Association between central neurotransmitters and inflammatory mediators in depressive state- An experimental study

A Thesis submitted to Gujarat Technological University

For the Award of

Doctor of Philosophy

In

Pharmacy

By

Mr. Shailendra Bhatt

Enrollment No: 119997290049

Under the supervision of

Dr. Sunita Goswami

GUJARAT TECHNOLOGICAL UNIVERSITY
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I declare that the thesis entitled **Association between central neurotransmitters and inflammatory mediators in depressive state- An experimental study** submitted by me for the degree of Doctor of Philosophy is the record of research work carried out by me during the period from 2011 to 2017 under the supervision of Dr Sunita Goswami and this has not formed the basis for the award of any degree, diploma, associateship, fellowship, titles in this or any other University or other institution of higher learning.

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Abstract

A number of recent studies have reported role of inflammation in depression. In this context, the aim of the present study was to examine the effects of anti- inflammatory drugs like Diclofenac sodium, aspirin, dexamethasone and the prototypical antidepressant, amitriptyline, in single dose studies in albino mice and SD rats and with repeated treatments in the interferon model and chronic mild stress (CMS) model of depression in SD rats. The study further examined the association between the central neurotransmitters and inflammatory mediators in depressive behavior.

A preliminary behavioral study using single dose of anti-inflammatory drugs and antidepressant was done in SD rats and albino mice. The drugs used were diclofenac sodium (10 mg/kg) aspirin (10 mg/kg, p. o.) dexamethasone (1mg/kg p. o.) and amitriptyline (10 mg/kg p. o., reference standard) respectively. Amitriptyline was also used in combination with aspirin and dexamethasone to inspect any synergistic effects. Tests performed were forced swim test (FST) in rats and tail suspension test (TST), elevated plus maze (EPM) and Light dark box. In the single dose studies, the rats treated with dexamethasone and aspirin showed decrease in immobility time in FST in rats and TST in mice. Further mice treated with dexamethasone showed an increased anxiolytic behavior in the Light dark box. Aspirin also showed decrease in immobility in FST and TST but no remarkable effect seen with the use of amitrytilline and Diclofenac sodium.

This was followed by studies using the repeated treatments of above drugs in interferon - α-2b model (21 days study) of depression CMS model (28 days study) in male SD rats. Tests performed in both the studies included Sucrose preference test, behavioural tests like Forced swim test, elevated plus maze test , light dark box test, locomotor activity using the photo-actometer. This was followed by biochemical tests like serum cortisol measurements and brain neurotransmitters estimation at the end of the studies. Disease control group (CMS treated) and interferon produced significant depressive behaviour in rats. The animals treated with aspirin showed increased sucrose preference, decreased immobility time in forced swim test, reduced serum cortisol and increased brain monoamines like serotonin, signifying antidepressant action in both the interferon model and the CMS model. There was aggravation of depressive behaviour in rats treated with dexamethasone on repeated treatment

in contrast to the single dose study wherein dexamethasone showed significant antidepressant behaviour.

Together, these findings suggest that there is inverse relation between inflammatory mediators such as interferon (cytokine) and neurotransmitters like serotonin in stress related behaviour. Further, the use of NSAIDS like aspirin can act as a potential antidepressant both individually and as adjunctive agent in the treatment of depression. In contrast, use of corticosteroids like dexamethasone worsens the depressive behavior when used for prolonged time.

Inhibition of the inflammatory mediators during stress procedures or any other potential physiological and biochemical mechanisms may be involved in the antidepressant effect produced by aspirin like NSAIDS.

Acknowledgement

Research work of Ph.D. is a long expedition is not possible without the support of numerous people. I would take this opportunity to express deep sense of gratitude to my beloved, enthusiastic and honourable guide Dr. Sunita Goswami, for her support and motivation throughout the journey of this research work. I am thankful to her to give me freedom and liberty to carry out the research work along with the required expertise and support. I sincerely express my gratitude towards her as my Teacher, Guide and Friend since I had been an undergraduate student in L.M. College of Pharmacy.

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I am grateful to the almighty for giving me direction and enthusiasm during the entire tenure of research work

Shailendra Bhatt

Abbreviations

5HT-3 Serotonin

ACTH: Adreno Corticotrophic Hormone

ANOVA: Analysis of varience

BDNF: Brain Derived Neurotrophic Factor

BSR: Brain Simulation Recor2ding

CMS: Chronic Mild Stress

CNS: Central Nervous System

COX: Cyclo Oxygenase

COX: Cyclo-oxygenase

COX1- Cyclo Oxygenase-1

COX-2: Cyclo-Oxygenase

CRF: Corticotrophin Releasing Factor

CRF-Corticotropin Releasing Factor

CRP: Corticotrophin releasing

CSF: Cerobro spinal fluid

CUMS: Chronic Unpredictable Mild Stress

CVD :Cardiovascular disorder

CD4 : cluster of differentiation 4

DA: Dopamine

ECT: Electroconvulsive Therapy

EDTA: Ethylene di amine tetra acetic acid

ELS: Early life stress

EPM: Elevated plus maze

FST: Forced Swim Test

GABA: Gamma Amino butyric acid

GIT: Gastro Intestinal Tract

GR: Glucocorticoid receptors

HPA: Hypothalamus Pituatory axis

I.U.: International Units

IDO: Indoleamine 2,3-Dioxygenase

IFN: Interferon

IFN-α- Interferon -α

IL-1: Interleukin -1

IL-2 :Interleukin -2

IL-6: Interleukin-6

IP: Intra Peritoneal

KC: Keratinocyte-Derived Cytokine

LD: Light Dark

LPS: Lipo Poly Saccharide

LTRP:L-Tryptophan.

MAO: Mono Amine Oxygenase

MCP-1: Monocyte Chemoattractant Protein-1

MD- Major Depression

MDD: Major Depressive disorder

MHPEG: 3-Methoxy-4-hydroxyphenylglycol

MIP-2: Macrophage Inflammatory Protein-2

mPGES : microsomal PGE2 synthase

MR: Mineralcorticoid receptors

NaCl: Sodium Chloride

NaHCO3: Sodium Bicarbonate

NE: Nor epinephrine

NSAIDS: Non Steroidal anti-inflammatory agents

NSC- Neural Stem Cells

P.O: Per Oral

PGE-2: Prostaglandin-2

SAD: Seasonal Affective Disorder

SD: Sprague Dawley

SEM:Standard Error Of Mean

SNRI: selective serotonin reuptake inhibitor

SPT: Sucrose Preference Test

SSRI: Selective Serotonin Reuptake Inhibitors

TCA: Tri-cyclic antidepressants

TGF-β: Transforming Growth Factor-β

TMS: Transcranial Magnetic Stimulation

TNF-α: Tumour necrosis factor--α

TST Tail Suspension Test

VNS: Vagus Nerve Stimulation

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List of Symbols

%	Percentage
µ	Micro
µl	Microliter
b.w.	body weight
C	Celsius
dl	Decilitre
g	Gram
h	Hour
IU	International Unit
Kg	Kilogram
m2	Meter square
mg	Milligram
min	Minute
ml	Milliliter
nm	Nano-metre
º	Degree
α	Alpha
β	Beta

Appendices

Appendix 1: Certificates of approval from Institutional Animal Ethics Committee

Appendix 2: Publication Copies

1. Introduction

Major depression or Major depressive disorder (MDD) is considered as commonly diagnosed neuropsychiatric disorder which is seen with a lifetime prevalence of 17% all over the world. It is predicted that major depression will be the second major cause of disability in the world by the year 2020 after cardiovascular diseases (1). The established treatment strategies for depression and other related disorders have principally focused on the medications that alter the activity of monoaminergic neurotransmitter systems. One of the widely accepted hypothesis is functional deficit of norepinephrine and serotonin in the brain which leads to depressive state (2). The emotional symptoms of depression include anhedonia, loss of motivation, apathy, misery, indecisiveness and suicidal tendency and the biological symptoms include sleep disturbance, loss of appetite or excessive eating and loss of libido. Most of the emotional symptoms are attributed to decrease in serotonin levels and biological symptoms are attributed to decrease in norepinephrine levels (3).

Recent research studies in the area of depression demonstrate the role of inflammation as well as dysfunction of immune system in the pathophysiology of depression (4, 5). Patients suffering from major depression are often found to show signs of the important characteristics of inflammation, such as increase in inflammatory cytokines, acute phase proteins, chemokines, adhesion molecules, and inflammatory mediators such as prostaglandins. Repeated exposure of Interleukin -1 (IL-1), Tumour necrosis factor-(TNF-α) and Interleukin-6 (IL-6) in the animals have been shown to produce depressive behavior (6-8). The mechanisms that contribute towards pathogenesis of depression include abnormal metabolism of neurotransmitters like norepinephrine, serotonin and dopamine, modified neuro-endocrine functions with increased activity of hyop-pituatory axis and changed neural plasticity (9, 10).

Studies demonstrate that on systemic administration of IL-1, IL-2 and IL-6 there is significant effect on serotonin and dopamine activity in the brain (11, 12). At a very low concentration, there is increase in dopamine release by IL-2, but at higher concentrations it inhibits dopamine release in the brain. Low level of dopamine in brain

is associated with depression (13). The pro-inflammatory cytokines also changes the norepinephrine activity in the key areas associates with the pathogenesis depression such as locus coeruleus, hippocampus and the hypothalamus leading to symptoms of depression (14, 15). Raised level of cytokines activates the HPA axis and cause activation of corticotropin releasing Factor (CRF) in the hypothalamus. The pituitary gland release ACTH (Adreno-corticotropin-releasing hormone) which in turn activates adrenal cortex and release cortisol in blood (16, 17). Under normal conditions, increased level of ACTH exerts negative feedback control on release of CRF, However in the patients of depression there is failure of suppression of cortisol secretion. This leads to raised serum cortisol in the patients of depression forms the foundation for dexamethasone suppression test (18, 19).

There is a plethora of evidence that suggest that the pro-inflammatory cytokines IL-1, and IL-6 and TNF- α along with their receptors are constitutively expressed in different regions of the brain. These inflammatory cytokines have an important role in regulating synaptic plasticity in major depressive disorder in rodent models of depression (20). The Increased IL-1, IL-6 and TNF activity in brain is linked with decreased hippocampal neurogenesis.

Most of the clinically used antidepressants are found to possess anti-inflammatory properties. Tricyclic antidepressants (TCA) like amitryptilline and desipramine, Selective serotonin reuptake inhibitors (SSRI) like fluoxetine and citalopram, (21,22) moclobamide mono amine oxidase -B inhibitor (23) and hyperforin which is a chemical constituent from herbal plant St John's wart (24) other herbal like withania somnifera and curcuma longa have been reported to possess anti-inflammatory properties and antidepressant action (25,26). Conversely, there has been use of antidepressant amitriptyline in therapy of chronic tension headache which can be a result of inflammatory processes in the body (27). Recently, it has been published that aspirin when added to antidepressant therapy with fluoxetine led to improvement in treatment resistant depressive rats (28). Further, use of selective COX -2 inhibitor Celecoxib enhanced the effect of reboxetine and fluoxetine on cortical noradrenaline and serotonin output in rats (29). Only fifty percent of the currently used antidepressants are clinically effective when compared with the effectiveness of placebo which is thirty percent (30).

Given the above-described distinct pathologic signatures of inflammatory mechanism in depression, it is reasonable to assume that use of anti-inflammatory drugs may have a different impact on the treatment of depression. In this context, the aim of the present study was to investigate the effects of aspirin, dexamethasone and the prototypical antidepressant, amitriptyline, in single dose studies in albino mice and SD rats and with repeated treatments in the Interferon model and chronic mild stress (CMS) model of depression in SD rats.

The primary objective of our study is to examine the antidepressant activity of anti inflammatory drugs using experimental models of depression. The secondary objective is to study association between central neurotransmitters and inflammatory mediators in depressive state.

2. Review of Literature

2.1 Introduction

Depression is currently witnessed as one of the most common psychological disorder prevalent in the society. It is a disorder of mood rather than disturbances of thought occurring in schizophrenia or cognition as seen in Alzheimer disease. It can range from a very mild borderline condition to severe (psychotic) depression which is accompanied by hallucinations and delusion. Depression is a major cause of disability and premature death worldwide. In addition to suicide risk, patients of depression are more prone to die from causes, such as cancer or heart disease.

The symptoms of depression can be divided into emotional symptoms and biological symptoms. Pessimistic behaviour, feeling of apathy, misery, guilt and ugliness are seen as emotional symptoms along with loss of decision making and motivation. In terms of biological symptoms the patient experiences retardation of thoughts and action, loss of libido, appetite & sleep disturbance (31).

2. 2. Types of Depression and associated symptoms (31, 32)

2.2.1 Unipolar depression:-

The patients with this type of depression always show depressive mood in contrast with bipolar depression in which the patients shows mood swing in both the directions i.e. depressive mood and maniac episodes.

Unipolar depression presents about 75 percents of all cases. It is distinctly connected with stressful events of life and non-familial in nature. It is also called as reactive depression and is generally associated with symptoms of anxiety and agitation

Other cases (about 25%, sometimes termed endogenous depression) show a genetic prototype, not linked to external stresses, and to some extent different symptoms. Although the difference is seen in patients clinically, the drug treatment in both the conditions somewhat remains the same when antidepressants treatment is considered.

Unipolar depression also called as **Major depressive disorder (**MDD) prevents the patients of depression from functioning normally because of its disabling nature.

MDD is divided in two types based on the symptoms (31).

A. MDD with psychotic behaviour often accompanied by delusions
B. MDD with atypical characteristics such as mood reactivity along with two of the symptoms listed below:
   Weight gain with raised appetite
   Hypersomnia
   Heavy feeling of arms and legs
   A feeling of Interpersonal rejection.

2.2.2 Bipolar depression

Bipolar mood disorder or manic depressive illness is a form of depressive disorder in which the patients experience extreme mood swings - from depression and sadness to elation and excitement. The mood swings are recurrent in nature, varying from mild to severe, and can be of different length of duration. The patient exhibits depressive behaviour with mania. Mania is generally the exact opposite of depressive symptoms in which the patients exhibits excessive excitement, self confidence and enthusiasm, clubbed with impulsive actions. These signs are seen with irritability, impatience and

violent behaviour, and sometimes with Napoleonic kind of delusions. There is a strong genetic association seen in bipolar depression but no specific genes have been identified.

It is further classified into two types

Type 1: It is characterized by full maniac episodes and major depressive episodes.

Type 2: Major depressive episodes with hypomanic episodes

2.2.3 Dysthymia

The symptoms of depression are chronic in nature rather than episodes of disturbance of mood. Symptoms are not severe and disabling as seen in major depression. The patient does not feel well and finds difficult to function normally. It is often less diagnosed and full dose of antidepressants are found to be effective in the treatment of dysthymia.

2.2.4. Cyclothymia

It is a less severe form of bipolar depression. The patient shows numerous period of hypomania and numerous periods of depressive symptoms which do not meet full criteria for maniac or major depression. Cyclothymia is often found in relatives of patient diagnosed with bipolar depression. Mood stabilizers are effective in the treatment of cyclothymia.

2.2.5. Mania

The characteristic features of mania are changes in feelings such as elated mood, heightened sensations and irritable feelings. The person would show extreme happiness and would feel on top all the times. In terms of increased sensations, the patient would perceive clearer sounds and more vivid colours. The person may become more angry and frustrated when opposed or criticized or prohibited from doing something. During the episode of mania the patient can show increased feeling activity, lack of sleep and impulsive and embracing behaviour.

2.2.6. Psychotic depression

It is severe form of depression in which the patients show symptoms like delusions, hallucinations and withdrawal from reality. The treatment may involve use of antipsychotic drugs.

2.2.7. Postnatal depression:

It is also termed as postpartum depression. It generally affects around 10% of women after pregnancy.

2.2.8 Seasonal affective disorder (SAD):

The incidence of SAD increases along with the distance from the equator. A person who develops a depressive illness during the winter months with symptoms that go away during spring or summer may have SAD (33).

2. 3. Causes of depression:

Based on the presence or absence of cause or a reason, depression can be divided into Reactive Type of depression and Endogenous type of depression. Reactive depression forms 75% of the cases of depression while Endogenous form which has an unknown aetiology and often genetic forms the other 25 percent of the population. Research related to the genetic correlation of depression suggests that a person would be 1.5 to 3 times more likely to suffer from depression if he or she has a parent or sibling that has major depression. The known causes of depression are as under (31, 32)

2.3.1. Events affecting life

Loss of a loved one, partner, family member or a friend can lead to depression. Child birth or menopause, relationship troubles or separation can also be a reason for this disorder. Natural disasters like earthquake or hurricanes can cause depression. Professional causes leading to depression are job changes or loss, financial difficulties and excessive work stress.

2.3.2. Drug treatments:

Use of medications like beta blockers, anti thyroid drugs, oral contraceptives, retinoid and cholesterol lowering drugs like statins are associated with depression. Interferon used in the treatment of Hepatitis C therapy also causes depressive symptoms.

2.3.3. Disease specific Causes of depression

Cancer, stroke and heart disease, infectious disease like Lyme disease, chronic pain and neurological disorders like multiple sclerosis have strong association with etiology of depression. Hormonal abnormalities like Addison disease and Hypothyroidism causes depression.

2.3.4. Deficiency Disorder

Depression is often linked with deficiency of folic acid, deficiency of vitamin 12 and deficiency of vitamin D. Patients with high levels of vitamin D have decreased risk of depression (34).

2.4. Hypothesis/ Theories of Depression (31)

2.4.1. Monoamine theory:

The most accepted theory accepted in the pathogenesis of depression is the mono amine theory. According to this theory depression is caused by functional deficit of amines like norepinephrine and serotonin and dopamine in the brain. The theory incorporates indirect evidence from presently used drugs in the treatment of depression such as MAO inhibitors, tricyclic antidepressant (TCA), Selective serotonin reuptake inhibitors (SSRI) and selective neurotransmitter reuptake inhibitors (SNRIs).

MAO inhibitors like Phenelizine, iproniazid and Isocarboxazide act by inhibiting the Mono amine oxidase which causes the metabolism of monoamines like catecholamine and serotonin. MAOIs were among the first clinically proven antidepressants. In the long term, MAOIs also causes desensitization and down-regulation of postsynaptic receptors.

Selective MAO –A inhibitors like moclobamide has selective affinity for enzyme like MAO-A increases the concentration of norepinephrine and dopamine. Drugs from the TCA like Amitryptiline, nortriptiline and Imipramine acts by inhibiting the uptake of monoamines at the synaptic cleft and cause increased actions of monoamines. SSRI like fluoxetine, flouvoxamine, paroxetine and sertraline acts by selectively inhibiting the reuptake of serotonin at synaptic cleft and produces antidepressant action. SNRI like venlafexine, selectively inhibits the reuptake of monoamines like norepinephrine and causes antidepressant effects. The monoamine theory is currently the most accepted hypothesis for action of antidepressants.

However, there are certain questions which are still unanswered by this theory. Firstly, there is no difference between the metabolites like MHPEG in the blood and urine of both the patients of depression and healthy individuals. According to antidepressant mechanisms listed above, there should be changes in the metabolites of monoamines in the patients of depression. Secondly, drugs like amphetamine and cocaine although acting through enhancement of monoamines have no antidepressant actions (31).

Thirdly, the currently used antidepressants along with the above listed drugs are effective in only 50 percent of the patients of depression compared with placebo which is effective in 30 percents (30).

2.4.2. Cholinergic /Adernergic imbalance

Depression is linked with increased cholinergic activity and decreased noradrenergic in the autonomic nervous system. This is supported by the observations that cholinesterase inhibition worsens depression and muscarinic antagonists like TCA and scopolamine have antidepressant action (31)

2.4.3. Involvement of Gama Amino Butyric Acid (GABA)

Depression is associated with reduced GABA neurotransmission in the cortical circuits of the brain. This is supported by low level of GABA in some MDD patients (35)

2.4.4. Glutamate dysfunction

Dysfunctional glutamate signalling in the brain leading to impaired neural plasticity is attributed to the pathogenesis of depression. Reduced expression of excitatory amino acid transporters in the tissues of brain have been found in the patients diagnosed with major depressive disorder. There are also changes observed in the cerebrospinal fluid (CSF) and brain levels of glutamate seen in patients with MDD. The clinical efficacy of ketamine further strengthens this hypothesis of depression (36).

2.4.5. Neural plasticity and trophic effects

Depression is attributed to impaired neural plasticity of the connection and circuits involving reduction in hippocampal neurogenesis, dendritic debranching and loss of dendritic synapses.

Increased neuronal plasticity has been observed in the preclinical studies with antidepressant treatments. Reduced hippocampal volume in brain and BDNF level in the serum, observed in MDD patients also supports the role of impaired neural plasticity and trophic effects in the pathogenesis of depression. Reduced neural and glial density in post mortem brain tissue of the subjects with MDD has also been witnessed. In animals, the same effect is produced by chronic stress of various kinds, or by administration of glucocorticoids, mimicking the rise in cortisol secretion in human depression. Excessive glucocorticoid secretion in humans often causes depression (37).

2.4.6. Role of opioid and reward pathway

Anehdonia is a major symptom of depression linked to dysfunctional reward circuitry pathway where the brain opioid system plays a significant role. Stress releases

dynorphin which in turn triggers kappa opiod receptors and down regulate dopamine levels. This is supported by the observation that a kappa agonist induces depression (38).

2.4.7. HPA axis and stress

Hypothalamic neurons controlling pituitary function receive noradrenergic and 5-HT inputs, which control the discharge of these cells. Hypothalamic cells release corticotrophin-releasing hormone (CRH), which stimulates pituitary cells to secrete adrenocorticotrophic hormone (ACTH), leading in turn to cortisol secretion.

