- Grid → output → save as gpf.
- Edit → delete → delete all molecules → continue.

Autodock calculation and creating 'dpf' file

- Docking → macromolecule → set rigid file name → open.
- Docking → ligand → open → accept.
- Docking → search parameters → genetic algorithm → accept.
- Docking → docking parameters → accept.

- Docking→output→ lamarckian genetic algorithm→save as dpf.

Programming of 'Auto Grid' and 'Auto Dock' execution

Open Cygwin64 and type as given below:

- cd C:
- cd cygwin64
- cd usr
- cd local
- cd bin

Program should list out the pdb, pdbqt, gpf and dpf files of an enzyme and ligand molecule.

Then type as:

- ./autogrid4.exe<space>–p<space>ligand.gpf<space> -l<space>ligand.glg

  If a ligand gets into the spacing of the grid, then the execution of this command was;

- ✓ '*Successful completion'*

Then type as:

- ./autodock4.exe<space> –p<space>ligand.dpf<space> -l<space>ligand.dlg

  If the ligand binds to the amino acids through 10 different conformations, then the execution of this command was;

- ✓ *'Successful completion'*

Step VI

Viewing docking results

Reading the docking log file.dlg

- Toggle the AutoDock Tools button.
- Analyse→Docking.
- Analyse→Conformations → Load.
- Double click on the conformation for to view it.

Visualizing docked conformations

- Analyse→Dockings→Play.
- Load dlg file.
- Choose the suitable conformations.
- Analyse→Docking→Show Interactions.

Obtaining snap shots of docked pose

- File→Read Molecule.
- Analyse→Dockings→Open dlg file.
- Analyse→Macromolecule→Choose pdbqt file.
- Analyse→Conformations→Load.
- Double click the desired conformation.
- Analyse→Docking→Show Interactions.

Proteins and ligand interaction was displayed. Zoom it and increase the contrast by holding right key and ctrl. Rapid energy evaluation was attained by pre-calculating the atomic resemblance potentials for each atom in the selected compounds. In the AutoGrid process, the target was enclosed on a three dimensional grid point and the energy of interface of the each atom in the compounds were encountered. The following docking factors were chosen for the Lamarckian genetic algorithm as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, and number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The probability of performing local search on a single compound in the population was set to 0.06. AutoDock was run various times to obtain various docked conformations, and used to calculate the predicted binding energy [119].

Table: 3 REAGENTS AND CHEMICALS USED

S.NO	Name of chemical/kit	Name of the supplier
1.	lipopolysaccharide from *E.coli*	Sigma Aldrich,USA
2.	3- Nitro –L- tyrosine	Sigma Aldrich,USA
3.	Lavender oil	P.Sridhar & co,ootty
4.	P-Nitroso-N,N-Dimethylaniline	Sigma Aldrich,USA
5.	Sulfosalicylic acid	Sigma Aldrich, USA
6.	5-Hydroxy tryptamine	Sigma Aldrich, USA
7.	Benzylamine	Sigma Aldrich, USA
8.	Agappe diagnostic kit for SGOT&SGPT	Agappe Diagnostic Ltd, Kerala
9.	Agappe diagnostic kit for triglycerides	Agappe Diagnostic Ltd, Kerala
10.	Agappe diagnostic kit for cholesterole	Agappe Diagnostic Ltd, Kerala

INSTRUMENTS / EQUIPMENTS USED

Semi auto-analyzer (Agappe Diagnostics Ltd., Mumbai), Centrifuge (Remi Instruments Ltd., Kolkata), Digital balance (Sartorius Ltd., USA), Eppendorf minispin, Incubator (Technico), Jasco FTIR-420 series, Shimadzu-Jasco V-630 UV/Vis Spectrophotometer, ELCO 1/27 pH meter, .High perfomance liquid chromatography, Reflex condenser , Coloumn chromatography, IR spectrometer[120,121]

PLANT MATERIAL

Collection and authentification

The seeds of Myristica fragrans have been collected from kerala. The plant was identified and authenticated by Dr.C Murugan, Scientist 'D', Botanical Survey of India, Tamilnadu Agricultural University (TNAU), Coimbatore, India and voucher specimen has been given the code BSI/SRC/5/23/2018/Tech/2418.

Isolation of myristicin from trimyristicin

A). Isolation of Trimyristin

About 30 g of crushed nutmeg and 200 ml chloroform were refluxed for 90 min on a water bath, filtered through a folded filter paper, and dried on calcium chloride. The chloroform solution was then filtered, and the solvent was distilled under reduced pressure, leaving a semisolid residue, which was dissolved in 200ml ethanol (95%). On cooling,

crystalline trimyristine precipitates and is filtered off with suction and washed with cold ethanol (95%). The crystals are colorless and odorless and melted at 54 to 55ºc and the yield determined 6g. The filtrate and washings were kept and used for the isolation of myristicin.[121]

B. Isolation of myristicin

On concentration of the mother liquor remaining after separation of trimyristin, a residue was obtained, which was dissolved in 20 ml petroleum ether and passed through a short column containing 10 g activated alumina. Elution with 150 ml petroleum ether and evaporation of solvent leaves an oil, which was fractionally distilled.[121]

***In Vivo* method**

Experimental animals

Male *Wistar* rats weighing 200-250 g were used for lipopolysaccharide and chronic mild stress induced depression study. The rats were procured from College of Veterinary and Animals Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India. The study protocol was approved by the Institutional Animal Ethical Committee (COPSRIPMS/IAEC/PG/PHARMACOLOGY/ 001/2018-19) and all procedures were performed in accordance with the recommendations for the proper care and use of laboratory animals.

Housing and feeding conditions

The animals were acclimatized at 12h light – dark cycle, temperature (22 ± 3 ^{O}C) 50-60% relative humidity. Animals were provided with standard pellet, feed and drinking water *ad libitum*.

Lipopolysaccharide and chronic mild stress - induced depression in rats

Male *wistar* rats were divided into 8 groups comprising 6 animals in each group.

Group 1: Treated with coconut oil 10 ml/kg

Group II : Treated with LPS i.p 50µg/ml for 2 week and CUMS for 4 week

Group 11I: Treated with impiramine (20 mg/kg) as standard for 6 weeks

Group IV: Treated with Lavender oil 300 mg for 6 weeks

Group V: Treated with Lavender oil 600 mg for 6 weeks

Group VI Treated with Myristicin 100 mg for 6 weeks

Group VII: Treated with Myristicin 200 mg for 6 weeks

Group VIII : Treated with combination of Lavender oil 300 mg and Myristicin 100 mg for 6 weeks

Group IX:. Treated with combination of Lavender oil 150 mg and myristicin 50 mg for 6 weeks

The dose for lipopolysaccharide was fixed as (50 µg/ml) based on works carriedout by various authors[122]. The dose for myristicin (100 and 200 mg/kg) [123] and lavender oil (300 and 600 mg/kg)[124] was fixed based on works reported.

Induction of depression

Wistar rat in group I-VIII was exposed to LPS (50 µg/kg i.p) over 2 weeks and CUMS protocol for 4 weeks with a total 6 weeks for induction of depression. Rats were examined for behavioral studies once weekly for 6 weeks.

Chronic Unpredictable Mild Stress (CUMS)

CUMS battery consisted of exposure to a variety of mild unpredictable stressors. Each stress regimen was carried out for 2 periods with following stressors: water deprivation for (24h), hot air stream for (10 min), cage tilt (45º) for 22 h, inverted light cycle (24 h), Physical restraint (20 min) and tail pinch (1h) and Soiled bedding for (22 h).all the animal was exposed to training session prior to start of the behavioural studies[125].

Table: 4 Chronic unpredictable mild stress regimens

Day	Water deprivation	Hot air stream	Cage tilt	Soiled cage	Inverted light cycle	Physical restraint	Tail pinch
1	24 h						
2		10 min					
3			22 h				
4				22 h			
5					24 h		
6						20 min	
7							1 h

Behavioral studies

1. **Sucrose preference test**

Sucrose preference test was carried out at the end of CUMS exposure. Before the test, rats were trained to adapt to sucrose solution (1% w/v): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation, rats were deprived of water and food for 24 h. Sucrose preference test was conducted by housing rats in individual cages and was allowed free to access to two bottles containing 100 ml of sucrose solution and water were recorded, and the sucrose preference was calculated.[126]

2. **Tail suspension test**

Animals were transported from housing room to the the testing area in their own cage and allowed to adapt to the new environment for 1h before testing. For the test animal are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 5 min. animals were considered immobile when they hang passively and completely motionless for at least 1 min[127].

3. **Spatial reference memory**

Morris water maze test

The water maze consist of a circular tank with 100 cm diameter and a wall 20 cm above the water level. A circular platform is hidden 2 cm below the water level. Training takes place on three consecutive days, with the rats receiving 4 consecutive trials per days, withan inter trial interval of 6-10 min. Each trial is started from one of four assigned polar position with different sequence each day , The lattency to find the platform is measured as the time of placement of rat in the water to the time it find the platform. If the animal fails to find the platform in any trial within 3 min it is placed on it for 10 s[123].

4. Depression like Behavior

Forced swimming test (FST)

Rats were placed in a vertical glass cylinder (diameter 22.5 cm, height 60 cm) containing 35 cm of water maintained at 25 °C. After OFT, rats were trained to swim for 15

min and 24 h later, they were re-exposed for 5 min. Behavior was videotaped and immobility time was measured (the time during which the animal floated with its front paws together and makes only those movements which were necessary to keep it afloat) Depressive behavior was inferred from "despair/helplessness" as indicated by increased duration of immobility. The test was performed on each rat only once and 24 h before sacrificing the animal[123]

5. Anxiety Behavior

Elevated plus maze (EPM)

The plus – maze consists of two open arm 50x10x40 cm and two closed arm 50x10x40cm with an open roof, The maze is elevated to a height of 50 cm. The rats ,aree housed in pairs for 10 days prior to testing in the apparatus. Group consist of 6 rats for each dose. 30min after i.p administration of test drug or the standard, the rat is placed in the center of the maze, facing one of the enclosed arms. During a 5 min test period the following measures are taken the number of entries into and time spent in the open and closed arms[123].

6. **Social interaction test**

Animals are housed in groups of 5 animals The apparatus used for detection of changes in social behavior and exploratory behavior consists of a perspex open topped box 51x51 cm and 20 cm hight with 17x17 cm marked areas on the floor . One hour prior to the test , two animals are separate housing cages are treated with test compound orally They are placed into the box (with 60 W illumination 17 cm above) and their behaviour is observed over 10 min period by remote video recording. Two type of behavior can be noted

1. Social interaction between the animals is determined by timing the sniffing of partner, crawling under or climbing over the partner.
2. Exploratory motion is measured as the number of crossings of the lines marked on the floor of the test box.[123]

ESTIMATION

On 43rd day of experiment blood was collected from the retro-orbital plexus using ketamine-xylazine anaesthesia using capillary tubes in fresh vials containing EDTA and Heparin Serum and plasma was seperated. Serum analyzed for ALT, AST, ALP, total cholesterol, triglycerides using standard commercial diagnostic kits. Rats were sacrificed by cervical dislocation under ketamine-xylazine anaesthesia on day 43 and brain tissue were removed and used for the preparation of homogenates.

Estimation of blood parameters

Plasma parameter:-

Determination of plasma corticosterone

Blood samples are collected, to separate the blood plasma by using centrifugation with 2500rpm for 10 min. To 2ml of plasma was taken and 15 ml of dichloromethane was added and shaken well. Two layers would be formed. The upper layer is seperated out. 10 ml of the lower layer is treated with 5 ml of the fluorescent reagent that is prepared as above and shaken well. Again the lower layer is separated and reading is taken at 530 nm.[124]

Serum parameter:-

Estimation of triglycerides

Principle:

Enzymatic colorimetric determination of triglycerides according to the following reactions.

