Effect of Ranolazine and Trimetazidine on Depression Paradigm in Male Swiss Albino Mice

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ABBREVIATIONS

WHO	:	World Health Organization
ANOVA	:	One way analysis of variance
CMDs	:	Common Mental Disorders
NCD	:	Non Communicable Disorders
IL-1β	:	Interleukin-1 beta
IL-6	:	Interleukin-6
TNF-α	:	Tumor necrosis factor-alpha
SSRI's	:	Selective serotonin receptor inhibitors
SNRI's	:	Selective noradrenaline receptor blockers
PPAR-α	:	Peroxisome Proliferator Activated Receptor alpha
iNOS	:	inducible nitric oxide synthase
NF- *κ*B	:	nuclear factor kappa B
AP-1	:	activator protein-1
GAS	:	*γ*-activation site
IL-1Ra	:	interleukin-1 receptor antagonist
MDD	:	Major Depressive disorder
DSM V	:	Diagnostic and statistical manual fifth edition
YLD	:	Years Lived with Disability
ICD-10	:	International classification of diseases
AMA	:	American Psychiatrist's Association
NAc	:	Nucleus Accumbens
Cg	:	cingulate
BDNF	:	Brain-Derived Neurotrophic Factor
VTG	:	ventral tegmental area
CREB	:	cyclic –AMP ‹adenosine mono phosphate›-response-element-binding protein
VEGF	:	Vascular Endothelial Growth Factor
LPS	:	lipopolysaccharide
IL-1	:	Interleukin-1
HPA	:	hypothalamic pituitary adrenal
CpG	:	5'—C—phosphate—G—3'

MeCP2	:	methyl-CpG binding protein 2
HDACs	:	histone deacetylases
GABA	:	Gamma amino butyric acid
5HT-T	:	5-Hydyroxytryptamine transporter protein
NMDA	:	N-Methyl, D-Aspartate
TRD	:	treatment resistant depression
RCT	:	randomized controlled trial
DXTM	:	deuterium-modified dextromethorphan
FST	:	Forced Swim Test
TST	:	Tail Suspension Test
EPM	:	Elevated Plus Maze
RZ	:	Ranolazine
TZ	:	Trimetazidine
FLU	:	Fluoxetine
MTORC1	:	mechanistic or mammalian target of rapamycin complex-1
PUFAs	:	Polyunsaturated fatty acids
NAC	:	N-acetyl cysteine
DMT	:	dimethyltryptamine
LSD	:	lysergic acid diethylamide
PRIDE	:	Prolonging Remission in Depressed Elderly
ECT	:	electroconvulsive therapy
sTMS	:	synchronized transient magnetic stimulation
dTMS	:	deep TMS
LFMS	:	low-frequency magnetic stimulation
CUMS	:	chronic unpredictable mild stress
CMS	:	chronic mild stress
HDL-C	:	low high density lipoprotein
apoC-III	:	apolipoprotein complement 3
LPL	:	lipoprotein lipase
VLDL	:	very low density lipoprotein
HDL-C	:	high density lipoprotein cholesterol
VCAM	:	vascular cell adhesion molecule
PECAM	:	platelet/endothelial cell adhesion molecule

Cyp4fs	:	Cytochrome P 450 4f's
LTB4	:	Leukotriene B4
PI3-K	:	phosphatidylinositol 3-kinase
SOD	:	superoxide dismutase
IAEC	:	Institutional Animal Ethics Committee constituted
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
EDTA	:	ethylene diamine tetra acetic acid
GSH	:	Reduced glutathione
DTNB	:	5,5- dithiobis 2-nitrobenzoic acid
NaOH	:	sodium hydroxide
m-RNA	:	messenger ribonucleic acid
cDNA	:	complementary Deoxyribonucleic acid
PCR	:	polymerase chain reaction
ABI	:	Applied Biosystems
MCP-1	:	monocyte chemoattractant protein-1
SYBR	:	synergy brands.inc
RT-PCR	:	real time real time PCR
S.E.M	:	standard error of mean
USA	:	United States of America
H&E	:	Hematoxylin and Eosin
CNS	:	Central Nervous System
CAD	:	Coronary Artery Disease
PRIDE	:	Prolonged Remission in Depressed Elderly
FORWARD	:	Focused on Results with A Rethinking of Depression trial
DALY	:	Disability-adjusted life years

ABSTRACT

Introduction:

Depression or Depressive Disorder is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. Recent advances in neurobiological research have shown a growing body of evidence indicating inflammatory pathways have a role in the genesis of depression. Depression is caused by a complex combination of behavioural, neurotropic, immunological, neuroendocrine, and other physiological components. Excessive secretion of proinflammatory cytokines, such as interleukin-1 beta (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-), causes sickness behavior, which has symptoms that are similar to those of depression, implying that cytokines have a role in depression. However, the current therapy available is with limited efficacy serious side effects.

Objective of the study:

1. To study the effect of Ranolazine and Trimetazidine on Depression paradigm.
2. To compare Ranolazine and Trimetazidine with that of standard anti-depressant drug Fluoxetine

Materials and Methods:

Animals were divided into 5 groups with n = 8 in each group. Animals were introduced to various stressors for 2 weeks. One day prior to the experimentation the Swiss Albino Mice were made to undergo training (first) session of 15 min. Next day onwards all the Animals undergone following behavioural tests for the establishment of Depression.

The behavioural tests done are Forced Swim Test, Elevated Plus Maze, Tail Suspension Test and Actophotometer.

Once the Depression is established animals were treated with Test drugs and Standard drugs for next 4 weeks Along with various stressors. After 6 weeks again animals were undergone above mentioned Behavioural tests and data were recorded. At the end of the study animals were sacrificed and blood was collected. Brain of mice was dissected out. Serum inflammatory markers, brain homogenate inflammatory stress markers and hippocampal histopathology analysis was carried out.

Results:

Ranolazine and Trimetazidine showed reduction in Depressive symptoms and inflammatory markers hippocampal cytokine and hisopathological study in comparison to control. However there is no improvement in Locomotors activity.

Conclusion:

The present study exhibited Ranolazine and Trimetazidine did exert significant anti-depressant effect. However, further research is requires in order to provide further insight and understanding into the mechanism of action and efficacy of this compound in Depression.

Keywords: Ranolazine, Depression, Coronary Artery Disease, Forced Swim Test, Inflammation, CUMS, FST, TST.

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INTRODUCTION

Depression or Depressive Disorder is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration.[1]

World Health Organisation (WHO) estimates a prevalence of more than 300 million people that suffer from depression. Long-lasting depression of moderate or severe intensity, is considered as a serious health condition. The affected person suffers greatly and performance is affected at work, school as well as in personal life. The worst outcome of depression is suicide. Approximately 800000 people commit suicide every year because of depression. Suicide is the second leading cause of death in 15-29-year olds.[2]

In INDIA the measured prevalence of depression for both current and life time was 2.7% and 5.2%, respectively in 2020, indicating that nearly 1 in 40 and 1 in 20 suffer from past and current depression, respectively. Depression was reported to be higher in females, in the age-group of 40-49 years and among those residing in urban metros. Equally high rates were reported amongst the elderly (3.5%).[3]

Common mental disorders (CMDs), including depression are closely linked to both, the causation and consequences of several non- communicable disorders (NCD) like hypertension, myocardial infarction, stroke, dementia, epilepsy and endocrinal disorders like diabetes and hypothyroidism.[4] Recently, it has been shown that these diseases being chronic inflammatory states, independently produce depressive symptoms in the patient.[5]

Treatment of such comorbidities, at times mandates concomitant use of multiple drugs which in turn leads to a drug-drug or a drug-disease interaction which can improve or worsen the primary disorder or comorbidity[6], in the latter case, thereby contributing to

a significantly increased healthcare burden.[3]

Several studies suggest that depression results from a complex interaction between behavioural, neuroendocrine, neurotrophic, immunological, and other physiological factors. Excessive secretions of proinflammatory cytokines, such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α), result in 'sickness behaviour', the symptoms of which resembles those of depression, pointing towards the role of pro-inflammatory cytokines in depression.[7]

Post chronic antidepressants therapy, levels of proinflammatory cytokines is significantly lower than pre-treatment levels. These observations suggest, that proinflammatory cytokines are a part of depression pathology and are modified by antidepressant drug treatment.[8-10]

The current treatment modalities for depression with selective serotonin receptor inhibitors (SSRI's), selective noradrenaline receptor blockers (SNRI's) and other older classes of drugs, are not proficient to provide complete cure in most of the patients and many patients remain refractory to treatment with these agents. Moreover, antidepressants produce significant adverse effects such as sedation, hypotension, anticholinergic symptoms and less frequently cardiac arrhythmias. Additionally, long latent period of 8-12 weeks is necessary to achieve therapeutic response. Unfortunately, only 30-40% of patients achieve remission with a single drug and most often the patients are switched to another agent or augmented by addition of another drug.[11] All these drawbacks drive the research in psychopharmacology to find a new drug, that is more safe and effective, and also lack the above problems. Thus, there is a great need for additional therapeutic options in depressive disorders.

According to a number of studies, depression is caused by a complex combination of behavioural, neurotropic, immunological, neuroendocrine, and other physiological components.[1, 2, 3] Excessive secretion of proinflammatory cytokines, such as interleukin-1 beta (IL-1), interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-), causes 'sickness behavior, which has symptoms that are similar to those of depression, implying that cytokines have a role in depression.[4-5] Proinflammatory cytokine levels are found to be considerably lower after persistent antidepressant therapy than before treatment.[6] Proinflammatory cytokines are a feature of depression pathogenesis and antidepressant therapy, according to these findings.

Coronary Artery Disease (CAD) is the major cause of morbidity and mortality, as well as an increased risk of depression.[7]

About 30% of depressed patients do not have an acceptable response to a first course of antidepressant medication, and the majority of them do not find relief from their symptoms with monotherapy. Most patients remain resistive to therapy with SSRIs (selective serotonin reuptake inhibitors) and SNRIs (selective noradrenaline reuptake inhibitors) in the current treatment paradigms for depression.

Antidepressants are psychopharmacological medicines having serious side effects include drowsiness, hypotension, and arrhythmias, as well as anticholinergic symptoms.[9]Furthermore, the disorder's pathogenesis is yet unknown. Monoamines have been the subject of intense investigation for decades. Alternatives to serotonin (5HT), norepinephrine (NE), and dopamine have been studied extensively in the literature. Despite substantial study on these neuro transmitter systems, the existing evidence is ambiguous, and monoamine does not appear to be adequate to describe the full scenario of depression.[10]

Current progresses in neurobiological research have shown a growing body of evidence indicating inflammatory pathways have a role in the genesis of depression.

Increased levels of many inflammatory markers, including pro inflammatory cytokines such as IL-1, IL-6, and TNF- alpha, consistently support the concept that inflammatory unique notion of employing cytokines as biomarkers of depression pathways are involved in depression. These discoveries have given rise to the, as well as the possibility of designing new antidepressants that block cytokine action[11].

Atherosclerosis is a pathogenesis implicated in angina pectoris and acute coronary syndrome (ACS). Inflammation is responsible for all stages of atherosclerosis, development, and consequences of coronary artery disease [9].

Newer antianginal medications (Ranolazine and Trimetazidine) have been proven to decrease inflammatory indicators such as CRP, IL-1, IL-6, IL-1, and TNF-alpha in studies. Inflammation has been linked to depression.

Ranolazine and Trimetazidine comparatively with antidepressants drugs has less side effects, if proved antidepressants, Ranolazine and TZ will be useful in management of depression along with Coronary artery disease.

Hence, this study is planned to evaluate the effect of Ranolazine and TZ on depression paradigm in Male Swiss Albino Mice.

OBJECTIVE OF THE STUDY:

Primary:

1) To study the effect of Ranolazine and Trimetazidine on Depression paradigm.

Secondary:

1) To compare Ranolazine and Trimetazidine with that of standard anti-depressant drug Fluoxetine.

REVIEW OF LITERATURE

Mental health is one of the most significant parts of an individual's total health. Mental diseases have plagued humanity for millennia. Mental illnesses are defined by aberrant behavior. Mental health is one of the most significant parts of an individual's total health. Mental diseases have plagued humanity for millennia. Abnormalities in cognition, emotion, mood, or the highest integrative components of behavior, such as social relations or planning future activities[20], describe mental diseases. A psychiatric condition, often known as a mental disorder, is a psychological pattern or aberration that is typically linked with suffering or incapacity and is not regarded to be part of a person's culture's normal development [13].

According to the Global Burden of Disease 2010 report, major depressive disorder (MDD) has risen from 15th to 11th place in the rankings of diseases that cause the most DALYs, representing a nearly 37 percent increase[1]. According to a survey undertaken by the World Health Organization (WHO), more than a third of people in most countries report issues that match criteria for diagnosis of one or more of the prevalent categories of mental disorders at some point in their lives. According to the same study, there are three types of mental diseases: anxiety, mood disorders, and drug addiction disorders, all of which have a role in the total estimated prevalence of mental disorders[10].

Mental and behavioral illnesses accounted for 7.4 percent of DALYs, with five distinct diseases causing more than 15 million DALYs apiece. Major depressive disorder (2.5%), anxiety disorders (1.1%)[1], drug abuse disorders (0.8%), alcohol abuse disorders (0.7%), and schizophrenia were the leading causes (0.6 percent) If present trends continue, the burden of depression is expected to rise to 5.7 % of the overall load of illness by 2020, making it the most common disease, and it will be the second greatest source of DALYs,

after only IHD.[1] Depression is the third biggest cause of worldwide illness problem, accounting for 4.3 % of total DALY in terms of public health importance. By 2030, if current situation continues, it will be the main source of illness burden[4]. Depression is a major contribution to the global burden of disease, affecting individuals from all walks of life all over the world. Around 350 million individuals are believed to be affected by depression today. The World Mental Health Survey, which was done in 17 countries, indicated that one out of every 20 persons had experienced depression in the preceding year[23]. Overall, psychiatric problems contribute significantly to morbidity, and their economic effect is enormous, with mental disorders predicted to account for roughly a third of the projected USS 47 trillion spent on non-communicable diseases[24].