Depression is ascribed to dysregulation of hypothalamic pituitary axis causing raised serum cortisol. Stress can precipitate depressive episode in some patients. Some depressed patients show elevated level of CRF in CSF and also exhibit increased response to stress (39).

2.4.8. Glial cells and depression.

Glial cells were first identified as non neural constituents in the nineteenth century and were believed to be non significant and no more than silent supportive glue for neurons. However in the last decades the research in this area has changed this perception and provided evidence for glia being important associates of neuronal cells actively taking part in metabolism in the brain, synaptic neurotransmission and neuronal communication.

There is a growing evidence for the involvement of glia in the neuropathology of neurological (40) and more recently, psychiatric disorders, such as major depression and manic-depressive (bipolar) illness. (41)

Types Of Glial Cells

Glia forms the highest composition of cells in the human brain outnumbering neurons by a ratio of ten to one. The ratio is one-to-one in rodents implying that increases in glia over neurons are associated with the progressive development of higher brain functions. Glia of the Central Nervous System (CNS), collectively called neuroglia, can be divided into three main types:.

a. Astrocytes b. Oligodendrocytes (both of ectodermal origin and together called macroglia) and c. Microglia (originating from monocyte-macrophage lineage)

Each type of glia is characterized by specific morphology and functions and they can be distinguished from each other by immunohistochemistry, metallic impregnation methods or electron microscopy.

The three types of CNS glial cells play a crucial role in the proper functioning of neurons. Some glial functions are more specialized and restricted to one morphological type of glia (e.g., oligodendrocytes produce myelin to insulate axons), while other functions are shared by two or three types of glia (e.g. both astrocytes and microglia participate in the response to neuronal injury and secrete neurotrophic factors).

Microglia are considered the "resident macrophages" of the brain. When in their resting state, microglia perform routine maintenance and immune surveillance. Once activated, either by injury or an immune stimulus, microglia secrete a variety of pro-inflammatory molecules, such as Nitric Oxide, superoxide, and inflammatory cytokines. Up-regulation of pro-inflammatory molecules is transient, and does not cause neurodegeneration. However, if up-regulation lasts for an extended period of time, neurodegeneration ensues. Activation of microglial cells in the CNS generate ROS and oxidize tetrahydrobioptin (BH4). BH4 is a critical cofactor for the synthesis of dopamine, noradrenaline, and serotonin, and its loss could explain some of the symptoms of depression. Use of antidepressants like monoamine uptake inhibitor, agomelatin and vagus nerve stimulation inhibit oxidation of BH4 and may thereby show antidepressant action (42).

In a separate study , Aspirin increased total Glutathione levels in microglia cells and enhanced their antioxidative capacity. It reduced production of the pro-inflammatory cytokines TNF-α and IL-6 induced through TLR-3 and TLR-4 activation Collectively, the findings highlight aspirin as a possible measure against inflammation of the nervous system, thus leading to protection against neurodegenerative diseases with an inflammatory etiology (43).

Glucocorticoids are found to one of the regulaters of glial responses during hippocampal neurodegeneration and regeneration. Further glucocorticoid effects on oligodendrocytes seem to be dual, ranging from protective to deleterious actions depending on doses, maturational stages or neural regions. (44)

2.5. Neuroimmunological basis for depression:

2.5.1. Major components of inflammation involved in depression

Inflammation is a vital protective mechanism engaged by the immune system. The Inflammation process prevents and isolates the damage to tissue and infections avoiding it spreading to all over the body. Fever and inflammation are reliable signs of immune system activation and are form of defenses mediated by cytokines.

Inflammation in general has two components, the cell derived components and plasma derived components of inflammation. The major components that can affect the brain function both physiologically and pathologically are:

Histamine:

It is released from the mast cells and basophiles and it causes increased venous permeability and arterial dilatation and a variety of effects on the other systems.

Eicosanoids: They include prostaglandins and leukotrienes. Prostaglandins are group of eicosanoids or lipids released from mast cells and causes vasodilatation, fever and pain. Leukotrienes are released by leukocytes, they can cause leukocyte adhesion and activation and helping them to attach to endothelium and migrate across it. It is also a potent chemo attractant and can trigger the formation of reactive oxygen species.

Cytokines: There are around 28 immune cytokines and each one of them have a wide range of actions. The main types of interleukins or pro-inflammatory cytokines implicated in depression are IL1, IL2, IL6 and TNF (45-47)

a. Interleukin 1: IL1 has significant effects on the brain. The IL1 receptors are found in hypothalamus, hippocampus, raphe nucleus and locus coeruleus which are the main structure of the brain. IL1 manages the most of the body's major neurotransmitters and hormones, Neurotransmitters like serotonin, norepinephrine and dopamine are majorly under its influence. Emotions, Instinctive behavior and reactions to physical and psychological stress, and basic drives such as thirst, hunger, sex, pain and pleasure are influenced by these neurotransmitters.

b. Interleukin 2: IL2 has powerful effects growth and survival of nerve growth, nerve impulses and action of neurotransmitter. The brain and has IL2 molecules and IL2 receptors all over and they can also cross the blood-brain barrier. Hence IL2 secreted by activated lymphocytes in lymph gland, gut and spleen can move to the brain and affect

its function. Along with the development of brain it is also involved in the functions like regulation of sleep, arousal and memory, movements, sickness behaviour and psychiatric disorders like schizophrenia and depression.

c. Interleukin-6: IL-6 potent pleiotrophic cytokine which is similar to IL1 and TNF but also has some differences in terms of significant amplification of IL1 and TNF by IL-6. The production of IL6 increases in the body with the age, in contrast to most cytokines which decline with age. This has vital impact on degenerative brain disorders Alzheimer's disease and like Parkinson's disease.

d. TNF-α: They are mainly secreted from macrophages, and they affect different cells to produce fever, chemotaxis, fibroblast activation, enthothelial regulations and leukocyte adherence. They are also responsible for the systemic effects of inflammation, such as increased heart rate and loss of appetite.

Differentiating neuro-inflammation and peripheral inflammation

A major difference in neuro-inflammation and CNS inflammation is microglial cells in cns which are involved in immunological processes are not lymphocytic cells (48,) in as seen in joints and muscle.

Minocycline, metformin, low-dose naltrexone,and pentoxifylline. Alendronate, a bisphosphonate that inhibits bone absorption, has recently been shown to attenuate microglial cells (49). Whereas the nonsteroidal anti-inflammatory drugs, such as ibuprofen and naproxen, do not do much clinically to reduce centralized pain and neuroinflammation. It may be due to some characteristic of neuroinflammation, perhaps the activation of the microglial cell, or failure to simply cross bbb (50). Corticosteroids dexamethasone and methylprednisolone to be very effective in the treatment of centralized pain and inflammation.

Treatment agents for peripheral inflammation are carried to the target solely by arterial blood where as Agents that target neuroinflammation may arrive at the pathologic site by arterial blood as well as by spinal fluid whose physiologic purposes include lubrication, waste removal, and nutrition. It may be that agents that effectively treat

neuroinflammation need not only to cross the blood-brain barrier but also to enter the spinal fluid to be transported to the inflamed tissue site. (50)

2.5.2 Role of inflammatory mediators in pathology of depression

The first findings that depression is characterized by cell-mediated immune activation and inflammation were published by Sluzewska et al. (51). Another study by Maes et al. reported that depression was found be associated with the raised plasma levels of TNF-α and IL-6 (52). Activation of Immune system by administration of endo-toxin caused an increase in depressive symptoms in a study in which the researchers witnessed a positive association between pro-inflammatory cytokines like IL-1, IL-6 and TNF-α and the depressive symptoms (53).

The results from clinical studies are also not different. Studies have shown raised levels of inflammatory markers (IL-6, IL-10, and CRP) in patients with major depression (54-59). Further in a separate study conducted by Dentino and colleagues, a positive correlation between depressive symptoms and plasma level of IL-6 was found (60). The findings in the study suggest that, in humans, changes in the production of the pro-inflammatory cytokines, TNF-α, IL-6 and IFN-γ, and negative immunoregulatory cytokines, IL-10 and IL-4, take part in the homeostatic response to psychological stress and that stress-induced anxiety is related to a T-helper-1-like response.

Both the absence and over-expression of the cytokines affects central nervous system functions such as neurogenesis, synaptic plasticity, and neuromodulation in different ways, including activity within basal ganglia and the frontal cortex (61, 62). Further they also affect the activity of the hypothalamus-pituitary axis (HPA), which is a key factor in the pathogenesis of MDD (63). While MDD is associated to the overall disturbance of the immune system homeostasis it can also cause other conditions like CVD, cancer and diabetes (64).

Increased expression of inflammatory mediators in depressed patients occurs which may lead to variability in response to antidepressant drug therapy. For example, depressed patients non-responsive to drug treatment are reported to have increased cell mediated immunity shown by elevated CD4+ T-cell activity, pro-inflammatory cytokine

expression, and stimulation of the acute phase response. This suggests a psycho-neuroimmunological approach may be required for optimal pharmacotherapy (65).

2.5.3 Association between neurotransmitters, cytokines and cortisol in depression

The major neurotransmitters implicated in the etiology of depression are noradrenaline and serotonin, the concentrations of which are generally decreased in brain of patients with depression. One reason for the decrease in the neurotransmitter concentration in the brain can be because of the increase in the inflammatory mediators during psychological stress (66).

Mechanisms through which inflammation may cause depression

Various inflammatory mediators like IL-1, IL-2, IL-6, TNF, IFN-γ stimulate the production of an enzyme called indoleamine 2, 3-dioxygenase (IDO). This enzyme degrades the tryptophan by converting it into another substance like kynurenine. Due to this enzymatic degradation less tryptophan is available for synthesizing 5-HT or serotonin (Fig- 2.1) .The degradation of tryptophan by IDO is believed to contribute to the reduced serotonin availability which leads to depression (67-68).

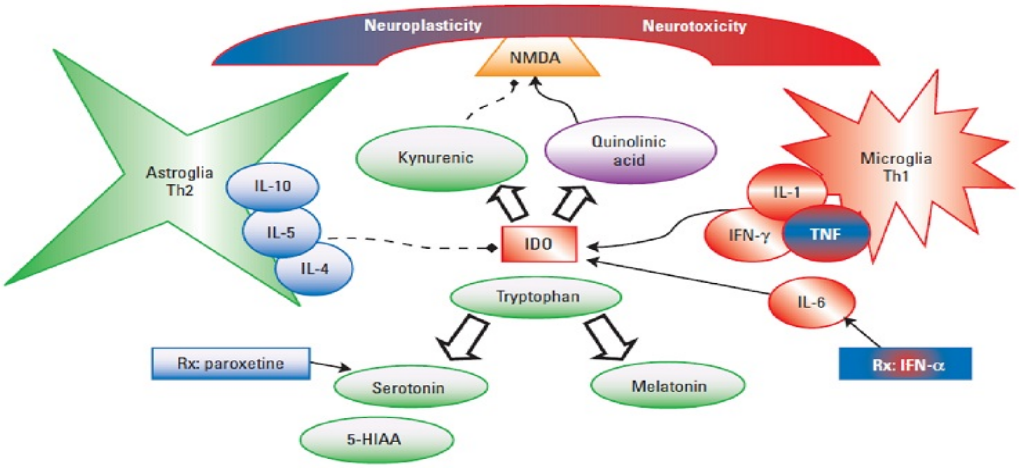

Figure 2.1: Correlation between IDO, Tryptophan interleukins and serotonins. [Adapted from Leah et al (66)]

Cytokines and norepinephrine

Cytokines like IFN-α, IL-1, and IL-2 have been found to be having a significant effect on the activity of the locus coeruleus in the brain. Locus coeruleus , hippocampus and hypothalamus have been associated with increased action of NE when challenged with the antigens activating the immune system and increased secretion of the cytokines. Peripheral administration of IL-1 to the animals stimulates both the turnover and the release of NE in the hypothalamus. The neurons which cause the release of NE into the hypothalamus originate in the locus coeruleus and influence the output of pituitary hormones including the stress hormones. Hence increased release of NE in the hypothalamus caused increase in the secretion of stress hormones. Apart from the extended release of the stress hormones it also stimulates the activity of tyrosine hydroxylase which causes the metabolism of the monoamines like NE and DA.

Cytokines and dopamine

Nor-epinephrine is synthesized from dopamine. Due to this close relation the patients of depression show less levels of dopamine. The steps in the biosynthesis of dopamine are:

Tyrosine → Dopamine → Norepinephrine → Epinephrine

Low levels of IL-2 are associated with increased dopamine release which higher dose of prolong treatment with IFN caused decrease in the release of dopamine.

Cytokines and Serotonin

Patients with increased immune activation and raised cytokine levels have been shown to exhibit low LTRP (L-Tryptophan) levels. Interferon stimulate the release of enzyme indoleamine 2,3-dioxygenase which degrades LTRP. Further cytokines like IL1 and IL6 also reduce LTRP. Low LTRP levels are associated with low serotonin in the patients of depression

Cytokines and HPA-axis Hyperactivity

In the case of depression, internal stressor such as, infection, trauma, autoimmune disease, cancer, dying tissue) causes of immune activation. Activated immune system secrete much higher amount of, IL-1, IL-2,IL-6 and TNF . These circulating cytokines in the blood stimulate the production of the corticotrophin- releasing hormone which

causes the activation of the HPA axis and results in hypercortisolemia. The symptoms of depression like anorexia, fatigue, sleep disturbances, anhedonia, weight loss and reduced psychomotor activity are linked with this increased function of adrenal gland through cytokines**.** Clinical and preclinical evidence supports the concept that impaired corticosteroid receptor signaling is a key mechanism in the pathogenesis of depression (63)

2.5.4. Transforming the current approach to the novel immune-cytokine approach leading depression

The Current model of depression provides limited incite to the etiology of depression. There is role of heredity but the mechanism remains unexplained. Mental stressors probably contribute towards pathogenesis of depression, but there is general agreement that mental stressors are not the principle causes of depression. This leaves unknown factors as the mysterious etiologic agents. Unfortunately, the Current explanatory model puts severe restrictions on the unknown factors. Physical stressors are not considered as causes of depression. The Current approach towards the pathogenesis of depression strongly restricts physical stressors which hinders the search for the other causes of depression (Fig 2.2)

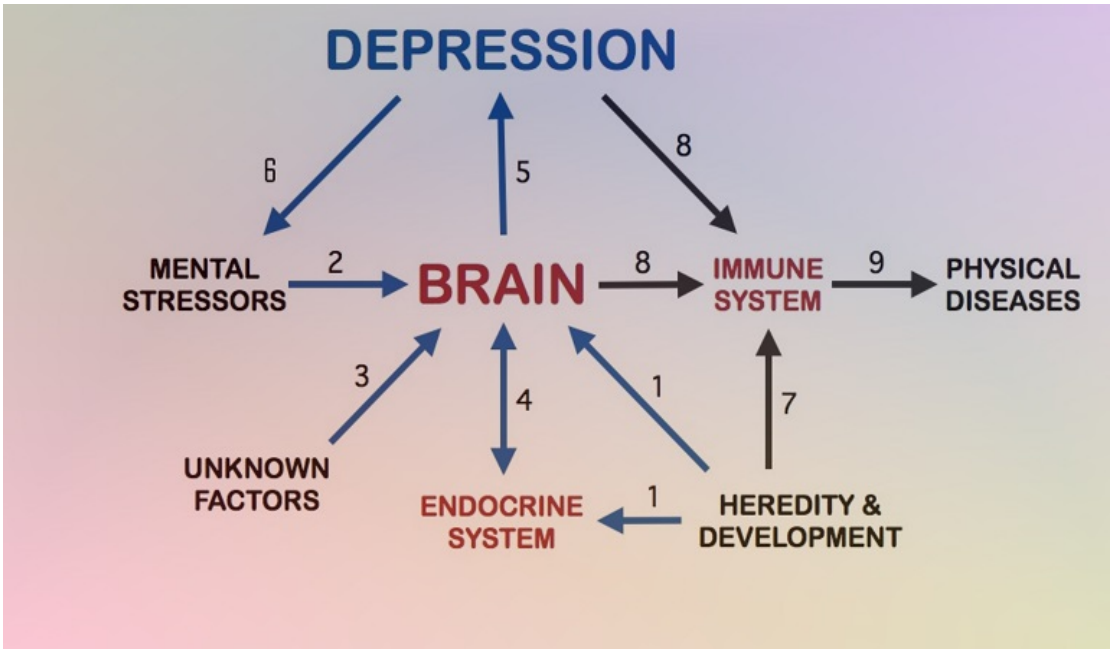

Fig 2.2: Current approach toward pathology of depression: Current approach fails to incorporate inflammation in depression. Main features: No interaction between immune system and endocrine system, No two way interaction between immune system and brain, Physical stressors not linked with depression and immune system [Adapted from Ronald Smith, 1997 (67)]

The Immune-Cytokine model

The Immune-Cytokine model provides a mechanism to search new areas for vast causes of depression. Although this model doesn't solve the problem of etiology, it does provide a mechanism to search novel widespread areas for the causes of depression. First, the effect of genes and development on the immune system is linked to establish the cause of depression. Second, the vast domain of physical diseases becomes available for exploration on the etiology of depression when physical stressors are incorporated. Of keen interest here are chronic disorders like arthritis, cancer, cardiovascular disorders which can now be explained by the immune cytokine model of depression along with chronic infections

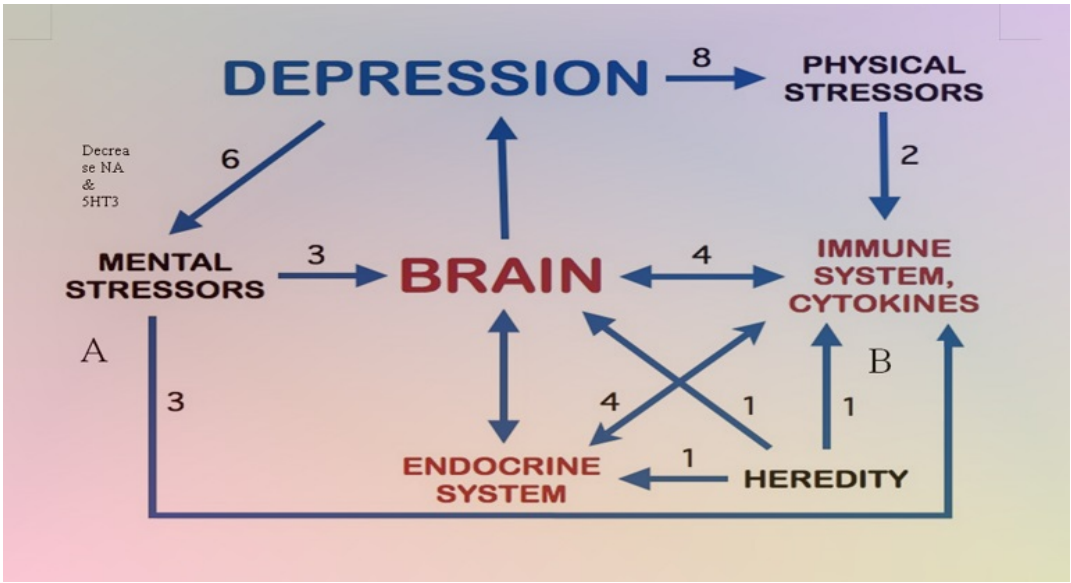

Figure 2.3: The Immune cytokine model explaining pathology of depression. It incorporates inflammation in depression. [Adapted from Ronald Smith, 1997 (67)]Main features: Two way interactions between immune system and endocrine system, two way interactions between immune system and brain, Physical stressors linked with depression and immune system.]

2.6. Evolutionary aspect of inflammatory role in depression

Previous evolutionary pressures were deduced from the interactions of the humans with predators, pathogens and their rival counterparts. Such interactions caused an inflammatory bias which incorporated assembly of immunological and behavioural responses which conserved energy for healing wounds and fighting infections concomitantly maintaining the vigilance against attack. This is generally inhibited by the activation of regulatory B (BReg) cells, regulatory T (TReg) cells and M2 macrophages as well as the production of the anti-inflammatory cytokines IL-10 and TGFβ. The sanitized urban environments of more developed societies in modern times lack in the infectious challenges as mentioned above. In the absence of the above triggers there is absence of immunological checks which leads to high prevalence of inflammatory disorder such as depression (Fig.2.4) (68).

FIGURE. 2.4: Evolutionary aspect of inflammatory role in depression. (Adapted from Miller et al. 2015) (55)

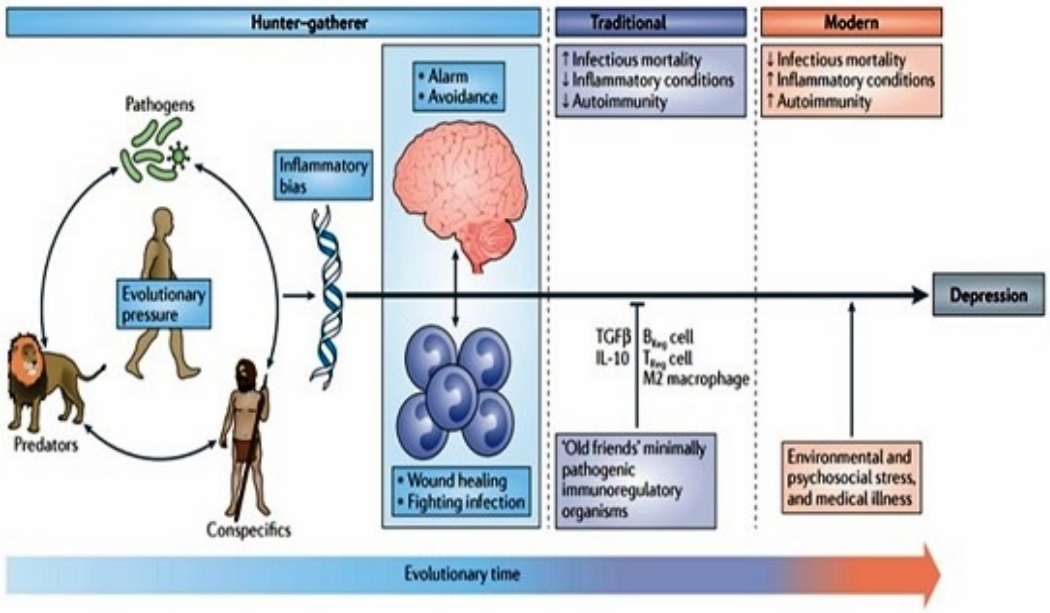

FIGURE. 2.4: Evolutionary aspect of inflammatory role in depression. (Adapted from Miller et al. 2015) (68)

2.7. Pharmacological evidences supporting that depression is an inflammatory disease

Overall activation of the inflammatory system causes lack of clinical therapeutic benefit of antidepressants (69). Further, antidepressants have been suggested to alleviate symptoms of depression via anti-inflammatory actions. Administration of anti-cytokines to patients with concurrent depression and inflammatory disease has resulted in relief of depressive symptoms.