Triglyceride + H_2O $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3- phosphate + ADP

Glycerol-3-PO_4 + $2O_2$ $\xrightarrow{\text{Glycerol-3- phosphate oxidase}}$ Dihydroxy acetone phosphate+ H_2O_2

$2H_2O_2$ + 4- Aminoantipyrine + p- Chlorophenol $\xrightarrow{\text{Peroxidase}}$ Violet quinone + $4H_2O$

Procedure

About 10 µL of serum was added to 1000 µL of working reagent [Pipes buffer, pH 7.0- 50 mmol/L, p-Chlorophenol- 5.3 mmol/L, Potassium ferrocyanate- 10 mmol/L, Magnesium salt- 17 mmol/L, 4- Aminoantipyrine- 0.9 mmol/L, ATP- 3.15 mmol/L, Lipoprotein lipase > 1800 U/L, Glycerol kinase > 450 U/L, Glycerol-3-phosphate oxidase > 3500 U/L, Peroxidase > 450 U/L] provided in the kit. 10 µL of the triglyceride standard (200 mg/dL) was also added to 1000 µL of the working reagent taken in another tube. Mixed well and incubated for 5 minute at 37 ^{O}C. Absorbance of both the sample and the standard was measured at 630 nm against reagent blank. The triglycerides concentration was expressed in mg/dL.

Estimation of total cholesterol

Principle:

Enzymatic colorimetric determination of total cholesterol according to the following reactions.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acids

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{4- Cholesten- 3- one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4- Aminoantipyrene} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4H_2O$$

Procedure:

About 10 µL of serum was added to 1000 µL of cholesterol reagent [Pipes buffer- (pH 6.7) 50 mmol/L, Phenol- 24 mmol/L, Sodium cholate- 0.5 mmol/L, 4- aminoantipyrene- 0.5 mmol/L, Cholesterol esterase > 180 U/L, Cholesterol oxidase > 200 U/L, Peroxidase > 1000 U/L] provided in the kit. 10 µL of the cholesterol standard (200 mg/dl) was also added to 1000 µL of the cholesterol reagent taken in another tube. Mixed well and incubated for 5 minute at 37 ^{O}C. Absorbance of both the sample and the standard was measured at 630 nm against reagent blank. The total cholesterol concentration was expressed in mg/dL.

Determination of serum glutamic pyruvic transaminase/alanine transaminase (SGPT/ALT) activity

SGPT catalyzes the transfer of amino group between L-alanine and α-ketoglutarate to form pyruvate and glutamate. The pyruvate formed reacts with NADH in the presence of Lactate dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT activity in the sample.

$$\text{L-Alanine α-Ketogutarat} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate LDH}$$

$$\text{Pyruvate} + NADH + H^+ \xrightarrow{\text{MDH}} \text{Lactate} + NAD^+$$

Procedure:

The working reagent was prepared by mixing 4 volume of Reagent1 [Tris buffer (110 mmol/L, pH 7.5), L-Alanine (660 mmol/L), LDH (1500 U/L)] with 1 volume of Reagent2 [α-Ketoglutarate (16 mmol/L, NADH (0.24 mmol/L)] provided in the kit. About 100 µL of serum was added to 1000 µL of the working reagent. Mixed well and incubated for 1 minute at 37 ^{O}C. The change in absorbance was measured per minute for 3 minutes at 340 nm and the SGPT activity was expressed in U/L.

Determination of serum glutamic oxaloacetic transaminase/aspartate transaminase (SGOT/AST) activity

Principle:

SGOT catalyzes the transfer of amino group between L-Aspartate and α-Ketoglutarate to form Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with NADH in the presence of Malate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT activity in the sample.

L-Aspartate + α-Ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+

Procedure:

The working reagent was prepared by mixing 4 volume of Reagent1 [Tris buffer (88 mmol/L, pH 7.8), L-Aspartate (260 mmol/L), LDH (1500 U/L), MDH (900 U/L)] with 1 volume of Reagent2 [α- Ketoglutarate (12 mmol/L, NADH (0.24 mmol/L)] provided in the kit. About 100 µL of serum was added to 1000 µL of the working reagent. Mixed well and incubated for 1 minute at 37 ^{O}C. The change in absorbance was measured per minute for 3 minutes at 340 nm and the SGOT activity was expressed in U/L.

Determination of serum alkaline phosphatase (ALP) activity

Principle:

In the presence of magnesium, p-Nitrophenyl phosphate is hydrolyzed by phosphatases to form phosphate and p-Nitrophenol. The release of this coloured p-Nitrophenol is proportional to the ALP activity and can be measured photometrically at 403 nm.

p-Nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ phosphate + p-Nitrophenol

Procedure:

The working reagent was prepared by mixing 4 volume of Reagent1 [Diethanolamine buffer (125 mmol/L, pH 10.2), Magnesium Chloride (0.625 mmol/L)] with 1 volume of Reagent2 [p-Nitrophenyl phosphate (50 mmol/L)] provided in the kit. About 20 µL of serum was added to 1000 µL of the working reagent. Mixed well and incubated for 1 minute at 37^{O}C. The change in absorbance was measured per minute for 3 minutes at 405 nm and the ALP activity was expressed in U/L[125].

Estimation of brain parameters

Measurement of MAO-A and MAO-B

Preparationofsample

Mouse brain mitochondrial fraction are prepared by cutting the brain sample in to small pieces and rinsed in 0.25M sucrose, 0.1 M tris, 0.02M EDTA (pH 7.41) to remove blood. The pieces were homogenized for 45 sec in a potter-elvehjem homogenizer with 400 ml of the same medium. The homogenate was centrifuged at 800rpm for 10min and the pellets were discarded. The supernatant was then centrifuged at 12,000 rpm for 20 min in the same medium. The precipitate was washed twice more with 100ml of sucrose tris EDTA and resuspended in 50ml of the medium. The protein concentration was adjusted to 1 mg/ml.

Procedur for estimation of MAO-A

250 µl of the homogenate was added to 250 µl of serotonin and 250 µl of buffer. The reaction tube was placed at 37°C for 20 minutes and the reaction was arrested by the addition of 200 µl of 1M HCl. The reaction product was extracted with 5 ml of Butyl acetate. The organic phase was separated and measured at 280 nm using a spectrophotometer. Blank samples were prepared by adding 1M HCl (200 µl) prior to reaction and the reaction was carriedout.The MAO-A is expressed in nmoles/ mg protein.

Procedure for estimation of MAO-B

250 µl of the homogenate was added to 250 µl of serotonin and 250 µl of buffer. The reaction tube was placed at 37°C for 20 minutes and the reaction was arrested by the addition of 200 µl of 1M HCl. The reaction product was extracted with 5 ml of Cyclohexane. The organic phase was separated and measured at 242 nm using a spectrophotometer. Blank samples were prepared by adding 1M HCl (200 µl) prior to reaction and the reaction was carried out.

The MAO-B activity is expressed in nmoles/ mg protein.

Estimation of norepinephrine, dopamine and 5-hydroxytryptamine

A weighed quantity of 1.55 mg of whole brain tissue was homogenized in 0.1 ml HCl-butanol mixture (O.85 ml 37% HCl in 1 liter n-butanol for spectroscopy) which will result in a total volume 0.105 ml. The total volume of sample was then centrifuged for 10 min at 2000 g. Resultant aliquot of the supernatant phase (0.08 ml) was removed and added to an Eppendorf reagent tube containing 0.2 ml heptane (for spectroscopy) and 0.025 ml 0.1 M HCl. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions

as above in order to separate the organic phase. From the separated aqueous phase, 0.02 ml was used for the assay of 5-HT, NA and DA assay respectively.

1) **Standard curve of norepinephrine hydrochloride**
    - The stock solution of norepinephrine hydrochloride is prepared at a concentration of 100 µg/ml in 0.1 N HCl solution.
    - Working standards was prepared in the range of 0.1 to 1.6µg/ml by appropriate dilution of the stock using 0.1N HCl
    - About 1ml of acetate buffer (pH3) was added to each of the above dilutions along with 0.1ml of iodine reagent.
    - After 6 minutes, 0.2ml sodium sulphite solution was added to remove the excess iodine followed by through mixing after each addition. Two minutes later 0.2ml of 5 N acetic acid was added and mixed well.
    - All tubes were placed in a boiling water bath for 2min, cooled in tap water and NE fluorescence was read.
    - The solution was activated at 380nm and the resulting fluorescence was measured at 480nm using Spectro-fluorimeter.
    - Standard curve of fluorescence vs. concentration was plotted.
2) **Standard curve of dopamine hydrochloride**
    - The stock solution of dopamine hydrochloride was prepared at a concentration of 100 µg/ml in 0.1 N HCl solution.
    - Working standards was prepared in the range of 0.25 to 4 µg/ml by appropriate dilution of the stock using 0.1N HCl
    - About 0.5 ml of 0.1 M phosphate buffer of pH (6.5) was added to each of the above dilutions followed by the addition of 0.05ml of 0.02N of iodine solutio.
    - After 5 minutes, 0.5 ml of alkaline sodium sulfite solution was added and about 5 minutes later, 0.6 ml of 5 N acetic acid was also added
    - The dilution was activated at 345nm and the resulting fluorescence was measured at 410nm using Spectro-fluorimeter.
    - Standard curve of fluorescence vs. concentration plotted.

3) Standard curve of 5-hydroxytryptamine (5-HT)

- The stock solution of 5-HT was prepared at a concentration of 10µg/ml in 0.1N HCl.
- Working standards were prepared in the range of 0.1 to 0.5µg/ml undertaking appropriate dilution of the stock with 0.1N HCl.
- About 1.2 ml of o-Phthalaldehyde (OPT) was added and mixed well, followed by which all tubes were placed in a boiling water bath for 10 min then cooled in tap water.
- The above dilutions were directly measured with activation maxima of 355 nm and fluorescence maxima of 470 nm. Blank was measured using 0.1N HCl and the values were substracted from the standard absorbance
- Standard curve of fluorescence vs. concentration was plotted.

Assay of norepinephrine

Blank preparation was carried out by adding 1ml of 0.2N acetic acid and 0.2ml alkaline sulfite followed by proper mixing. 0.1ml of 0.1N iodine and 0.2ml of 5N acetic acid was added mixed well. To 0.02ml of brain homogenate, 0.1ml of 0.1N iodine was added and mixed well. After 2 min 0.2ml of 5N acetic acid was added. Finally 0.2 ml EDTA was added to all tubes and were placed in boiling water bath for 2min followed by cooling under tap water. Absorbance of norepinephrine was measured by fluorimetry with excitation at 380nm and emission at 480nm.

Assay of dopamine

About 0.02 ml of whole brain homogenate was mixed with 0.5 ml 0.1M phosphate buffer followed by further addition of 0.05ml of 0.02N iodine solution. After 5min 0.5ml of alkaline sulfite solution was added to test and 0.5ml of 2.5N NaoH and 0.6ml of 2.5N acetic acid was added to both test and blank solutions. Absorbance of dopamine was measured by fluorimetry with excitation at 335nm and emission at 410nm.