Major Depressive disorder (MDD) is a severe kind of sorrow, despair, or melancholy that impairs an individual's health and social functioning. For a diagnosis of MDD, the patient must exhibit symptoms of either depressed mood or anhedonia (loss/ lack of pleasure in normally/ previously pleasurable activity) for at least two weeks, in addition to at least five other symptoms, according to the Diagnostic and Statistical Manual fifth edition (DSM V) criteria. Feelings of overwhelming sadness or emptiness, loss of interest or pleasure in daily activities, weight loss (or gain), sleep disturbances, feelings of worthlessness, loneliness, and or anxiety, psychomotor agitation or retardation, fatigue, or loss of cognition and memory functioning are all examples of these symptoms[25].

Pathophysiology of depression

Despite the fact that depression is common and has a substantial influence on health, little is known about its aetiology. This mismatch can be caused by a number of factors. To begin with, pathogenic alterations in the brain are less well understood than in other organs. Existing methods for recording brain functions rely on post-mortem research

or neuroimaging techniques, both of which trust on identifying changes in neuronal action via indirect indicators of initiation. Second, the bulk of depression cases are idiopathic. Finally, there are no animal models that can accurately describe or reproduce human depression[26].

Neural circuitry of depression[26]

The pathophysiology of depression has been linked to several brain areas. The following diagram can help to illustrate the following figure-1.

(a) The Nucleus Accumbens (NAc) is engaged in depression's reward and hedonic impairments. Stimulation of the subgenual cingulate (Cg) cortex (Cg25) 31 or the NAc32, which ameliorate depression in those with treatment-resistant depression, backs this up. This impact is assumed to be caused by depolarization blockage or stimulation of crossing axonal fibers affecting the activity of certain locations.

(b) Increased activity-dependent release of (BDNF) within the mesolimbic dopamine circuit mediates vulnerability to community stress, which is likely mediated in part by phosphorylation of the transcription factor cyclic AMP adenosine monophosphate-response-element-

(c) Neuroimaging studies clearly link the amygdala (red pixels indicate active regions) to the processing of emotional cues like 'frightful faces.'

(d) In the hippocampus (HP), stress increases the concentrations of neurotrophins-BDNF, the amount of neurogenesis, and the intricacy of neuronal activity, effects that are arbitrated in part by higher cortisol and decreased level of CREB action.

(e) Metabolic hormones such as ghrelin and leptin, which are produced peripherally in addition to cortisol, causes mood shifts through their actions on the hypothalamus (HYP) and other limbic areas.

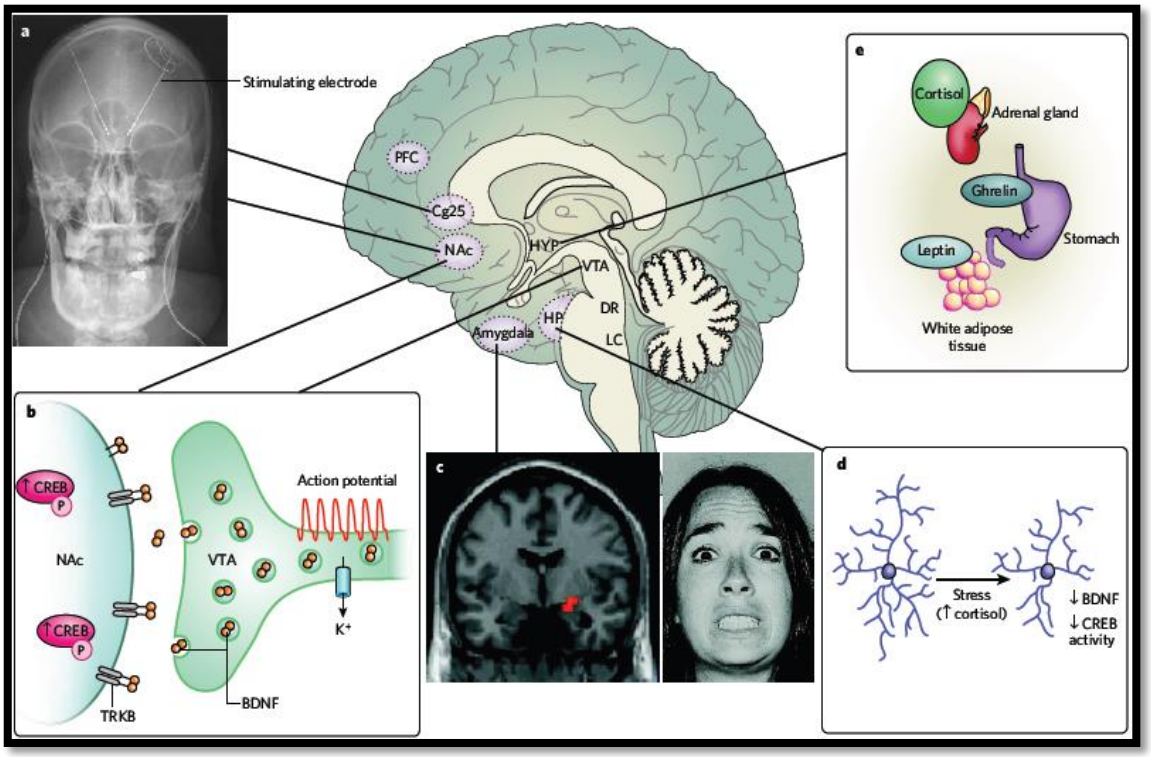


Figure 1: Neural circuitry of depression[26]

DR -dorsal raphe LC-locus coeruelus PFC-prefrontal cortex TRK-tyrosine kinaseβ

2. Role of Monoamines[16]

Following the unintentional discovery that two structurally unrelated compounds, Iproniazid and Imipramine, showed strong antidepressant effects in humans and were exposed to increase central serotonin and transmission of noradrenaline, the 'monoamine hypothesis' of depression states that depression is the result of decreased monoamine meaning inside the brain. In a minority of individuals, reserpine, an older antihypertensive drug that lowers central monoamine reserves, caused depressed symptoms. The majority of today's antidepressants work by increasing monoamine transmission.

Though monoamine hypothesis-based antidepressants are the first-line therapy for depression, their extended time to produce therapeutic effect and poor remission rates (30%) are not explained by this theory, despite an initial rise in central monoamines levels upon medication commencement. Now the thought is that antidepressant-induced acute increases in synaptic monoamine levels cause subsequent neuroplastic alterations that persistently influence molecular and cellular plasticity via transcriptional and translational mechanisms[28].

3. Neurotrophins and neurogenesis theory[27,29]

A reduction in the size of the hippocampus and other parts of forebrain regions in a subgroup of depressed individuals has been reported in several investigations. This discovery backs with a prevalent theory that low levels of neurotropic factors, which govern plasticity in the adult brain, play a role in depression. These researches have mostly focused on BDNF, which is abundantly expressed in adult limbic regions. The BDNF theory is supported by preclinical evidence showing various kinds of stress impair BDNF-mediated signaling in HP, whereas persistent antidepressant therapy increases BDNF-mediated signaling.

Recent investigations using the "BDNF hypothesis" reveal, however, that the present formulation of the Brain Derived Neurotropic Factor hypothesis is overly simple.[29,30] Although BDNF-Directed signaling is implicated in neuroplastic responses to stress and antidepressants, these are region effects and antidepressant specific, and they operate in the shadow of other powerful environmental genetic and modifiers[18].

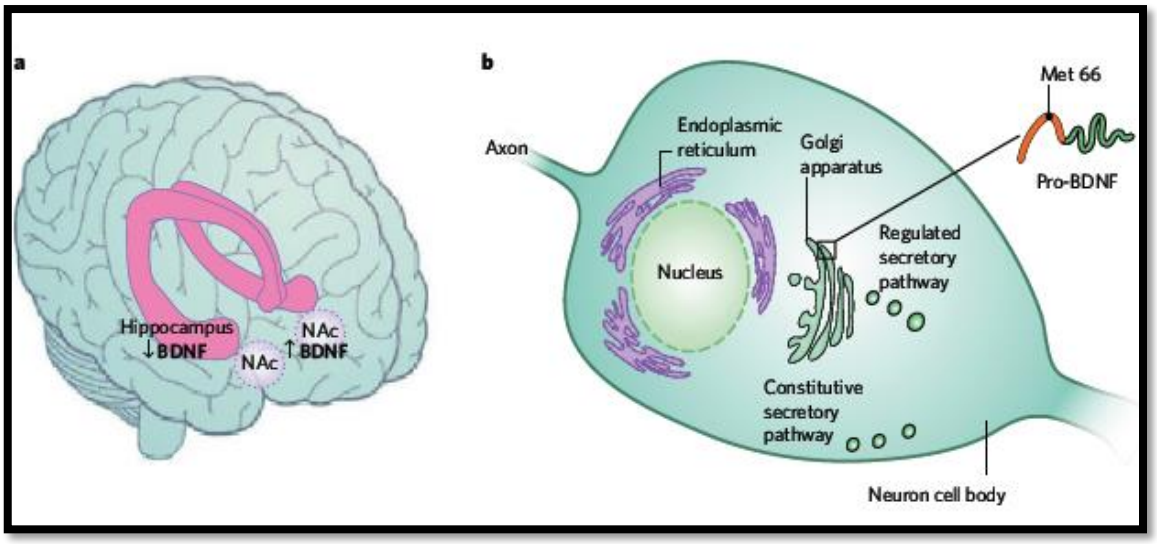


Figure 2: Depression and Neurotrophin-BDNF

a. E.g. BDNF-induced neuroplastic alterations in a particular location.

b. BDNF secretion via controlled and constitutive secretory pathways

In the adult hippocampus, several antidepressants can promote neurogenesis.[31] In rodent models, inhibiting hippocampus neurogenesis diminishes the therapeutic efficacy of most antidepressant drugs[17]. Antidepressant therapy has been shown to raise the levels of many growth factors that control hippocampus neurogenesis, potentially through CREB and transcriptional regulators. BDNF and Vascular Endothelial Growth Factor (VEGF), both of which have antidepressant and pro-neurogenic characteristics in rats, are among them. The processes by which newly generated neurons correct mood alterations, on the other hand, are unknown.

4. Neuroendocrine hypothesis [29,32]

Glucocorticoids levels in the blood have been found to rise with depression in several clinical investigations.[33,34] Atrophic alterations in the Hippocampus can be caused by high glucocorticoid levels in the body, which are mediated by the glucocorticoid receptor. Hypercortisolemia is virtually always present in severely depressed individuals evaluated

in an in-patient environment, according to recent investigations.[24] Atypical depression, on the other hand, appears to be linked to hypocortisolemia.[25]

5. Neuroimmune Theory-Cytokine hypothesis [29,32]

Cytokines, which play a role in both innate and adaptive immunity, have been linked to mood fluctuations. A 'sickness behaviour' is induced in rodents after a low dose injection of lipopolysaccharide (LPS) or Interleukin-1 (IL-1) through the release of pro-inflammatory cytokines such as Interferon-, Tumour Necrosis Factor-, IL-6, and IL-1, which in turn activate the hypothalamic pituitary adrenal (HPA) axis 42 The findings of recent preclinical research that demonstrate that inhibiting pro-inflammatory cytokine-mediated signaling can have antidepressant benefits add to this conclusion.[26]

As shown in fig 3, antidepressant therapies can be controlled by particular enzymes that are further dependent on gene transcriptional potential.

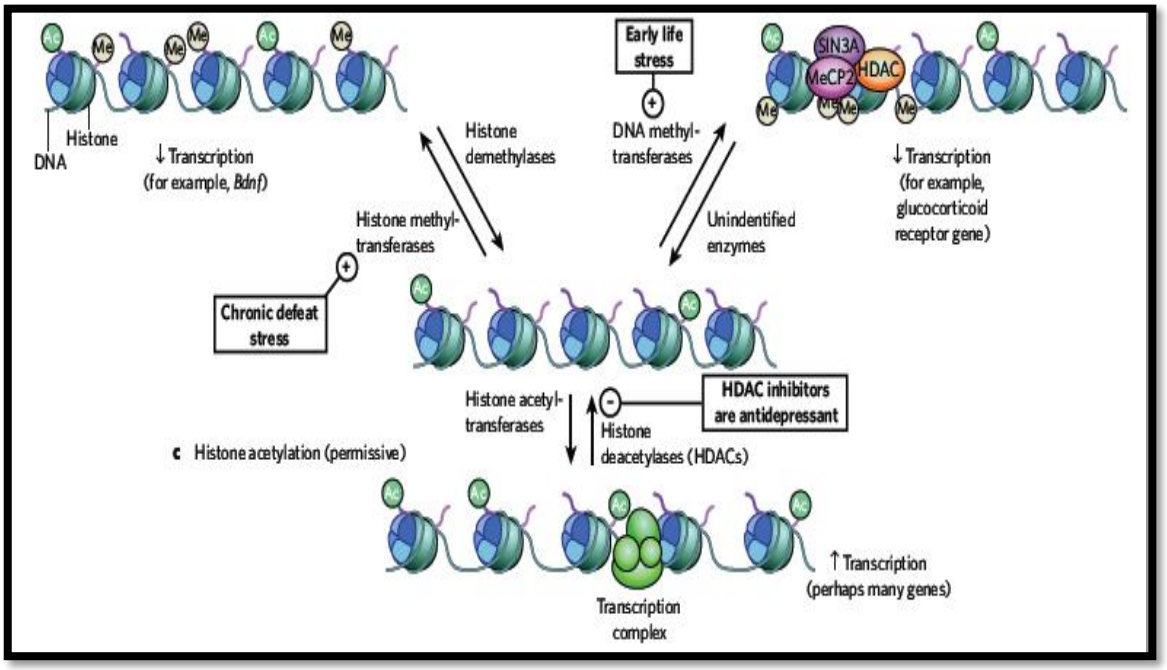


Figure 3: Epigenetic regulation in depression.

a) Methylation of histones on certain lysine residues is required for BDNF production in

the hippocampus, which can occur after social defeat. The processes of activation and inhibition are represented by the addition and subtraction, respectively.

Histone acetylation, performed by histone acetyltransferases, increases the activity of transcriptional units in euchromatin. HDAC inhibitors have been demonstrated to have antidepressant characteristics in several studies.