A number of antidepressants, currently prescribed in clinical practice are found to have anti-inflammatory effects and some of the medicines from herbal source are found to have anti-inflammatory and antidepressant effects. Most of the antidepressants used in the treatment of depression have significant anti inflammatory Activity. Tricyclic antidepressants like Amitriptyline, desipramine, trimipramine, doxepin SSRI fluvoxamine, fluoxetine , and Norepinephrine reuptake inhibitor maprotiline are found to have significant anti-inflammatory activity (21-23). Evidence from herbal pharmacology is also not different. Hyperforin, an antidepressant constituent from St. John's Wart has considerable ant inflammatory activity (24). Herbal drugs like curcuma and ashwgandha also have both anti inflammatory and antidepressant action (25, 26).

2.8 Management of depression: Pharmacological Treatment (31)

2.8.1 Selective Serotonin Reuptake Inhibitors (SSRIs)

Fluoxetine, sertraline, Paroxetine, Fluvoxamine The major adverse effects associated with them are nausea, dry mouth, insomnia, diarrhea, nervousness, agitation or restlessness dizziness, sexual problems, such as reduced libido or erectile dysfunction, headache and blurred vision.

2.8.2 Tricyclic Antidepressants (TCAs)

The major drugs used in this class are Amitriptyline, Clomipramine, desipramine and doxepin. The major side effects are dizziness dry mouth constipation,weight gain and tremors They are usually reserved for depression that doesn't respond to other class of antidepressants.

2.8.3. Monoamine oxidase inhibitors (MAOIs)

They include drugs like Phenelzine and Tranylcypromine. The major side effects are stomach upset, dizziness dry moth constipation, tremor, blood pressure changes and nausea. MAOIs can cause serious drug interactions and also major food drug interaction. They are usually reserved for depression that doesn't respond to other class of antidepressants.

2.8.4. Serotonin Norepinephrine Reuptake Inhibitors (SNRI)

The main drugs from this class are Venlafaxine and Dulexatine. They have similar adverse effect like SSRI and also show changes in appetite, muscle weakness palpitation, increased blood pressure, and increased heart rate.

2.8.5. Serotonin antagonist and reuptake inhibitors

They include drugs like Trazodone, and Nefazodone. They share similar adverse effect profile with that of SSRI along with muscle weakness, numbness and tingling sensations.

2.8.6. Norepinephrine- dopamine reuptake inhibitor

Norepinephrine- dopamine reuptake inhibitor like Buproprion is used a antidepressant. It is also used for treatment of alcohol abuse. It causes headache drowsiness, insomnia, GIT side effects like aches, nausea, vomiting and constipation along with redness and pain in the mouth and feeling drunk and difficulties in concentration.

2.8.7. Noradrenergic and specific serotonergic antidepressant

These include drugs like Mitrazepine. It causes CNS side effects like drowsiness, dizziness, vivid dreams, dry mouth and constipation along with increased appetite and weight gain.

2.8.8. Melatonin receptor agonist

Agomelatin is s a novel antidepressant. It may cause excessive sweating, back pain and GIT adverse effects like nausea, vomiting diarrhea and constipation.

2.9. Management of depression: Non Pharmacological Treatment

2.9.1 Talk therapy:

The talk therapy includes therapies like cognitive behavioural therapy, interpersonal therapy and problem-solving therapy. Generally a trained therapist or a psychologist can help in this type of treatment. It includes:

Cognitive behavioural therapy: It helps in finding how the patient thinks and their behaviours which plays a key role in the pathogenesis of depression. The therapy helps in identifying and changing the unhealthy patterns and help in effective treatment of depression.

Interpersonal therapy: It primarily focuses on the relationships of the patient with other people and how it affects them. The therapy helps in targeting it and changing unhealthy habits.

Problem-solving therapy: It focuses on the specific problems that the patients faces and helps in finding the solutions.

2.9.2. Electroconvulsive Therapy (ECT)

It is generally preferred in patients who are resistant to multiple medications. It is typically recommended when the patients show severe symptoms of depression. The patient is under general anaesthesia and the doctor then gives short controlled seizure with small electric current. This type of treatment is done over a few weeks influences the brain areas which affect mood and 70 to 90 percent of the patients show improvement. Temporary memory loss is the major side effect seen with ECT.

2.9.3. Transcranial Magnetic Stimulation (TMS)

It uses an electromagnetic device above the top of head and causes a smaller electric current in the brain region controlling mood. This method doesn't require sedation or anaesthesia and it generally causes very mild side effects like discomfort at the site of

application and headache. It generally used for 4 to 5 times a week for one to two months.

2.9.4. Vagus Nerve Stimulation (VNS)

It is used in the patient with treatment resistant depression. It is a process in which a small electrical device is attached with wires to the vagus nerve by implanting a electrical generator. The pulses are delivered via vagus nerve to the brain areas associated with mood control and relieve depression.

2.9.5. Other Therapies

They include strategies like yoga and meditation. Exercise also help in causing the relief of symptoms and herbal drugs like St John's wart is also considered for the treatment.

2. 10. Animal Models of depression

Exposure to stress is a main environmental risk factor associated with the occurrence of depression. Experimentally, the effect of stress exposure is influenced by several variables, including the nature of the stress, the severity of the stress, and exposure parameters. Different neural circuits are activated by different types of stressors. For example, differential involvement of limbic pathways is thought to occur for the processing of stressors that differ in their systemic versus cognitive/psychological nature (70, 71).

Based on the duration of exposure of the stress the models can be acute or sub-chronic stress methods which include learned helplessness (LH), forced swim test (FST), and tail suspension test (TST), where rodents are exposed to relatively acute or sub-chronic stress. The chronic stress models that include modalities such as chronic unpredictable mild stress exposure, early-life stress (ELS) paradigms, and social defeat/conflict models are considered more naturalistic in the induction of a depressive-like state and are suggested to have better potential homology to the human situation (72, 73)**.**

Learned helplessness: The Learned helplessness principle utilizes a stress-exposure timeline in which rats or mice are exposed to stress which is unavoidable (e.g., electrical footshock) in one or more sessions. In following session, the animals are examined for their performance in an active avoidance test. In a prototype active avoidance test, animals are restricted to one part of box chamber where foot-shocks are given but the animal has the chance of actively escaping the foot-shock. Animals exposed previously to inescapable stress depict decreased abilities to get away in this model. The decreased ability to escape the shock is re-established by treatment with different types of antidepressant, including selective serotonin reuptake inhibitors, tri-cyclic antidepressants, electroconvulsive shock therapy and monoamine oxidase inhibitors (74). This model for assessing antidepressant activity has established validity for the prediction of antidepressant action and efficacy (75). It has been utilized to show the importance of stressor control as an important psychological aspect in causing the behavioural deficit. Animals assessed by the learned helplessness model show a number of features that have similarity with the symptomology of depression such as weight

loss, decreased motivation, altered sleep, reduced motor activity , and increased stress hormones (76).

The timeline for the induction and treatment in learned helplessness model is better in the subchronic rather than the acute response of forced swim test and the tail suspension test. The models based on the learned helplessness can differentiate subgroups of stress exposed animals which are more susceptible to become helpless. Hence, using the learned helplessness model, vulnerable and resistant subgroups of depression can be identified and mechanisms causing differential susceptibility can be studied in as it has similarity with the human depression. The major limitation of this model is lack of reproducibility and the methods for the induction (77).

Forced swim test (FST): Tests like FST in rats and tail suspension test (TST) in mice are generally performed to ascertain the antidepressant action using learned helplessness model. The FST involves the recording of active behaviour like swimming and climbing and passive behaviour like immobility when rats are made to swim forcefully in a cylinder filled with water from which it is not able to escape. A pre-test or pre training session of 15 minutes or a 10-min pre-test with success (without frequent drowning of animal) is included, as this depicts the diverse behaviors in the 5-min swim test after drug treatments. Reduction in the immobility is considered as antidepressant like effect, while taking in consideration that it doesn't increase the motor activity, which can lead to a false positive result in forced swim test. Being is active behaviour means the animal is continuously swimming and climbing which involves immense physical stress and can be painful to the animal (continuous movements to try to not to immerse in water) (78). The immobility has been found be dose dependently reduced by MAO inhibitors ,TCA and other antidepressants (79).

In another modification of the FST method, the treatment response by norepinephrine selective drugs is distinguished from the SSRI by quantification of swimming and climbing behaviour. The climbing behaviour predominantly attributed to the Serotonin selective drugs and the swimming behaviour to nor epinephrine selective drugs (80). However, false positive results may be observed with the drugs like amphetamine which increase the locomotor activity.

Tail suspension test: Another screening test for antidepressant drugs, called as the tail suspension test is also based on the learned helplessness model. It is based on the principle that mice exposed to the short-term, will develop an immobile posture if

suspended by their tail (inescapable stress) It is takes root from hypothesis that animal will forcefully try to escape from a hostile and stressful situation. If the circumstances are inevitable, the animal will give up eventually.

Longer periods of immobile behaviour are characteristic of depressive behaviour. The use of antidepressant will decrease the immobility time. The acute antidepressant treatment prior to the TST decreased the immobility time and is considered to have a good predictive validity (81). Different strains of mice respond differently to the basal immobility in the tail suspension test. (82). The TST is not used in rats due to larger size and weight which sometimes damage the tail of the rats. TST has similar limitations as the FST i.e. false positive responses to psychostimulants.

2.10.2 Chronic stress models

Chronic Mild Stress: The chronic mild stress model is based on more chronic effects in comparison with the FST, TST or LH which are based on short term aversive behaviour. In this method the rats and mice are exposed to series of different stressors for several weeks. Typical stressors that are applied include overnight illumination, cage tilts, isolation of animal, paired housing, crowded housing, food or water restriction or housing in damp sawdust. This type of arrangements does not allow the animals to habituate to any one of the reoccurring condition (83, 84).

The gradual development of anhedonia or decrease in reward sensitivity is the main core of CMS paradigms. Anhedonia is common characteristic of all forms of depression. Along with the anhedonia the other features such as changes in sleep, decreased self care and increased HPA activation with immune system abnormalities are also observed in this model (85).

The validity of this model is mainly based on the development of reduced sucrose preference which occurs which shows the presence of anhedonia in the animals.

The anhedonia in the CMS model responds to chronic but not acute treatments with the different classes of antidepressant drugs (86). The CMS model is more time consuming in nature but the occurrence of other symptoms of depression along with the validity of using anhedonia as an endpoint has lead to rise in the use of CMS models.

Determining the consumption of sweetened fluids like (sucrose or saccharin) is the most commonly used method for examining chronic unpredictable stress effectiveness in rats and mice. Rats which are previously habituated to sucrose are given an option of drinking sucrose versus water in a two-bottle test. Rats exposed to CMS loose this preference while control rats typically show the preference for drinking weak sucrose solutions,

The progress of this effect can be shown by frequent sucrose preference testing during the CMS exposure. The time-dependent reversal of this effect with chronic antidepressant treatment can also be demonstrated by repeated testing. The reliance of sucrose consumption/ preference on several experimental variables such as test duration and sucrose concentration (87, 88) can be performed. Stress-sensitive changes in sucrose preference are more difficult to establish in mice in comparison with rats.

In brain simulation recording (BSR) models, animals with implanted electrodes are trained to perform a specific task, completion of which results in a form of reward. This causes electrical stimulation into a specific area of the brain which can be quantified and recorded. BSR models can be used to evaluate stress effects on reward function, including effects of CMS (89).

However, because they require complicated processes like surgeries and also training they are less preferred than sucrose testing.

Early-life stress: Early life adverse experiences or events are an important predisposing factor for depression in humans. Stress or adversity in the early life along with genetic risk factors increases the risk of depression (90). Experimental models have been developed in an effort to mimic model of ELS, and these models make use of stress exposure during critical periods of development and result in stable phenotypic changes (91). During the developments in humans parental care is very important modifier of effects of stress. One of the most widely preferred methods is maternal separation and during the developments of animal models of maternal deprivation is an important for predisposition of affective disorders. In this method the pubs are separated for three to six hours during the first two weeks of birth. These animals are than developed in normal conditions throughout adulthood (92).

Social defeat: Social setup is a key factor in the development of in many species and plays a vital roe in the development of depression and other psychological disorders in

humans (93). The rodent models of depression often use a conflict situation which results in one of the animal holding dominant status and another ending up subsidiary or 'defeated'. A phenotype trait seen in the defeated animals is social avoidance which resembles to the social withdrawal trait in human depression. Social withdrawal is a key symptom of depression. (94, 95)

In this model, social conflicts are produced between the male animals. This is done by carefully considering the body weight, strain and social status of the animal before introducing as intruder into another resident's home cage. The prototype used may vary in the number of session of conflicts or the type of conflict. Either physical attack or threat of attack or combination of both can be utilized. The key traits observed in the defeated animals are anhedonia and social isolation. The other behavioural changes include decreased sexual behaviour and increased anxiety along with decreased locomotor and exploratory activity. Alteration in feeding, body weight, sleep and circadian rhythms are also observed. Activation of HPA axis along with impaired immune functions is also witnessed in the defeated animals (96). Social defeat is a helpful paradigm for exploring mechanisms at molecular level which can cause stable changes which mimicking the depressive pathology.

10.3 Interferon-α model of depression in rats

The interferon induced depressive behavior in rat model (97,98) is a new model for assessment of depressive behaviour. Rats treated with Interferon-α2b for two to four weeks show significant increase in depressive and anxiety like behavior. Symptoms of Interferon-induced depression in human are depressed mood, irritability, agitation, fatigue, apathy, anhedonia, anorexia, psychomotor retardation, sleep disturbance, sexual dysfunction, memory impairment, and diminished ability to concentrate (99,100). This cluster of nonspecific symptoms is often referred to as ''sickness behavior'' when modeled in animals (101). The range of Interferon-α doses (1000-100000 units/ kg) which vary with the duration of treatment and route of administration (102-104).

3. Material and Methods

Study protocol:

The study was divided into four phases in which the first two phases (study -1 and study -2) focused on single dose effect and the last two phases focused on effects in the established animal model of depression like interferon induced (study- 3) and CMS induced model of depression (study-4).

Study 1 – Preliminary single dose study using diclofenac, dexamethasone and amitriptyline in **SD rats** with FST.

Study 2- Single dose study using aspirin, dexamethasone and amitriptyline in **albino mice** and tests performed included TST, EPM and LD box.

Study 3:-

Interferon - α- 2b model of depression in Sprague Dawley rats - The animals in different groups were treated for 21 days with aspirin (10 mg/kg, po) dexamethasone (1mg/kg po) and amitriptyline (10 mg/kg po). Amitriptyline was used as reference standard, and was also used in combination with aspirin and dexamethasone to examine any synergy. Interferon-α-2b (6000 I.U./ k.g, ip) was administered in above groups daily, except normal control. Tests performed included Sucrose preference test, behavioural tests like forced swim test, elevated plus maze, light dark box and locomotor activity and biochemical estimations like serum cortisol and brain neurotransmitters.

Study 4:

Chronic mild stress (CMS) model of male Sprague–Dawley rats- All the animals in different groups, except the normal control group were exposed to CMS procedure for 28 days and concurrently treated with aspirin (10 mg/kg, p.o.) dexamethasone (1mg/kg p.o.) and amitriptyline (10 mg/kg p.o., reference standard) respectively. Amitriptyline was used as reference standard, and was also used in combination with aspirin and dexamethasone to examine any synergy Tests performed included Sucrose preference test, behavioural tests like forced swim test, elevated plus maze, light dark box and locomotor activity and biochemical estimations like serum cortisol and brain neurotransmitters.

All experiments were conducted after the approval from Institutional Animal Ethical Care Committee **(Protocol number-LJIP/IAEC/12-13/75).**

3.1 Study 1: Preliminary single dose forced swim test using diclofenac, dexamethasone and amitriptyline in SD rats.

3.1.1: Animals

Male Sprague-Dawley rats (200–250g) were obtained from the Department of Laboratory Animal Science, Zydus Research Centre (Ahmedabad, India). They were housed under standard conditions (23_1 °C; relative humidity, 55 ± 5%), maintained under a 12-h light/dark cycle and ad libitum food and water. They were allowed to acclimatize to the colony for at least 7days before any experimentation. All experiments were carried out during the light phase of the light/dark cycle. All experimental procedures were performed in compliance with the Institutional Animal ethics committee. All efforts were made to reduce the number of animals used and their suffering.

3.1.2: Pharmacological treatments and experimental protocol

The animals were divided into six groups (6 in each group) and were treated with drugs specified in Table 3.1, which included Diclofenac sodium (105) dexamethasone(106-107) and amitriptyline (108) were given orally one hour before conducting forced swim test.

Table 3.1.Treatment of drugs and their doses in different groups in the single dose study in SD rats

Group No.	Drug Treatment	Dose
Group 1	Normal	Saline
Group 2	Amitryptilline	10 mg/kg p.o.
Group 3	Dexamethasone	1 mg/kg p.o.
Group 4	Diclofenac sodium	10 mg/kg p.o.
Group 5	Amitryptilline Dexamethasone	10 mg/kg p.o. 1mg/kg p.o.
Group 6	Amitryptilline Diclofenac	10 mg/kg p.o. 10 mg/kg p.o.

3.1.3. Forced swim test:

The Forced swim test was performed as described in a previous study conducted by Porsolt et al. (78). The rats were placed singly in a cylinder (45 cm height, 20 cm diameter and 30 cm depth) in the experimental room. They were kept at 25±1 ◦C. The Rats in the training phase were made to swim for 15 minutes forcefully. At the end of the training session they were dried and placed back to their corresponding home cages. The rats were made to swim again for a session of 5 minutes after 24 hours of training period.

The rats in the different groups were given the treatments orally with the drugs drugs, doses and routes as described in the table one hour before the commencement of the force swim test. Care was taken in cleaning and disinfecting the cylinders before performing the forced swim test because water from the previous trials has been associated with alteration in the behavior of animals (78).

All experiments were videotaped and assessments were one by the observers who were blind to the drug treatments to the animals. Climbing, immobility and swimming were assessed for 5 minutes period.

Upright movement of the front paws of the rats positioned towards the wall of the container were considered to be climbing movement and mild swimming was described as horizontal movement. Immobility was considered to be absence of all movements except the movements necessary to keep the heads above the surface of water. Only the observations recorded during the test period were considered for the study.

3.2 Study 2: Single dose study using TST, EPM and LD box to study effect of aspirin, dexamethasone and amitriptyline in albino mice.

3.2.1: Animals

Male mice (30-35 g) were obtained from the Animal Science Laboratory department, Zydus Research Centre, ZRC, Ahmedabad, Gujarat). The animals were housed under standard conditions (Temperature 23±1 °C; RH, 55 ± 5%), maintained in 12 hour light/dark cycle and ad libitum water and food in the animal house facility of L.J. Institute of Pharmacy. They were allowed to adapt to the new conditions for 7days before carrying out any experiments. The experimental procedures were done during light phase. All the experiments and assay procedures were done in accordance with the Institutional Animal ethics committee guidelines. Efforts were made to reduce pain and suffering to the animals and also minimal number animal were utilized for the study.

3.2.2: Pharmacological treatments and experimental protocol

The mice were divided into six groups (6 animals each) and were treated with drugs specified in Table 3.2, which included aspirin (109, 110) dexamethasone (106, 107) and amitriptyline (108) were given orally one hour before conducting behavioral tests.

Table 3.2: Treatment of drugs and their doses in different groups in the single dose study in albino mice.

Group No.	Drug Treatment	Dose
Group 1	Normal	Saline
Group 2	Amitryptilline	10 mg/kg p.o.
Group 3	Dexamethasone	1 mg/kg p.o.
Group 4	Aspirin	10 mg/kg p.o.
Group 5	Amitryptilline Dexamethasone	10 mg/kg p.o. 1mg/kg p.o.
Group 6	Amitryptilline Aspirin	10 mg/kg p.o. 10 mg/kg p.o.

3.2.3: Tail suspension test: The tail suspension test was done according to the method explained in previous study (111). Mice were suspended by tail 50 cm above the surface and the experiment were conducted in acoustically isolated experimental area. Approximately one cm from the tip of the tail an adhesive tape was applied and the observations were video recorded for a period of five minutes for each mouse.