Assay of 5-hydroxytryptamine

To samples and reagent and blank 0.2 ml of 0.1N HCL add 1.2 ml o- phthaldehyde was added and mixed well. All tubes were placed in boiling water bath for 10min and cooled under tap water. Absorbance of 5-hydroxytryptamine was measured by fluorimetry with excitation at 355nm and emission at 470nm.[127]

Histopathology

At the end of study, brains hippocampus region was quickly removed and washed with ice-cold saline. Fixed in 10% formaldehyde solution . After fixation tissues were five micrometer thick sections were cut and stained with hematoxylin and eosin. The slides were observed under light microscope, photomicrograph was taken and examined the histopathological changes.[127]

Stastical analysis:-

Satistical analysis of the result should be carried out by One – way ANOVA followed by Dunnett's test. Results would be expressed as mean ±SEM from six rats in each group. P value < 0.05 would be considered significant.[127]

RESULT

Table 1: Effect of myristicin and lavander oil on sucrose preference test in depressed rats exposed to LPS followed by CUMS

Group	Percentage of sucrose taken by the rats						
	0^{th} day	1^{st} week	2^{nd} week	3^{rd} week	4^{th} week	5^{th} week	6^{th} week
I	49.18	51.84	53.92	55.53	58.36	62.00	76.50
II	51.26	50.61	48.63	44.17	41.17	35.27	28.07
III	50.92	55.05	59.23	64.44	66.05	69.36	74.63
IV	51.86	51.61	55.37	59.00	63.71	65.45	68.74
V	50.71	55.90	58.33	58.66	64.00	66.61	71.94
VI	49.76	52.91	54.33	56.36	58.33	62.13	66.65
VII	50.12	55.00	59.51	62.86	66.06	70.81	73.28
VIII	49.64	51.17	52.12	53.82	56.33	61.51	65.40
IX	49.05	50.63	51.66	54.44	57.20	60.45	63.33

Table2 : Percentage of time spent in open arm in EPM in depressed rats exposed to LPS followed by CUMS

Group	Time spent in open arm in EPM in sec						
	0th day	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
I	246.08±2.31	247.12±2.29**	249.11±2.74**	250.14±2.85*	253.09±3.12*	254.13±3.39*	255.10±3.61*
II	248.17±3.12 ns	246.06±17.68 ##	245.50±14.75 ##	240.11±21.53 ##	237.12±4.97##	235.17±2.08###	231.19±2.21##
III	246.12±1.71 ns	247.06±14.69**	249.91±14.67**	250.53±25.96**	251.37±17.25**	253.26±15.89**	254.17±17.36**
IV	247.06±2.31 ns	247.13±17.38*	248.10±17.15*	248.29±11.07*	249.25±5.24**	250.21±2.73**	251.18±8.21**
V	249.04±3.21 ns	248.10±9.04**	248.90±19.06**	249.24±7.08**	250.82±8.59**	251.93±5.35**	252.12±6.31**
VI	248.13±1.75 ns	248.0±10.47*	247.18±15.03*	249.06±8.21**	250.01±4.50**	250.93±17.38**	251.95±6.07**
VII	247.15±2.21 ns	247.19±15.89**	248.20±15.89**	248.95±6.29**	250.93±12.66**	251.03±6.29**	252.00±9.21**
VIII	246.10±2.71 ns	246.25±10.09*	247.21±12.68*	247.14±6.31*	248.28±22.07*	248.15±19.29**	250.32±11.41**
IX	246.09±1.17 ns	247.31±11.07*	247.27±2.73*	248.17±8.23*	248.93±22.57*	249.27±10.75**	249.46±3.91**

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression

One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns, $P<0.05$, normal control compared with CUMS control; ##$P<0.01$,**$P<0.01$, treatment groups compared with CUMS control

Table3: Percentage of time spent in closed arm in EPM in depressed rats exposed to LPS followed by CUMS

Group	Time spent in closed arm in EPM in sec						
	0th day	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
I	53.92±1.31	52.88±45.61*	50.89±47.51*	49.86±22.89*	46.91±6.02*	45.87±2.15*	44.49±3.51*
II	51.83±7.15	53.94±14.05##	54.91±21.02##	59.89±20.08##	62.88±26.71##	64.83±2.07##	68.81±23.15##
III	53.88±2.31	52.92±10.20**	50.09±6.76**	49.47±15.6**	48.63±3.65**	46.74±3.83**	45.83±3.21**
IV	52.94±1.21	52.87±31.12**	51.09±15.06**	51.71±19.53**	50.75±9.13**	49.79±7.02**	48.82±8.06**
V	50.96±2.14	51.09±12.96**	51.01±24.10**	50.76±24.10**	49.18±4.13**	48.07±5.89**	47.88±5.93**
VI	51.87±3.21	51.97±23.85**	52.82±20.96**	50.94±14.34**	49.99±8.81**	49,07±6.59**	48.07±9.39**
VII	52.85±1.31	52.81±16.27**	51.8±12.67**	51..05±20.96**	49.07±8.59**	48.71±5.83**	47.39±6.37**
VIII	53.9±2.15	53.75±16.27**	52.79±15.58**	52.79±26.83**	51.72±5.24**	51.85±6.34**	49.68±3.86**
IX	53.91±1.21	52.69±31.12**	52.73±23.85**	51.83±22.89**	51.07±4.16**	50.73±6.78**	50.54±6.27**

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression

One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns, $P<0.05$, normal control compared with CUMS control; ##$P<0.01$,**$P<0.01$, treatment groups compared with CUMS control

Table 4: Effect of myristicin and lavander oil on social interaction test in depressed rats exposed to LPS followed by CUMS

Group	Social interaction time in sec						
	0th day	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
I	83.21±3.27	85.03±1.21*	86.14±1.31*	88.18±1.67*	92.19±2.34*	95.21±2.97*	97.04±1.27*
II	77.14±4.23	82.07±2.31##	82.03±1.72##	79.26±2.78##	76.34±1.23##	70.67±4.53##	65.86±2.19##
III	78.24±1.09	88.16±3.17**	89.12±3.24**	91.53±3.27**	92.87±1.74**	93.12±1.89*	96.12±3.65*
IV	79.34±4.23	85.12±3.21**	87.23±1.09*	88.34±4.16*	90.06±3.21*	91.34±2.03**	93.19±4.09*
V	82.61±2.91	87.34±3.94*	88.04±1.21**	90.21±1.76*	91.17±3.81**	92.04±8.13**	95.04±3.08**
VI	81.09±2.34	87.23±1.07*	88.54±2.21*	88.43±2.21*	89.04±2.45	90.11±1.59	92.34±1.50*
VII	78.67±3.56	86.07±12.09*	87.05±3.21*	89.45±1.94*	90.67±1.23**	92.76±3.89**	96.15±3.85**
VIII	79.45±2.30	84.04±1.78*	85.18±3.78*	85.34±1.45*	86.23±1.48*	87.32±4.53*	89.45±2.34*
IX	81.08±1.92	85.63±1.38*	86.24±4.07*	86.04±2.94*	87.56±2.23*	88.05±2.90*	90.18±3.32*

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression

One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns,* P<0.05, normal control compared with CUMS control; ##P<0.01,**P<0.01, treatment groups compared with CUMS control

Table 5: Effect of myristicin and lavander oil on duration of immobility in FST in depressed rats exposed to LPS followed by CUMS

Group		Duration of immobility in sec on FST					
	0th day	1st week	2nd week	3rd week	4th week	5th week	6th week
I	200.13±2.14	217.06±2.76	213.21±3.26	220.17±3.15	211.08±2.19	209.24±2.24	206.13±2.36
II	200.24±3.98	210.24±2.58##	218.31±2.64##	226.07±2.78##	245.12±3.22##	258.71±3.21##	265.15±3.7##
III	197.43±5.8	189.61±2.01**	173.36±1.89**	160.15±1.79**	147.53±2.05**	144.21±2.21**	138.17±3.17**
IV	201.35±4.56	191.52±2.47**	182.25±2.25**	171.22±2.19**	167.11±3.16**	153.23±3.02**	145.27±3.14**
V	198.52±3.45	192.08±2.66**	185.11±2.48**	162.34±2.12**	143.5±2.11**	138.73±2.75**	130.23±1.71**
VI	203.34±2.45	195.31±2.31**	190.15±1.71**	187.25±1.83**	173.10±2.06**	166.17±3.19**	151.29±3.17**
VII	195.44±1.43	183.17±1.07**	172.15±2.17**	166.12±2.17**	152.5±2.79**	147.82±13**	132.19±2.15**
VIII	205.67±8.56	199.21±2.76**	190.31±3.01**	182.18±1.74**	176.13±1.76**	166.17±12.23**	158.23±3.24**
IX	202.45±3.45	197.18±1.03**	181.21±2.17**	180.21±2.24**	173.23±2.89**	161.42±1.98**	162.34±2.45**

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression
One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns, $P<0.05$, normal control compared with CUMS control; ##$P<0.01$,**$P<0.01$ treatment group compared with CUMS control.

Table 6: Effect of myristicin and lavander oil on duration of swimming in FST in depressed rats exposed to LPS followed by CUMS

Group		Duration of swimming in sec on FST					
	0th week	1st week	2nd week	3rd week	4th week	5th week	6th week
I	82.46±1.46	85.17±1.34	86.32±1.38	85.57±1.36	88.44±1.42	89.53±1.27	93.35±1.37
II	83.09±2.89	77.03±2.45#	68.34±2.32#	62.16±2.24#	43.26±2.12ns	40.17±2.27ns	38.26±2.75ns
III	88.23±1.05	96.41±1.22**	104.03±1.28**	120.51±1.36**	137.2±1.48**	141.32±1.56**	153.71±1.03**
IV	83.89±1.67	89.31±1.51**	95.05±1.56**	107.43±1.78**	123.5±2.01**	138.21±2.17**	145.23±2.31**
V	85.45±1.98	86.11±1.01**	99.36±1.13**	115.30±1.17**	123.05±1.20**	138.07±1.76**	152.05±1.07**
VI	84.34±3.98	88.17±3.02**	96.15±2.17**	109.15±2.13**	126.15±1.32**	139.11±2.31**	149±2.73**
VII	81.22±2.56	84.02±1.12**	99.27±1.61**	110.72±2.23**	133.77±2.05**	141.06±1.37**	151.02±1.71**
VIII	85.76±1.67	90.17±1.17**	94.31±2.17**	104.21±1.91**	123.21±2.17**	136.18±1.43**	143.23±1.32**
IX	86.54±2.56	93.21±2.17**	98.05±1.41**	107.32±1.73**	117.23±1.19**	129.21±1.56**	138.53±1.41**

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression

One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns, P<0.05, normal control compared with CUMS control; #P<0.05,**P<0.01 treatment group compared with CUMS control.