6. Role of Substance P, Gamma Amino Butyric Acid (GABA), Glutamate and Enkephalins[18]

Neurokinin antagonists (substance P antagonists) have previously been demonstrated to have antidepressant properties. Depression can be caused by a lack of gamma amino butyric acid (GABA) and neuroactive peptides (particularly vasopressin and endogenous opiates). Overactivity of acetylcholine, corticotrophin releasing factor, and glutamate is hypothesized to cause depression.[38]

Risk factors for development of depression[39]

1. **Genetic factors:**

Although no particular anomalies in genes affecting neurotransmitter or hormone production or release have been found, familial, twin, and adoption studies have revealed that both severe depression and bipolar illness are highly heritable. Genes and stress are thought to impact the magnitude and scope of neuronal processes, the generation of new neurons, and neuronal repair in severe depression. When confronted with a stressful environment, people having a variation in the proximal 5' regulatory protein of the gene encoding the 5-Hydyroxytryptamine transporter protein (5HT-T) have been observed to suffer from significant depression.[40]

2. **Psychosocial factors:**

• Social and familial interactions are disrupted

• Pessimism and poor self-esteem are examples of abnormal thinking.

• Gender — women are more likely than males to suffer from emotional disorders (attributed to social, occupational roles, biological and psychological changes).

• Socioeconomic status — a poor socioeconomic level appears to be linked to an increased occurrence of mood disorders.

• Behaviour and temperament.

3. **Risk factors for disease:**

Many diseases, particularly those with a long and severe course and/or outcome, are frequently linked to depression in varying degrees. [41-42]

Pharmaceuticals used to treat depression:

Table 2 includes the drugs that are currently utilized in depression pharmacotherapy.

Table 1: Drugs for treatment of MDD[43]

Modulation of Monoaminergic system.	
TRICYCLIC ANTIDEPRESSANTS	Amitriptyline (Elavil), Nortriptyline (Pamelor), Imipramine (Tofranil), Desipramine (Norpramin), Doxepin (Sinequan), Clomipramine (Anafranil)
MONOAMINE OXIDASE INHIBITORS	Phenelzine (Nardil), Tranylcypromine (Parnate), Isocarboxazid (Marplan), Transdermal selegiline (Emsa)
SELEECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIs)	Fluoxetine (Prozac), Sertraline (Zoloft), Paroxetine (Paxil), Fluvoxamine (Luvox), Citalopram (Celexa), Escitalopram (Lexapro)
MIXED NOREPINEPHRINE/SEROTONIN REUPTAKE INHIBITORS (SNRI) AND RECEPTOR BLOCKERS	Venlafaxine (Effexor), Desvenlafaxine (Pristiq), Duloxetine (Cymbalta), Mirtazapine (Remeron), Vilazodone (Viibryd), Vortioxetine (Brintellix), Levomilnacipran (Fetzima).
MIXED-ACTION DRUGS	Bupropion (Wellbutrin), Trazodone (Desyrel), Trazodone extended release (Oleptro), Amoxapine (Asendin)

New treatments are being developed.

1. **New glutamatergic drugs for the treatment of MDD**

Esketamine, a NMDA receptor antagonist and dopamine reuptake inhibitor, is an enantiomer of ketamine. It has been in phase 3 clinical studies funded by Janssen Pharmaceuticals since 2017, and volunteers are now being sought. In March 2019 drug is approved by FDA for individuals with treatment-resistant depression (TRD), the SUSTAIN-3 trial compares intranasal esketamine to placebo (NCT02782104).[44]

The FDA has authorised esketamine for the treatment of MDD with suicidal thoughts. In general, intranasal esketamine has a lot of promise for treating MDD since it is more tolerable than ketamine.[45]

Treatment of MDD with opioid receptor agonists

Buprenorphine: In a Phase 2 study, buprenorphine will be compared against venlafaxine extended release and placebo effects in older people with MDD (NCT02181231).[33]

ALKS 5461 is made up of two identical molecules (buprenorphine and samidorphan). The Focused-on Benefits with a rethinking of Depression experiment (FORWARD)-5 discovered that a 2 mg/2 mg dose provided substantial antidepressant results in a phase 3 study. [44]

Modulation of the cholinergic system

Scopolamine works by stimulating synaptogenesis in the mechanistic or mammalian target of rapamycin complex-1 (MTORC1) complex via the mechanistic or mammalian target of rapamycin (mTOR) pathway, resulting in an antidepressant effect.[48]

Antidepressants that are anti-inflammatory

MDD has been treated with PUFAs, N-acetyl cysteine (NAC), infliximab, aspirin, and COX-2 inhibitors like celecoxib.[44] PUFAs, celecoxib, and aspirin exhibited statistically significant impacts for the treatment of MDD, according to a meta-analysis of anti-inflammatory drugs in unipolar depression. [49]

2. Miscellaneous agents

Clobenpropit, a histamine (H3) antagonist, has antidepressant properties and helps to prevent memory loss.[50] Dopamine turnover is influenced by captodiamine, a 5HT2C antagonist and agonist at sigma-1 and D3 dopamine receptors.[44] Effects on hypothalamic CREB phosphorylation and subsequent BDNF transcription were seen in a preclinical research.[51] Human trials, on the other hand, have yet to be conducted. NSI-189, a new benzylpiperizine-aminiopyridine chemical compound created by Neuralstem, stimulates hippocampal neurogenesis in vitro and in vivo in mice. Clinical studies are presently being conducted (NCT02695472 and NCT02724735)[44] SAGE-21, a GABA receptor positive allosteric modulator created by SAGE Therapeutics, is now in phase 3 testing (NCT02942017 and NCT02942004).[44]

3. Antidepressant properties of psychedelics

Psilocybin, N, N-dimethyltryptamine (DMT), and D-lysergic acid diethylamide (LSD), all naturally occurring hallucinogens, have showed promise in modest trials for treating MDD and anxiety disorders.[52] More RCTs are needed to investigate the potential of these substances while balancing their medicinal and recreational misuse risk.

4. Neurostimulation

The PRIDE trial found that combining ultra-brief right unilateral electroconvulsive treatment

(ECT) with Venlafaxine improved effectiveness.[47,53,54] New discoveries in the field of neurostimulation, such as Synchronised transient magnetic stimulation (sTMS), deep transient magnetic stimulation (dTMS), and low-Frequency magnetic stimulation, were mentioned in a recent study (LFMS).[55] A multisite study to investigate The effectiveness of LFMS is now underway (NCT01654796).

Screening methods for antidepressant activity:

Table 2: Animal models for screening antidepressant activity.[56]

Sl.No	Model	Advantage	Disadvantage
1	**Catalepsy Antagonism**	The specificity of the method has been tested. The test can be considered as specific for central stimulants allowing the possibility to distinguish between antidepressants and central stimulants of the amphetamine type.	Common laboratory animals cannot be used.
2	**1.Forced swim test (FST)**	Sensitive to antidepressants Easy to perform High reproducibility	Sensitive to acute treatment Validity for MAOIs uncertain Risk of hypothermia
	2.Modified FST	Sensitive to antidepressants Easy to perform	Sensitive to acute treatment Validity for MAOIs uncertain Risk of hypothermia
	3.Tail Suspension Test (TST) in mice	Sensitive to antidepressants Easy to perform High reproducibility	Not applicable in rats Applicable only in certain mouse strains
3	**Intracranial self-stimulation**	Measures affective state and motivation Responds to chronic antidepressants	Further validation required in models of depression
4	**Learned Helplessness in**		Time consuming and its specificity questionable.

	Rats	Can be regarded as an additional measure for antidepressant activity in addition to other tests	The major drawback of the model is that most of the depression-like symptomatology does not persist beyond 2–3 days following cessation of the uncontrollable shock.
5	**Muricide Behavior in Rats**	A selective inhibition of mouse-killing behavior in rats by antidepressants. The test can be used to evaluate antidepressants such as tricyclics and MAO inhibitors.	The mouse-killing behavior is inhibited not only by antidepressants but also by central stimulants like d-amphetamine, some antihistamines and some cholinergic drugs
6	**Behavioral Changes After Neonatal Clomipramine Treatment**	Might correlate well with childhood depression. Studied by many researchers.	Specificity of the procedure to evaluate potential antidepressant compounds remains to be established.
7	**Chronic Stress Model of Depression**	Simulates in animals the symptom of anhedonia, a major feature of depression.	Time consuming and observations may vary subjectively.
8	**Novelty-Induced Hypophagia Test**	Chronic, but not acute antidepressant treatment alters behavior	Time consuming
9	**Reduction of Submissive Behavior**	Submissive behavior for one subject can be objectively measured.	Time consuming
10	**Elevated plus maze (Anxiolytics Screening)**	Easy to perform, Reliable measures of anxiolytic activity. Does not required any sophisticated instrument.	Time consuming

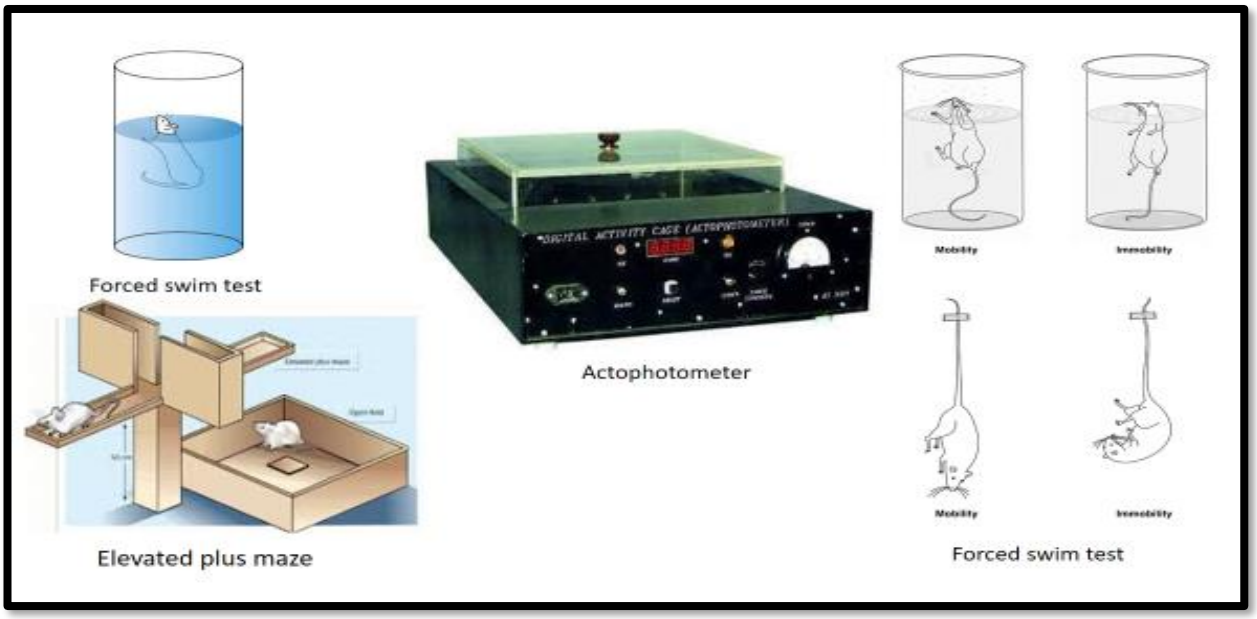


Figure 4: commonly used screening methods for antidepressant activity-I

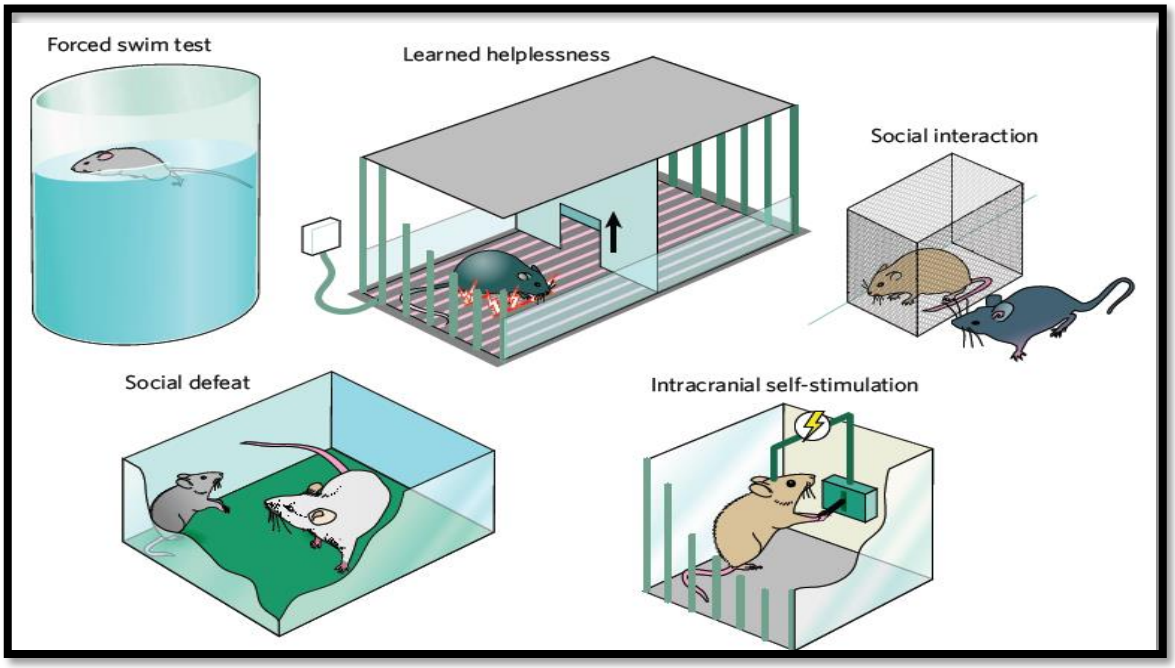


Figure 5: Commonly used screening methods for antidepressant activity -II

The current study employed the CUMS or CMS (chronic unpredictable mild stress or chronic mild stress) depression paradigm. More than 20 years ago, the CUMS model was created as an animal model of depression. The premise of this paradigm was that animals acquire anhedonia after long-term exposure to a succession of modest, but unexpected

stresses. Since its introduction, this model has been employed in a number of research looking at neurobiological characteristics linked to depression.

• Animals would experience a condition of diminished reward salience following the CUMS procedure, similar to the anhedonia seen in major depressive illness.

• The mild chronic stress paradigm:

(1) Elicits a variety of neurobiological changes that are similar to those seen in depressive disorders, and

(2) Is a useful tool for investigating novel systems that may be disrupted in depression, thereby assisting in the development of novel targets for the treatment of depression in major depressive disorder.[57]

DRUGS USED IN PRESENT STUDY:

Ranolazine and Trimetazidine are second-line antianginal agent for the treatment of chronic angina.