3.2.4: Elevated plus-maze The open arm close arm or Elevated Plus-Maze apparatus as described in (112) was utilized for the current study. It is based on the principle that the animal in the presence of an anti- anxiety drug would spent more time in the elevated plus maze in open arm and would not consider risk of fall. The EPM test was done one hour after the administrating the drugs in mice. The animals were placed alone at the centre of the apparatus and the observation of the animal behavior was recorded for 5 minutes. Total time spent by animal in each of the open arms was noted. The EPM was cleaned by the use of 5% alcohol solution while performing the experiment.

3.2.5: Light/dark box test: The Light/dark box test and the method used for the study was performed as described in (113). This test relies on the observation that rodents avoid lit places and prefer dark chamber in anxious state. The rats in the different groups were treated with the drugs mentioned in the table 1. After one hour the rats were placed individually in the lit chamber of the light dark box. The time used up by rat in each chamber and parallel activity i.e. the total number of transitions between the lit and dark chamber was recorded for a period of four minutes.

3.3: Study 3: Interferon model of depression.

3.3.1: Animals and experimental methology

Healthy Male Sprague Dawley Rats (200- 250 grams) were divided into seven groups (6 in each group) and were treated with approximately 6000 international units (I.U.)/kg (97,98,114)) reliferon (Human recombinant interferon alpha-α 2b, Reliance life science) ip and normal control rats were treated with vehicle 0.2 ml (0.9% w/v NaCl) ip once daily for 21 days. Other drug treatments were given orally half an hour before interferon treatments (Table 3.3) After 14 days of treatment; sucrose preference test was done to measure anhedonia. Behavioral tests were performed on 17^{th} to 20^{th} day and observations were recorded. On 21st day, the blood samples were obtained for the measurement of cortisol and the rats were sacrificed to collect brain for the estimation of neurotransmitters.

Table 3.3 : Treatment of drugs and their doses in different groups in the interferon-α-2b model of Sprague Dawley rats.

Group 1	Normal	Saline *po*
Group 2	Interferon-α-2b	6000 (I.U.)/kg *ip*
Group 3	Amitryptilline Interferon-α-2b	10 mg/kg *po* 6000 (I.U.)/kg *ip*
Group 4	Dexamethsone Interferon-α-2b	1 mg/kg *po* 6000 (I.U.)/kg *ip*
Group 5	Aspirin Interferon-α-2b	10 mg/kg *po* 6000 (I.U.)/kg *ip*
Group 6	Amitryptilline Dexamethasone Interferon-α-2b	10 mg/kg *po* 1mg/kg *po* 6000 (I.U.)/kg *ip*
Group 7	Amitryptilline Aspirin Interferon-α-2b	10 mg/kg *po* 10 mg/kg *po* 6000 (I.U.)/kg *ip*
po :per oral, ip: intra peritoneal, I.U. : -α-2b International Units		

3.3.2. Sucrose preference test

The sucrose preference test was performed as described in (115) at the end of 14th day of the study. Rats were initially trained to drink 1% sucrose solution (11th day). Three days later, rats received sucrose preference test, preceded by 14 hour food and water deprivation. Each rat was provided simultaneously with both sucrose (1%) and water. Sucrose intake was calculated by measuring the bottle at 60 min.

3.3.3. Forced swim test

Forced swim test was carried out as explained in 3.1.3.

3.3.4. Elevated plus-maze

The Elevated Plus-Maze test was done as explained in 3.2.4

3.3.5. Light/dark box - choice paradigm

The light/dark test was done as explained in 3.2.5

3.3.6. Locomotor activity

Each animal was placed in the photoactometer chamber equipped with light sensitive photocells. The observations were taken for a period of 5 minutes and the values obtained were expressed as counts per 5 minutes. The apparatus was placed in a darkened, light and sound attenuated and ventilated test room. (116)

3.3.7 Serum cortisol measurements

Blood samples were collected around 8 am on 21st day of the study just before collection of brains. Cortisol levels were measured in serum using a cortisol assay (117) on the Diagnova Elisa reader. All reagents, working standards, and samples as directed in the procedure manual outlined in the Kit manual. To determine the number of wells to be used the Assay Layout Sheet was referred. Blank well was set without any solution. 50µl of standard and sample per well and 50µl Antibody (1x) were added to each well immediately (not to Blank well). The plate was shaken gently for 60 seconds. After covering with the adhesive strip provided it was incubated for 40 minutes at 37°C. Each well was aspirated and washed with wash buffer (200µl) using a squirt bottle. The plate was inverted and blotted against clean paper towels. 100µl HRP-conjugate (1x)

was added to each well immediately (not to Blank well). It was then covered with the adhesive strip provided and incubated for 30 minutes at 37°C. The process of washing was repeated for five times. Then 90µl of TMB Substrate was added to each well and incubated for 20 minutes at 37°C, protecting from light. 50µl of Stop Solution was added to each well, gently tapping the plate to make sure thorough mixing. The optical density of each well was done within 5 minutes, using a microplate reader set to 450 nm.

3.3.8. Neurotransmitter estimations

Tissue extracts were prepared by the method of Schulmpf (118) & Ciarlone (119) for the estimation of the serotonin, norepinephrine, and dopamine neurotransmitters by spectroflourimetry method.

Reagents: Acidified N-butanol, N-heptane, sodium hydroxide, acetic acid, iodine, , EDTA, NaHCO3 and alkaline sulphite, Dopamine (DA) hydrochloride, norepinephrine (NE) hydrochloride, serotonin (5-HT), and hydrogen oxalate (obtained from Sigma-Aldrich, St. Louis, USA) were used in the experiments. The chemicals used in the study were of analytical grade. Care was taken to store reagents in dark glass bottles with glass stoppers to prevent the leaching of fluorescent contaminants.

Extraction and Separation: The estimation of DA, NE, and 5-HT levels in the selected rat tissues was carried out according to the fluorometric method described by (118,119). Each tissue sample was homogenized in 10 volumes of cold acidified N-butanol (3 mL) using a glass homogenizer. For plasma samples, 3 mL of acidified N-butanol was added to 0.3 mL of plasma.

Duplicate internal standard tubes were carried in parallel with the tissue homogenates. The homogenate and internal standard tube were centrifuged at 2000 rpm for 5 min after treatments described as per method, 2.5 mL of the supernatant fluid was transferred to tubes, placed on a vortex mixer for 30 s, and the phases were separated by centrifugation at 2000 rpm for 5 min. 5-HT, NE, and DA were assayed in the aqueous phase.

Assay of Serotonin, Dopamine and NA : The aqueous phase (0.2 mL) was pipetted into test tubes. External standard was prepared in duplicate in 0.2-N acetic acid to a total volume of 0.2 mL. The blank consisted of 0.2 mL of 0.2-N acetic acid. To all tubes, 1.2 mL of 4 mg/dL OPT was added and mixed well. All tubes were placed in a boiling water bath for 10 min, cooled under tap water, and read in a spectrofluorometer at excitation and emission wave lengths of 295 and 355 nm, respectively. For the assay of DA and NE, the aqueous phase (1 mL) was transferred to a tube for the assay of DA and NE. External standards were prepared for NE and DA in duplicate in 0.2-N acetic acid and a total volume of 1.6 mL per tube. After performing the treatments described in procedure all solutions were returned to their original test tubes, reheated in a boiling water bath for 5 min, cooled under tap water, and analyzed for DA fluorescence at excitation and emission wavelengths of 320 and 375 nm, respectively.

3.3.9. Statistical Analysis

Data were expressed as mean + SEM. Analysis was performed with Prism version 3.0 software using one-way analysis of variance (ANOVA) followed by Tukey`s comparison test where results showing $p< 0.05$ were considered statistically significant.

3.4. STUDY 4: Study in CMS model of depression in SD rats.

3.4.1 Animals and housing

Male Sprague-Dawley rats (200–250g) were obtained from the Department of Laboratory Animal Science, Zydus Research Centre (Ahmedabad, India).The animals were maintained under standard laboratory conditions (12/12 h light/dark cycle with lights on at 8:00 A.M., 22 ± 2 ◦C with a relative humidity at 50 ± 10%, food and water ad libitum). The animals were allowed to adapt to laboratory conditions for at least one week. All stress exposed rats were singly housed and non-stressed rats grouped (five/cage). All experiments were conducted after the approval from Institutional Animal Care Ethical Committee.

3.4.2. Experimental protocol

In the present study, chronic mild stress (CMS) model of male Sprague–Dawley rats was used to evaluate the antidepressant effect of aspirin and dexamethasone.

Drug treatments

Drug treatments were given every morning between 10 am and 11 am. The animals were divided into 7 groups and the drug treatments were done as per (Table 3.4).

Table.3.4 Treatment of drugs and their doses in different groups in the CMS model of rats.

Group	**Stress procedure**	**Drugs/Dose**
Group 1	Nil	Saline *p.o.*
Group 2	Chronic mild stress (CMS)	Saline *p.o.*
Group 3	CMS	Amitriptyline (10 mg/kg) *p.o.*
Group 4	CMS	Dexamethasone (1 mg/kg) *p.o.*
Group 5	CMS	Aspirin (10 mg/kg) *p.o.*
Group 6	CMS	Amitriptyline (10 mg/kg) *p.o.* Dexamethasone (1mg/kg) *p.o.*
Group 7	CMS	Amitriptyline (10 mg/kg) *p.o.* Aspirin (10 mg/kg) *p.o.*

3.4.3 CMS procedure

The unpredictable chronic mild stress was applied for 4 weeks. The stress regime used in this study was a modified version of models previously described in (120-121). The stress consisted of repeated mild physical and psychological stressors. Six different stressors were presented. The stress procedure consisted of: two span of food or water deprivation; two periods of 45° cage tilt; two span of intermittent illumination (lights on and off every 2 hours); two span of soiled cage (250 ml water in sawdust bedding); two span of paired housing (3h); Confinement in a small tube (1 h) and two periods of no stress. Control animals were kept in separate rooms and in isolation from stressed animals. Stressors were not applied during the periods of sucrose intake measurement, and food and water deprivations were never made before the sucrose consumption test. The animals were deprived of food and water for 14 h preceding each sucrose test, but otherwise food and water were freely available in the home cage .Stressors were scheduled according to the CMS procedure described in (Table 3.5). The stress sequence was changed every week in order to make the stress procedure unpredictable.

Sucrose preference test was done at the end of first, second and third week of the study. The observations of sucrose preference test were utilized as a measure of anhedonia. (Indicator for depressive behaviour) Behavioral tests were performed on 25th, 26th 27th day and the observations were recorded. On 28st day, the blood samples were collected for cortisol measurements and the rats were sacrificed to collect brain for neurotransmitter study.

Table 3.5: CMS procedure in the Chronic Mild Stress model of SD rats.

Duration of stress	Start of the stress	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 [a]
Week 1	8.00 a.m.	Water and food deprivation (3 h)	intermittent illumination (12 h)	Confinement in a small tube (1 h)	intermittent illumination (12 h)	Paired housing (3h)	Confinement in a small tube (1 h)	No stress
	8.00 p.m.	Paired housing (3h)	45º cage tilt (12-15 hr)	Housing in mild damp sawdust[b,c] (20 h)	Water and food deprivation (3 h)	Housing in mild damp sawdust (20 h)	45º cage tilt (12-15 hr)	No stress
Week 2	8.00 a.m.	45º cage tilt (12-15 hr)	intermittent illumination (12 h)	Paired housing (3h)	Confinement in a small tube (1 h)	Water and food deprivation (15 h)	intermittent illumination (12 h)	No stress
	8.00 p.m.	Water and food deprivation (3 h)	Confinement in a small tube (1 h)	Housing in mild damp sawdust (20 h)	Housing in mild damp sawdust (20 h)	Paired housing (3h)	45º cage tilt (12-15 hr)	No stress
Week 3	8.00 a.m.	Intermittent illumination (12 h)	intermittent illumination(12 h)	Water and fooddeprivation (3 h)	Confinement in a small tube (1 h)	45º cage tilt (12-15 hr)	Housing in mild damp sawdust (20 h)	No stress
	8.00 p.m.	Paired housing (3h)	Confinement in a small tube (1 h)	Housing in mild damp sawdust (20 h)	Paired housing (3h)	Water and food deprivation (3 h)	intermittent illumination (12 h)	No stress
Week 4	8.00 a.m.	Confinement in a small tube (1 h)	No stress	Water and food deprivation (3 h)	Paired housing (3h)	No stress	45º cage tilt (12-15 hr)	Blood & brain collection
	8.00 p.m.	Water and food deprivation (3 h)	Housing in mild damp sawdust (20 h)	45º cage tilt (12-15 hr)	Housing in mild damp sawdust (20 h)	Paired housing (3h)	45º cage tilt (12-15 hr)	

[a] At the end of day 7th (week -1-3) Sucrose preference test was performed.
[b] 200 ml of water for 100 gms of saw dust
[c] Test time was modified for housing in mild damp sawdust

3.4.4. Sucrose preference test

The sucrose preference test was performed as documented previously with minor modifications (115) at the end of 7th, 14th and 21st day of study of the study. Only the results of SPT done on 21st day were presented as the previous results of day 7 and 14 were statistically insignificant. The test was performed as described in 3.3.2.

3.4.5. Forced swim test

Forced swim test was carried out as explained in 1.1.

3.4.6 Light/dark box - choice paradigm

The light/dark test was done as explained in 2b

3.4.7. Elevated plus-maze

The Elevated Plus-Maze test was done as explained in 2c

3.4.8. Locomotor activity

3.4.9. Light/dark box test

The Light/dark box test was performed as described in 6 c

3.4.10. Serum cortisol measurements

Blood samples were collected around 8 am on 21st day of the study just before collection of brains. Cortisol levels were measured in serum using a cortisol assay 44 on the Diagnova Elisa reader as described in 3.3.7.

3.4.11. Neurotransmitter estimation

Neurotransmitter estimation was done on 28th day as described in 3.3.8 in the study using interferon model

3.4.12. Statistical Analysis

Statistical analysis were done as described in 3.3.9

4. Results

4.1 Study 1: Preliminary study using single dose of drugs on forced swim test in SD rats.

FIGURE 4.1: Immobility time after single dose treatment in various groups in SD rats.

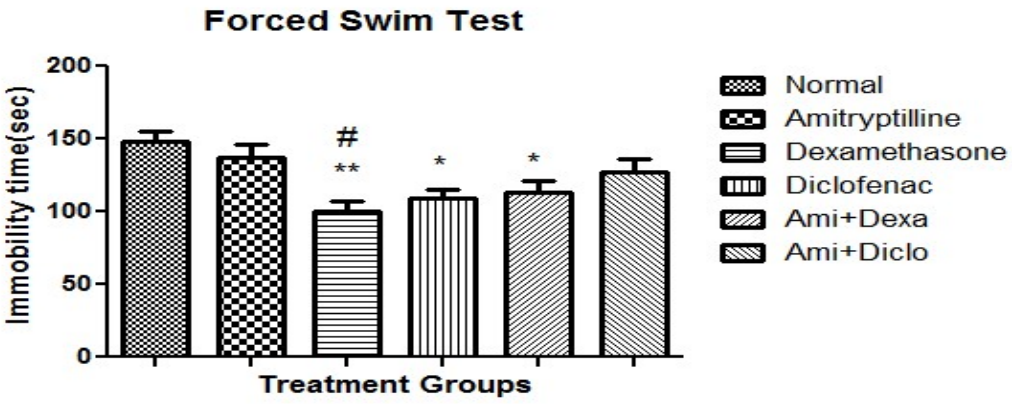

FIGURE 4.1.: Immobility time after single dose treatment in various groups as observed in forced swim test. Bar diagram represents total immobility time out of 300 seconds swim test. Results are represented as mean ± SEM with n=6 rats in each group, # $p<0.05$ when compared with the Amitriptyline treated group. * $p < 0.05$,** $p< 0.01$ when compared with the normal group

TABLE 4.1.1: Effect of various treatments on immobility time in SD rats.

Parameter	Normal	Dexamethasone	Diclofenac	Amitriptyline
Immobility time	148.16± 7.23	100.33± 6.8 **	109.33 + 6.21 *	136.66±9.611

* p<0.5, ** p< 0.01 when compared with the normal group

TABLE 4.1.2: Comparative study of concomitant treatment groups (Normal Vs Anti-inflammatory + antidepressant)

Parameter	Normal	Amitriptyline + Dexamethasone	Amitriptyline + Diclofenac
Immobility time	148.16± 7.23	113.33 ± 7.49 *	127.16 ± 9.06

* p<0.5 when compared with the normal group.

TABLE 4.1.3: Inter group comparison of concomitant treatments. (Anti-inflammatory Vs anti-inflammatory + antidepressant)

Parameter	Dexamethasone	Amitriptyline + Dexamethasone	Diclofenac	Amitriptyline + Diclofenac
Immobility time	100.33± 6.8	113.33 ± 7.49	113.33 ± 7.49	127.1 ± 9.06

Result Summary of study 1: In the forced swim test, the immobility time in the dexamethasone, diclofenac and concomitant treatment of amitryptilline and dexamethasone groups decreased significantly when compared with the normal control group (Fig.4.1.). However, in the group treated with amitryptilline, no effect in immobility was observed. Dexamethsone significantly reduced the immobility time when compared with amitryptilline treatment group.

4.2: Study 2- Single dose study using aspirin, dexamethasone and amitriptyline in albino mice.

FIGURE: 4.2.1. Effect of single dose treatment on immobility time in TST

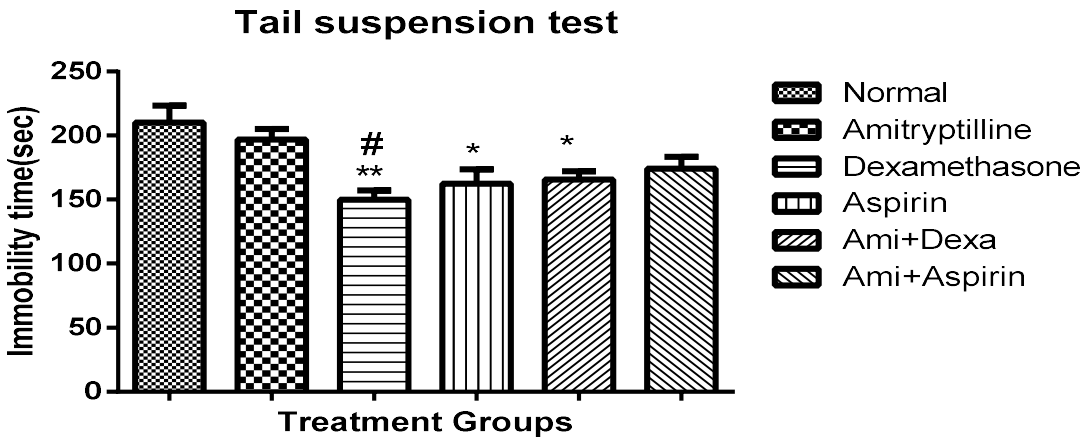

FIGURE 4.2.1: Immobility time on single dose treatment as observed in TST. Bar diagram represents total immobility time out of 300 seconds of Tail suspension test. Results are represented as mean ± SEM with n=6 mice in each group, * p< 0.05, ** p< 0.01, when compared with the normal group. # p<0.05 when compared with amitriptyline treated group

FIGURE 4.2.2. Effect of single dose treatments on number of transitions in the Light Dark Box test

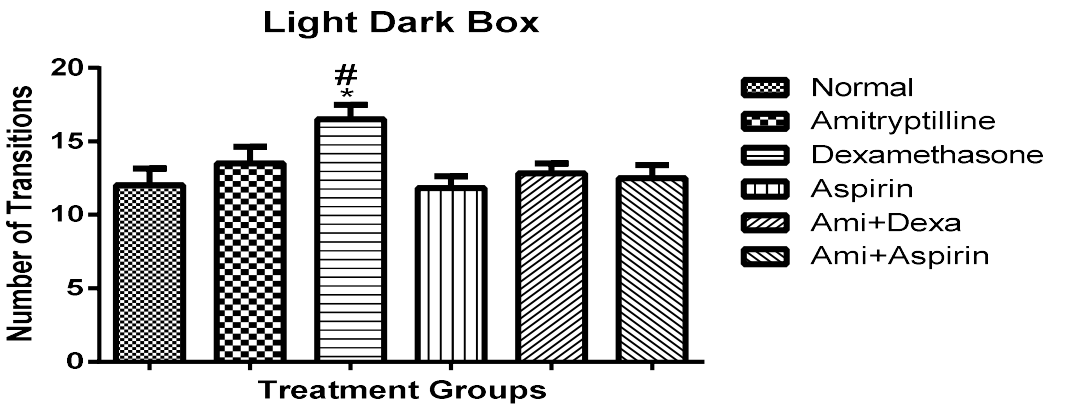

FIGURE 4.2.2. Number of transitions observed in light dark box after single dose treatments in different groups. Bar diagram represents total number of transitions in the light dark box. Results are represented as mean ± SEM with n=6 mice in each group, * p<0.05 when compared with the normal group. # p < 00.05 when compared with the aspirin treated group.