Table 7: Effect of myristicin and lavander oil on duration of immobility in tail suspension test in depressed rats exposed to LPS followed by CUMS

Group		Duration of immobility in tail suspension test					
	0th day	1st week	2nd week	3rd week	4th week	5th week	6th week
I	223.24±2.90	215.33±12.17	209.71±14.12	193.36±20.81	186.71±12.58	170.53±15.27	161.65±13.22
II	228.13±7.88	231.5±12.94##	243.83±11.14##	258.5±9.75##	268.83±18.81##	286.83±12.07##	301.71±10.21##
III	224.43±4.32	218.83±7.36**	195.66±12.07**	183.31±10.23**	176.62±6.05**	162.5±6.59**	155.25±6.81**
IV	227.21±4.76	220.23±7.83**	212.15±9.35**	196.66±12.22**	191.82±7.36**	186.16±7.73**	180.17±7.82**
V	226.15±7.65	219.31±10.32**	196.61±6.76**	190.72±3.21**	182.21±6.61**	175.21±6.75**	168.85±11.93**
VI	225.08±6.43	220.62±5.82**	215.82±13.12**	205.17±6.61**	196.5±12.66**	183.24±17.15**	176.51±19.29**
VII	223.32±9.45\	217.16±20.96**	198.13±8.81**	187.70±3.21**	173.12±17.31**	160.15±19.06**	153.19±17.38**
VIII	225.22±2.56	219.13±6.76**	208.62±5.24**	196.36±3.51**	193.08±14.21**	186.25±34.95**	179.81±10.17**
IX	223.34±3.56	216.85±8.56**	210.5±4.31**	202.91±8.06**	196.11±12.32**	183.17±22.07**	180.23±9.04**

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression
One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns, $P<0.05$, normal control compared with CUMS control; ##$P<0.01$,**$P<0.01$, treatment groups compared with CUMS control

Table 8: Effect on escape latency in Morris water maze in depressed rats exposed to LPS followed by CUMS

Group		Escape latency in morrris water maze in sec					
	0th day	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
I	103.21±3.23	98.66±4.50	95.35±2.76	86.37±2.88	79.12±8.08	63.23±12.7	47.66±6.50
II	105.13±1.23	113.50±0.838$^{\#\#}$	121.66±3.29$^{\#\#}$	138.33±29.03$^{\#\#}$	143.16±13.12$^{\#\#}$	158.66±17.72$^{\#\#}$	161.66±45.72$^{\#\#}$
III	103.32±2.45	98.50±17.85**	96.83±11.77**	89.33±13.39**	68.02±33.10**	48.83±13.34**	52.16±5.49**
IV	106.43±7.12	103.60±0.81**	100.66±8.35**	92.66±21.64**	89.16±17.32**	79.33±15.06**	65.83±2.71**
V	105.13±5.23	102.60±7.78**	99.50±6.28**	88.50±36.59**	79.16±23.72**	68.16±16.81**	58.16±13.60**
VI	100.12±3.21	89±30.16**	80.50±7.36**	74.03±5.72**	61.5±20.13**	53.5±7.09**	48.33±5.53**
VII	101.23±4.15	98.5±8.80**	96.33±4.96**	87.50±90.67**	73.66±19.03**	63.83±11.05**	51.66±11.13**
VIII	102.56±2.23	93.83±2.04**	89.16±4.02**	80.16±2.14**	85.16±7.65**	78.33±11.75**	64.43±11.17**
IX	104.13±5.06	95.33±3.26**	92.33±3.50**	89.66±10.46**	82.33±23.22**	79.16±7.25**	73.5±10.36**

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression
One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns, P<0.05, normal control compared with CUMS control;$^{\#\#}$P<0.01, the treatment groups compared with CUMS control **P<0.01

Table9: Effect of myristicin and lavander oil on total cholesterol, triglycerides and plasma corticosterone in rats exposed to CUMS and LPS

Group	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Corticosterone (µg/dl)
I	91.3 ±2.11	81.23 ±1.21	83.23±1.67
II	189.47 ± 15.75$^{\#\#}$	138.16±17.50$^{\#\#}$	137.14±3.05$^{\#\#}$
III	125.76 ± 8.21**	108.53±3.55**	95.66±6.13**
IV	158.13±1.05*	124.47±4.46*	129.85±2.50**
V	137.85±0.92**	113.79±5.35**	120.31±2.38**
VI	154.31±1.44*	116.30±4.17*	126.87±2.16*
VII	128.08±2.18**	106.91±2.78**	101.74±2.64**
VIII	175.29±1.37*	125.87±5.01**	124.84±3.64**
IX	106.94±2.48*	127.68±1.12**	131.20±1.43*

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression
One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns,P<0.05, CUMS control compared with Standard ; ##P<0.01, *P<0.05 treatment groups compared with CUMS control.

Table10: Effect of myristicin and lavander oil on AST/ALT/ALP

Group	SGOT/AST	SGPT/ALT	ALP
I	936.23± 34.12	874.85±21.23	1023.32±323.21
II	1714.07±54.69$^{\#\#}$	1776.42±23.40$^{\#\#}$	3125.37±369.46$^{\#\#}$
III	1292.23±97.47**	911.19±200.95**	2282.54±149.61**
IV	1556.93±38.69**	1448.16±45.33**	2714.84±32.37**
V	1424.41±76.15**	1153.59±161.22**	2372.24±90.31**
VI	1561.52±100.19*	1509.16±64.59**	2717.64±19.76**
VII	1310.73±82.45**	884.44±71.36**	2092.22±73.57**
VIII	1495.69±94.81**	1255.04±110.33**	2501.62±72.98**
IX	1581.56±65.24*	1570.13±32.63*	2716.95±29.86**

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression

One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns,P<0.05, CUMS control compared with standard control;$^{\#\#}$P<0.01, **P<0.05, treatment groups compared with CUMS control

Table11: Effect of myristicin and lavander oil on the measurement of brain monoamine (NE, DA and 5HT)

Group	Norepinephrine(µg/g)	Dopamine(µg/g)	Serotonin (µg/g)
I	0.20±1.16	0.25±2.11	0.33±1.67
II	$0.05\pm0.02^{\#\#}$	$0.043\pm0.015^{\#\#}$	$0.03\pm0.010^{\#\#}$
III	0.88±0.30**	0.50±0.16**	1.92±1.09**
IV	0.17 ± 0.015^{ns}	0.08 ± 0.010^{ns}	0.17 ± 0.005^{ns}
V	0.24 ± 0.36^{ns}	0.18 ± 0.010^{ns}	0.22 ± 0.03^{ns}
VI	0.16 ± 0.010^{ns}	0.086 ± 0.005^{ns}	0.73 ± 0.38^{ns}
VII	0.33±0.015*	0.21±0.04*	1.12±0.48*
VIII	0.18 ± 0.020^{ns}	0.13 ± 0.011^{ns}	0.44 ± 0.04^{ns}
IX	0.14 ± 0.010^{ns}	0.086 ± 0.006^{ns}	0.15 ± 0.02^{ns}

From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression

One-way ANOVA followed by Dunnett's test. All the values are Mean±SEM,. ns, P<0.05, negative control compared with standard ; $^{\#\#}$P<0.01, **P<0.05, treatment groups compared with CUMS control.

Table12: Effect of myristicin and lavander oil on the measurement of monoamineoxidase and monoamineoxidase B

Group	MAO-A(µg/g)	MAO-B(µg/g)
I	0.20±0.04	0.25±0.07
II	1.21±0.009	1.49±0.02
III	0.34±0.008	0.41±0.003
IV	0.73±0.05	0.86±0.05
V	0.60±0.05	0.57±0.04
VI	0.71±0.014	0.88±0.08
VII	0.53±0.03	0.51±0.008
VIII	0.79±0.03	0.73±0.04
IX	0.93±0.03	0.94±0.04
From the group II-IX was exposed to LPS(50 µg/kg i.p) over 2 weeks and CUMS for 4 week with total 6 week for induction of depression		

Effect of behavioural parameters

SUCROSE PREFERENCE TEST

Sucrose preference test was evaluated for the experimental animals for 42 successive days (Table1). The rats preference to sucrose solution during the last day of training session before respective drug treatment was found to be 49.18% and after drug treatment in the 1st, 2nd, 3rd, 4th, 5th, & 6th week (1 hour after the dose) of study there was a significant increase in consumption of sucrose solution and decrease in sucrose preference in negative control group. The CUMS group showed 28.07% consumption of sucrose less than vehicle-treated group 76.50%. Administration of Imipramine 20 mg/kg showed 74.63%, Myristicin 200 mg/kg attributed to 73.28% and lavander oil 600 mg/kg showed 71.94% by the virtue of which an increment in the sucrose consumption levels were observed when compared with negative control. Combination- 1 consisting of lavander oil 300mg/kg+ myristicin 100 mg/kg showed 65.40% consumption of sucrose. Combination 1 and 2 did not have much effect and it shows antagonistic effect since the volume of sucrose consumed was less than the individual drug administration **Table 1**.

ELEVATED PLUS MAZE (EPM) TEST:

The anxiolytic activity of myristicin and lavander oil was determined by elevated plus maze test wherein parameters like the time spent in the open arm and time spent in the closed arm were evaluated **Table 2&3**. Animals in all the groups were observed in EPM model on the 0th day (pretreatment) and on the 1st, 2nd, 3rd, 4th, 5th & 6th weeks of drug treatment (1hour after the dose) (table 2,3). In the pretretment period, the time spent (in seconds) by the animals in all the groups in the open arm (246.08±2.31 to 249.04±3.21) and closed arm (50.96±2.14 to 53.92±1.21) was recorded. On the 42nd day animals in group 1 (vehicle-treatment) spent 255.10±3.61 in the open arm and 44.49±3.51 in the closed arm. On the other hand, animals in group 2 (negative control) spent 231.19±2.21 in open arm and 68.81±23.15 in the closed arm which showed significant increase ($p< 0.01$) in time spent in closed arm and decrease in open arm when compared to group 1. Group 3 (Standard) showed significant ($p< 0.01$) increase in occupancy in the open arm when compared with group 2. Animals in group 5 (lavander oil 600 mg/kg) and Group 7 (myristicin 200 mg/kg) brought about a significant ($p<0.01$) decrease in the time spent in the closed arm when compared with group 2. Similarly the animals in group 3 (imipramine 20mg/kg) showed a decreased preference for the closed arm.

SOCIAL INTERACTION TEST

Animals in all the groups were observed in social interaction on the 0^{th} day (pretreatment) and on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} &6^{th} weeks of drug treatment (1hour after the dose) (table4). In the pretretment period, social interaction test revealed a range of 77.14±4.23 to 83.21±3.27 percentage. In group 2, decreased time of active social interaction from 1^{st} to 6^{th} week (65.86±2.19) was observed when compared with the group 1 (control) (97.04±1.27). Whereas animals in group 3 (Imipramine 20 mg/kg) (96.12±3.65) and group 7 (myristicin 200mg/kg) (96.15±3.85) showed improvement in the social interaction time when compared with negative control LPS-CUMS treated group **Table4**.

FORCED SWIM TEST (FST):

The depression like behaviour was observed in all the experimental animals on the 0^{th} day (pretreatment) and on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} &6^{th} weeks of drug treatment (1hour after the dose) in all the groups (Table: 5,6). In the pretretment period, the immobility time spent by the animals in all the groups (195.44±1.43 to 205.67±8.56) and swimming time (81.22±2.56 to 88.23±1.05) was recorded from 1^{st} week to 6^{th} week of drug treatment (1 hour after the dose). Animals in group 1(vehicle treated) spent 206.13±2.36 sec in immobility state and 93.35±1.37 sec swimming time and animals in group 2 (LPS-CUMS) spent 265.15±3.07 sec in immobility state and 38.26±2.75 sec of swimming time. Moreover, the time spent by animals in group 7 (myristicin 200mg/kg) was found to be 151.02±1.71 and group 5 (lavender 600 mg/kg) showed 152.05±1.07 sec in swimming. Group 3 animals (imipramine 20 mg/kg) spent (153.71±1.03) sec in swimming and the result were found to be increase significantly, on the other hand animals in group 6 (myristicin 100 mg/kg) spent 153.71±1.03 sec which was found to be non-significant in the immobility time**Table 5&6**.

TAIL SUSPENSION TEST

Animals in all the groups were observed in tail suspension on the 0^{th} day (pretreatment) and on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} & 6^{th} weeks of drug treatment (1hour after the dose) (table7). In the pretretment period, tail suspension test revealed that animals (223.24±2.90 to 228.13±7.88) immobility time was significantly ($P<0.01$) increased in the animals in group 2 (CUMS) after LPS administration. Pretreatment in group 7 (myristicin

200 mg/kg) and group 3 (imipramine 20 mg/kg) significantly reversed the effect of LPS-CUMS on immobility time in TST. The vehicle- treated rat was immobile for 161.65±13.22 sec. The CUMS rat showed immobility time of 301.71±10.21 sec and group 7 (myristicin 200mg/kg) and group 5 lavender oil treatment (600 mg/kg) showed immobility time for 153.19±17.38 and 168.65±11.93 sec respectively. Animals in group 8 (Combination 1- lavander oil 300mg/kg+ myristicin 100mg/kg and group 9 combination 2- lavander oil 150 mg/kg and myristicin 50 mg/kg) compared to other individual groups did not have much effect and shows antagonistic effect. Group 3 (imipramine 20 mg/kg) showed immobility time 155.25±6.81 sec and the results were found to be significant**Table 7**.