Ranolazine -

Ranolazine is being be used with a number of different drugs, including blockers, Ca2+ channel blockers, ACEIs, ARBs, and lipid-lowering and platelet-aggregation-reducing medicines.[9]

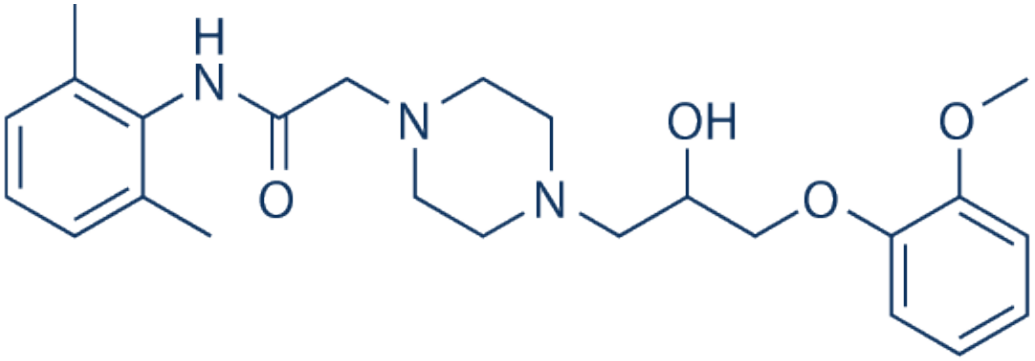


Figure 6: Ranolazine Structure

Mechanism of action: Ranolazine works by inhibiting a late sodium current that allows calcium to enter through the sodium-calcium exchanger. Ranolazine lowers diastolic tension, cardiac contractility, and workload by decreasing intracellular calcium concentration.[9]

Pleotropic effects

1. Anti-inflammatory effects

Studies conducted show that Ranolazine inhibits the inflammatory markers such as IL1β and TNF-α, and increases anti-inflammatory PPAR-γ.

1. Anti-Oxidant effects

Furthermore, antioxidant proteins Cu/ZnSOD and Mn-SOD significantly increased after Ranolazine addition in cultured astrocytes.[61]

Pharmacokinetics: The medicine has a 75 percent oral bioavailability; Pgp inhibitors can enhance ranolazine absorption and exposure to both ranolazine and the competing drug. The terminal t 1/2 of ranolazine is approximately 7 hours; with repeated doses, a steady-state Cp is established in 3 days. Ranolazine is metabolised primarily by CYP3A4 and to a lesser amount by CYP2D6; both the unmodified drug and its metabolites are eliminated

in the urine (5 percent). Strong CYP3A4 inhibitors (e.g., macrolide and imidazole antibiotics, HIV protease inhibitors) should not be used with Ranolazine, and dosages should be reduced when moderate CYP3A4 inhibitors (e.g., Verapamil, Diltiazem, and Erythromycin) are taken simultaneously. CYP3A4 inducers (e.g., Rifampin, Carbamazepine, and Hypericum) can lower Ranolazine plasma levels, necessitating dosage adjustments.[64]

Adverse effects and drug interactions: Additional CYP3A4 substrates, such as simvastatin and its active metabolite, can be affected by Ranolazine, necessitating dosage adjustments; dose reduction may be required for other CYP3A4 substrates (e.g., lovastatin), especially for those having a limited therapeutic range (e.g., cyclosporine, tacrolimus, sirolimus). Ranolazine may enhance exposure to other CYP2D6 substrates, such as tricyclic antidepressants and antipsychotics, when used together.[64]

Dizziness, headache, nausea, and constipation are the most common side effects. Some CNS effects resemble those of class I antiarrhythmic (e.g., dizziness, fuzzy vision, and disorientation). Although QT prolongations must be noted, there have been no reports of torsade des pointes arrhythmias or associated occurrences.[64]

Therapeutic use: Ranolazine is a drug that is used to treat chronic angina. It comes in extended-release tablets and is taken twice a day without regard for meals at 500 to 1000 mg twice daily;

Trimetazidine-

Trimetazidine is used to treat stable angina in individuals who are unable or unwilling to respond to first-line antianginal therapy. It improves exercise tolerance and decreases angina in adults with diabetes and heart failure.[9]

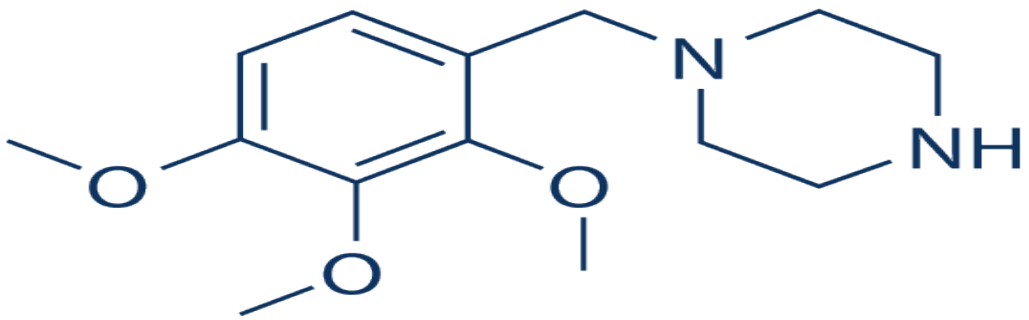


Figure 7: Trimetazidine Structure

Mechanism of action: Trimetazidine is supposed to work by preventing the long-chain 3-ketoacyl coenzyme from forming. The last enzyme in the FFA-oxidation pathway is a thiolase. This causes a partial switch in the heart from FFA to glucose oxidation, which produces less ATP but uses less oxygen, which may be advantageous in ischemia.[64]

Pleotropic effects

1. Anti-inflammatory effects

Trimetazidine, a metabolic modulator and antianginal medication, has been shown in trials to reduce inflammatory indicators such as CRP, nitric oxide products, IL-1, IL-6 and TNF alpha.[62] Study conducted on carrageenan-induced pain and inflammation rats show that Trimetazidine decrease inflammation.

Trimetazidine has been shown to be beneficial in lowering interleukin 6 (IL-6) in previous trials.[63] as a result, with increased cytokines (proinflammatory indicators) involved in the pathogenesis of depression and Trimetazidine's demonstrated anti-inflammatory and antioxidant actions,

Adverse effects and drug interactions: Trimetazidine has been linked to GI distress, nausea, and vomiting, as well as thrombocytopenia, agranulocytosis, and liver failure in rare cases. Trimetazidine, furthermore, may raise the risk of movement disorders like Parkinson's disease, especially in elderly individuals with impaired renal function.[64]

Therapeutic use: Second line Antianginal drugs, recommended dose is 20 mg thrice daily or 35 mg MR (Modified Release) twice a day.

Relation between Ranolazine and Trimetazidine with inflammation:

Ranolazine reduces inflammatory indicators such as IL1 and TNF- and elevates anti-inflammatory PPAR-, according to Aldasoro M et al. In addition, after Ranolazine treatment, the antioxidant proteins Cu/ZnSOD and Mn-SOD significantly increased in cultured astrocytes.[61] Trimetazidine is a second-line treatment for stable angina in individuals who have failed to respond to or are intolerant to first-line antianginal therapy. It improves exercise tolerance and decreases angina in adults with diabetes and heart failure9.

Interestingly Kuralay F et.al in his studies have reported that metabolic modulator trimetazidine, which is an antianginal drug, inhibits the inflammatory markers like CRP, IL-1, IL-6 and TNF alpha.[62]

Study conducted on carrageenan-induced pain and inflammation rats show that Trimetazidine decrease inflammation.

Martins GF et.al In his studies proved that Trimetazidine to be effective for reducing interleukin 6 (IL-6).[63] So, in the view of raised cytokines (proinflammatory markers) levels being implicated in the pathophysiology of depression and documented anti-inflammatory and antioxidant effects of Ranolazine and Trimetazidine, can be beneficial in the management of depression.

Relationship between Depression and inflammation:

Major depression is associated to inflammation and greater levels of proinflammatory cytokines, diminished neurogenesis and resultant neuroprogression (pathological reorganisation of the central nervous system), mitochondrial dysfunction, and HPA axis dysfunction.

Reduced antioxidant levels and increased oxidative stress have also been discovered. Increased amounts of proinflammatory cytokines TNF-alpha, IFN, and IL may induce oxidative damage. Increased levels of proinflammatory cytokines such TNF-alpha, IL-1a, IL-1B, IL-4, IL-5, IL-6, IL-12, IFN-y, and C-reactive protein have been demonstrated to enhance inflammatory response and moderate chronic inflammation in depressed people. When proinflammatory cytokines or their signal pathways are suppressed, antidepressants are more effective, and patients' depression levels are lowered. In recent decades, considerable evidence has accumulated associating depression with increased immune system activation.[47-48] Ole Köhler et al in his study mentioned that anti-inflammatory treatment for depression has positive results, and later meta-analyses have backed up the positive effects of anti-inflammatory treatment on depression, which might be a proof-of-concept.[49-50] These are impressive findings, especially in light of the continual focus on the need for new and improved treatment choices for depression sufferers. On the other hand, the inflammatory cascade and its possible links to depression are exceedingly intricate. Ole Köhler proposed three pillars that link inflammation and depression in his studies.

1) Depression is increased by inflammation and somatic illnesses involving inflammatory processes.[51]

2) Depressed people have higher levels of pro-inflammatory markers than healthy people,.[52]

3) Antidepressants can be used to alleviate depression symptoms caused by pro-inflammatory substances.[53]

Several unsolved questions should be answered through further study. Antidepressants' effects on several aspects of oxidative and nitrosative damage in people with mental disorders are unknown. Is oxidative stress more detrimental in the early stages of illness or later stages? Can various oxidative and nitrosative damage indicators be used to predict mental disorders? Can these indicators tell the difference between clinically distinct mental diseases. Another issue is the uncertainty of data validation derived from blood and urine samples when compared to levels in the brain or diseased brain areas. Major depression has pathophysiological similarities with not just mental illnesses, but also a variety of other diseases, such as cardiovascular ailments. Understanding the link between oxidative stress and depression is unquestionably a step toward a better understanding of the pathophysiology of depression. All of these contradictory findings from numerous research add to our grasp of the complexities of depression pathogenesis.

Histopathological Changes in Depression.

In depression research, the hippocampus is the most widely investigated brain area. Furthermore, the hippocampus has a high concentration of glucocorticoid receptors and glutamate, both of which regulate the HPA axis, making it more vulnerable to stress and depression. Stress and other negative stimuli can cause changes in hippocampus plasticity. Stress can activate the hypothalamic-pituitary-adrenal axis, raising corticosteroid levels and inhibiting hippocampus neurogenesis.[54]

Depression and Hippocampal Apoptosis

Depression and stress have been shown to cause hippocampus apoptosis in rodents, non-human animals, and humans in several studies, while hippocampal apoptosis has also been shown in non-depressed mice. Chronic depression has longer-lasting apoptosis-promoting effects in the hippocampus than acute depression in animal models and human studies. The apoptosis-inducing properties acute depression can be entirely recovered in one day; however, the negative consequences of chronic depression might take up to three weeks to heal. It's unclear when sadness and stress begin to drive apoptotic progression at lower levels.[55]

Furthermore, fluoxetine, a 5-hydroxytryptamine reuptake inhibitor, controls hippocampal plasticity by reducing depression-induced upregulation of synaptosomal polysialic neural cell adhesion protein and eliciting an ant apoptotic response in the hippocampus.[56]

As a result of the above reports, there is no studies regarding the effect of Ranolazine and Trimetazidine on depression in animal models. Therefore, in view of scarcity of information the study was conducted to evaluate the effects of Ranolazine and Trimetazidine in CUMS (Chronic Unpredictable Mild Stress) model of depression in Male Swiss Albino mice.

METHODOLOGY

The complete course of the experiment was conducted by using healthy 40 Adult Male Swiss Albino mice. Animals were obtained from central animal house. The mice were housed under standard laboratory conditions and acclimatized to 12-h light/dark cycle for 10 days prior to the day of experimentation. They were maintained at constant room temperature (22°-25°) and on free access to food (standard chow pellet) and water *ad libitum*. The animals were housed ingroups in polypropylene cages with 8 animals per cage.

The study was approved by IAEC as per the guidelines of CPCSEA. For CUMS 40 mice were divided into 5 groups. Normal Control group received 0.2ml of Normal saline, while test groups received Ranolazine and Trimetazidine. Standard group received a dose of Fluoxetine.

DRUGS AND DOSES

The clinically equivalent human doses of drugs were converted into rat equivalent doses by the conversion table by Paget and Barnes.[65]

1. **Tab. Ranolazine** (Tablet RANCAD 500mg LUPIN Limited) the following mice doses were administered:

Drug dose: 130 mg/kg (per oral)/0.2ml (3.9 mg/30 gm. of mice).

2. **Tab. Trimetazidine** (Tablet Trivedon20 mg Cipla Limited) the following mice doses were administered:

Drug dose: 5 mg/kg (per oral)/0.2ml (0.15 mg/30 gm. of mice).

All the drugs were administered after dissolving in dissolved 2% gum acacia suspension. Drugs were administered orally at 24 hours.

Table 3: Study drugs and dosages

Groups (n=8)	Dose for mice (mg/kg) (per oral)
1.	Normal Control
2.	CUMS (Disease Control)
3.	Fluoxetine (Standard) – 2.7 mg/kg (0.3 mg/30 gm. of mice)
4.	Ranolazine 130 mg/kg (3.9 mg/30 gm. of mice)
5.	Trimetazidine 5 mg/kg (0.15 mg/30 gm. of mice)

Table 4: STUDY PROTOCOL

CUMS	Day 1 – Day 14
Behavioral tests (screening for the establishment of depression)	Day 15,16 ,17 and18
CUMS (established depression animals) + Treatment	Day -19 to Day 39
Behavioral tests	Day 40,41,42 and 43
Sacrificing of animals	Day 44 euthanasia, collection of blood, dissection and isolation of brain tissues for histopathology andinflammatory markers TNF-α, IL-1β, From brain Homogenate and blood serum.
Results and statistical analysis	

Table 5: DAILY STRESS PROTOCOL (Duration 6 weeks)

Day-1	Monday	Tilt cages at 30 degrees (4h)
Day-2	Tuesday	Placing mice in an empty cage with water at the bottom (4h)
Day 3	Wednesday	Tilt cages at 30 degrees (4h)
Day-4	Thursday	Placing mice in cages with wet sawdust (4h)
Day 5	Friday	Placing mice in soiled cages of other mice (4h)
Day 6	Saturday	Space reduction (4h)
Day-7	Sunday	Reversal of light dark cycle

To prevent habituation and ensure the unpredictability of the stressors, all the stressors were randomly scheduled over a 1-week period and repeated throughout the 6 -week experiment.