FIGURE 4.2.3. Time spent in open arm box after single dose treatments in different groups

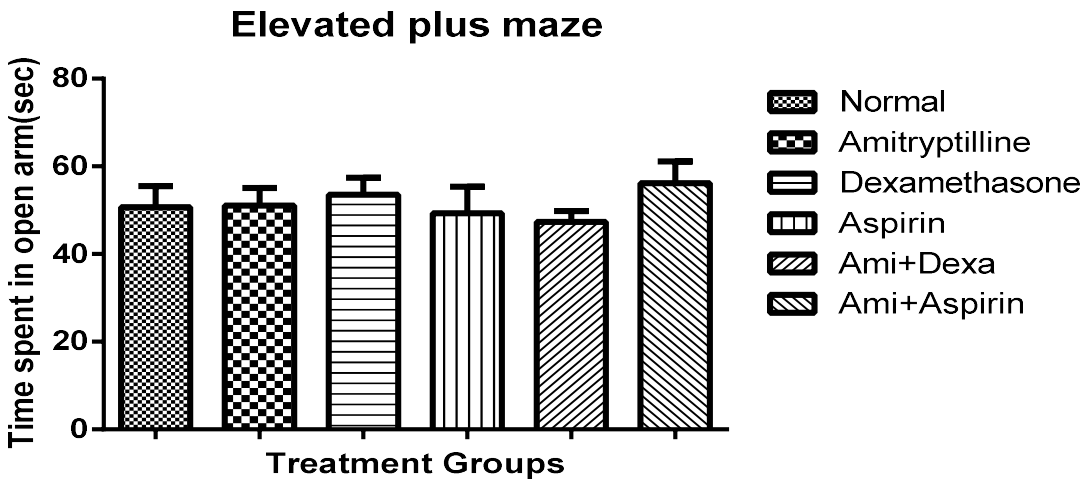

FIGURE 4. 2.3: Time spent in open arm observed after single dose treatment in various groups. Bar diagram represents total time spent in seconds in open arm of 300 seconds duration. Results are represented as mean $\pm$ SEM with n=6 mice in each group

TABLE: 4.2.1. Effect of single dose treatments of various drugs on behavioral tests in the albino mice

Parameters	Normal	Dexamethasone	Aspirin	Amitriptyline
Immobility time (sec)	210 ± 13.4	149.8±7.29 **	162.3±11.2 *	196.66±8.33
Number of transitions	12±1.2	16.5±0.9 *	11.8± 0.8	13.5±1.1
Time spent in open arm (sec)	50.6 ±4.0	53.5±3.9	49.3±6.0	51±4

*** p<0.5, ** p< 0.01 when compared with the normal group**

TABLE: 4.2.2. Inter group comparison of concomitant treatments (Normal Vs anti-inflammatory + antidepressant)

Parameters	Normal	Amitriptyline + Dexamethasone	Amitriptyline + Aspirin
Immobility Time (sec)	210 ± 13.4	165.5±6.6	174±9.3
Number of transitions	12±1.2	12.8±0.65	12.5±0.9
Time spent in open arm (sec)	50.6 ±4.0	47.3±2.4	56.1±4.9

TABLE: 4.2.3. Inter group comparison of concomitant treatments (Anti-inflammatory Vs anti-inflammatory + antidepressant)

Parameters	Dexamethasone	Amitriptyline + Dexamethasone	Aspirin	Amitriptyline + Aspirin
Immobility Time (sec)	149.8±7.29	165.5±6.6	162.3±11.2	174±9.3
Number of transitions	16.5±0.9	12.8±0.65	11.8± 0.8	12.5±0.9
Time spent in open arm (sec)	53.5±3.9	47.3±2.4	49.3±6.0	56.1±4.9

Result Summary of Study 2: In the tail suspension tests, the immobility time in the groups treated with dexamethasone, aspirin and dexamethasone + aspirin decreased significantly on comparison with the group A (normal control group) (Fig.4.2.1). On the other hand, no effect on immobility was observed in group treated with amitriptyline.

There was significant increase in the number of transitions in the animals which were treated with dexamethasone when compared with the normal untreated or the normal control group (Fig.4.2.2).

No important and statistically significant results could be concluded from elevated plus maze experiment (Fig.4.2.3).

4.3: STUDY 3: Study in the Interferon model of depression in rats.

FIGURE 4.3.1. Effect of various treatments on Sucrose preference test in the Interferon-α model in SD rats

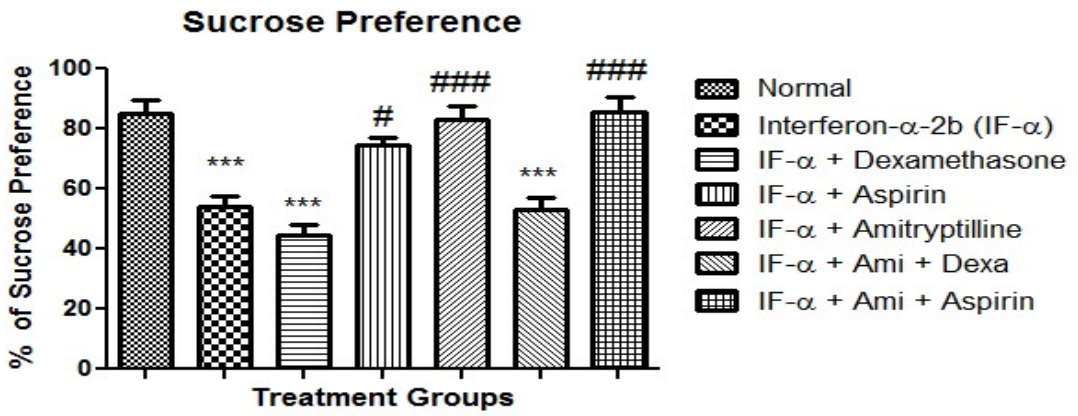

FIGURE. 4.3.1 Percentage of sucrose preference on 14th day of treatment of interferon-α-2b. Bar diagram represents percentage of sucrose preference (%). Results are represented as mean ± SEM with n=6 rats in each group, #p<0.05 , ###p<0.01 when compared with the Interferon -α-2b treated group. *** p< 0.05 when compared with the normal group

FIGURE 4.3.2. Effect of various treatments on Forced swim test in the Interferon-α model in SD rats

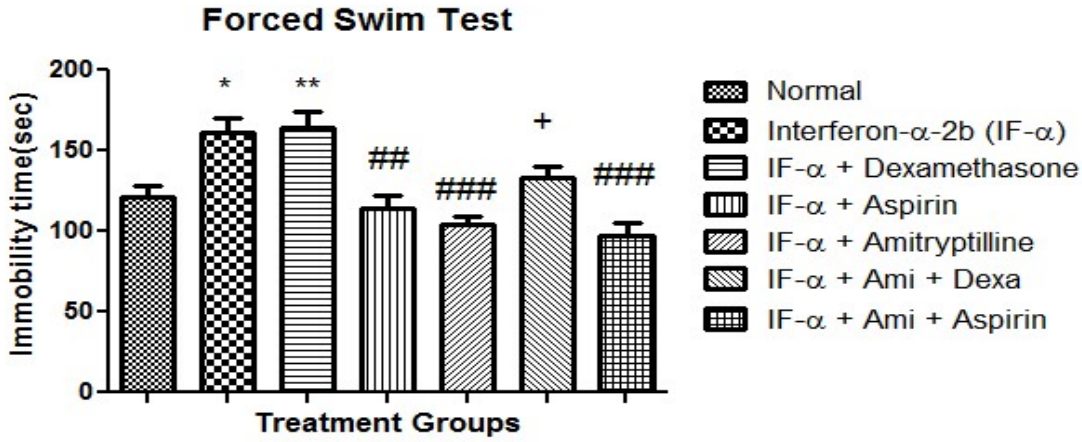

FIGURE. 4.3.2. Immobility time on 17th day of treatment of interferon-α-2b as observed in forced swim test. Bar diagram represents total immobility time out of 300 seconds swim test. Results are represented as mean ± SEM with n=6 rats in each group,## p<0.01, ###p<0.001 when compared with the Interferon -α-2b treated group. *p< 0.05 ,** p< 0.001 when compared with the normal group. ,+p< 0.05 when compared with the interferon-α-2b + amitriptyline +aspirin treated group

FIGURE 4.3.3.Number of transitions observed in the Light Dark box test in in the Interferon-α model in SD rats

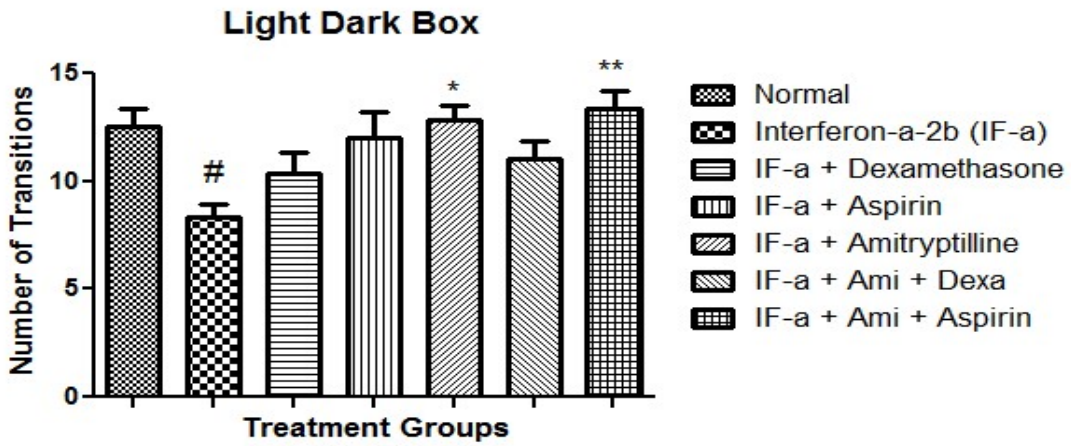

FIGURE. 4.3.3. Number of transitions observed in light dark box on the 18th day. Bar diagram represents total number of transitions in the light dark box for four minutes. Results are represented as mean ± SEM with n=6 rats in each group, # $p<0.05$ when compared with the normal group. ** $p< 0.01$,* $p< 0.5$ when compared with the interferon-α-2b treated group.

FIGURE4.3.4: Time spent in the open arm in plus maze in the Interferon-α model in SD rats.

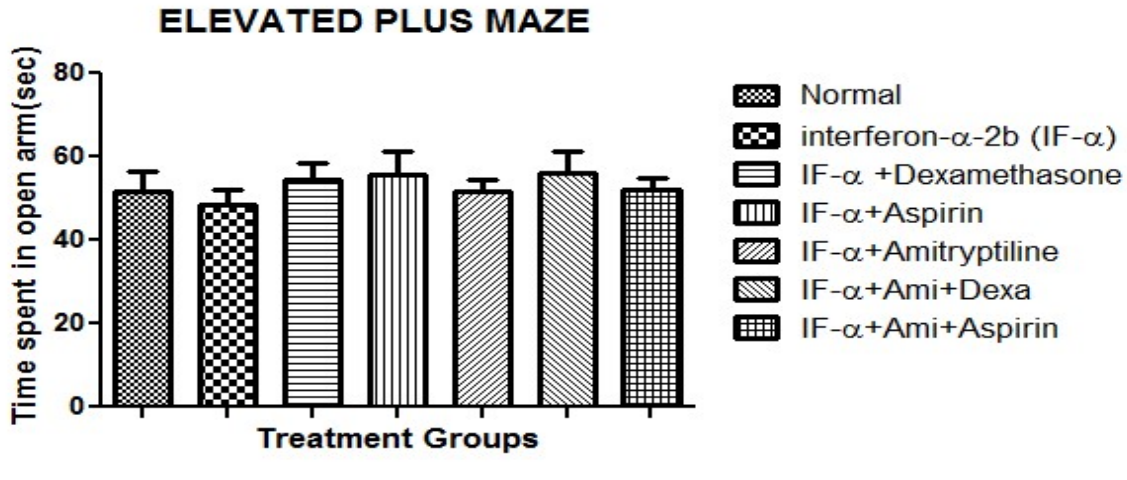

FIGURE. 4.3.4: Time spent in open arm observed on 19th day. Bar diagram represents total time spent in seconds in open arm of 300 seconds. Results are represented as mean ± SEM with n=6 rats in each group

FIGURE: 4.3.5. Number of Cut-offs observed in the photo-actometer in the Interferon-α model in SD rats

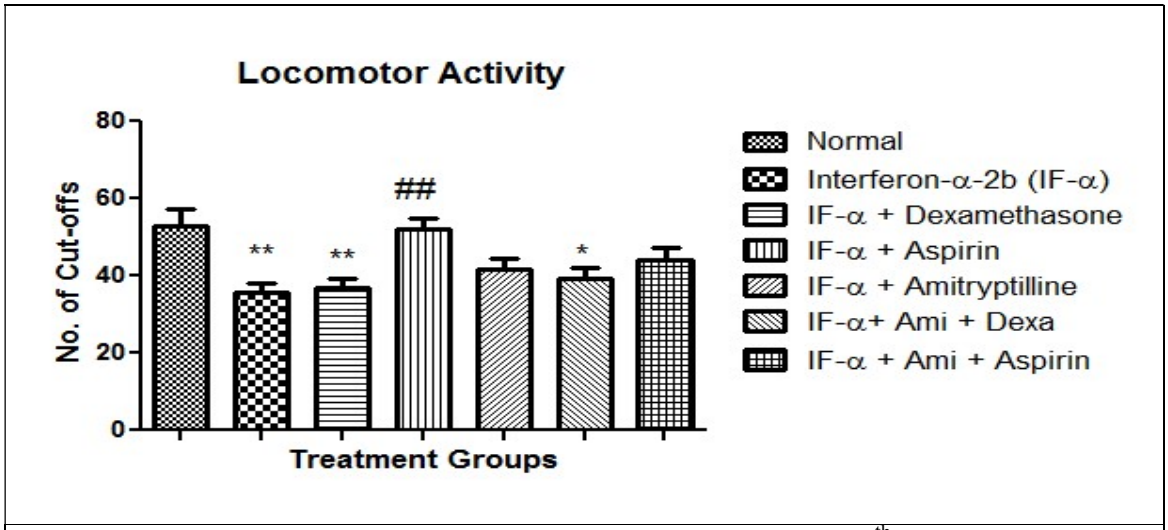

FIGURE 4. 3.5. Number of cut-offs observed in photoactometer on 20th day of treatments in various groups. Bar diagram represents number of cut-offs in photoactometer in five minutes. Results are represented as mean ± SEM with n=6 rats in each group, * $p < 0.05$, ** $p< 0.01$, when compared with the normal group. ## $P < 0.01$. when compared with the Interferon--α-2b group

FIGURE 4.3.6. Serum cortisol levels in the Interferon-α model in SD rats

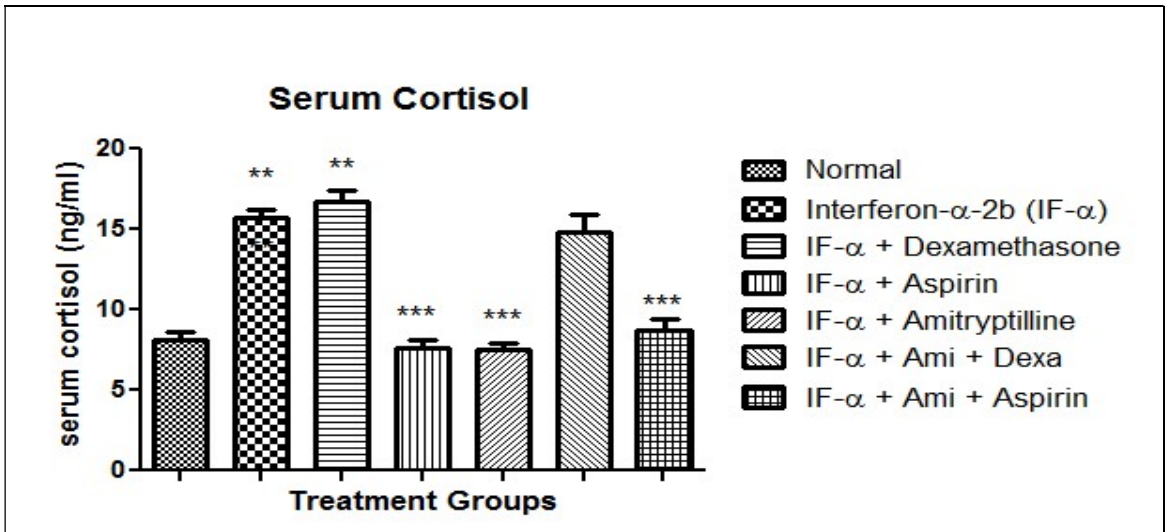

FIGURE 4.3.6. Serum Cortisol levels on 21st day of treatment of interferon-α-2b. Bar diagram represents Cortisol in ng/ml. Results are represented as mean + SEM with n=6 rats in each group, *** $p<0.05$ when compared with the Interferon -α-2b treated group. ** $p< 0.05$, when compared with the normal group

FIGURE.4.3.7. Brain serotonin levels in the Interferon-α model in SD rats

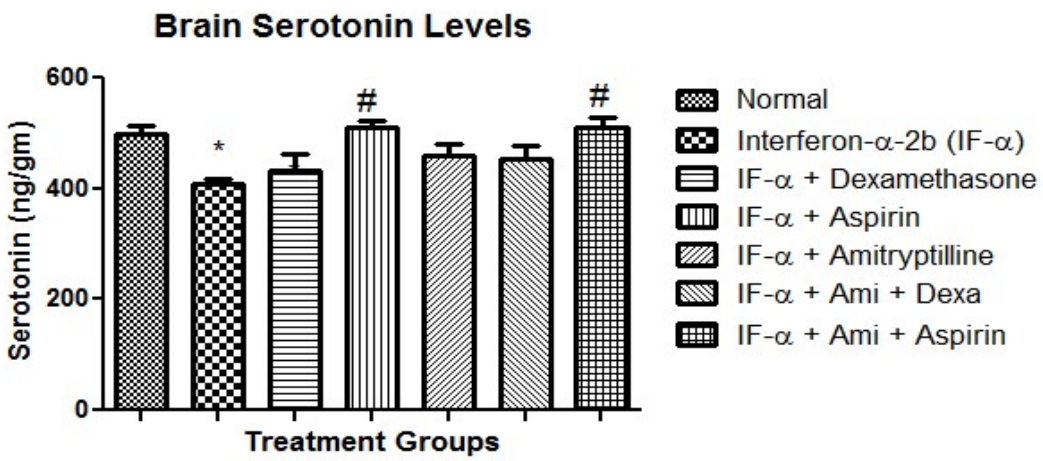

FIGURE. 4.3.4.1. Estimation of Serotonin in brain homogenate after 21 days of administration of drugs in various groups. Bar diagram represents serotonin in ng/ gm of brain tissue. Results are represented as mean ± SEM with n=6 rats in each group. *p <0.05 when compared with the normal group. #p< 0.05, when compared with IF-α treated group

FIGURE 4.3.8. Brain norepinephrine levels in the Interferon-α model in SD rats

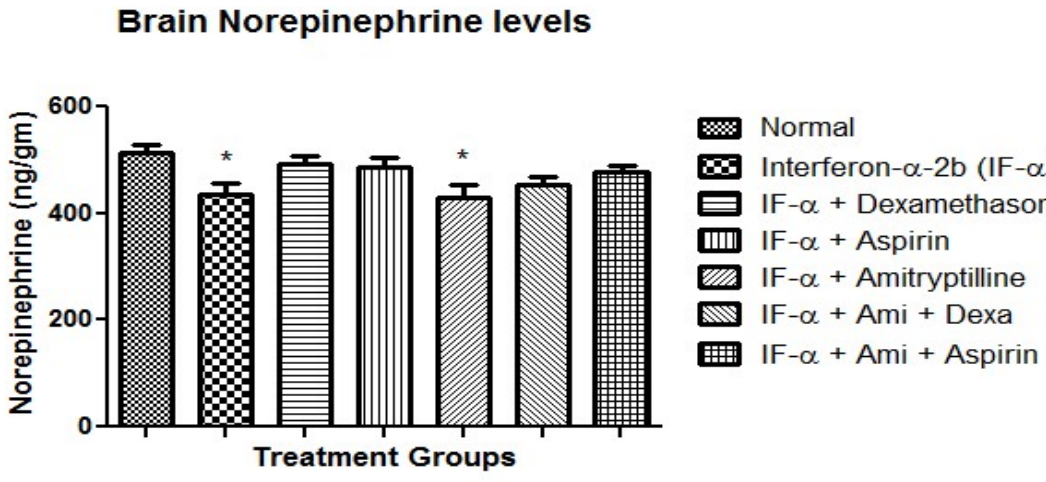

FIGURE 4.3.8. Estimation of norepinephrine in brain homogenate after 21 days of administration of drugs in various groups. Bar diagram represents dopamine in ng /gm of brain tissue. Results are represented as mean ± SEM with n=6 rats in each group. *p<0.05, when compared with the normal group

FIGURE 4.3.9. Brain dopamine levels in the Interferon-α model in SD rats

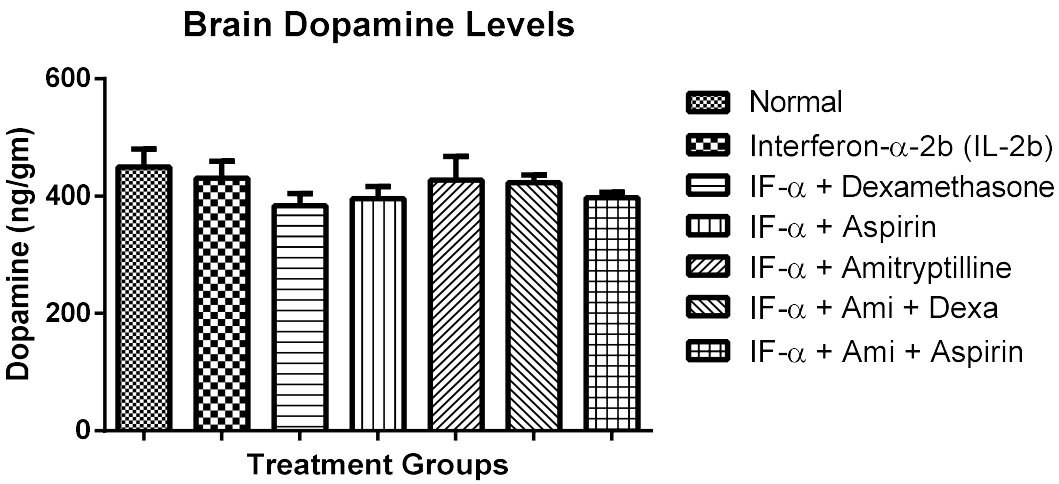

FIGURE.4.3.9: Estimation of dopamine in brain homogenate after 21 days of administration of drugs in various groups. Bar diagram represents dopamine in ng/gm of brain tissue. Results are represented as mean ± SEM with n=6 rats in each group