Spatial Reference Memory

MORRIS WATER MAZE TEST

Animals in all the groups were observed for morris water maze on the 0^{th} day (pretreatment) and on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} & 6^{th} weeks of drug treatment (1 hour after the dose) (Table 8). In the pretretment period, escape lattency in morris water maze test revealed 101.23±4.15to106.43±7.12 sec. The treatment groups showed the escape latency (EL) on the first day which reflected learning behaviour of animals whereas, EL of second day reflected retention of information or memory. Animals in various groups were administrated with drugs for 42 successive days. The escape latency was evaluated using Morris water maze model from 1^{st} to 6^{th} week of drug treatment (1hours after the dose). Vehicle-treated animals in group 1 took 47.66±6.50 sec to find out hidden platform. The group 2 consisting of negative control CUMS took 161.66±45.72 sec and showed significant increase in time to find out the hidden platform. Animals in group 3 (Imipramine 20mg/kg) has shown significant decrease in the escape latency (52.16±5.49) when compared with group 2. Similarly, treatment with myristicin and lavender oil alone or in combination a significant decrease in escape latency was observed **Table 8**.

EFFECT ON BLOOD PARAMETES

Table no.9 describes about the increment in cholesterol levels in stress control group when compared with normal control group. Animals in group 3 treated with imipramine 20mg/kg (108.53±3.55) showed significant decrease ($P<0.01$) in choleasterol level compared to group 2 (negative control). Group 6 treated with myristicin 100 mg/kg (116.30±4.17) and group 4 lavender oil 300 mg/kg (124.47±4.46) treated animals showed less significant decrease in cholesterol level compared to stress control group 2 (138.16±17.50). Group 7

animals treated with myristicin 200 mg/kg (106.91±2.78) and group 5 animals treated with lavender oil 600 mg/kg (113.79±5.35) showed significant decrease (P<0.01) cholesterol levels compared to stress control group 2.

Rats in negative control group (189.47 ± 15.75) showed an increase in triglyceride level when compared to normal control (91.3±2.11) whereas, standard imipramine (125.76±8.21) treated animals showed significant decrease ($^{\#\#}$p<0.01) in triglyceride level when compared to stress control. Group 6 (154.31±1.44) , group 4 (158.13±1.05) and group 8 (175.29±1.37) treated animal showed moderate significant decrease (p<0.05) in triglyceride level when compared to group 2 (stress control). Group 7 (128.08±2.18) and group 5 (137.85±0.92) animals showed significant decrease (p<0.01) in tryglyceride level compared to negative control group 2 **Table 9**.

Standard imipramine 20 mg/kg (95.66±6.13) showed decrease in corticosterone levels when compared to stress control group (137.14±3.05). Plasma corticosterone levels in group 2 (137.14±3.05) were found to be increased when compared to group 1(83.23±1.67). Group 7 (101.74±2.64), group 5 (120.31±2.38) and group 8 (124.84±3.64) animals showed significant decrease (p<0.01) in corticosterone level when compared to stress control. Group 4 (120.85±2.50), group 6 (126.87±2.16) and group 9 (131.20±1.43) rats showed moderate significant decrease (p<0.05) when compared to stress control animals of group 4.

LPS has the tendency to alter the liver enzymes since it has the potential to damage liver and induce hepatotoxicity. In our studies, levels of AST, ALT and ALP were measured to confirm whether drug treatment have the potential to reduce these enzymes level and offer protection to liver. Table 10 shows the significant increase in the levels of AST, ALT and ALP in group 2 rats when compared with the normal control animals. After treatment in the group 6 and 7 animals with myristicin (100 and 200 mg/kg) and group 4 and 5 with lavender oil (300 & 600 mg/kg) and standard resulted in moderate fall of these liver enzymes when compared to LPS treated group 2. The AST level in group 7 (1310.73±82.45) and group 5 (1424.41±76.15) showed significant (p<0.01) decrease when compared with the stress control group. The standard imipramine 20 mg/kg shows (1292.23±9.47) significant decrease when compared with stress control (1714.07±54.69). ALT levels in the group 7 (884.44±71.36) shows significant decrease (##P<0.01) compared with negative control group 2 (1776.42±23.40). Group 5 (1153.59±161.22) and group 8 (1255.04±110.33) shows moderatly significant decrease compared with negative control. The ALP levels for animals in group 7

(2092.22±73.57) decreased significantly when compared with group 2. The standard imipramine 20 mg/kg (2282.54±149.61) showed decrease in ALP compared with stress control group (137.4±3.05) **Table 10**.

EFFECT ON BRAIN PARAMETERS

Table 11 shows details about the level of brain neurotransmitters after a consecutive 42 days treatment. The results describes the decrease in the level of brain monoamines, norepinephrine (0.05±0.02µg/ml), dopamine (0.043±0.015 µg/ml) and seotonin (0.03±0.010 µg/ml) levels in negative control group when compared with the normal control. Group 3 treated with imipramine showed significant (P<0.01) increase in brain norepinephrine (0.88±0.30), dopamine (0.50±0.16) and serotonin (1.92±1.09) levels. Group 5, 6, 8 and 9 did not significantly prevent the reduction of NE, dopamine and 5-HT. Where as group 7 animals showed fall in levels of NE (0.33±0.015) dopamine (0.21±0.04) and 5-HT (1.12±0.48) which was found moderately significant (*P<0.05) **Table 11**.

Significamt elevation was noted in the levels of monoamine oxidase A (1.21±0.009) & B (1.49±0.02) in LPS + CUMS induced group when compared with the normal control animals. The standard drug treated group imipramine 20 mg/kg showed decrease in monoamine oxidase A (0.34±0.008) and B (0.41±0.003) significantly when compared with group 2. Group 6 myristicin - 100 mg/kg (0.71±0.014&0.88±0.08), group 4 lavander oil 300 mg/kg (0.73±0.05&0.86±0.05), group 8 combination 1 (myristicin 100mg/kg and lavander 300 mg/kg) (0.79±0.03&0.73±0.04) shows moderate decrease in monoamine oxidase A nd B whereas group 7 myristicin 200 mg/kg (0.53±0.03 & 0.51±0.08), group 5 lavander oil 600 mg/kg (0.60±0.05 & 0.57±0.04) shows decrease in monoamino oxidase A and B level **Table 12**.

Table.13 Drug likeness properties of the Myristicin and Active constituents in Lavander oil using molinspiration software

S.No.	Compound	Drug relevant properties				
		M.W	Mi Log P	No. of H Bond Acceptors	No. of H Bond Donors	No. of Violations
1	Myristicin	192.21	2.44	3	0	0
2	Alpha pinene	136.24	3.54	0	0	0
3	Limonene	136.24	3.62	0	0	0
4	Linalool	154.25	3.21	1	1	0
5	Camphor	152.24	2.16	1	0	0
6	Linalyl acetate	192.29	3.92	2	0	0
7	Cis- ocimene	136.24	3.99	0	0	0
8	3-octanone	128.22	2.81	1	0	0
9	Imipramine	280.42	4.16	2	0	0
10	Camphene	136.24	3.33	0	0	0
11	β-Pinene	136.24	3.33	0	0	0
12	Sabinene	136.24	3.10	0	0	0
13	Myrcene	136.24	3.99	0	0	0
14	α-terpinene	136.24	3.36	0	0	0
15	Terpinolene	136.24	3.67	0	0	0
16	Vinyl amyl carbol	128.22	2.76	1	1	0
17	Bormeol	154.25	2.35	1	1	0
18	Neryl acetate	196.29	3.91	2	0	0

Table. 14 Predicted bioactivity score of myristicin and constituents in lavander oil using molinspiration software

S.No.	Compound	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
1	1.Myristicin	-0.71	-0.42	-0.12	-0.90	-1.10	-0.43
2	2.Alpha pinene	-0.48	-0.43	-0.50	-0.62	-1.85	-0.34
3	3.Limonene	-0.91	-0.27	-0.01	-0.34	-0.38	0.21
4	4.Linalool	-0.73	-0.07	-0.26	-0.06	-0.94	0.07
5	5.Camphor	-0.79	-0.56	-0.12	-0.21	-0.95	-0.52
6	6.Linalyl acetate	-0.49	0.11	-0.01	-0.06	-0.57	0.36
7	7. Cis- ocimene	-0.11	-0.38	-0.55	-0.51	-0.38	-0.05
8	8.3-octanone	-0.23	-0.60	-0.40	-0.07	-0.19	-0.42
9	9. Imipramine	0.25	0.16	0.10	0.00	-0.08	-0.13
10	10.Camphene	-0.02	-0.55	-0.85	-0.15	-0.40	-0.82
11	11.β-Pinene	-0.53	-0.32	-0.45	-0.50	-0.80	-0.34
12	12.Sabinene	-0.15	-0.33	-0.79	-0.69	-0.78	-0.60
13	13.Myrcene	-0.11	-0.33	-0.51	-0.45	-0.31	-0.07
14	14.α-terpinene	-0.96	-0.24	-0.29	-0.24	-0.52	-0.11
15	15.Terpinolene	-0.88	-0.41	-0.61	-0.50	-0.74	-0.26
16	16.Vinyl amyl carbol	-0.78	-0.24	-0.59	-0.68	-0.86	-0.09
17	17.Bormeol	-0.47	-0.51	-0.57	-0.84	-0.80	-0.23
18	18.Neryl acetate	-0.50	0.04	-0.11	-0.12	-0.80	0.21

Table. 15 ADMET properties of the Myristicin and constituents in lavander oil

S. No.	.Compound	BBB	Buffer_solubility_mg/L	Caco2	CYP_2C19_inhibition	CYP_2C9_inhibition	CYP_2D6_inhibition	CYP_3A4_inhibition	CYP_3A4_substrate	HIA	MDCK	Plasma_Protein_Binding	Pure_water_solubility_mg/L	Skin_Permeability	SKlogP_value
1	Myristicin	1.29867	69.5785	57.4357	Inhibitor	Inhibitor	Non	Inhibitor	Weakly	100.00000	356.957	96.977921	139.841	-2.42497	2.621310
2	Alpha pinene	1.01496	3560.94	23.6343	Non	Inhibitor	Non	Non	Substrate	100.00000	1.57213*	97.150175	250.205	0.971564	0.521330
3	Limonene	6.91802	114.923	23.637	Inhibitor	Inhibitor	Non	Non	Substrate	100.00000	12.1852	97.150176	254.102	0.992546	3.433610
4	Linalool	6.12506	63.4132	29.355	Inhibitior	Inhibitior	Non	Non	Weakly	100.00000	2521.39	97.150175	250.205	0.895645	2.749420
5	Camphor	6.39547	3849.57	22.3034	Non	Inhibitior	Non	Inhibitor	Substrate	100.00000	286.668	100.000000	76.5371	-1.3965	3.156000
6	Linalyl acetate	1.05126	97.3768	25.2511	Inhibitor	Inhibitor	Non	Inhibitor	Substarte	100.00000	189.299	100.000000	367.6	-0.816564	3.513370
7	Cis- ocimene	8.86332	26.0443	23.6338	Inhibitor	Inhibitor	Non	Non	Substrate	100.00000	222.654	100.000000	151.682	-0.654122	4.050040
8	.3-octanone	1.12734	2646.4	51.0019	Inhibitor	Inhibitor	Non	Inhibitor	Substrate	100.00000	62.3049	100.000000	737.131	-1.54872	2.653970
9	Imipramine	2.1134	1021.07	49.103	Inhibitor	Non	Inhibitor	Non	Non	100.00000	79.1031	21.957318	14676.2	-2.72347	2.830520