ANTIDEPRESSANT ACTIVITY STUDY

The four models of depression that have been employed in the current study namely

Forced swim test, Tail suspension test, Elevated plus maze and Locomotor activity test in mice.

FST: [66,67,68,69,70] **It** comprises of a vertical Plexiglas cylinder measuring 21cms in height with 12cms diameter, containing 10 cms of water column maintained at 25° C.

Principle: When mice are forced to swim in a situation from which there is no escape, will, after an initial period of vigorous activity, eventually cease to move altogether making only those movements necessary to keep its head above water. This characteristic and readily identifiable behaviour of immobility indicates a state of despair in which the mouse has

learned that escape is impossible and resigns itself to the experimental conditions and such behaviour is said to be equivalent to clinical depression. Obviously, drugs that decrease immobility would be antidepressants.

Procedure: Mice weighing 20-30g were plunged vertically into the cylinder and left for 15mins. The duration of immobility is scored. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water making only those movements necessary to keep its head above water. 1he airmails were subjected to test after 24hrs.After administration of a single oral dose of the test drug/vehicle, mice were placed in the cylinder and left for 6 mins to note the duration of immobility (in seconds). The animals were then allowed to dry before returning them to their individual cages.

Tail suspension test:[66,68,71] It consist of two metallic rods 35cms apart connected with a horizontal rod to suspend a nylon thread from its centre. A hook was attached to the free end of the thread to enable suspension of the animal by its tail. The threaded length was adjusted to provide a distance of 35cms from the ground to hook. (Photograph 3)

Principle: A normal animal submitted to an aversive situation alternates between two kinds of behaviours: agitation and immobility. These can be named as Searching behaviour (characterized by intense motor activity and expense of energy) and Waiting behavior (immobility and energy saving). lt is named as searching-waiting strategy. Antidepressant drugs modify the balance between these forms of behavior in favour of searching.

Procedure: The mouse pre-treated with drug/vehicle was suspended from the hook

hanging at the centre or a horizontal rod by an adhesive tape stuck 2 cm proximal the tail tip. The mouse is said to be immobile when it stops moving and hangs motionless.

Elevated Plus Maze:[66,67,73,74] The elevated plus maze apparatus consists of two open arms (I6cm x >cm) and two closed arms (16cmx 5cm x 12cm) and an open root with the entire maze elevated 25cms from the floor.

Principle: Exposure of mice to such novel stimuli can evoke both exploratory drive and fear. Elevation of the maze causes greater fear. Open arms are more fear provoking and animals tend to spend time in the closed arm. Antidepressants would be expected to increase the entries and time spent in the open arms. In addition, the rearing behaviour is also increased by antidepressants. (Photograph 3)

Procedure: The pre-treated animals were placed individually for 5 mins at the centre of the elevated plus maze with the head facing towards an open arm. The number of entries into open or closed arm and the time spent in each arm were recorded.

Locomotor activity:[66,67] Effect of all the drugs used in the present study on locomotor activity was tested by using the an Actophotometer (M/S INCO). The apparatus consists of a metal box measuring 68 cm in length, 8 Cm in breadth and 45 cm in height. Here, a count is recorded It is equipped with photocells sensitive to infrared light when the beam of light falling on the photocell is cut off by the movement of a mouse.

Principle: This test provides Simultaneous measures of locomotion, exploratory behaviour and anxiety. This test helps to differentiate between sedative and stimulant drugs. It also

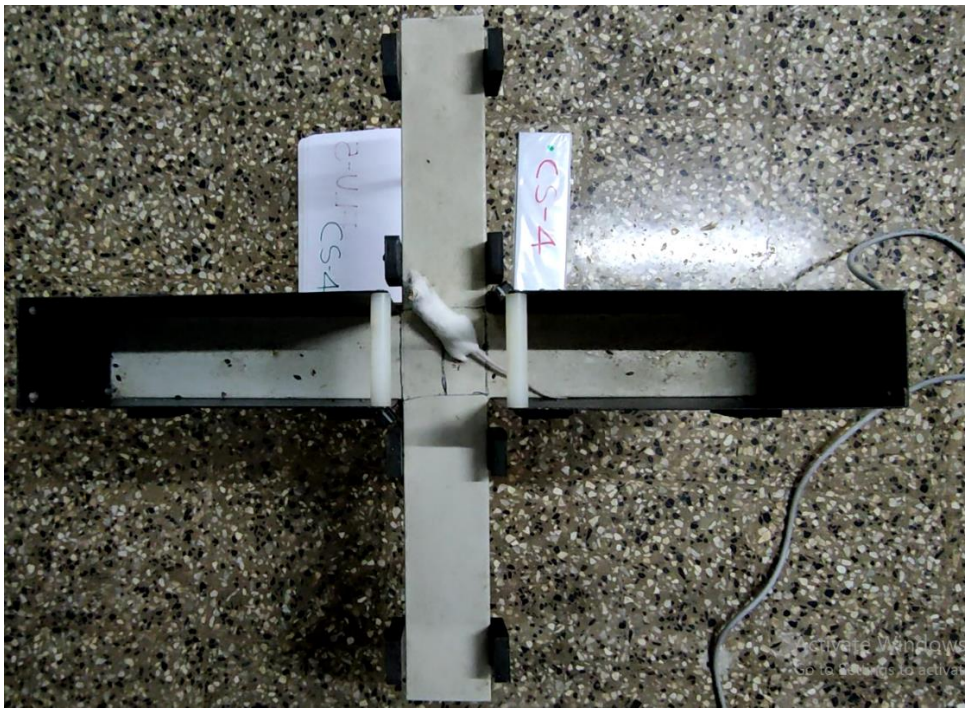
helps to rule out any influence of the drugs on locomotor system which in turn may affect immobility (in antidepressant tests).

Procedure: Pre-treated mice were placed in the centre of the apparatus for a period of 5 minutes. The device electronically counts the number of times the infrared beams are interrupted by movement of the animal. This Actophotometer count is a measure of the locomotor activity.

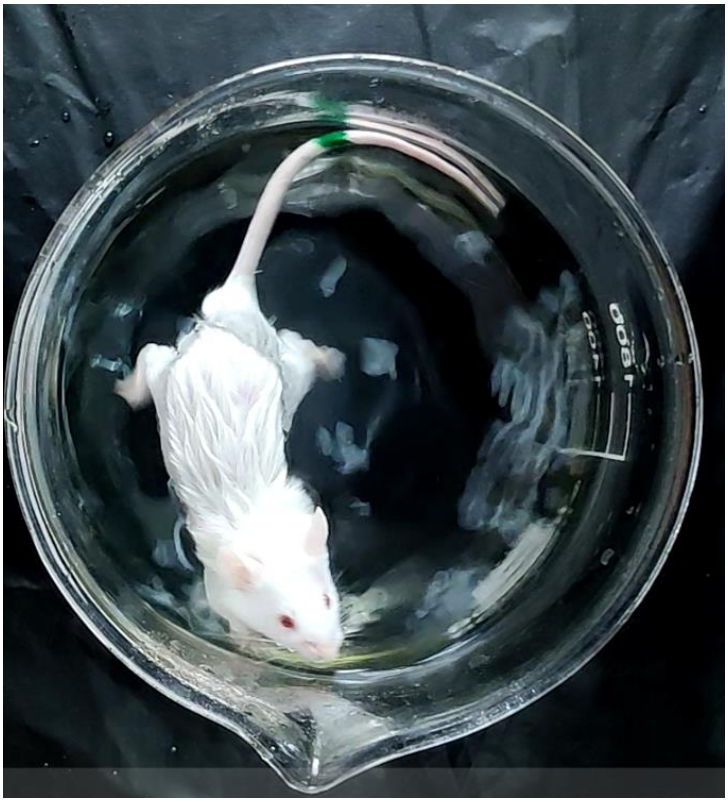
Statistical analysis:

The results presented here are the means +/- SD of 6 mice in each group. Tukey's multiple comparisons test and Kruskal- Wallis test was used Assess the discrete values. $P \leq 0.05$ was considered statistically significant. All data as analysed using statistical software Graph Pad Prism (version 9.0).

Photographs



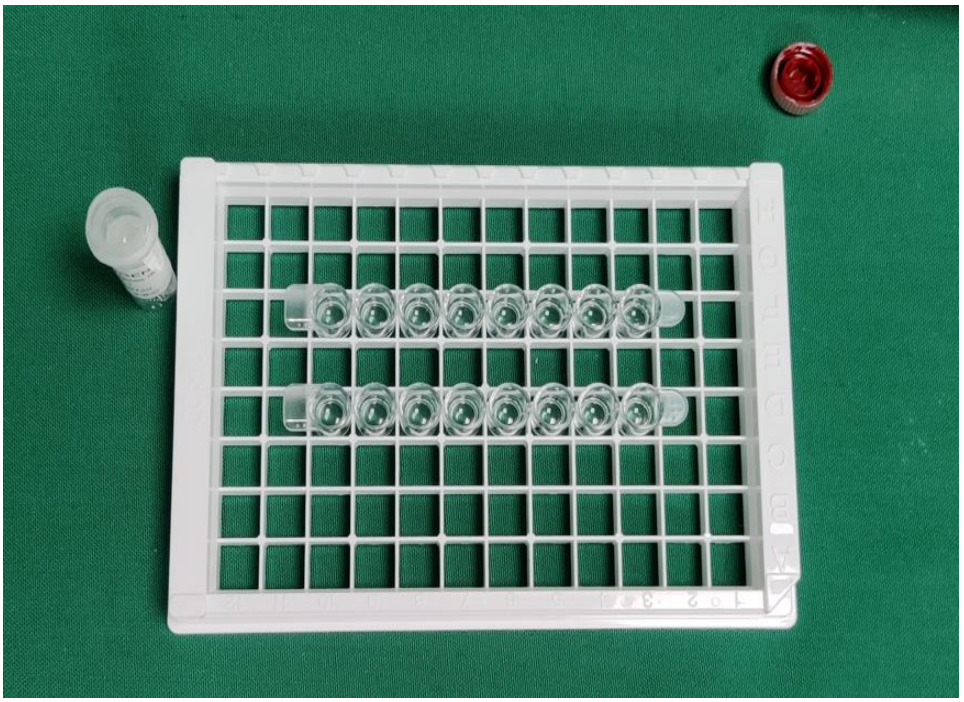
1. Elevated Plus Maze



2. Forced Swim Test

3. Tail Suspension Test

4. Photoactometer



5. ELISA kits Mouse TNF alpha and IL-1 beta

ELISA kits Mouse TNF alpha and IL-1 beta

RESULTS

The present study, investigated for the possible antidepressant activity of Ranolazine and Trimetazidine as test drugs, using a depression paradigm in Adult male Swiss Albino mice. Antidepressant activity was studied using Forced Swim Test, Tail suspension Test, Elevated plus Maze and Locomotor Test at the end of 2 weeks and following 6 weeks of Chronic Unpredictable Mild Stress.

Antidepressant activity which was studied at the end of 2 weeks is to know about the establishment of depression by (CUMS) Significant result indicative of established CUMS.

Forced swim test: The duration of immobility time in second was noted over a period of 6 minutes.

The mean duration of immobility at the end of 2 weeks in the Normal control group was 34.50± 07.91while it was 93.33± 19.66, 85.00±33.91, 94.17±14.97 and 91.67±40.21 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively.

The mean duration of immobility at the end of 6 weeks in the Normal control group was 68.67 ± 48.05 while it was96.33 ± 10.13, 45.00±14.14, 68.33±23.80and 73.33±32.66 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively. (Mention table and graph)

There was statistically significant difference ($p < 0.05$) in the duration of immobility When Normal control compared with Fluoxetine and ($p < 0.01$) with Chronic Unpredictable Mild Stress, Ranolazine and Trimetazidine groups at the end of 2 weeks and 6 weeks respectively.

Tail suspension test: The duration of immobility time in second was noted over a period of 6 minutes.

The mean duration of immobility at the end of 2 weeks in the Normal control group was 45.00± 10.00while it was 137.7± 60.69, 130.5±36.34, 103.8±23.41and 131.2±27.50 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively.

The mean duration of immobility at the end of 6 weeks in the Normal control group was 75.00± 37.28 while it was 149.5± 52.00, 76.50±27.08, 117.5±19.94and 112.5±39.97 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively. (Mention table and graph)

There was statistically significant difference ($p < 0.01$) in the duration of immobility with Trimetazidine, Fluoxetine and CUMS group when compared with Normal control at the end of 2 weeks.

There was statistically significant difference ($p < 0.05$) in the duration of immobility with Fluoxetine treated groups and Normal control at the end of 6 weeks when compared with the CUMS groups.

Elevated Plus Maze: In the Elevated plus maze number of entries and time spent in open are were observed over a period of 5 minutes.

Time spent in open arm:

The mean percentage of time spent in the open arm at the end of 2 week (% time spent in open arms of total 300 seconds) in the Normal control group was 143.5±30.06 while it was 62.67±25.55, 62.50±13.87, 67.00±13.96 and 57.83±27.66 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU),Ranolazine (RZ) and Trimetazidine (TZ) groups respectively.

The mean percentage of time spent in the open arm at the end of 6 week in the Normal control group was 103.3±30.77while it was 45.00±30.17, 185.0±31.46, 184.5±48.06 and 123.3±56.45 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively.

There was statistically significant difference ($p < 0.0001$) in the percentage of time spent with Normal control and CUMS, Fluoxetine, Ranolazine and Trimetazidine groups at the end of 2 weeks.

There was statistically significant difference ($p < 0.05$) in the percentage of time spent with Fluoxetine treated groups and Normal control and Trimetazidine and CUMS groups and difference ($p < 0.0001$) in the Fluoxetine treated groups and CUMS and Ranolazine treated groups and CUMS at the end of 6 weeks.