TABLE 4.3.1 Effect of various treatments in interferon- α model of depression in rats

Parameters	**Interferon-α**	**Interferon-α +Dexamethasone**	**Interferon-α +Aspirin**	**Interferon-α + Amitriptyline**
Immobility time (sec)	161.5±8.7	163.7±10.5	114.7±7.6**	104.2±4.7***
Number of transitions	8.3±0.6	10.3±1	12±1.2	12.8±0.6*
Time spent in open arm (sec)	48.3±3.9	54.3±4	55.6±5.5	51.5±2.9
Number of cut-offs	35.8±2.28	36.6±2.4	52±2.8**	41.6±2.6
% of Sucrose Preference	54 ± 3.6	44.6±3.1	74.6±2.2*	83.16±4.6***
Serum cortisol (ng/ml)	15.7±0.54	16.7±0.6	7.6±0.4***	7.4±0.4***
Brain Serotonin (ng/g)	409.5 ± 7.1	431.6±32	511.5±12.3*	459.6±21.7
Brain Nor epinephrine (ng/g)	434.8±21.1	491.8±15.2	487.3±18.5	430±21.8
Brain Dopamine (ng/g)	430.7±28.6	383.7±20.7	395.8±20.3	427.4±40.2

* $p<0.5$, ** $p< 0.01$, $p<0.001$ when compared with the interferon-α group

TABLE: 4.3.2. Inter group comparison of concomitant treatments (Interferon-α Vs Interferon-α +Anti-inflammatory + antidepressant)

Parameters	**Interferon-α**	**Interferon-α Amitriptyline +Dexamethasone**	**Interferon-α +Amitriptyline +Aspirin**
Immobility time (sec)	161.5±8.7	133±6.7	96.6±8.4***
Number of transitions	8.3±0.6	11.0±0.85	13.3±0.84**
Time spent in open arm (sec)	48.3±3.9	56.1±4.9	52.1±2.6
Number of cut-offs	35.8±2.28	39.3±25	44.1±2.9
% of Sucrose Preference	54 ± 3.6	53.3±3.7	85.5±5.2***
Serum cortisol (ng/ml)	15.7±0.54	14.8±1.0	8.6±0.7***
Brain Serotonin (ng/g)	409.5 ± 7.1	452±25.9	511.6±15.3*
Brain Nor epinephrine (ng/g)	434.8±21.1	453.3±14	476±13.4
Brain Dopamine (ng/g)	430.7±28.6	422.6±13.3	397±9.6

* $p<0.5$, ***$p<0.001$ when compared with the interferon-α group

TABLE 4.3.3. Inter group comparison of interferon treatments. (Anti-inflammatory Vs anti-inflammatory + antidepressant)

Parameters	**Interferon-α +Dexamethasone**	**Interferon-α Amitriptyline +Dexamethasone**	**Interferon-α +Aspirin**	**Interferon-α +Amitriptyline +Aspirin**
Immobility time (sec)	163.7±10.5	133±6.7	114.7±7.6	96.6±8.4
Number of transitions	10.3±1	11.0±0.85	12±1.2	13.3±0.84
Time spent in open arm (sec)	54.3±4	56.1±4.9	55.6±5.5	52.1±2.6
Number of cut-offs	36.6±2.4	39.3±25	52±2.8	44.1±2.9
% of Sucrose Preference	44.6±3.1	53.3±3.7	74.6±2.2	85.5±5.2
Serum cortisol (ng/ml)	16.7±0.6	14.8±1.0	7.6±0.4	8.6±0.7
Brain Serotonin (ng/g)	431.6±32	452±25.9	511.5±12.3	511.6±15.3
Brain Nor epinephrine (ng/g)	491.8±15.2	453.3±14	487.3±18.5	476±13.4
Brain Dopamine (ng/g)	383.7±20.7	422.6±13.3	395.8±20.3	397±9.6

Summary of results in the Interferon model of depression in rats

4.3.1: Sucrose preference test

The sucrose preference test was conducted after 14 days of administration of drugs. The animals treated with dexamethasone both individually and along with amitriptyline showed significantly lower preference of sucrose as that of disease control and that of normal untreated group $p<0.001$. The groups treated with amitriptyline, aspirin and amitriptyline + aspirin showed significant rise in sucrose preference when compared with the disease control group $p<0.01$ (Fig.4.3.1).

4.3.2: Behavioral tests

The Phase 2 studies involved long term exposure to drugs in the interferon model of depression. The immobility time in forced swim tests in the groups treated with amitriptyline, aspirin, aspirin + amitriptyline, decreased significantly when compared with the interferon treated group (Fig.4.3.2.). However, in the group treated with dexamethasone, significant increase in immobility was observed as compared to that of disease control group.

In the Light dark box, the number of transition significantly reduced in the interferon treated group when compared with the normal control group. In the groups treated with amitriptyline and amitriptyline + aspirin, the number of transitions were significant ($p<0.5$ & $p< 0.01$) when compared with the interferon treated disease control group (Fig.4.3.3). The observations were statistically insignificant in the open arm and close arm (Fig.4.3.4). In the groups treated with dexamethasone and amitriptyline + dexamethasone, the locomotor activity was significantly low, $p< 0.01$ and $p< 0.05$ when compared with Aspirin treated group (Fig. 4.3.5).

4.3.3. Cortisol measurement in rat blood serum

The serum cortisol levels increased significantly in Interferon and Interferon +Dexamethasone when compared group with the normal control group. In the groups treated with aspirin, amitriptyline and concomitant treatment of amitriptyline and aspirin reduced the cortisol levels significantly (Fig.4.3.6).

4.3.4. Neurotransmitter measurements in rat brain

In the interferon-α2b treatment group, serotonin and norepinephrine levels were significantly reduced when matched with the normal. Both serotonin and norepinephrine was found increased in aspirin treated group when compared with interferon-α2b treatment group (Fig.4.3.7 and Fig.4.3.8). The observations of dopamine in different groups were not remarkably significant (Fig.4.3.9.).

4.4. Study 4: Effect of various treatments on the CMS model in rats

FIGURE 4.4.1. Effect of various treatments on Sucrose preference test in the chronic mild stress model in SD rats

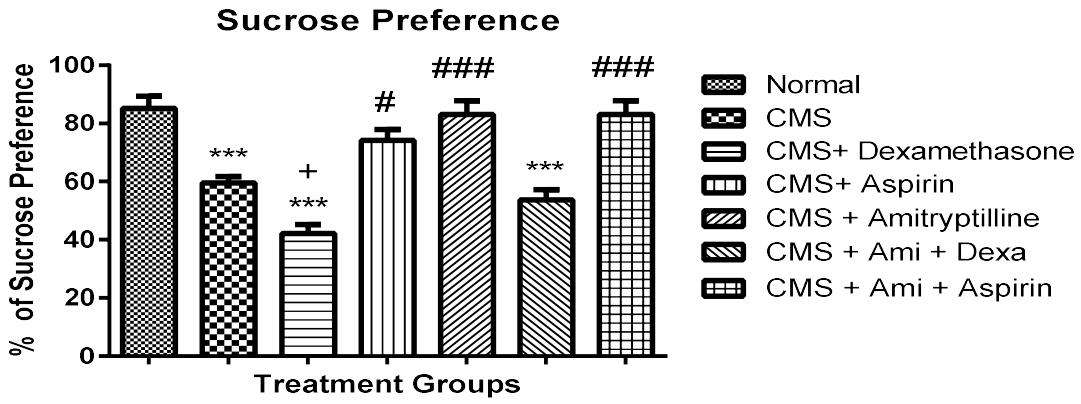

FIGURE 4.4.1. Percentage of sucrose preference on 21st day of CMS exposure. Bar diagram represents percentage of sucrose preference (%). Results are represented as mean ± SEM with n=6 rats in each group, ***p<0.001 when compared with the Normal control group. # p< 0.05, ### p< 0.001 when compared with the CMS group. + p<0.05 when compared with CMS group.

FIGURE 4.4.2. Effect of various treatments on forced swim test in the chronic mild stress model in SD rats

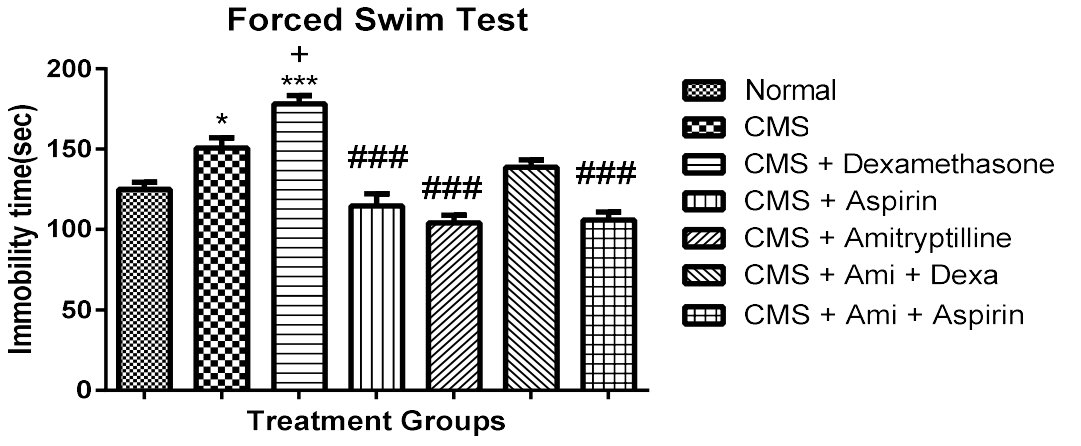

Fig. 4.4.2: Immobility time as observed in forced swim test. Bar diagram represents total immobility time out of 300 seconds swim test. Results are represented as mean ± SEM with n=6 rats in each group.* p< 0.05, *** p< 0.001 when compared with the normal group. ### p< 0.001, when compared with the CMS group. +p<0.05 when compared with CMS group

FIGURE 4.4.3. Number of transitions observed in the Light Dark box test in the chronic mild stress model in SD rats

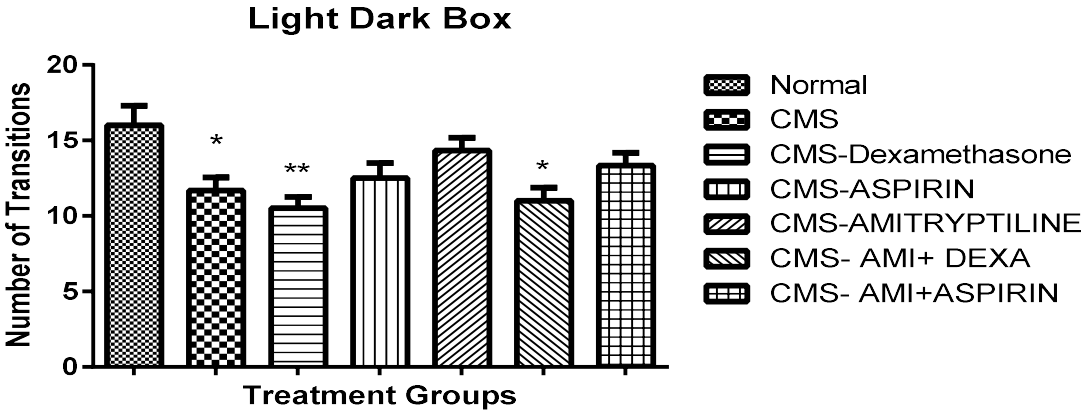

FIGURE 4.4.3: Number of transitions observed in light dark box. Bar diagram represents total number of transitions in the light dark box for four minutes. Results are represented as mean ± SEM with n=6 rats in each group, * p< 0.5, ** p< 0.01 when compared with the normal control group

FIGURE 4.4.4: Time spent in the open arm in plus maze in the chronic mild stress model in SD rats.

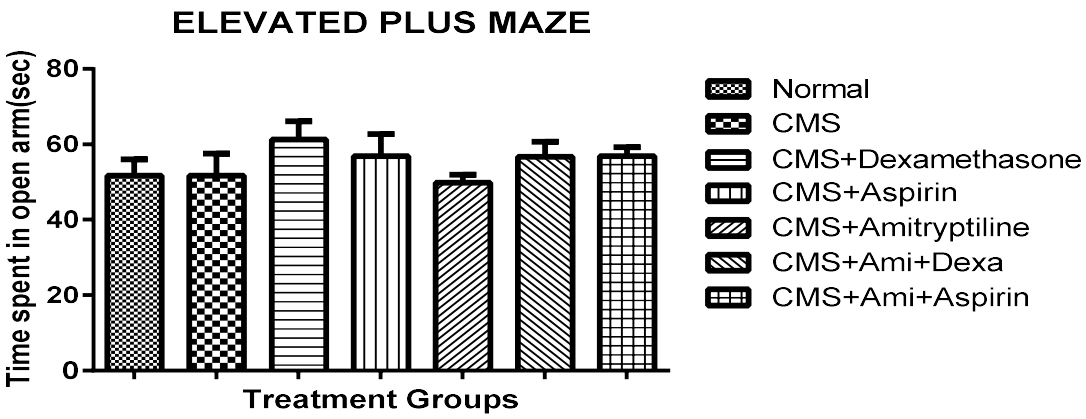

FIGURE 4.4.4: Time spent in open arm observed on 19th day. Bar diagram represents total time spent in seconds in open arm of 300 seconds. Results are represented as mean ± SEM with n=6 rats in each group

FIGURE 4.4.5. Number of Cut-offs observed in the photo-actometer in the chronic mild stress model in SD rats

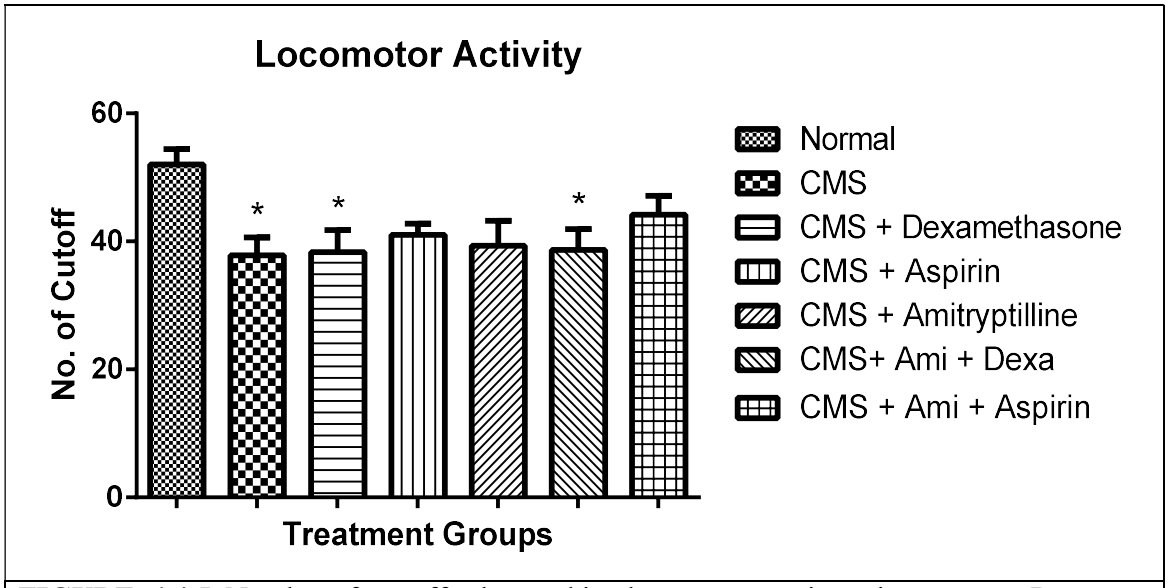

FIGURE. 4.4.5. Number of cut-offs observed in photoactometer in various groups. Bar diagram represents number of cut-offs in photoactometer in five minutes. Results are represented as mean ± SEM with n=6 rats in each group. *p<0.05 when compared with the normal group

FIGURE 4.4.6. Serum cortisol levels in the chronic mild stress model in SD rats

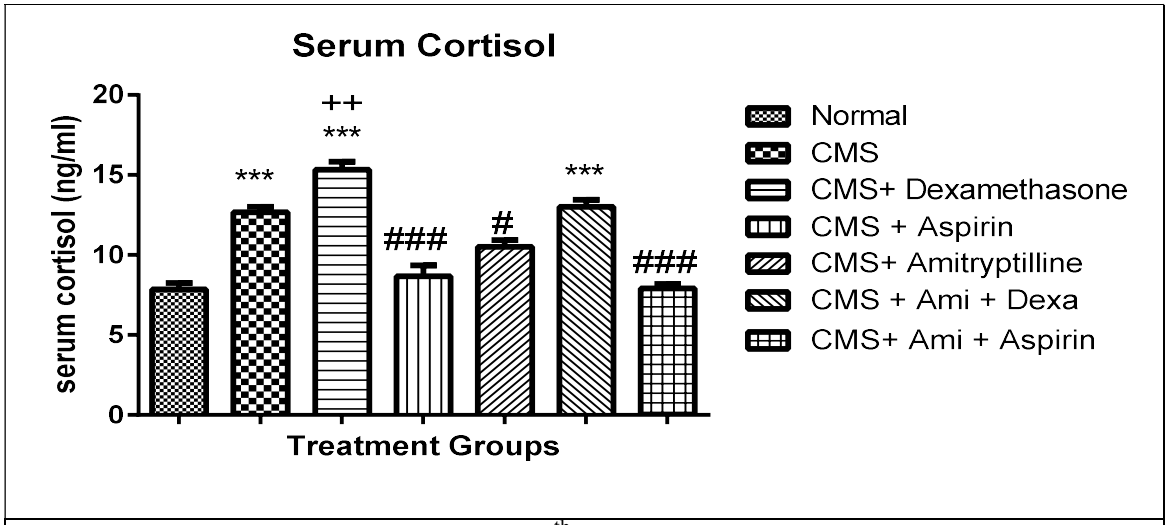

FIGURE 4.4.6. Serum Cortisol levels on 28th day of treatment of CMS. Bar diagram represents Cortisol in ng/ml. Results are represented as mean ± SEM with n=6 rats in each group, *** p<0.001 when compared with the normal group. #p< 0.05, ### p< 0.001 when compared with the CMS group. ++ p< 0.01 when compared with the CMS group

FIGURE.4.4.7. Brain serotonin in the chronic mild stress model in SD rats

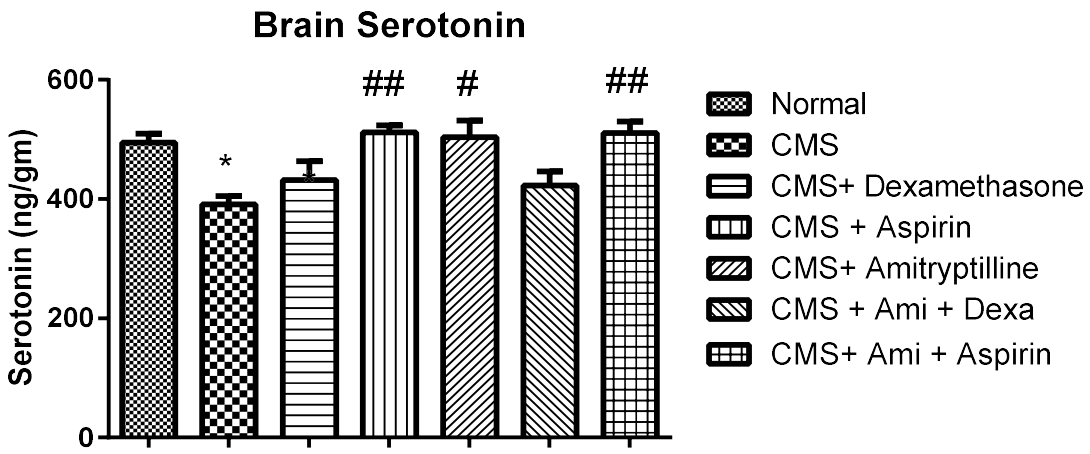

FIGURE. 4.4.7. Estimation of Serotonin in brain homogenate on 28th day of administration of drugs in various groups. Bar diagram represents serotonin in ng/ gm of brain tissue. Results are represented as mean ± SEM with n=6 rats in each group. *p <0.05 when compared with the normal group. # p<0.05 and ##p< 0.01 when compared with CMS group

FIGURE 4.4.8. Brain norepinephrine levels in the chronic mild stress model in SD rats

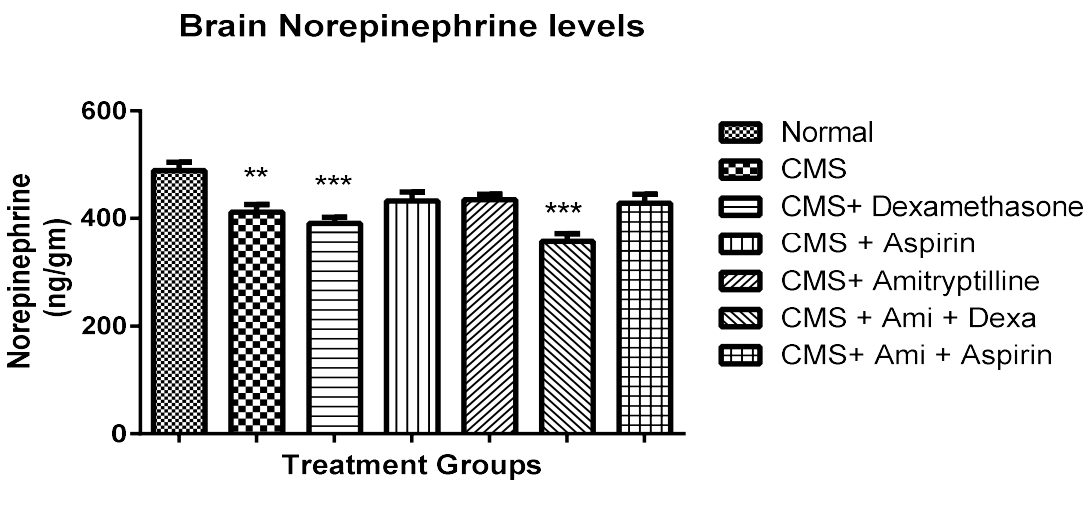

FIGURE. 4.4.8: Estimation of norepinephrine in brain homogenate after 28 days of administration of drugs in various groups. Bar diagram represents norepinephrine in ng/ gm of brain tissue. Results are represented as mean ± SEM with n=6 rats in each group. **p<0.01, ***p<0.001 when compared with the normal group.