10	Camphene	5.75623	164.943	23.4938	Non	Inhibitor	Non	Inhibitor	Substrate	100.00000	300.517	100.000000	113.777	-1.33638	2.952770
11	β-Pinene	5.75623	164.943	23.4942	Non	Inhibition	Non	Inhibitor	Substrate	100.00000	300.517	100.000000	113.777	-1.33638	2.952770
12	Myrcene	9.1018	3.13858	23.6306	Inhibitor	Inhibitor	Non	Non	Substrate	100.00000	216.252	100.000000	129.408	-0.63298	4.084600
13	α-terpinene	8.03745	116.738	23.45555	Inhibitor	Inhibitor	Non	Non	Substrate	100.00000	118.03	100.000000	129.406	-0.88226	3.634740
14	Terpinolene	8.90455	11.1059	23.6372	Inhibitor	Inhibitor	Non	Non	Substrate	100.00000	221.545	93.161811	98.4766	-0.88394	3.882260
15	Vinyl amyl carbol	5.47447	753.763	43.3131	Inhibitor	Inhibitor	Non	Inhibitor	Non	100.00000	60.5384	100.000000	1014.76	-1.33388	2.576870
16	Bormeol	3.80575	9139.02	24.2343	Non	Inhibitor	Non	Inhibitor	Substrate	100.00000	91.738	100.000000	574.037	-1.88173	2.076990
17	Neryl acetate	1.45295	160.552	44.5132	Inhibitor	Inhibitor	Non	Inhibitor	Substrate	100.00000	232.699	100.000000	249.302	-0.99539	3.571090
18	Sabinene	6.7413	106.201	8.75688	Inhibitor	Inhibitor	Non	Non	Substrate	100.00000	271.032	100.000000	665.548	-1.05921	3.223480

Table.16 *In Silico* docking of myristicin and phytoconstituents in lavender oil against Indoleamine 2, 3-dioxygenase

Phytoconstituents	Binding energy	Inhibition constant	Intermolecular energy
Trimyristin	-4.49	0.0	-5.58
Myristicin	-4.82	293.41µM	-5.18
Camphor	-5.94	444.28 µM	-5.94
Limonene	-5.31	128.3 µM	-5.47
Linalool	-4.82	292.94 µM	-6.00
α – pinene	-5.26	139.39 µM	-5.26
Cis-ocimene	-4.7	357.7 µM	-5.12
Linalyl acetate	-4.56	455.16 µM	-5.81
3- octanone	-4.14	927.96 µM	-5.63
Imipramine	-6.66	13.04 µM	-7.86

Table.17 *In Silico* docking of myristicin and phytoconstituents in lavender oil against Tryptophan 2,3-dioxygenase

Phytoconstituents	Bindingenergy(Kcal/mol)	Inhibition constant	Intermolecularenergy(Kcal/mol)
Myristicin	-4.03	1.11mM	-4.41
α-pinene	-4.98	222.84µM	-4.98
Camphene	-4.57	447.26µM	-4.57
β- pinene	-4.4	596.47µM	-4.4
Sabinene	-4.45	545.6µM	-4.64
Myrcene	-3.28	**3.92µM**	-4.11
Borneol	**-5.53**	88.7µM	-5.83
Neryl acetate	-5.12	178.0µM	**-6.91**
Camphor	-5.23	146.04µM	-5.23
Limonene	-4.4	599.09µM	-4.56
Terpinolene	-4.58	441.57µM	-4.58
Vinylamyl carbol	-3.03	16.06mM	-4.51
Cis – linalool oxide	-5.25	141.15µM	-5.07
α – Terpinene	-4.56	457.84µM	-4.71
Linalyl acetate	-4.16	894.7µM	-5.95
Cis – ocimene	-4.26	757.78µM	-5.45
3-Octanone	-3.78	1.7mM	-5.27
Linalool	-3.47	2.79mM	-4.61
Imipramine	-6.96	18.07mM	-7.32

Docking orientations

The docking orientations of myristicin and 25 constituents present in lavander oil and standard drugs with the enzyme target were as follows:

Table 18: Docking orientations of the myristicin and constituents present in lavander oil and imipramine using enzyme indoleamine 2,3-dioxygenase

Compounds	Binding interaction with amino acid residue
Trimyristin	TYR 353,ILE 341, ILE354,PHE 214, ILE 217,PHE 163, VAL 170, SER 167, GLU 171, PHE 270, TYP 126, ALA 264, LEU 384, GLY 265, PHE 387, PHE 291, HIS 287, LYS 238
Myristicin	ARG 334, GLU 133, LEU 132, ASN 327, MET 331
Camphor	VET 108, LEU 168, GLN 169, **GLU 105**, **PRO 307**,ALA22,SER 355
Limonene	TYR 254, **ARG 303**, ILE 217, **GLU 105**, **VAT 108**,GLY 26
α-pinene	ILE 354, PHE 226, PHE 163, VAL 350, ILE 349, HIS 346, TYR 353, ILE 217
Linalool	LYS 233**, ARG 303**, **GLU 105**, **ARG 103**, PHE 303, GLY 24, SER 355
Cis-ocimene	SER 143, LEU 17, VAL 32, LEU 336
Linalyl acetate	PHE 72, LEU 147, PRO 149, ALA 150, PHE 153,LEU 338, VAL 332
3- octanone	LEU 204, ARG 211, PHE 97, VAL 102, **ARG 103**, TRP 208
Imipramine	**ARG 303,PHE 304, PRO 307, VET 108, GLU 105, ARG 103**

Table. 19 Docking orientations of the myristicin and constituents present in lavander oil and imipramine using enzyme Tryptophan 2,3-dioxygenase

Compound	Binding interaction with amino acid residue
Camphene	LEU 168, GLN 169, VAL 167, ASN 163, GLU 196,THR 200
Sabinene	MET 108, **GLU 105**, PRO 30, TRP 208, **ARG 103**,**PHE 304**, **ARG 303**, PRO 213
Beta-pinene	PRP 208, ARG 211,PHP 97,**ARG 103**,VAL 102
AlphaTerpinene	**PRO 307**, **PHE 304**,PRO 213, MET 108, **ARG 103**
Myrecene	VAL 332, VAL 329, THR 254, **PRO 307**, **GLU 105** , SER 355,
Terpinolene	PHE 97, LEU 204, TRP 208, VAL 109, **ARG 103**,ARG 211
Neryl acetate	PHE 304,ALA 22, THR 254, **VET 108**, SER 355, **GLU 105**, **ARG 103**
Vinyl amyl	LEU 160,LYS 164,LEU 87,ASP 91, TRP 88
Borneol	GL 105, ARG 303, TYR 298, THR 254, SER 256,PRO 307
Imipramine	**AEG 303,PHE 304,PRO 307, VET 108, GLU 105, ARG 103**

Binding of selected constituents in lavander oil and myristicin With Indoleamine 2,3-dioxygenase

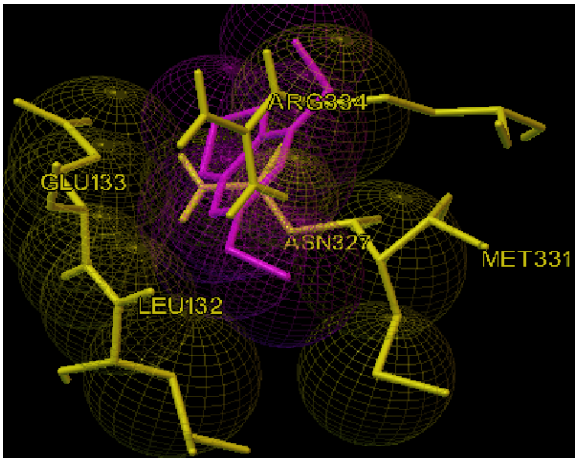

Fig:1 Myristicin

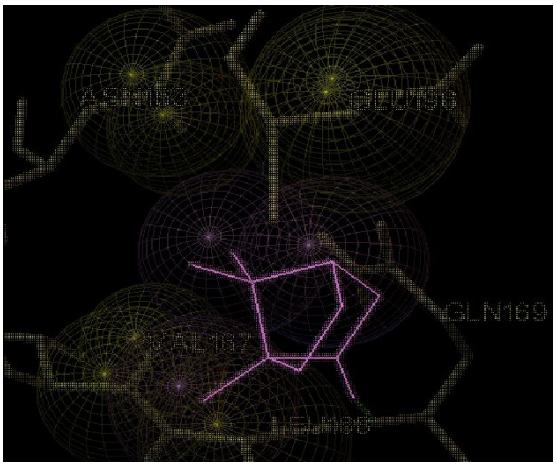

Fig 2: Camphor

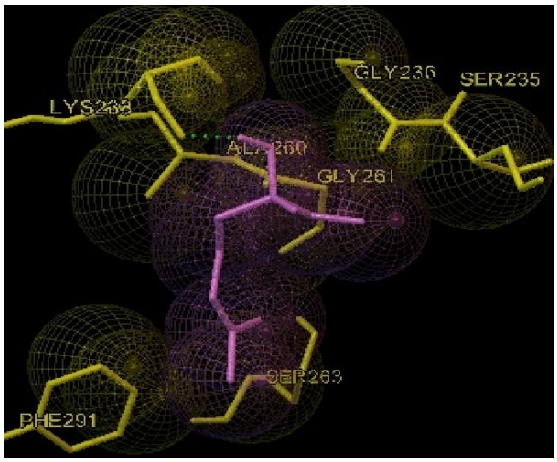

Fig 3: Linalool

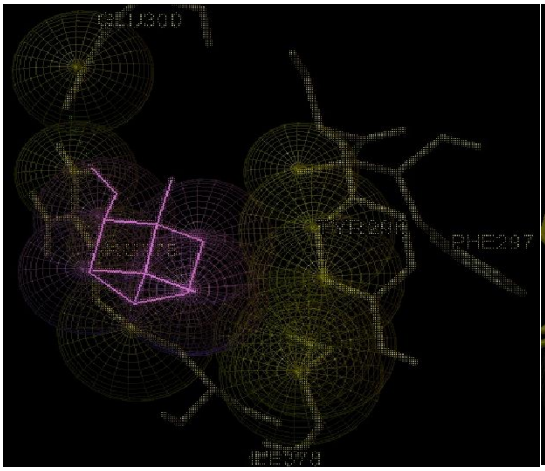

Fig 5: Myrecene

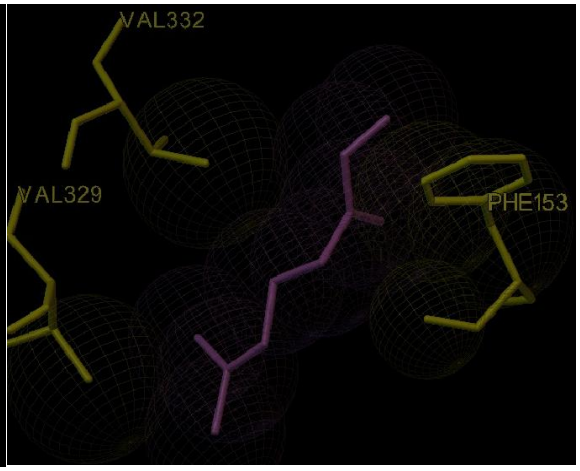

Fig 6: Bomeol

IN SILICO DOCKING STUDIES

Predicted bioactivity score and drug liknesse property of the myristicin and lavander oil constituents using molinspiration software

According to Lipinski's rule of five, molecules should have log P values of ≤ 5 in order to be readily bioavailable. Lipinski's rule also states that the molecular weight should be below 500 dalton, the number of hydrogen bond acceptors should not be more than 10 and the number of hydrogen bond donors should not be more than 5. These numbers are the upper limits for the biomolecules to be able to penetrate through biomembranes. As shown in Table 13, all the compounds had molecular weight below 500, hydrogen bond donor index was in range of 0 to 2 and hydrogen bond acceptor index was in range of 3 to 5. The calculated log P values were in the range of 2.16 to 4.16 which indicates that these compounds have no violations according to Ro5.