Number of entries in open arm:

The effect of various treatment on number of entries into open arm were calculated for each animal and mean was calculated. The mean of number of entry into the open arms at the end of 2 weeks in the Normal control group was 15.17± 4.35, 4.33±1.86, 6.50±1.37, 5.50±2.25, and 4.50±1.87 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively.

The effect of various treatment on number of entries into open arm were calculated for each animal and mean was calculated. The mean of number of entry into the open arms at the end of 6 weeks in the Normal control group was 9.66± 3.61, 1.66±0.51, 10.00±3.95, 10.33±6.40, and 10.00±2.60 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively.

There was statistically significant difference ($p < 0.0001$) in the number of entries into open arm when Normal control compared with CUMS, Fluoxetine, Ranolazine, and Trimetazidine groups at the end of 2 weeks.

There was statistically significant difference ($p < 0.05$) in the number of entries into open arm with Normal control and CUMS and difference ($p < 0.001$) Fluoxetine, Ranolazine and Trimetazidine groups when compared with CUMS groups at the end of 6 weeks.

Locomotor activity:

The mean of difference of locomotor activity at the end of 2 weeks in the Normal Control was 689.0±65.60 while it was 449.8±110.0, 519.8±92.28, 491.7±29.90 and 494.8±84.37 in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively.

The mean of difference of locomotor activity at the end of 6 weeks in the Normal Control was 519.0±302.9while it was 396.8±180.5, 562.52±54.20, 443.2±99.22and 684.5±274.5in the Chronic Unpredictable Mild Stress (CUMS), Fluoxetine (FLU), Ranolazine (RZ) and Trimetazidine (TZ) groups respectively.

There was statistically significant difference ($p < 0.05$) in the Locomotion when Normal control compared with Fluoxetine and ($p < 0.01$) Ranolazine, and Trimetazidine groups and ($p < 0.001$) with CUMS groups at the end of 2 weeks. At the end of 6 weeks p Value non-significant and there was homogeneity among six groups.

Serum and Brain homogenate TNF-α

Serum TNF-α

There was statistically significant difference ($p < 0.001$) in the serum TNF-α when CUMS compared with Normal control, Fluoxetine, Ranolazine and Trimetazidine groups at the end of the study.

Brain homogenate TNF-α

There was statistically significant difference ($p < 0.0001$) in the Brain homogenate TNF-α when CUMS compared with Normal control, Fluoxetine, Ranolazine and Trimetazidine groups at the end of the study.

Serum and Brain homogenate IL-1β

Serum IL-1β

There was statistically significant difference ($p < 0.05$) in the serum TNF-α when CUMS compared with Normal control and ($p < 0.01$) with Fluoxetine and ($p < 0.001$) with Trimetazidine groups at the end of the study

Brain homogenate IL-1β

There was statistically significant difference ($p < 0.01$) in the serum TNF-α when CUMS compared with Ranolazine and Trimetazidine groups and ($p < 0.001$) with Fluoxetine group at the end of the study.

TABLE 6: FORCED SWIM TEST (Effect of various treatments on duration of immobility in seconds)

a) Mean of immobility time (seconds)

	Immobility time					ANOVA Result		
Groups	NC	CUMS	FLU	RZ	TZ	$F_{4,25}$	p-value	significant
After 2 weeks								
Mean	34.50	93.33	85.00	94.17	91.67	5.689	0.0021	**
SD	7.918	19.66	33.91	14.97	40.21			
After 6 weeks								
Mean	68.67	96.33	39.17	45.00	73.33	4.259	0.0092	**
SD	48.05	10.13	8.612	14.14	32.66			

Abbreviations: NC- Normal Control; CS Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

b) Tukey's multiple comparisons test (after 2 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	-58.83	-103.3 to -14.36	0.0055	**
NC vs. FLU	-50.50	-94.98 to -6.023	0.0205	*
NC vs. RZ	-59.67	-104.1 to -15.19	0.0048	**
NC vs. TZ	-57.17	-101.6 to -12.69	0.0072	**
CUMS vs. FLU	8.333	-36.14 to 52.81	0.9809	ns
CUMS vs. RZ	-0.8333	-45.31 to 43.64	>0.9999	ns
CUMS vs. TZ	1.667	-42.81 to 46.14	>0.9999	ns
FLU vs. RZ	-9.167	-53.64 to 35.31	0.9729	ns
FLU vs. TZ	-6.667	-51.14 to 37.81	0.9917	ns
RZ vs. TZ	2.500	-41.98 to 46.98	0.9998	ns

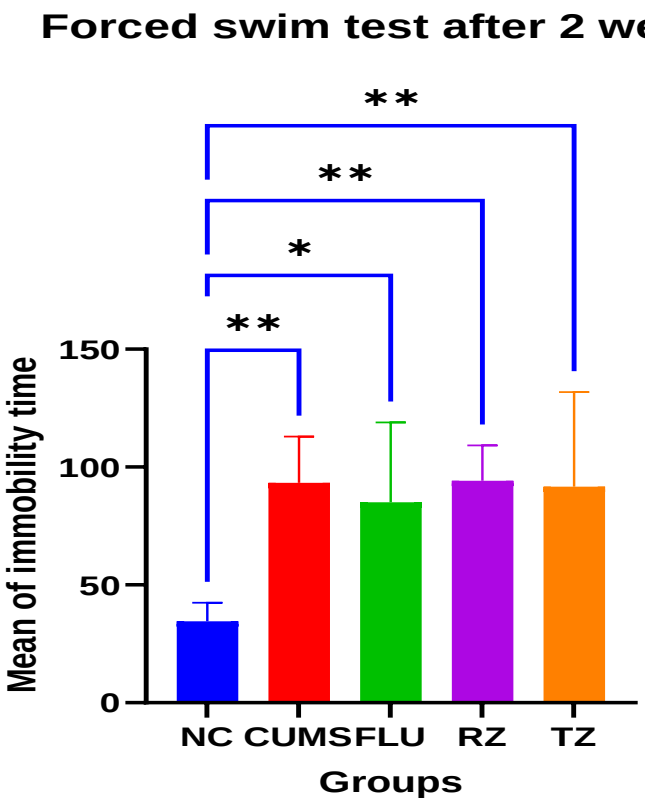
p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

c) Tukey's multiple comparisons test (after 6 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	-27.67	-74.12 to 18.78	0.4238	ns
NC vs. FLU	29.50	-16.95 to 75.95	0.3610	ns
NC vs. RZ	23.67	-22.78 to 70.12	0.5742	ns
NC vs. TZ	-4.667	-51.12 to 41.78	0.9982	ns
CUMS vs. FLU	57.17	10.72 to 103.6	0.0106	*
CUMS vs. RZ	51.33	4.884 to 97.78	0.0251	*
CUMS vs. TZ	23.00	-23.45 to 69.45	0.6001	ns
FLU vs. RZ	-5.833	-52.28 to 40.62	0.9958	ns
FLU vs. TZ	-34.17	-80.62 to 12.28	0.2273	ns
RZ vs. TZ	-28.33	-74.78 to 18.12	0.4004	ns

p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

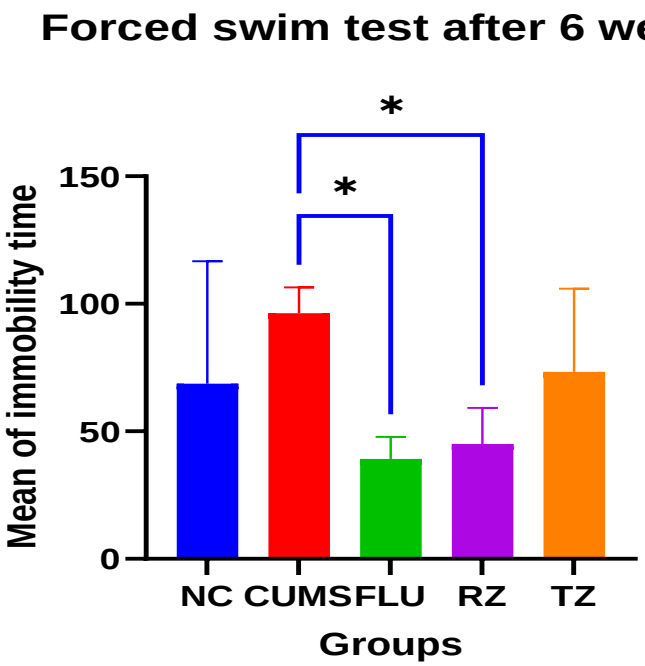
Graph -1 Forced Swim Test, Effect of Various Treatment on duration of immobility (seconds)



p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001;

Abbreviations: NC- Normal Control; CUMS - Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Graph -2 Forced Swim Test, Effect of Various Treatment on duration of immobility (seconds)



p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001;

Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Table 7: TAIL SUSPENSION TEST (Effect of various treatments on duration of immobility in seconds)

a) Mean of immobility time (seconds)

	Immobility time					ANOVA Result		
Groups	NC	CUMS	FLU	RZ	TZ	$F_{4,25}$	p-value	significant
After 2 weeks								
Mean	45.00	137.7	130.5	103.8	131.2	6.900	0.0007	***
SD	10.00	60.69	36.34	23.41	27.50			
After 6 weeks								
Mean	75.00	149.5	76.50	117.5	112.5	4.285	0.0089	**
SD	37.28	52.00	27.08	19.94	39.97			

p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001;****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant.

b) Tukey's multiple comparisons test (after 2 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	-92.67	-153.4 to -31.96	0.0012	**
NC vs. FLU	-85.50	-146.2 to -24.80	0.0029	**
NC vs. RZ	-58.83	-119.5 to 1.871	0.0607	ns
NC vs. TZ	-86.17	-146.9 to -25.46	0.0027	**
CUMS vs. FLU	7.167	-53.54 to 67.87	0.9967	ns
CUMS vs. RZ	33.83	-26.87 to 94.54	0.4890	ns
CUMS vs. TZ	6.500	-54.20 to 67.20	0.9977	ns
FLU vs. RZ	26.67	-34.04 to 87.37	0.6995	ns
FLU vs. TZ	-0.6667	-61.37 to 60.04	>0.9999	ns
RZ vs. TZ	-27.33	-88.04 to 33.37	0.6803	ns

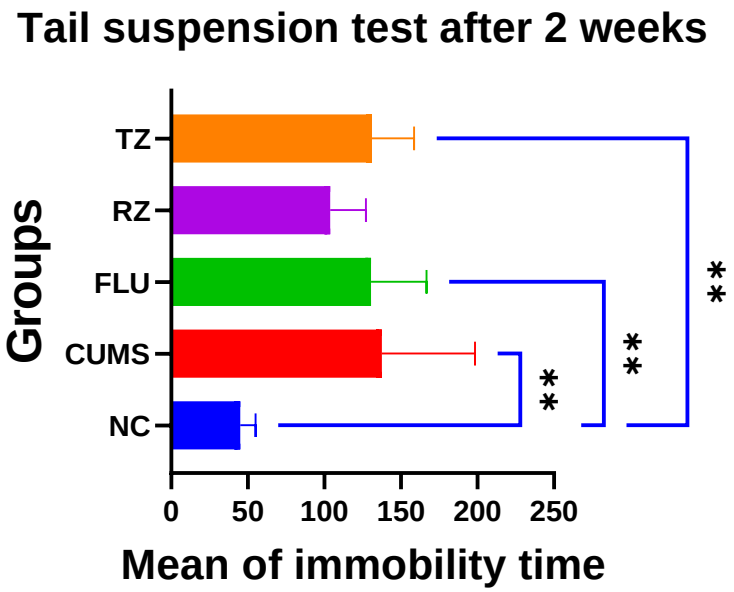
p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

c) Tukey's multiple comparisons test (after 6 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	-74.50	-137.1 to -11.87	0.0141	*
NC vs. FLU	-1.500	-64.13 to 61.13	>0.9999	ns
NC vs. RZ	-42.50	-105.1 to 20.13	0.2983	ns
NC vs. TZ	-37.50	-100.1 to 25.13	0.4187	ns
CUMS vs. FLU	73.00	10.37 to 135.6	0.0166	*
CUMS vs. RZ	32.00	-30.63 to 94.63	0.5716	ns
CUMS vs. TZ	37.00	-25.63 to 99.63	0.4319	ns
FLU vs. RZ	-41.00	-103.6 to 21.63	0.3320	ns
FLU vs. TZ	-36.00	-98.63 to 26.63	0.4589	ns
RZ vs. TZ	5.000	-57.63 to 67.63	0.9993	ns

p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

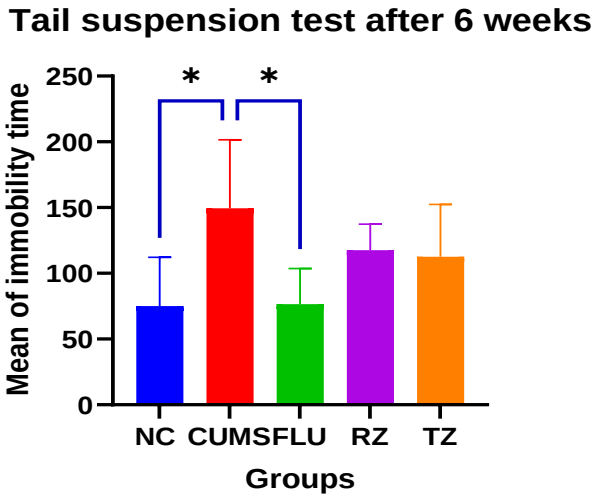
Graph: 3 TAIL SUSPENSION TEST (Effect of various treatments on duration of immobility in seconds)



p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;

Abbreviations: NC- Normal Control ;CUMS- Chronic Unpredictable Mild Stress;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Graph: 4 TAIL SUSPENSION TEST (Effect of various treatments on duration of immobility in seconds)



p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;

Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Table 8: ELEVATED PLUS MAZE (Effect of various treatments on the time spent in open arms)

a) Mean of time spent in open arms of the total 300 seconds

	Time spent in seconds					ANOVA Result		
Groups	NC	CUMS	FLU	RZ	TZ	$F_{4,25}$	p-value	significant
After 2 weeks								
Mean	143.5	62.67	62.50	67.00	57.83	14.65	<0.0001	****
SD	30.06	25.55	13.87	13.96	27.66			
After 6 weeks								
Mean	103.3	45.00	185.0	184.5	123.3	12.55	<0.0001	****
SD	30.77	30.17	31.46	48.06	56.45			

p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001;
Abbreviations: NC- Normal Control; CUMS Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

b) Tukey's multiple comparisons test (after 2 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	80.83	41.37 to 120.3	<0.0001	****
NC vs. FLU	81.00	41.54 to 120.5	<0.0001	****
NC vs. RZ	76.50	37.04 to 116.0	<0.0001	****
NC vs. TZ	85.67	46.20 to 125.1	<0.0001	****
CUMS vs. FLU	0.1667	-39.30 to 39.63	>0.9999	ns
CUMS vs. RZ	-4.333	-43.80 to 35.13	0.9975	ns
CUMS vs. TZ	4.833	-34.63 to 44.30	0.9962	ns
FLU vs. RZ	-4.500	-43.96 to 34.96	0.9971	ns
FLU vs. TZ	4.667	-34.80 to 44.13	0.9967	ns
RZ vs. TZ	9.167	-30.30 to 48.63	0.9585	ns

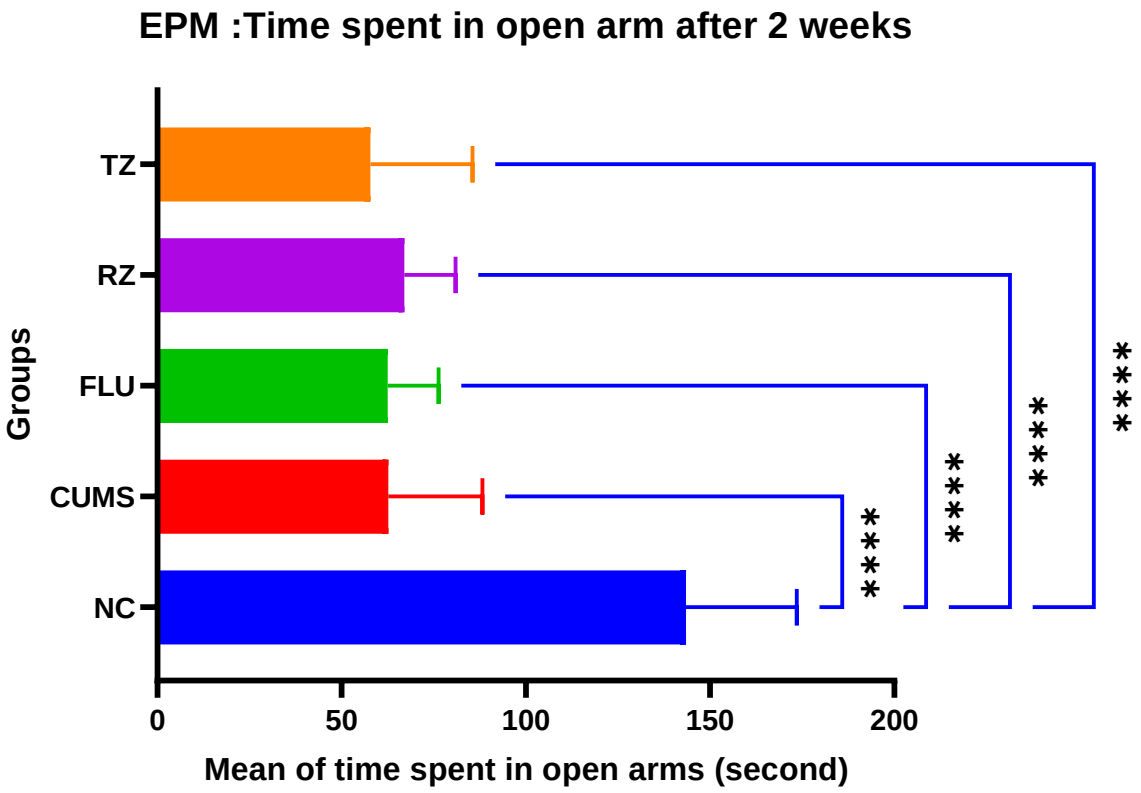
p< 0.05: statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;
Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

c) Tukey's multiple comparisons test (after 6 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	58.33	-10.93 to 127.6	0.1291	ns
NC vs. FLU	-81.67	-150.9 to -12.40	0.0152	*
NC vs. RZ	-81.17	-150.4 to -11.90	0.0159	*
NC vs. TZ	-20.00	-89.26 to 49.26	0.9128	ns
CUMS vs. FLU	-140.0	-209.3 to -70.74	<0.0001	****
CUMS vs. RZ	-139.5	-208.8 to -70.24	<0.0001	****
CUMS vs. TZ	-78.33	-147.6 to -9.071	0.0211	*
FLU vs. RZ	0.5000	-68.76 to 69.76	>0.9999	ns
FLU vs. TZ	61.67	-7.596 to 130.9	0.0979	ns
RZ vs. TZ	61.17	-8.096 to 130.4	0.1021	ns

p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

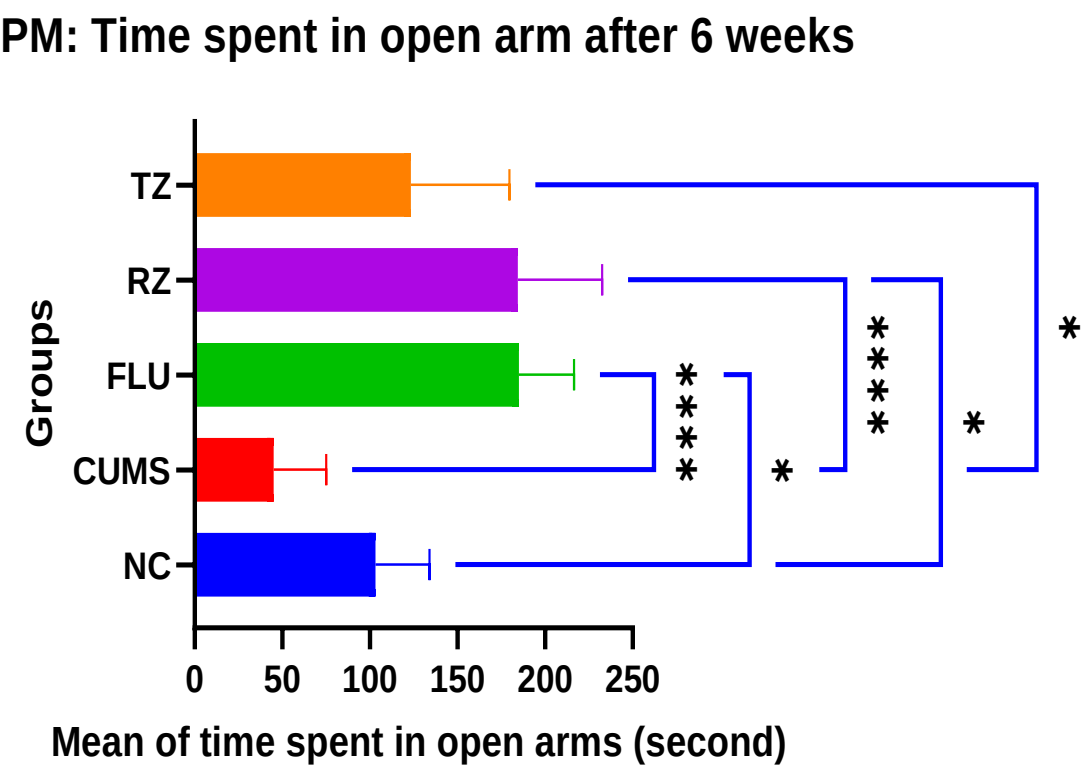
Graph: 5; ELEVATED PLUS MAZE (Effect of various treatments on the time spent in open arms)



p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001;

Abbreviations: NC- Normal Control; CUMS Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Graph: 6; ELEVATED PLUS MAZE (Effect of various treatments on the time spent in open arms)



p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001;

Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Table 9: ELEVATED PLUS MAZE (Effect of various treatments on entries into the open arm)

a) **Mean of no. entries in both open arm**

	Entries into open arm					ANOVA Result		
Groups	NC	CUMS	FLU	RZ	TZ	$F_{4,25}$	p-value	significant
After 2 weeks								
Mean	15.17	4.333	6.500	5.500	4.500	18.76	<0.0001	****
SD	4.355	1.862	1.378	2.258	1.871			
After 6 weeks								
Mean	9.667	1.667	10.00	10.33	10.00	5.447	0.0027	**
SD	3.615	0.5164	3.950	6.408	2.608			

p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

b) Tukey's multiple comparisons test (after 2 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	10.83	6.482 to 15.19	<0.0001	****
NC vs. FLU	8.667	4.315 to 13.02	<0.0001	****
NC vs. RZ	9.667	5.315 to 14.02	<0.0001	****
NC vs. TZ	10.67	6.315 to 15.02	<0.0001	****
CUMS vs. FLU	-2.167	-6.518 to 2.185	0.5951	ns
CUMS vs. RZ	-1.167	-5.518 to 3.185	0.9319	ns
CUMS vs. TZ	-0.1667	-4.518 to 4.185	>0.9999	ns
FLU vs. RZ	1.000	-3.352 to 5.352	0.9601	ns
FLU vs. TZ	2.000	-2.352 to 6.352	0.6638	ns
RZ vs. TZ	1.000	-3.352 to 5.352	0.9601	ns

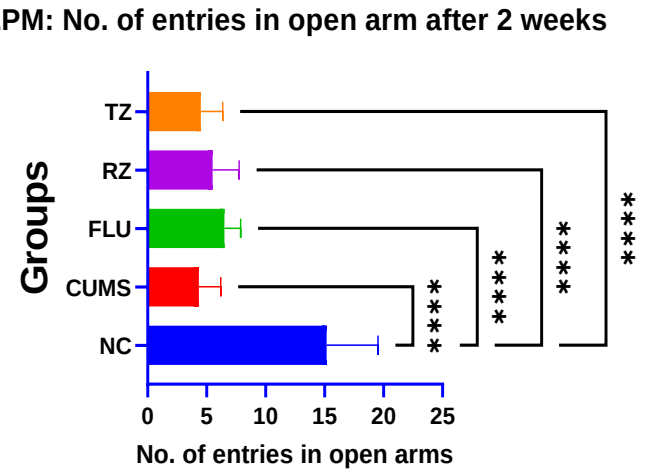
p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

c) Tukey's multiple comparisons test (after 6 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	8.000	1.355 to 14.65	0.0128	*
NC vs. FLU	-0.3333	-6.979 to 6.312	0.9999	ns
NC vs. RZ	-0.6667	-7.312 to 5.979	0.9982	ns
NC vs. TZ	-0.3333	-6.979 to 6.312	0.9999	ns
CUMS vs. FLU	-8.333	-14.98 to -1.688	0.0090	**
CUMS vs. RZ	-8.667	-15.31 to -2.021	0.0063	**
CUMS vs. TZ	-8.333	-14.98 to -1.688	0.0090	**
FLU vs. RZ	-0.3333	-6.979 to 6.312	0.9999	ns
FLU vs. TZ	0.000	-6.645 to 6.645	>0.9999	ns
RZ vs. TZ	0.3333	-6.312 to 6.979	0.9999	ns

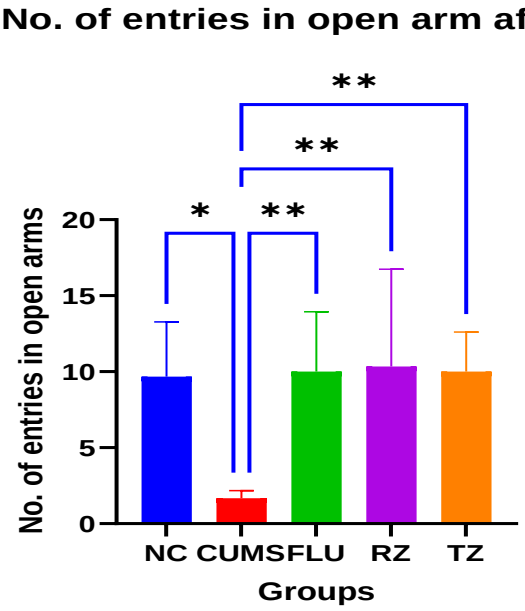
p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

Graph: 7; ELEVATED PLUS MAZE (Effect of various treatments on no. of entries in open arms)



p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Graph: 8; ELEVATED PLUS MAZE (Effect of various treatments on no. of entries in open arms)



p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001;

Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Table 10: Effect of various treatments on Locomotors activity

a) Mean of various treatments

	Locomotors activity					ANOVA Result		
Groups	NC	CUMS	FLU	RZ	TZ	$F_{4,25}$	p-value	significant
After 2 weeks								
Mean	689.0	449.8	519.8	491.7	494.8	7.861	0.0003	***
SD	65.60	110.0	92.28	29.90	84.37			
After 6 weeks								
Mean	519.0	396.8	562.5	443.2	684.5	1.763	0.1679	ns
SD	302.9	180.5	54.20	99.22	274.5			

p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

b) Tukey's multiple comparisons test (after 2 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	239.2	101.6 to 376.8	0.0003	***
NC vs. FLU	169.2	31.56 to 306.8	0.0107	*
NC vs. RZ	197.3	59.73 to 334.9	0.0024	**
NC vs. TZ	194.2	56.56 to 331.8	0.0029	**
CUMS vs. FLU	-70.00	-207.6 to 67.60	0.5756	ns
CUMS vs. RZ	-41.83	-179.4 to 95.77	0.8969	ns
CUMS vs. TZ	-45.00	-182.6 to 92.60	0.8700	ns
FLU vs. RZ	28.17	-109.4 to 165.8	0.9736	ns
FLU vs. TZ	25.00	-112.6 to 162.6	0.9830	ns
RZ vs. TZ	-3.167	-140.8 to 134.4	>0.9999	ns

p< 0.05: statistically significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Abbreviations: NC- Normal Control; CUMS- Chronic Unpredictable Mild Stress; FLU- Fluoxetine; RZ- Ranolazine; TZ- Trimetazidine; CI- Confidence Interval; ns - not significant.