FIGURE 4.4.9. Brain dopamine levels in the chronic mild stress model in SD rats

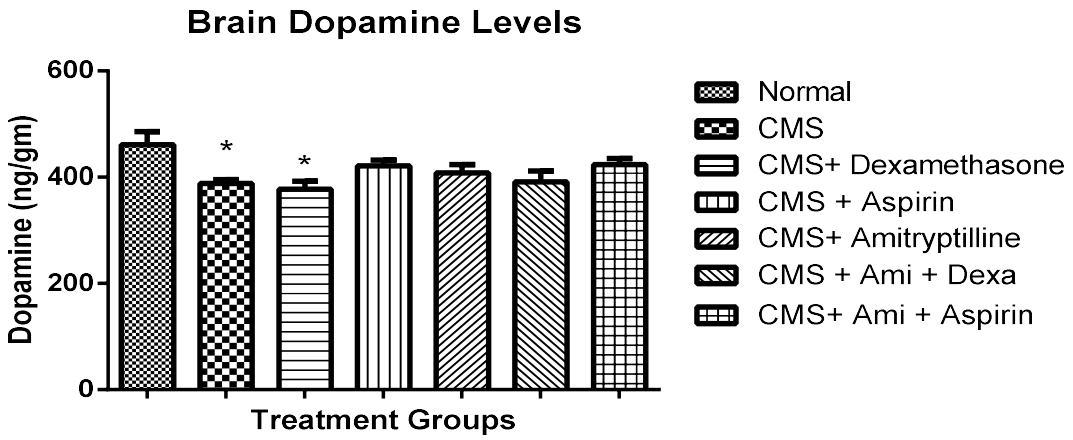

FIGURE. 4.4.9: Estimation of Dopamine in brain homogenate after 28 days of administration of drugs in various groups. Bar diagram represents dopamine in ng/gm of brain tissue. Results are represented as mean $\pm$ SEM with n=6 rats in each group. *p <0.05 when compared with the normal group

TABLE 4.4.1. Effect of various treatments in CMS model of depression in rats

Parameters	**CMS**	**CMS +Dexamethasone**	**CMS +Aspirin**	**CMS + Amitriptyline**
Immobility time (sec)	152.8±4.4.	160.3±5.1	114.6±7.6***	104±4.7***
Number of transitions	11.6±0.8	10.5±0.7	12.5±0.9	14.3±0.8
Time spent in open arm (sec)	51.6±5.8	61.3±4.7	56.8±5.8	49.8±2.05
Number of cut-offs	37.8±2.7	38.3±3.4	42.1±2.4	37.1±3.8
% of Sucrose Preference	59.8±2.6	45.8±2.6	74.1±3.7*	83.1±4.6***
Serum cortisol (ng/ml)	12.6±0.3	15.3±0.5	8.6±0.6***	10.5±0.42*
Brain Serotonin (ng/gm)	391.1±14.1	431±32	511.5±12.3**	503.5±28.4*
Brain Nor epinephrine (ng/gm)	411.5±14.1	390.6±11.6	422.8±12.07	426.1±6.08
Brain Dopamine (ng/gm)	387±7.4	377±15.2	421±10.5	407.5±15.5

* $p<0.5$, ** $p< 0.01$, ***$p<0.001$ when compared with the CMS group

TABLE.4.4.2. Inter group comparison of concomitant treatments (CMS Vs CMS+ anti-inflammatory + antidepressant)

Parameters	**CMS**	**CMS +Amitriptyline +Dexamethasone**	**CMS +Amitriptyline +Aspirin**
Immobility time (sec)	152.8±4.4.	140.8±3.1	105.8±5.0***
Number of transitions	11.6±0.8	11.0±0.8	13.3±0.8
Time spent in open arm (sec)	51.6±5.8	56.6±3.9	56.8±2.3
Number of cut-offs	37.8±2.7	38.6±3.2	44.1±2.9
% of Sucrose Preference	59.8±2.6	53.6±3.5	83.1±4.6***
**Serum cortisol (ng/ml)	12.6±0.3	13.0±0.4	7.9±0.3***
Brain Serotonin (ng/gm)	391.1±14.1	422.5±23.5	510.8±19.5**
Brain Nor epinephrine (ng/gm)	411.5±14.1	357±13.9	415±10.6
Brain Dopamine (ng/gm)	387±7.4	391.5±20.8	423.6±11.4

** p< 0.01, ***p<0.001 when compared with the CMS group

TABLE.4.4.3. Inter group comparison of treatments in CMS model (Anti-inflammatory Vs anti-inflammatory + antidepressant)

Parameters	**CMS +Dexamethasone**	**CMS+ Amitriptyline +Dexamethasone**	**CMS +Aspirin**	**CMS +Amitriptyline +Aspirin**
Immobility time (sec)	160.3±5.1	140.8±3.1	114.6±7.6	105.8±5.0
Number of transitions	10.5±0.7	11.0±0.8	12.5±0.9	13.3±0.8
Time spent in open arm (sec)	61.3±4.7	56.6±3.9	54.8±5.8	56.8±2.3
Number of cut-offs	38.3±3.4	38.6±3.2	42.1±2.4	44.1±2.9
% of Sucrose Preference	45.8±2.6	53.6±3.5	74.1±3.7	83.1±4.6
Serum cortisol (ng/ml)	15.3±0.5	13.0±0.4 *	8.6±0.6	7.9±0.3
Brain Serotonin (ng/gm)	431±32	422.5±23.5	511.5±12.3	510.8±19.5
Brain Nor epinephrine (ng/gm)	390.6±11.6	357±13.9	422.8±12.07	415±10.6
Brain Dopamine (ng/gm)	377±15.2	391.5±20.8	421±10.5	423.6±11.4

* $p < 0.5$ when compared with the CMS group

Summary of results in the CMS model of depression in rats

5.4.1. Sucrose preference test

Only the results of sucrose preference test conducted at the end of the 3rd week of the study were considered for the study as no statistically significant conclusions could be derived from the sucrose preference test conducted at the end of 1st and 2nd week of the study. The animals treated with CMS for three weeks showed significantly lower preference of sucrose when compared with the normal control group ($p<0.001$). The groups treated with aspirin showed significant increase in sucrose preference ($p< 0.05$), and the groups treated with amitriptyline alone and concomitant treatment of aspirin with that of amitriptyline showed significant increase in sucrose preference when compared with the disease control group ($p<0.001$). The group treated with CMS and dexamethasone showed significant decrease in sucrose preference ($p< 0.05$) when compared with the CMS group (Fig. 4.4.1).

5.4.2. Behavioural tests

In forced swim test, the immobility time in the CMS and CMS and dexamethasone treated rats decreased significantly ($p<0.05$ and $p< 0.001$ respectively) when compared with the normal control group. Further, treatments in the groups receiving aspirin, amitriptyline and aspirin plus amitriptyline showed significant decrease in immobility time ($p< 0.001$) when compared with that of disease control (CMS) group. The dexamethasone treatment group showed significant decrease in immobility time ($p< 0.05$) when compared with the CMS group (Fig. 4.4.2).

In the Light dark box test, the number of transitions significantly reduced in the CMS group ($p<0.05$). There was also significant reduction in number of transitions in the groups receiving dexamethasone and concomitantly treatement of dexamethasone and amitriptyline treated groups ($p< 0.01$ and $p<0.05$ respectively) on comparing with the normal control group (Fig. 4.4.3).

In the elevated plus maze test, on comparing the time spent in open arm of CMS group with normal group and the other treatment groups with the CMS group, the results were found statistically insignificant and inconclusive (Fig. 4.4.4.).

The locomotor activity observed using photoactometer was significantly reduced in the groups treated with CMS, dexamethasone and amitriptyline plus dexamethasone ($p< 0.05$) when compared with the normal group (Figure 4.4.5).

5.4.3. Cortisol measurement in rat blood serum

The serum cortisol levels increased significantly in CMS, dexamethasone and dexamethasone plus amitriptyline ($p<0.001$) when compared with the normal control group. Paradoxically, the cortisol levels reduced significantly in the aspirin and aspirin plus amitriptyline treated groups ($p<0.001$) and amitriptyline ($p<0.05$) when compared with the disease control (CMS) group. However, there was significant increase in serum cortisol levels in the rats treated with CMS and dexamethasone when compared with the CMS treatment group ($p<0.01$) (Fig. 4.4.6)

5.4.4. Neurotransmitter measurement in brain

Serotonin levels in the CMS treatment group reduced significantly when compared with the normal group ($p<0.05$). There was significant increase in the brain serotonin levels in the groups treated with aspirin and amitriptyline ($p<0.01$, $p< 0.05$ respectively). Further, significant rise in brain serotonin levels was also observed in the group receiving concomitant treatment of aspirin with amitriptyline ($p< 0.01$) (Fig. 4.4.7).

Brain norepinephrine levels reduced significantly in the CMS group when compared with the normal control group ($p<0.05$). There was also reduction of brain norepinephrine in the groups treated with dexamethasone and group treated with dexamethasone + amitriptyline (($p<0.05$) on comparison with the normal control group. However, there was no effect of aspirin or amitriptyline treatment on brain norepinephrine levels, when compared to the CMS treatment group (Fig. 4.4.8).

Brain dopamine levels reduced significantly in the CMS group and the CMS group treated with dexamethasone ($p<0.05$) when compared with the normal group. There was no effect of aspirin or amitriptyline treatment on brain dopamine levels, when compared to the CMS treatment group (Figure 4.4.9).

4.5. Summary of Correlation And Regression in interferon and CMS model

4.5.1 Interferon Model

TABLE 4.5. Correlation Inter Groups (42 ANIMALS)

	R	**p (0.05)**
Serrum Cortisol Vs Neurotransmittors		
Serum Cortisol VS 5HT	-0.45	**0.002**
Neurotransmitters Vs Immobility Time		
Immobility vs 5HT	-0.39	**0.01**
Sucrose Preference Vs Neurotransmitters		
SUC PRE VS 5HT	0.53	**0.0002**

4.5.2 CMS Model

TABLE 4.6. Correlation Inter Groups (42 ANIMALS)

	R	**p (0.05)**
Serrum Cortisol Vs Neurotransmittors		
Serum cortisol vs 5HT	-0.64	3.7
Serum cortisol vs NE	-0.53	**0.000251**
Serum cortisol vs DA	-0.66	1.27
Neurotransmitters Vs Immobility Time		
IMT VS DA	-0.53	**0.0002**
IMT VS 5HT	-0.71	7.93
Sucrose Preference Vs Neurotransmitters		
SUC PRE VS NE	0.53	2.11
SUC PRE VS DA	0.62	1.33
SUC PRE VS 5HT	0.62	5.86 ns

5. Discussion

The present study was divided into four phases in which, study 1 and study 2 principally focused on the single dose effects of the drugs under study. Drugs used included, diclofenac sodium, aspirin (NSAIDs) and dexamethasone (corticosteroid) individually and in combination with amitriptyline in depression based tests and anxiety based tests. Study 3 included Interferon model of depression for 21 days and Study 4 was carried out using CMS model of depression for 28 days.

In study 1, rats treated with diclofenac sodium (10 mg/kg dose) showed significant reduction in the immobility time when used alone. However, when used in combination with amitriptyline, it failed to show any significant effect on immobility. Based on the results obtained in study 1 and on the basis of further review of literature, diclofenac was replaced by aspirin.

In phase 2, aspirin treatment reduced immobility time in the tail suspension test when used alone in the mice. The combination of aspirin and amitriptyline did not produce any effect on the immobility time. Aspirin also did not show any noteworthy effects in the anxiety based tests such as open arm close arm and light dark box.

Amitriptyline did not show any remarkable effect in FST although it is an antidepressant. The results are in line with the previous study conducted by Menzes et al., in which the antidepressant action of amitryptyline were studied in forced swim test and it failed to show reduction in immobility time. (122). The reason for the negative result could be due to its use as a single dose in study.

As per the earlier reports, NSAIDS like meloxicam, lornoxicam, sodium metamizole, ketorlac had been evaluated for their antidepressant effect using forced swim test (FST) (123). Only Ketorolac 5 mg/kg showed an antidepressant effect amongst all. None of drugs altered the cage locomotor activity. Further, the depressant or antidepressant effect of these drugs was not due to locomotor impairment (sedation) or psychomotor stimulation.

Use of dexamethasone both individually and in combination with the amitriptyline showed significant antidepressant action that was evident from reduction in the immobility time in forced swim test and the tail suspension test methods. Further, dexamethasone in the open arm and the close arm increased the number of transition of mice between the lit and dark chamber showing anti-anxiety action.

The forced swimming test is a behavioral test performed in rodents and it estimates the efficacy treatment of antidepressants. The rodents are placed in water filled chamber for a period of time, and the time for which rats remains immobile and making only the movements necessary to keep its head above water, are measured (122). The immobilization is a sign of desperation in the rodents, which is an indicator seen in depression. The use of antidepressants reduces the immobility time which results in increased activity, and is directly related with the efficacy of the antidepressants.

Both the forced swim test and tail suspension tests involve a procedure in which, the animals undergo stringent physical stress. This involves continuous physical efforts of the rats to constantly swim in order to stay afloat. The same is applicable in tail suspension test in which the mice are suspended by tail which produces immense stress due to upside down position. During such stress, there is a release of acute mediators of inflammation and activation of lipoprotein lipase pathway and COX pathway which may be blocked by dexamethasone and aspirin respectively. In this study, dexamethasone showed decreased immobility time whereas aspirin did not have any effect on the immobility time. The results are contradictory to the results in study 4 conducted in CMS model (124) and study 3 conducted in interferon model (125) where use of dexamethasone showed increase in immobility time.

Dexamethasone acts by activating glucocorticoid receptors (GR) while cortisol binds to both mineralocorticoid receptors (MR) and GR in the central nervous system. The results obtained from a previous study by Plihal et al. suggest that glucocorticoid receptors show and energizing effect for short term and with extended activation causes a dysphoric influence on mood. The predominant activation of mineralcorticoid receptors causes euphoric mood (126). In addition to this, the corticosteroids also act by inhibiting uptake-2, a high-capacity monoamine transport system originally described in peripheral tissues. Studies show that expression of uptake -2 transporters is seen in the brain and it has a significant role in clearance of the monoamines like NE, DA and 5HT indication that they influence both the

physiology and behavioral processes. In the CNS uptake -2 is inhibited by both synthetic and natural corticosteroids, and puts forward that blockade of uptake-2-mediated monoamine clearance may bring about some behavioral and physiological effects of corticosteroids (127).

One of the study has focused on investigation of the effect of proinflammatory cytokines on fibrocytes and regulation of the same by dexamethasone, it was found that dexamethasone suppressed the inflammatory response in spiral ligament fibrocytes by suppressing the levels of keratinocyte-derived cytokine (KC), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), and interleukin-6 (IL-6) (128)When exposed to stress, there is increased secretion of cortisol in the body. The elevated levels of cortisol initially produced, could be a compensatory mechanism against raised inflammatory mediators seen during depression. There are also studies indicating antianxiety effect of dexamethasone might be attributed to its interaction with opoidergic neurons (129).

Aspirin treatment in the study showed reduction in the immobility time in both TST in mice and FST in rats. Indicators of inflammation process, particularly raised levels of prostaglandine E2 (PGE2), have frequently been described in major depressive disorder (130). 'Sickness behavior' is an animal model of depression showing symptoms such as lack of drive, lack of appetite, decrease of weight, and fatigue etc. 'Sickness behavior' is mainly induced by pro-inflammatory cytokines such as IL-1 and IL-6, but also PGE -2 seems to be directly involved in sickness behavior. Inhibition of COX-2 could therefore be hypothesized to exert antidepressant action COX-2 inhibitors inhibit the Prostaglandin E2, and advocate a positive role in depression (131).

Further, in depression, opposite patterns of type-1 - type-2 immune response seem to be associated with differences in the activation of the enzyme indoleamine 2,3-dioxygenase (IDO) , decreased production of kynurenic acid in depression. The immunological imbalance results in depression which can be due to increased Prostaglandin E2 (PGE2) production and probably also in an increased Cyclo-oxygenase-2 (COX-2) expression as witnessed in depression (132). Blocking of the pain pathway by inhibition of COX could be a reason for the decrease in immobility time in rats and mice during FST and TST respectively.

Therefore, the data from preliminary study in rats (study 1) and single dose study in mice (study 2) suggests that the anti-inflammatory and analgesic effect of drugs needs to be

considered in tests like FST and TST particularly on the immobility time and number of escape efforts. Use of anti-inflammatory drug or the anti-inflammatory property of the antidepressant drug, under investigation can affect the behavior of animal in Forced swim test and Tail suspension test. There can be false positive results because of anti inflammatory property of the drug leading to the antidepressant action of the drug. Additionally the CNS stimulant effects of corticosteroids need to be carefully evaluated before going for behavioural test like open arm close arm and mobility behaviour in the photo-actometer.

Phase 3 and 4 studies were based on repetitive exposure of the experimental drugs for longer period of times. The models used in the study were Interferon model of depression and CMS model of depression.

In phase three study, Interferon alpha model of depression was used. The study required a model, in which use of inflammatory cytokine was utilized to produce depressive behavior and association between the central neurotransmitters and the inflammatory mediator (interferon alpha) was explored using anti-inflammatory drugs. The phase four study used the CMS model of depression which mimics the natural model of depression based on chronic unpredictable mild stress.

Interferon treatment for 21 days produced increase in immobility time in forced swim test and serum cortisol levels in the rats, decreased sucrose preference, monoamines like serotonin. The behavioral changes seen in the rats treated with Interferon like ptosis, piloerection, lethargy, and sleep coined as 'sickness behavior' were replicated as described in previous studies (112). In humans, Interferon alpha (IFNalpha) is used for the treatment of several disorders, such as chronic hepatitis or malignant melanoma. During the therapy, IFN alpha may cause severe neuropsychiatric syndromes including depression with suicidal ideation, paranoid psychoses, or confusional states (133). Acute or repeated infusion of IFN-α into the lateral ventricles have been found to induce depressive-like behavior and concomitant changes in serotonin (5-HT) and mRNA expression of particular 5-HT receptors and pro-inflammatory cytokines (134).

Our study reproduced the results of previous studies (103, 135) in which interferon treatment increase immobility time in the FST in the animals and reduced the sucrose preference in the rats and raised serum cortisol with reduction in brain serotonin levels. The interferon-

induced treatment increased in the immobility time by 30 % and results were in agreement with previous study in which Human interferon alpha has been found to prolong immobility time in rats in the Forced swimming test (85). The probable mechanism by which use of human interferon have shown increase in immobility time is found be either through opioid receptors (135), or through involvement of increased cerebral tryptophan level and serotonin turnover (116).

Previous studies have shown raised plasma concentration of adenochorticotrophic hormone and cortisol on exposure of subcutaneous dose of of 3 x 10^6 I.U. (136). Another study examined the acute effects of IFN-α on cortisol and IL-6 release, and the time course of any changes in these variables. Serum cortisol and plasma IL-6 were assessed in healthy volunteers over an 8-h period following 3 million units subcutaneous IFN-α or placebo using a double-blind, placebo-controlled crossover design. IFN-α treatment have resulted in a significant increase in both cortisol and IL-6 levels (137).

The newly generated neurons by the neural stem cells (NSCs) in the hippocampus play an vital role in regulation of emotional control and act in response to the antidepressants action. The commonly present and severe side effect of IFN-α therapy is depression. It was found in a recent study that mice exposed to IFN-a treatment presented a depression-like behavior phenotypes. IFN-α treatment caused a direct suppression of neural stem cells proliferation, causing reduced generation of new neurons. Brain-specific mouse knockout of the IFN-α receptor inhibited the depressive behavior and caused the inhibition of neurogenesis. The results suggest that IFN-α suppresses neurogenesis in the hippocampal region and induces depression via IFN receptor in the brain (138). Increased cortisol may play a role by inducing tryptophan 2,3 dioxygenase, the main metabolizing enzyme of tryptophan. Elevated cortisol level could explain lowered plasma tryptophan levels. Lowered plasma tryptophan levels is associated with decreased serotonin turnover(139). Concomitant treatment of dexamethasone for 21 days in interferon- treated group further raised cortisol levels, decreased sucrose preference and reduced the brain mono amines. Short-term corticosteroid therapy associated with disturbance of mood, cognition, sleep, and behavior as well as psychosis. Long-term therapy of corticosteroids tends to induce depressive symptoms (140,141). The decrease in sucrose preference can be a manifestation of behavioral changes like anhedonia, seen with long term use of corticosteroids as described above.