Bioactivity of a compound was decided based on the bioactivity score. If bioactivity score is >0, it is biologically active, < -5.0 is inactive and range between -0.5 to 0.0 is moderately active compound. As shown in Table 14, all the compounds had bioactivity scores within the range of -0.5 to 0.0 indicating these compounds are moderately active. Based on the drug likeness properties and bioactivity scores, all the constituents in lavander oil and myristicin were selected for the further prediction of *in silico* biological activity using AutoDock 4.2.

ADMET studies

ADMET studies were carried out using PreADMET software for the compounds selected for synthesis and the results were tabulated in Table 15. Numerous *in vitro* methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, Caco2-cell model and MDCK (Madin-Darby canine kidney) cell model has been recommended as a reliable *in vitro* model for the prediction of oral drug absorption. In absorption, this module provides prediction models for *in vitro* Caco2-cell and MDCK cell assay. Additionally, *in silico* HIA (human intestinal absorption) model and skin permeability model can predict and identify potential drug for oral delivery and transdermal delivery. In distribution, BBB (blood brain barrier) penetration can give information of therapeutic drug in the central nervous system (CNS), plasma protein binding model in its disposition and efficacy. In order to build these QSAR models, genetic functional approximation was used to select relevant descriptors from all 2D descriptors that calculated by Topomol module, followed by Resilient back-propagation neural network to develop

successful nonlinear model.

The 18 compounds exhibited optimum ADMET values. The leads were found to have good oral absorption, intestinal absorption, solubility and less interaction. Hence the phytoconstituents present in nutmeg and lavanderoil.

***In silico* docking of myristicin and phytoconstituents in lavender oil against Indoleamine 2, 3-dioxygenase**

In *silico* docking study was carried out to identify the inhibiting potential of **myristicin and 17 different phytoconstituents in lavender oil against indoleamine 2, 3-dioxygenase (PDB ID: 4PK5) and tryptophan 2, 3-dioxygenase (PDB ID: 4PW8) .** The docking studies were performed by the use of Autodock 4.2 In the docking studies, if a compound shows lesser binding energy compared to the standard it proves that the compound has higher activity. The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding pose. The best docked structure should have lower binding energies. The binding sites and the active sites are shown in the snapshots in fig 1 to 8.

Table16 phytoconstituents present in nutmeg and lavander oil trimyristicin, myristicin, camphor, limonene, linalool,alpha-pinene, cis ocimene, linalyl acetate, 3-octanone showed binding energy ranging from -5.94 Kcal/mol to -4.14 Kcal/mol with indoleamine 2,3-dioxygenase and that of standard (imipramine) showed -6.66kcal/mol. Inhibition constant (kI) and intermolecular energy is directly proportional to binding energy and the constituents showed inhibition constant ranging from 444.28µM to 927.96µM and for standard drug it was found to be 13.04µM . The phytoconstituents showed the intermolecular energy of -5.94 to -5.63kcal/mol respectively and that of standard (imipramine) showed -7.86 kcal/mol. Among the phytoconstituents present in lavender oil, camphor was found to possess excellent (low) binding energy (-5.94), inhibition constant was good in limonene (128.3µM) and linalool showed excellent intermolecular energy (-6.00µM) and 3-octanone possess poor (high) binding energy, inhibition constant and Cis-ocimene showed poor intermolecular energy. The binding energy,inhibition constant and intermolecular energy for myristicin was found to -4.82 Kcal/mol, 293.41 µM and -5.18 kcal/mol.

***In silico* docking of myristicin and phytoconstituents in lavender oil against tryptophan 2,3-dioxygenase**

Table17 The phytoconstituents present in nutmeg and lavander oil myristicin,alpha pinene, camphene, beta pinene, sabinene, myrcene, borneol, neryl acetate, camphor,limonene, terpinolene,vinylamyl carbol,cis linalool oxide, alpha terpinene, linalyl acetate, cis-ocimene, 3-octanone, linalool showed binding energy ranging from -5.53 Kcal/mol to -3.03Kcal/mol withTryptophan 2,3-dioxygenase and that of standard (imipramine) showed -6.96 kcal/mol. Inhibition constant (kI) and intermolecular energy is directly proportional to binding energy and the constituents showed inhibition constant ranging from 3.92µM to 16.06 mM and for standard drug it was found to be 18.07mM . The phytoconstituents showed the intermolecular energy of -6.91kcal/mol to -4.2kcal/mol respectively and that of standard (imipramine) showed -7.32 kcal/mol. Out of 18 phytoconstituents present in lavender oil, borneol was found to possess excellent (low) binding energy (-5.53 Kcal/mol), inhibition constant was excellent in myrcene (3.92µM) and Neryl acetate showed excellent intermolecular energy(6.91Kcal/mol) Hexyl-butyrate possess poor (high) binding energy, inhibition constant and Vinylamyl carbol showed poor intermolecular energy. The binding energy, inhibition constant and intermolecular energy for myristicin was found to (-4.03 Kcal/mol), (1.11 mM) and (-4.4 kcal/mol).

Discussion

There are many prepetrator interacting pathways that are involved in the pathogenesis of depression due to its complexity and heterogeneity. Depressive illness is closely associated with chronic inflammatory pathway, which is manifested by elevated levels of proinflammatory cytokines, chemokines, and adhesion molecules in the periphery and central nervous system.[3] There are ample evidences that central or peripheral injection of LPS (a potent immune activator) induces oxidative stress, neuroinflammation, depressive and anxiety like behaviour in rodents.[7] The chronic unpredictable mild stress (CUMS) protocol has been widely used to study the impact of stress exposure in several animal models and consist of random, intermittent, and unpredictable exposure to a variety of stressors during several weeks.[15]

Experimental studies have demonstrated that myristicin and lavander oil exhibit neuroprotective activity via inhibition of inflammation and oxidative stress in rat brain exposed to stress and also inhibits chronic inflammation induced by lipopolysaccharide .Based on these facts, The present study was designed to evaluate the protective effect of myristicin (100 and 200mg/kg), lavander oil (300 and 600mg/kg) and the combination of myristicin 100 mg/kg and lavander oil 300 mg/kg and combination of myristicin 50 mg/kg and lavander oil 150 mg/kg against LPS - CUMS induced behavioural and biochemical alterations in rat.This study evaluated different behavioural and biochemical parameter, which demonstrated that 42 days treatment with myristicin and lavander oil prevented the augmentation of pro-inflammatory cytokines, oxidative stress, depressive and anxiety like behaviour .

Converging evidence indicates that LPS induced brain IDO activation and this depends on brain expression of cytokines, particularly TNF-α and IL-6 in the hypothalamus and IFN-ɣ in the hippocampus.Depression disorders associated with altered level of this proinflammatory cytokines.[127] *In silico* docking with indoleamine 2,3-dioxygenase exhibited potential IDO inhibitory activity for the selected phytoconstituents and it was compared with standard Imipramine. The phytoconstituents showed better binding interactions and docking parameters (binding energy, inhibition constant and intermolecular energy). Therefore the combination of myristicin and phytoconstituents present in lavender oil will be a better therapeutic tool for the prevention and treatment of depression and anxiety related diseases

Acute activation of the peripheral innate immune system in laboratory animals, through the administration of the cytokine induce lipopolysaccharide, induces depression like behaviour as measured by increased immobility in the forced swim test and tail suspension test, decreased consumption of sweetened solution and suppression of social interaction, the decrease the time taken to identify the hidden platform in morris water maze test and the anxiety like behaviour in elevated plus maze test.[121] The present study was designed to evaluate the protective effect of myristicin (100 and 200mg/kg), lavander oil (300 and 600mg/kg) and the combination of myristicin 100 mg/kg and lavander oil 300 mg/kg and combination of myristicin 50 mg/kg and lavander oil 150 mg/kg against LPS - CUMS induced behavioural and biochemical alterations in rat.

. Imipramine is a tricyclic antidepressant; it blocks the reuptake of neurotransmitters serotonin and NE almost equally. Our studies suggested that Imipramine ameliorated LPS-induced depressive like behaviour in rat and decreased anhedonia, reduced social interaction, locomotor and exploratory behaviour.[112]

LPS (50µg/kg i.p.) and CUMS challenged animal (negative control) exhibited significant decrease in sucrose solution consumption during test period i.e.24 h. In contrast, myristicin (200 mg/kg) and combination of myristicin 100mg/kg+ lavander oil 300mg/kg treatment group showed more preference for the sucrose solution as compared to negative control.

The elevated plus maze is a test measuring anxiety in laboratory animals that usually uses rodents as a screening test for putative anxiolytic or anxiogenic compounds. In the elevated plus maze test an increase in the open arm activity (duration and number of entries) reflects anti anxiety behavior, LPS - CUMS treated group showed anxiety as revealed by significant reduction in the number of entries in open and closed arm, time duration in open arm and closed-arm explorations as compared to the vehicle treated group. Experimental models treated with myristicin (100 and 200 mg/kg), lavander oil (300 and 600 mg/kg) and the combination of myristicin and lavander oil (100 and 300 mg/kg) shows a dose-dependent decrease in duration and number of entries in closed arm and time duration and number of entries in open arm increases.

Social interaction time of LPS exposed rat was significantly less as compared to vehicle treated..Experimental animals treated with myristicin (100 and 200 mg/kg), lavander

oil (300 and 600 mg/kg) and the combination of myristicin and lavander oil (100 and 300 mg/kg) shows a dose-dependent decrease in social interaction time.

Depressive like behavior is associated with latency to float and the duration of immobility in the forced swim test, it is a reliable behavior despair test and has been widely used to screen new antidepressant drugs.[108] The myristicin 200 mg/kg and imipiramine 20 mg/kg induced significant increase in the swimming time. The lavander oil 600 mg/kg, combination 1 (myristicin 100 mg/kg + lavander oil 300 mg/kg) produced less significant increase in swimming time.

TST is similar in some ways to the FST, but animals are suspended by tail in such a way that they cannot escape or reach anything nearby. They struggle initially but eventually become immobile.[119] Myrisicin 200 mg/kg and combination 1 treated group showed a significant decrease in immobility. FST and TST are the most widely used animal models for assessment of antidepressant activity

The Morris water maze is a behavioral task to test hippocampal – dependent learning and memory. It has been widely used in the study of neurobiology, neuropharmacology and neurocognitive disorders in rodent mode, It is a relatively simple procedure typically consisting of six day trial the main advantage being the differentiation between spatial (hidden- platform) and non – spatial (visible platform), the rat must swim to a hidden escape platform [125]. The LPS+CUMS rat took more time to find out hidden platform and increase the escape tendency. Imipramine treated group showed significant decrease in the escape latency. Myrisicin 200 mg/kg and combination 1 induced group showed a significant decrease in the escape latency.

An overactive hypothalamic-pituitary-adrenal (HPA) axis is also a feature of a maladaptive response to chronic stress. Chronic stress can be associated with an effective negative feed back system that is able to shut down the excessive production of glucocorticoid occuring in response to stress. In our study we observed 4 week long CUMS protocol led to a presistent increase on circulating corticosterone level and increased adrenals weight, features consistent with a hyperactive HPA axis. We observed that 4 week CUMS study induced a hyperactive HPA axis, had a negative impact on emotional behavior. Specifically, an enhanced anxious like behavior, we observed in our study and was revealed by an increased time spent in the closed arm, and a decreased time in the open arm in EPM.[85] In animal treated with myristicin 100 and 200 mg/kg and lavander oil 300 and 600

mg/kg and combination of myristicin 100 mg/kg and lavander oil 300 mg/kg the time spent in open arm increased with a decrease in time spent in closed arm. These animals also displayed behavior despair, a symptom of depressive like behavior, as they spent more time immobile in Forced Swim Test. This was further confirmed by performing Tail Suspension Test, another validated test for depressive like behavior assessment.Increased concentration of corticosterone in the plasma is an indication of stress. The experiment conducted as a part of this study showed decreased corticosterone levels in treated group compared to stress control group. This shows the anti-stress potential of myristicin (100 and 200 mg/kg) and lavander oil (300 and 600 mg/kg) and the combination of myristicin and lavander oil (100 and 300 mg/kg) .