Treatment of dexamethasone for 21 days produced raised cortisol levels in the animals receiving interferon and interferon with dexamethasone. A dysregulated hypothalamic-pituitary-adrenal axis (HPA) has been implicated in major depressive disorder and most commonly used animal models of depression have been shown to elevate circulating levels of plasma cortisol. Moderately elevated cortisol for a prolonged period is sufficient to induce cellular changes in the hippocampus (142) that may be prevented by chronic administration of antidepressants. The repetitive administration of dexamethasone produced worsening of depressive behavior as seen in the sucrose preference test. The reduction in the sucrose preference by the animals indicates that dexamethasone worsened anhedonia in the groups receiving interferon and dexamethasone. Anhedonia or failure to experience the pleasure is one of the key symptoms of depression.

Corticosteroid exposure in the human studies have been found to be be associated with changes in hippocampal volume and functioning in humans. The patients are found to manifest the symptoms of depression in conjugation with the hippocampal changes due to corticosteroid therapy (143).

Aspirin treatment in interferon-α-2b model of depression for about 21 days, showed significant antidepressant effect through the parameters such as sucrose preference test, forced swim test, serum cortisol levels and brain monoamines. There was increase in sucrose preference and reduction in serum cortisol in the interferon group treated with aspirin. Further, there was noteworthy decrease in immobility in the group treated with interferon-α-2b with aspirin. The brain serotonin levels were raised when compared with the interferon treatment group. These findings are in line with the observations seen in previous studies which focus on the beneficial effect of aspirin in affective disorders (144,145).

The first evidence relating NSAIDS to depression arose from the undesirable psychiatric effects caused by some of them, such as the appearance of depressive symptoms on withdrawal of indomethacin (146), the increase in the undesirable effects of imipramine due to acetyl salicylic acid (147) or various depressive manifestations after the administration of NSAIDS (148,149). A range of doses of aspirin (100 to 300 mg/day) reduced the plasma levels of inflammatory biomarkers such as CRP, IL-6 and TNF-α in patients with cardiovascular metabolic syndrome (150). Aspirin was shown to reduce the levels of inflammatory cytokines, such as TNF-α and IL-8, but not those of negative

immunoregulatory cytokines, such as IL-4 and IL-10 (151). In the same study, there did not seem to be any effect of aspirin on IL-1b, and the suppressant effects of aspirin on IL-6 did not reach significance. Aspirin also reduced the production of TNF-α in rats with streptozotocin-induced type -2 diabetes (152).

The increase in cortisol levels might decrease serotonin levels by diminishing the activity of tryptophan 2, 3 dioxygenase (139).Signs of an inflammatory process, in particular increased levels of prostaglandine E2 (PGE2), have repeatedly been described in major depression and inhibition of PGs by Cyclooxygenase inhibitors and its beneficial role in depression has been shown. In study conducted by De Pavia et.al. (153), it was reported that Prostaglandins mediate depressive-like behaviour induced by endotoxin in mice. The study evaluated the possible participation of prostaglandins in lipopolysaccharide-induced sickness behaviours. The mice were submitted to the tail suspension test (TST), forced swim test (FST), open field test and dark–light box test and it was found that lipopolysaccharide (LPS, 100 g/kg; i.p.) administration increased the time spent immobile in the TST, increased the time spent floating in the FST, and depressed locomotor activity in the open field. These findings not only confirmed previous observations that have reported LPS-induced sickness behaviours but also provided evidence that the synthesis of prostaglandins is necessary for changes in depressive-like and exploratory behaviours in mice (153).

Increased pro-inflammatory cytokines and prostaglandine E2 (PGE2), have been consistently shown in major depression . Incread PGE2 in the saliva in the patients of major depression (154,155)., the serum (156) and the cerebrospinal fluid (157) in depressed patients has previously been reported in literature. In a study conducted by Muller and associates in 2006 (131), it was found that in comparison with the group treated with reboxetine alone the group treated with celecoxib (selective cox-2 inhibitor) and reboxitine there were significant positive effects on considering the symptoms of depression. Further this study also strengthened the hypothesis that inflammation in related to the pathogenesis of depression. Similar observations have been witnessed in study study involving COX 1 inhibition through NSAIDS (158).

Apart from implication of COX inhibition as mechanism for antidepressant action, the other reason by which aspirin may decrease depressive behavior is by reduction of plasma homocysteine. A recent study published by Almeida et al., reported that aspirin decrease risk

of depression in patients with high plasma homocysteine levels. The study involved 513 subjects and the results of this study indicate that older men with high homocysteine who use aspirin have lower risk of depression, and suggest that anti platelet therapy with aspirin may be an effective preventive or management strategy for these cases (159). Furthermore, one of the studies (160) showed use of aspirin as augmentation agent in fluoxetine treatment resistant depressive rats. The study showed that the rats which were resistant to fluoxetine treatment when given adjunctive aspirin treatment significantly improved the depressive behaviours and downregulated the COX-2 level and PGE2 concentration in the hippocampus. The results suggest that aspirin can be served as an effective adjunctive agent in the treatment resistant depression mediated by inhibition of the COX-2 level and PGE2 concentration. Increased activity of cyclooxygenase-2 produces prostaglandin D2 (161) leading to subsequent inhibition of serotonin in the brain. Inhibition of such eicosanoids brought by aspirin may result in its antidepressant action. Supportive evidence to this is role of hyperforin, a constituent from St. John's wart inhibits prostaglandin synthesis, which is an established antidepressant agent. Hyperforin, the key active constitutent from St. John's wort possesses anti-inflammatory effects that were ascribed among others to the inhibition of 5-lipoxygenase. The study investigated whether Hyperforin also interferes with prostanoid generation in biological systems, particularly with key enzymes participating in prostaglandin (PGE2) biosynthesis, i.e., cyclooxygenases (COX)-1/2 and microsomal PGE2 synthase (mPGES)-1 which play key roles in inflammation.

It was found that Intraperitoneal administration of Hyperforin in 4mg/kg dose to rats decreased exudate volume and leukocyte numbers in carrageenan-induced pleurisy associated with reduced PGE2 levels, and Hyp (i.p.) inhibited carrageenan-induced mouse paw edema formation. The study concluded that the suppression of PGE2 biosynthesis in vitro and in vivo by acting on mPGES-1 critically contributes to the anti-inflammatory efficiency of Hyperforin or St John's Wart (162).

One of the recent findings shows that Patients with treatment resistant depression have elevated levels of cortisol along with high levels of Interleukin -6, Interleukin-10. The result from this study supports the hypothesis that decreasing the inflammatory status in the patient with depression by using anti-inflammatory drugs can help in relieving depression (163).
Studies also show that anti-nociceptive effects of non-opioids or NSAIDS like aspirin could be due to their effects on the serotonergic and noradrenergic like mono amine pathways

(164). The control of the nociceptive processing by serotonin may facilitate or inhibit nociception due to the different types of serotonin receptors and their location on facilitating sites like projection neurons, primary afferent fibers, excitatory interneurons and inhibitory inter neurons like the one in the superficial laminae of the spinal cord (165). The participation of the serotonergic system in the antinociceptive effects of NSAIDS has been comprehensively studied. In the rats, NSAIDS like aspirin increases serotonin levels in the areas of the brain such as pons and cerebral cortex (166,167) Similar results with acetaminophen in the striatum, posterior cortex, hypothalamus, hippocampus and brain stem have been witnessed but not the spinal cord of rats.

On the level of neurogenesis, aspirin has been found to decrease spontaneous recurrent seizures and inhibited hippocampal neuronal loss, mossy fiber sprouting and aberrant neurogenesis in pilocarpine-induced status epilepticus in rats (168). The loss of the neurons of hippocampus, mossy fiber sprouting alongwith abnormal neurogenesis lead to the alteration in the functioning of hippocampus which can cause depressive symptoms. Thus, one of the mechanisms for antidepressant action of aspirin in the present can be attributed to effect of aspirin on the neurons in the brain.

The phase 4 of the study was done in chronic mild stress model of rats in which, the rats were exposed to different types of stress for 28 days. The stressors were applied for a different periods and were unpredictable in terms of sequence in which the given type of stressor was applied. The chronic mild stress model is a natural model for depression and uses various inescapable stressors mimicking human stress-induced depression.

The rats treated with chronic mild stress after 28 days produced increase in immobility time in forced swim test, serum cortisol levels, and decreased sucrose preference, monoamines like serotonin. Chronic sequential administration of a variety of mild stressors causes a decrease in responsiveness to rewards in rats, which is reversed by chronic administration of antidepressant drugs. The behavioural changes seen in the rats treated with CMS were replicated as described in previous studies (169). Our study showed parallel results with that of previous studies in which exposure to CMS increased immobility time. In this study, exposure of 3-week chronic mild stress (CMS) decreased sucrose preference of rats and increased immobility in the forced swim test. It also induced social avoidance and increased grooming (170), reduced the sucrose consumption in the rats (76).The reduction of

sucrose preference was seen after 21 days of CUMS procedure. The study replicated the reduction of sucrose preference by chronic mild stress procedure and the restoration of the same as shown in a previous study.

Simultaneous treatment of dexamethasone in CMS group, further increased cortisol levels in the groups receiving interferon and dexamethasone, along with reduction in sucrose preference and brain monoamine like serotonin. Long term exposure of corticosteroids is associated with a range of actions leading to depressive behavior (141,171). Changes in the hippocampus of the brain caused by the extended use of corticosteroids (140,142) could be attributed to depressive behaviour. The decrease in sucrose preference can be a manifestation of behavioural changes like anhedonia, seen with chronic use of corticosteroids as documented in the earlier research (139). Further, the decrease in the brain serotonin levels may possibly be a result of induction of tryptophan 2, 3 dioxygenase (139) causing decrease in brain serotonin levels as observed in the current study. There was overall deterioration of depressive behaviour seen by use of dexamethasone for 28 days. There was no effect of anti-inflammatory treatments in the anxiety based parameters like number of transitions in the Light dark box, number of cut-offs in photoactometer and time spent in open arm in the elevated plus maze.

In a separate study conducted by Cox et. al., using the chronic unpredictable stress (CUS) paradigm (2 stressors per day for 10 days) in adult Sprague-Dawley rats, the study showed no effect on anxiety-behavior as measured by the elevated plus maze (EPM). Similar results were obtained in our study in EPM (172). In another study wherein, the delayed effects of on anxiety measures were determined. There were no significant effects of CUS on behavioral measures in the unconditioned response tasks like the elevated plus-maze or light-dark box, at any time point following exposure to CUS (171). The overall results were ambiguous in anxiety based test after the induction of CMS procedure and non conclusive and were in agreement with the previous studies (172,173).

In the groups treated with aspirin alone and the groups treated with the aspirin and amitryptilline, the animals showed restoration of sucrose consumption in the sucrose preference test. Further, there was significant reduction in the immobile behavior in the forced swim test comparable to that of the normal untreated groups. The results were similar to the one observed in the interferon model study. The serum cortisol levels reduced and the

brain serotonin levels increased significantly when compared with the CMS treatment group signifying the antidepressant action produced by aspirin treatment alone and in combination with amitryptilline.

The study investigated the association of inflammatory mediators with central neurotransmitter levels using the anti-inflammatory treatments. The two models used in the study for repetitive and longer exposure of the drugs. Interferon alpha in phase 3 and CMS model in phase 4 of the study. Interferon model used in the study was based on the use of a cytokines (Mediator of inflammation) to produce depressive like behavior and see the effect on antidepressant and anti-inflammatory drugs along with the effects on the monoamines like 5HT, NE and DA.

The interferon belong to the large class of proteins known as cytokines which are used for communication between cells to trigger the protective defence system of the immune system which helps to eradicate pathogens. Certain symptoms of infections, such as fever, muscle pain and "flu-like symptoms", are also caused by the production of IFNs and other cytokines. They are typically divided among three classes: Type I IFN, Type II IFN, and Type III IFN. IFNs belonging to all three classes are important for fighting viral infections and for the regulation of the immune system. Interferon alpha belongs to TYPE 1 IFN. Inverse correlation was observed between the interferon exposure and 5HT. Positive association was observed between the interferon exposure and stress related behavior and the symptoms of depressive behavior like sucrose preference and immobility aspects were inhibited by use of NSAID like aspirin.

The CMS model of depression was used in the phase 4 of the study. Studies conducted in this model have already established increased expression of pro-inflammatory cytokines like Interleukin 1 interleukin 6 and TNF-Alpha in rat. The use of the CMS model fulfilled the two important aspects of the study. Firstly, use of a natural model mimicking depression and secondly having an already established modality with elevated expression of pro-inflammatory cytokines. Raised pro-inflammatory cytokines like Interleukin 1 interleukin 6 and TNF-Alpha in rat brain have been already been reported in the CMS model of rats (174,175).

In the correlation and regression studies conducted after evaluating the data obtained from the various parameters like FST, SFT, Serum cortisol and brain neurotransmitters. It was found that positive correlation existed between the inflammatory mediators (The groups receiving Interferon and CMS) and depressive behavior which was confirmed by SFT and immobility time in FST. Further, inverse correlation was found between the brain neurotransmitters like serotonin and cortisol (an indicator for stress). There was a positive correlation observed between the use of NSAID like aspirin and negative correlation with dexamethasone treatment in CMS model of depression in rats.

In summary, the data from the preliminary studies in rats and mice suggests that the anti-inflammatory and analgesic effect of drugs needs to be considered in tests like FST and TST particularly on the immobility time and number of escape efforts. Use of anti-inflammatory drug or the anti-inflammatory property of the antidepressant drug, under investigation can affect the behavior of animal in Forced swim test and Tail suspension test. In the interferon and CMS model,our findings propose targeting anti-inflammatory drugs in the treatment of depression. The deterioration of depressive behavior seen with long term use of dexamethasone supports the use of novel antidepressants like Antalarmin CRHR-1 (Corticotropin-releasing hormone receptor-1) receptor blocker in the treatment of depression (176). These observations reinforce the possibility of aspirin intervention in the pathogenesis of depression. Aspirin may provide a potential adjunctive therapy for patients suffering from depression.

Studies based on the results and observations evidenced in current study can be conducted in future in numerous directions. Depression being a complex, multi factor disorder, it becomes difficult to generalize same treatment for all the patients. The effect of aspirin and other aspirin like drug can be studied directly on the enzymes like mono amine oxidase enzyme (MAO), Catechol o methyl transferase (COMT) and tryptophan 2,3 dioxygenase. Effect of aspirin like drugs can be studied on the micro-glial cells which in turn can help in correlating it effects on components of the brain other than neurons such as astrocytes. Further studies can also be conducted on agents like antalarmin (CRHR-1) (Corticotropin-releasing hormone receptor-1) antagonist, investigating its effect on COX enzyme and inflammatory cytokines in depression models

6. Conclusion

The NSAIDS like anti-inflammatory drugs can play a vital role in the treatment of affective disorders like depression particularly when depression is considered to psycho neuroimmunological in nature. Drugs like aspirin can play a vital role in controlling the symptoms of depression. In contrast use of potent anti-inflammatory drug like dexamethasone for longer terms worsens the symptoms of depression.

Further, there is inverse correlation between neurotransmitters like serotonin and inflammatory mediators which can be one of the reasons for pathogenesis of depression.

The exact mechanism responsible for the antidepressant action of aspirin is unknown.

However, it may be attributed to one of the mechanism or mechanisms such as inhibition of the Cyclo-oxygenase pathway, reduction in the serum cortisol, and mechanism leading to neuro-protection during stress induced depression, or any other potential physiological and biochemical mechanisms.

Regardless of the mechanism(s) involved, the use of aspirin appears to have a beneficial effect on psychopathology of depression. Further studies are required in both pri-clinical and clinical setup to establish antidepressant action of different NSAIDs and their mechanism involving antidepressant action.

7.References

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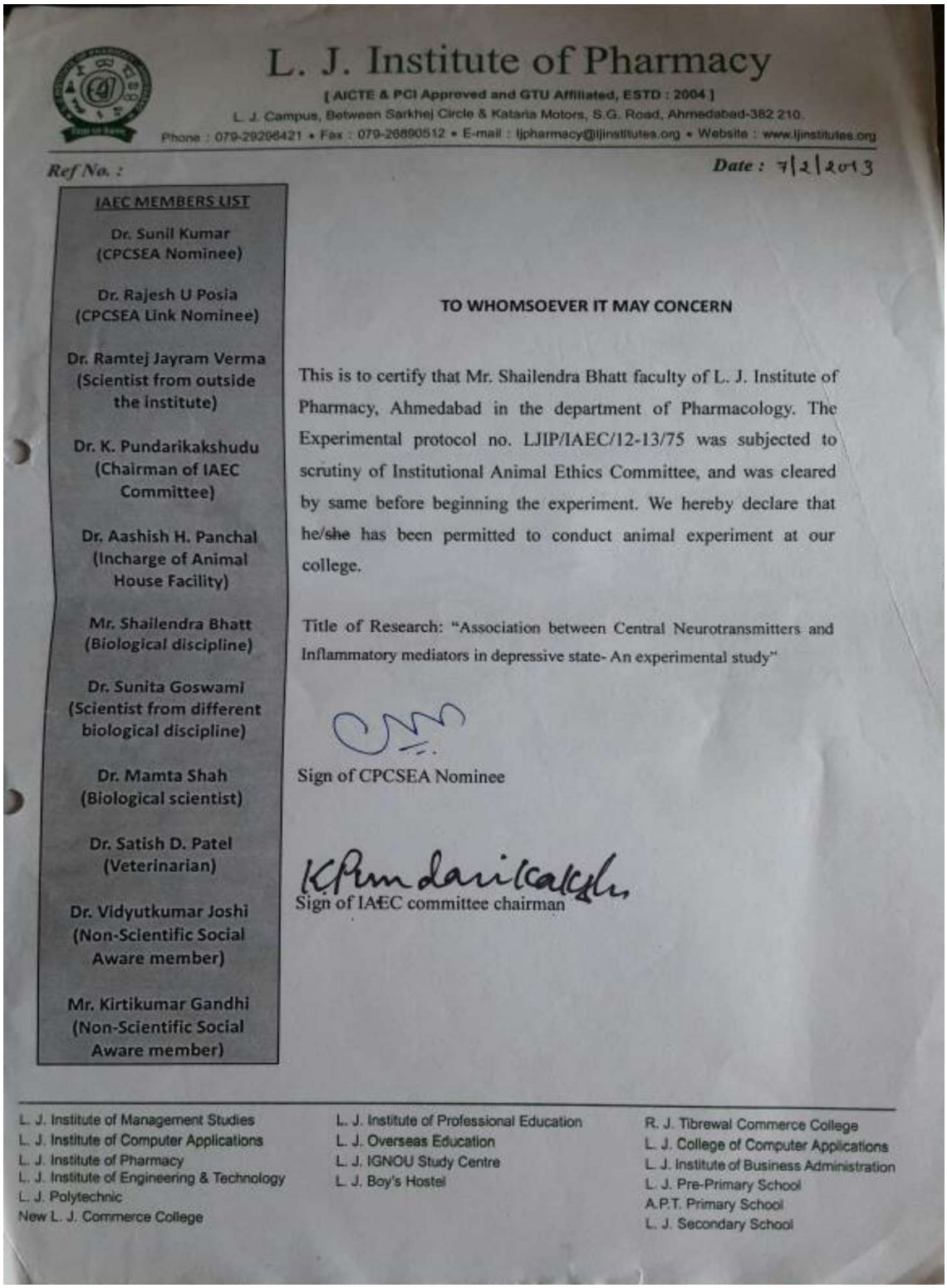
APPENDIX-1

Certificate of approval for expereiment- IAEC



L. J. Institute of Pharmacy

[AICTE & PCI Approved and GTU Affiliated, ESTD : 2004]

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Date : 7/2/2013

IAEC MEMBERS LIST

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Dr. Rajesh U Posia
(CPCSEA Link Nominee)

Dr. Ramtej Jayram Verma
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(Chairman of IAEC Committee)

Dr. Aashish H. Panchal
(Incharge of Animal House Facility)

Mr. Shailendra Bhatt
(Biological discipline)

Dr. Sunita Goswami
(Scientist from different biological discipline)

Dr. Mamta Shah
(Biological scientist)

Dr. Satish D. Patel
(Veterinarian)

Dr. Vidyutkumar Joshi
(Non-Scientific Social Aware member)

Mr. Kirtikumar Gandhi
(Non-Scientific Social Aware member)

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Mr. Shailendra Bhatt faculty of L. J. Institute of Pharmacy, Ahmedabad in the department of Pharmacology. The Experimental protocol no. LJIP/IAEC/12-13/75 was subjected to scrutiny of Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment. We hereby declare that he/~~she~~ has been permitted to conduct animal experiment at our college.

Title of Research: "Association between Central Neurotransmitters and Inflammatory mediators in depressive state- An experimental study"

Sign of CPCSEA Nominee

Sign of IAEC committee chairman

L. J. Institute of Management Studies
L. J. Institute of Computer Applications
L. J. Institute of Pharmacy
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L. J. College of Computer Applications
L. J. Institute of Business Administration
L. J. Pre-Primary School
A.P.T. Primary School
L. J. Secondary School

APPENDIX-2

Copy of Publications

LIST OF PUBLICATIONS

Bhatt S, Shukla P, Raval J, Goswami S (2016).Role of Aspirin and Dexamethasone against Experimentally Induced Depression in Rats. Basic and Clinical Pharmacology and Toxicology. 119 (1):10-8 .

Bhatt S, Pundarikakshudu K, Patel P, Patel N, Panchal A, Shah G, Goswami S. (2016) Beneficial effect of aspirin against interferon-α-2b - induced depressive behavior in Sprague Dawley rats. Clinical and Expeirmental Pharmacology and Physiology. ;43(12):1208-1215