It is observed that during stress, there is an increase in the cholesterol level to meet the extra metabolic demands of the tissues. The level of number of hormones like corticosterone, epinephrine and nor-epinephrine in the blood increases. These hormones mobilize the lipid store of adipose tissue[74]. Experimental models treated with myristicin (100 and 200 mg/kg), lavander oil (300 and 600 mg/kg) and the combination of myristicin and lavander oil (100 and 300 mg/kg) shows a dose-dependent decrease in cholesterol levels signifying its anti-stress potential.

Stress also increases the serum triglyceride levels to meet the increased metabolic demands of the body. It could be suggested that the changes in the levels of serum triglyceride is possibly mediated via adrenal medullary secretion and through activation of sympathetic nervous system.[76] Treatment with myristicin, lavander oil and its combination shows significant reduction in triglyceride level due to the decreased adrenal medullary secreation may be due to the decreased activation of sympathetic nervous system.

In neurochemical study for depression we measured excitatory neurotransmitter levels, 5HT, DA and NE in hippocampus and cerebral cortex, as deficieny of these monoamines in brain is thought to be manifested in to induction of depression like behaviors.Various depressive symptoms like, altered mood, anhedonia, pessimism and feeling of worthlessness are associated with impared 5HT and NA neurotransmission. Most of the currently used antidepressants exert their action probably by elevation of these monoamine in several brain area particularly in hippocampus[123]. In the present study LPS - CUMS induced group showed a decrease in the levels of brain monoamines, norepinephrine, dopamine and serotonin. Pretreatment of animals for 42 consecutive days with Myristicin

(100 and 200 mg/kg) and lavander oil (300 and 600 mg/kg) and the combination of myristicin and lavander 100 & 300 mg/kg showed a increase in the levels of brain monoamines, norepinephrine, dopamine and serotonin. The standard drug treated group showed significant increase in brain norepinephrinne, dopamine and serotonin respectively.

Monoamine oxidase A and B play an important role in the metabolism of biogenic amines in the central nervous system and in the periphery. MAO-A shows greater affinity for hydroxylated amines such as NA, 5-HT whereas MAO-B shows greater affinity for non-hydroxylated amines such as phenylethylamine. The amine DA and tyramine show similar affinity for each enzyme form[52]. Elevated level of monoamine oxidase A and B observed in LPS + CUMS induced groups. The standard drug treated group imipramine 20 mg/kg showed decrease in monoaminooxidase A and B respectively. Myristicin 100 mg/kg, lavander oil 300 mg/kg,combination1 (myristicin 100mg/kg and lavander 300 mg/kg) shows moderate decrease in monoamino oxidase A nd B whereas Myristicin 200 mg/kg, lavander 600 mg/kg shows decrease in monoamino oxidase A and B level.[114]

Conclusion

This study highlights the possible interaction between stress and immune-inflammatory pathways in the pathogenesis of depression and suggests an animal model that addresses these pathways. The result of the present study showed that 2 week of LPS and 28 days of CUMS, resulted in depression like behavior in rats, as indicated by the significant decrease in sucrose consumption and increase in immobility time in the forced swim test. Long term treatment with myristicin and lavander oil significantly prevented these behavioral changes, suggesting the antidepressant like effects of myristicin lavander oil similarly, in theMorris water maze test, CUMS significantly suppressed the social interaction , suggesting a loss of exploration and interest in a novel environment. The decreased mobility in tail suspension test caused by LPS-CUMS was prevented by long-term pre-treatment with myristicin and lavander oil. Taken together results obtained from the behavioral test indicated that myristicin lavander oil administration could produce anti depressant like effect in the CUMS induced rat models of depression.

The exact mechanism for antidepressant activity of myristicin and lavander oil is still unknown and the protection level was found to be at considerable range. Hence, further studies are needed to isolate, characterize the active principles and to find out the exact mechanism responsible for its antidepressant activity.

In silico docking with myristicin and phytoconstituents present in lavender oil were docked using Autodock 4.2. with the enzyme indoleamine 2,3-dioxygenase . The docked pose of the phytoconstituents with IDO were analyzed and discovered their binding sites and interaction with amino acid residues. The binding sites were found to be similar with the residual amino acid series indicate the common binding sites for the standard compounds and phytoconstituents tested. This data implies that the phytoconstituent have similar or better interaction with the enzymes.

In conclusion, the results of molecular docking with indoleamine 2,3-dioxygenase exhibited potential IDO inhibitory activity for the selected phytoconstituents and it was compared with standard imipramine. The phytoconstituents showed better binding interactions and docking parameters (binding energy, inhibition constant and intermolecular energy). Therefore the combination of myristicin and phytoconstituents present in lavender oil will be a better therapeutic tool for the prevention and treatment of depression and anxiety related disease.

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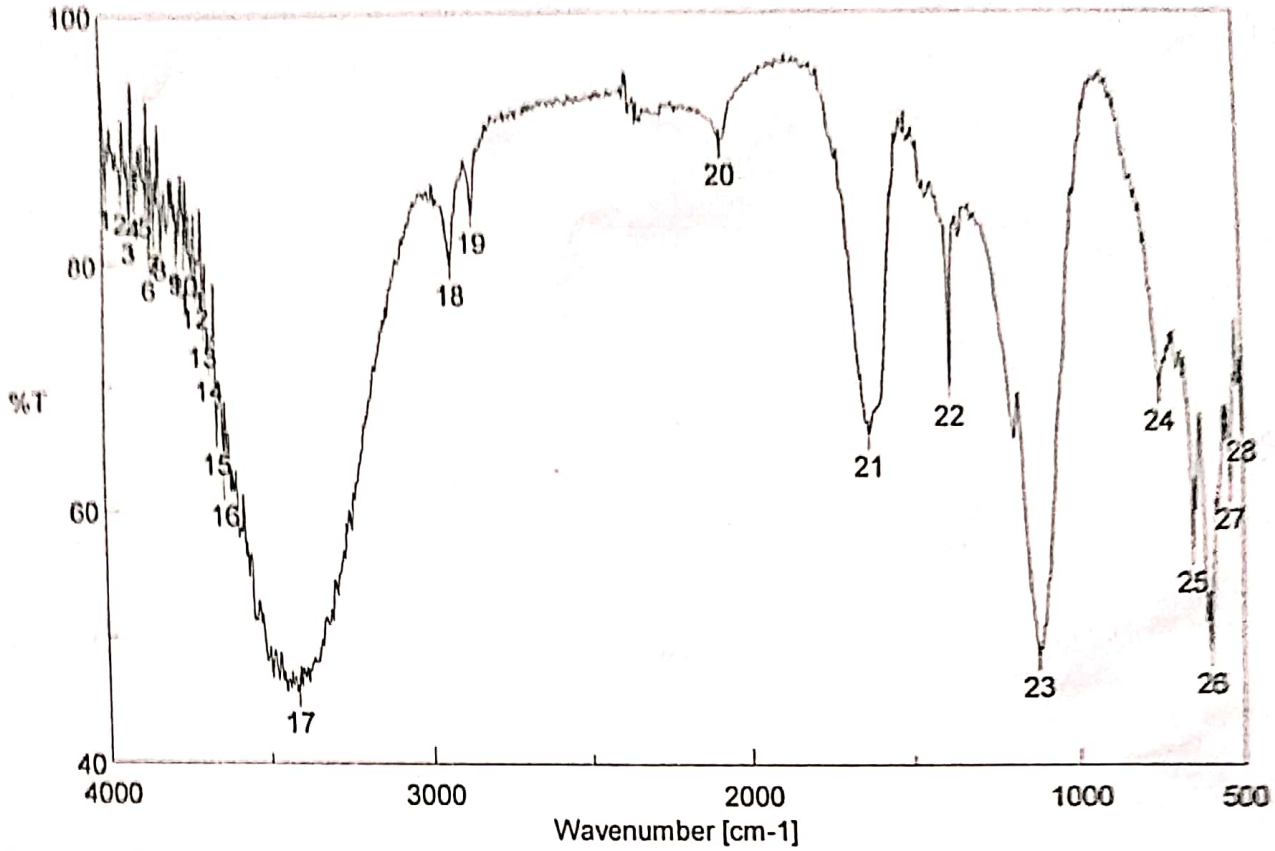
MYRISTICINE



[Comments]
Sample name
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3989.03	86.1163	2	3939.86	85.7591
3	3913.82	83.333	4	3897.43	85.3635
5	3862.72	85.5323	6	3851.15	80.2252
7	3836.68	82.4018	8	3813.54	81.8916
9	3766.3	80.7968	10	3742.19	80.6156
11	3718.08	79.3368	12	3707.48	77.9401
13	3685.3	74.7317	14	3666.02	72.0246
15	3645.77	66.131	16	3625.52	62.0606
17	3412.42	45.7195	18	2918.73	79.5999
19	2850.27	83.8519	20	2074.07	89.3034
21	1631.46	66.0745	22	1384.64	69.9807
23	1121.4	48.6595	24	764.031	69.4878
25	656.643	56.7688	26	600.717	48.895
27	539.971	61.7222	28	503.33	67.0222

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी,एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2018/Tech. /2418

दिनांक/Date: 6th December 2018

पौधे प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen brought by you for authentication is identified a *Myristica fragrans* Houtt. - MYRISTICACEAE. The identified specimen is returne herewith for preservation in their College/ Department/ Institution Herbarium.

6/12/20

डॉ सी मुरुगन/Dr. C. Murugan
वैज्ञानिक 'डी' एवं कार्यालय अध्यक्ष
Scientist 'D' & Head of Office

वैज्ञानिक 'डी' एवंकार्यालयअध्यक्ष
SCIENTIST 'D' & Head of Office
भारतीय दनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयम्बत्तूर / Coimbatore - 641 003.

सेवा में / To

Ms. Arya P Anil
M. Pharm. (2nd Year)
Department of Pharmacology
College of Pharmacy
Sri Ramakrishna Institute of Paramedical Sciences
Coimbatore – 641 044

COMMITTEE FOR THE PURPOSE OF CONTROL AND SUPERVISION OF EXPERIMENTS ON ANIMALS (CPCSEA)

INSTITUTIONAL ANIMAL ETHICS COMMITTEE (IAEC)

College of Pharmacy, SRIPMS, Coimbatore

(CPSCEA Registration # 1559/PO/Re/S/11/CPCSEA)

IAEC PROTOCOL APPROVAL CERTIFICATE

Principal Investigator	:	**Arya P Anil**
Title of the Project	:	Combination of lavender oil and myristicin for treatment of depression in rats exposed to lipopolysaccharide repeated challenge followed by chronic unpredictable mild stress.
Proposal Number	:	COPSRIPMS/IAEC/PG/Pharmacology/001/2018-2019
Approval date	:	19/09/2018
Animals	:	*Wistar* rats
No. of animals sanctioned	:	Male: 48 Female: Nil
Expiry date (Termination of the Project)	:	18/09/2019
Name of IAEC chairperson	:	Dr. T. K. Ravi
Name of CPCSEA Main Nominee	:	Dr. G. Arihara Sivakumar

Signature
IAEC Chairperson

CHAIRMAN
IAEC

29/1119

Signature
CPCSEA Main Nominee

MAIN NOMINEE
CPCSEA