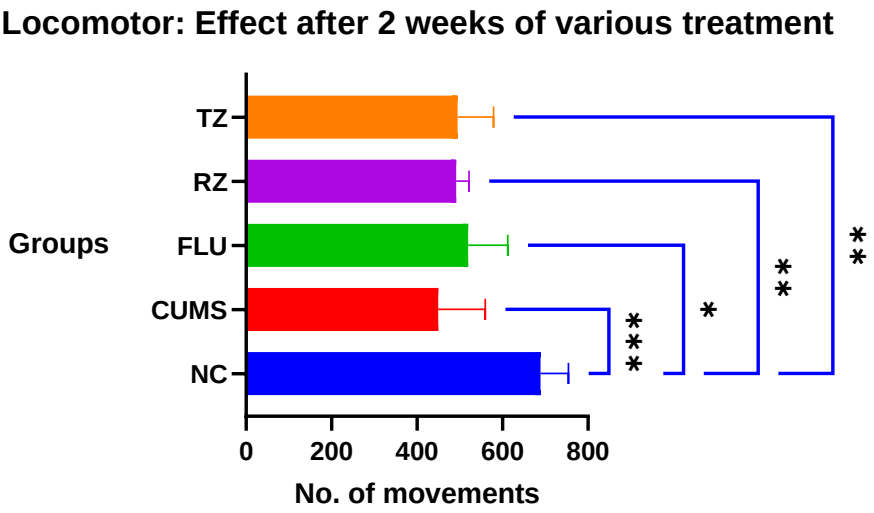
c) Tukey's multiple comparisons test (after 6 weeks)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	122.2	-227.3 to 471.6	0.8407	ns
NC vs. FLU	-43.50	-393.0 to 306.0	0.9959	ns
NC vs. RZ	75.83	-273.6 to 425.3	0.9674	ns
NC vs. TZ	-165.5	-515.0 to 184.0	0.6389	ns
CUMS vs. FLU	-165.7	-515.1 to 183.8	0.6381	ns
CUMS vs. RZ	-46.33	-395.8 to 303.1	0.9948	ns
CUMS vs. TZ	-287.7	-637.1 to 61.81	0.1436	ns
FLU vs. RZ	119.3	-230.1 to 468.8	0.8516	ns
FLU vs. TZ	-122.0	-471.5 to 227.5	0.8413	ns
RZ vs. TZ	-241.3	-590.8 to 108.1	0.2823	ns

p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;

Abbreviations: NC- Normal Control ; CUMS- Chronic Unpredictable Mild Stress;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant.

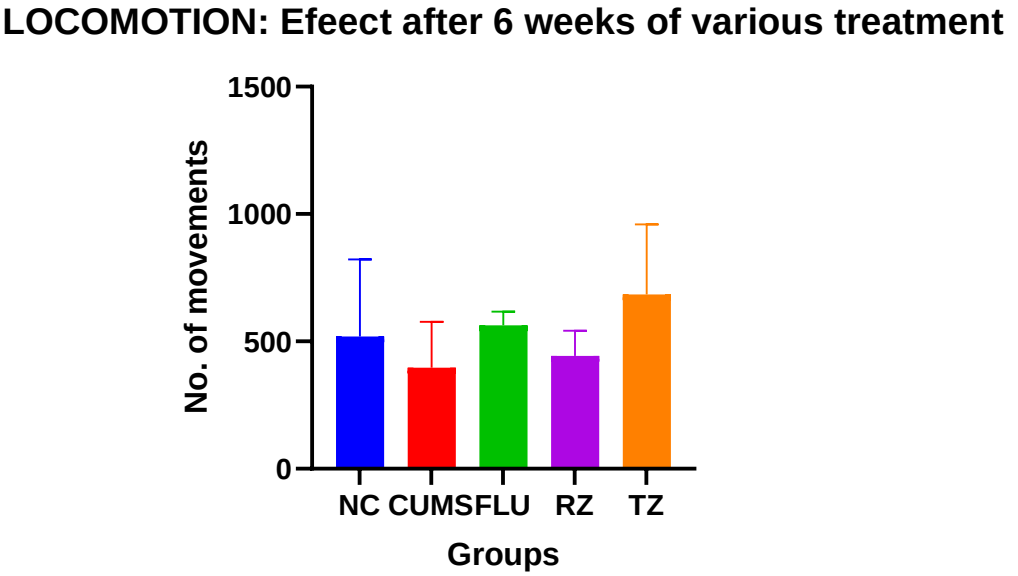
Graph: 9; Effect of various treatments on Locomotors activity



p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;

Abbreviations: NC- Normal Control ;CUMS- Chronic Unpredictable Mild Stress;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Graph: 10; Effect of various treatments on Locomotor activity



p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;
Abbreviations: NC- Normal Control ;CUMS- Chronic Unpredictable Mild Stress;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Table 11: Effect of various treatments on Serum and Brain homogenate TNF-α

a) Mean of various treatments

	Serum and Brain homogenate TNF-α					ANOVA Result		
Groups	NC	CUMS	FLU	RZ	TZ	$F_{4,25}$	p-value	significant
Serum								
Mean	25.37	59.47	21.70	24.08	19.72	9.996	<0.0001	****
SD	3.310	26.39	4.370	6.885	7.129			
Brain homogenate								
Mean	449.6	5710	375.6	385.7	355.6	129.2	<0.0001	****
SD	120.6	1136	63.24	49.29	54.64			

p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001; Abbreviations: NC- Normal Control ; CUMS Chronic Unpredictable Mild Stress; FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant.

b**) Tukey's multiple comparisons test (Serum TNF-α)**

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	-34.10	-55.88 to -12.32	0.0009	***
NC vs. FLU	3.667	-18.11 to 25.45	0.9872	ns
NC vs. RZ	1.283	-20.50 to 23.06	0.9998	ns
NC vs. TZ	5.650	-16.13 to 27.43	0.9391	ns
CUMS vs. FLU	37.77	15.99 to 59.55	0.0003	***
CUMS vs. RZ	35.38	13.60 to 57.16	0.0006	***
CUMS vs. TZ	39.75	17.97 to 61.53	0.0001	***
FLU vs. RZ	-2.383	-24.16 to 19.40	0.9975	ns
FLU vs. TZ	1.983	-19.80 to 23.76	0.9988	ns
RZ vs. TZ	4.367	-17.41 to 26.15	0.9755	ns

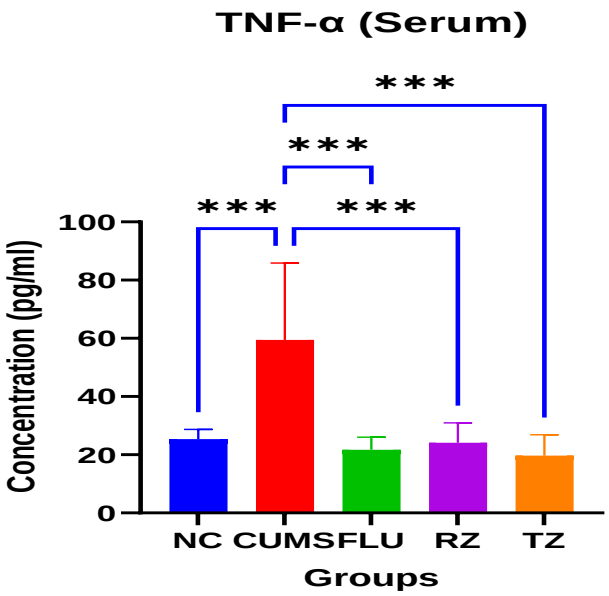
p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001; Abbreviations: NC- Normal Control ; CUMS- Chronic Unpredictable Mild Stress ;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant.

c) Tukey's multiple comparisons test (Brain Homogenate TNF-α)

Group Comparison	Mean diff.	95.00% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	-5261	-6130 to -4391	<0.0001	****
NC vs. FLU	73.93	-795.3 to 943.2	0.9991	ns
NC vs. RZ	63.83	-805.4 to 933.1	0.9995	ns
NC vs. TZ	94.00	-775.3 to 963.3	0.9976	ns
CUMS vs. FLU	5335	4465 to 6204	<0.0001	ns
CUMS vs. RZ	5325	4455 to 6194	<0.0001	****
CUMS vs. TZ	5355	4485 to 6224	<0.0001	****
FLU vs. RZ	-10.10	-879.4 to 859.2	>0.9999	****
FLU vs. TZ	20.07	-849.2 to 889.3	>0.9999	ns
RZ vs. TZ	30.17	-839.1 to 899.4	>0.9999	ns

p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;
Abbreviations: NC- Normal Control ; CUMS- Chronic Unpredictable Mild Stress ;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant.

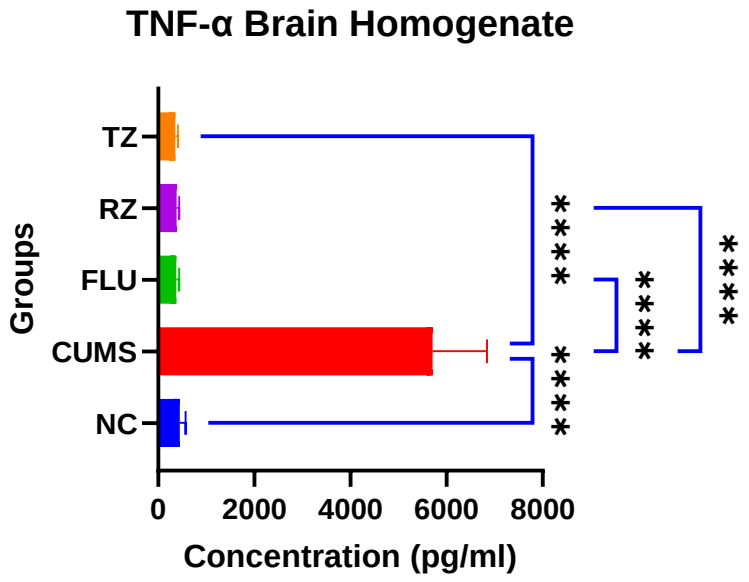
Graph 11: Effect of various treatments on Serum and Brain homogenate TNF-α



p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;

Abbreviations: NC- Normal Control ;CUMS- Chronic Unpredictable Mild Stress;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Graph 12: Effect of various treatments on Serum and Brain homogenate TNF-α



p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;

Abbreviations: NC- Normal Control ;CUMS- Chronic Unpredictable Mild Stress;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Table 12: Effect of various treatments on Serum and Brain homogenate IL-1β

a) Mean of various treatments

	Serum and Brain homogenate IL-1β					ANOVA Result		
Groups	NC	CUMS	FLU	RZ	TZ	$F_{4,25}$	p-value	significant
Serum								
Mean	0.3850	0.5750	0.3417	0.5917	0.2600	11.89	<0.0001	****
SD	0.07064	0.02168	0.08085	0.1216	0.1655			
Brain homogenate IL-1β								
Mean	0.6083	0.8933	0.3767	0.4517	0.5183	7.736	0.0003	***
SD	0.2854	0.06121	0.1782	0.1248	0.1505			

p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001; Abbreviations: NC- Normal Control ; CUMS- Chronic Unpredictable Mild Stress;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant.

b) **Tukey's multiple comparisons test (Serum IL-1β)**

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	-0.1900	-0.3665 to -0.01351	0.0304	*
NC vs. FLU	0.04333	-0.1332 to 0.2198	0.9496	ns
NC vs. RZ	-0.2067	-0.3832 to -0.03017	0.0160	*
NC vs. TZ	0.1250	-0.05149 to 0.3015	0.2597	ns
CUMS vs. FLU	0.2333	0.05684 to 0.4098	0.0055	**
CUMS vs. RZ	-0.01667	-0.1932 to 0.1598	0.9986	ns
CUMS vs. TZ	0.3150	0.1385 to 0.4915	0.0002	***
FLU vs. RZ	-0.2500	-0.4265 to -0.07351	0.0028	**
FLU vs. TZ	0.08167	-0.09483 to 0.2582	0.6583	ns
RZ vs. TZ	0.3317	0.1552 to 0.5082	<0.0001	****

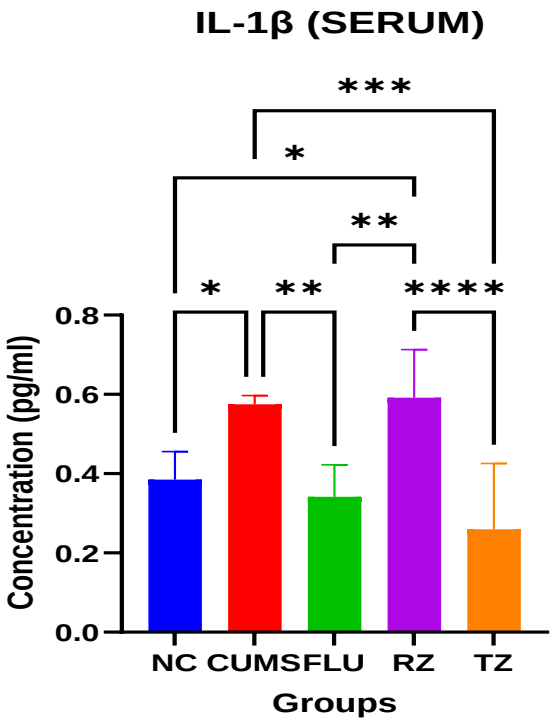
p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001; Abbreviations: NC- Normal Control ; CUMS- Chronic Unpredictable Mild Stress ;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant.

c) Tukey's multiple comparisons test (Brain homogenate IL-1β)

Group Comparison	Mean diff.	95% CI of diff.	Adjusted p value	Summary
NC vs. CUMS	-0.2850	-0.5837 to 0.01372	0.0667	ns
NC vs. FLU	0.2317	-0.06705 to 0.5304	0.1854	ns
NC vs. RZ	0.1567	-0.1421 to 0.4554	0.5473	ns
NC vs. TZ	0.09000	-0.2087 to 0.3887	0.8998	ns
CUMS vs. FLU	0.5167	0.2179 to 0.8154	0.0003	***
CUMS vs. RZ	0.4417	0.1429 to 0.7404	0.0018	**
CUMS vs. TZ	0.3750	0.07628 to 0.6737	0.0089	**
FLU vs. RZ	-0.07500	-0.3737 to 0.2237	0.9456	ns
FLU vs. TZ	-0.1417	-0.4404 to 0.1571	0.6377	ns
RZ vs. TZ	-0.06667	-0.3654 to 0.2321	0.9640	ns

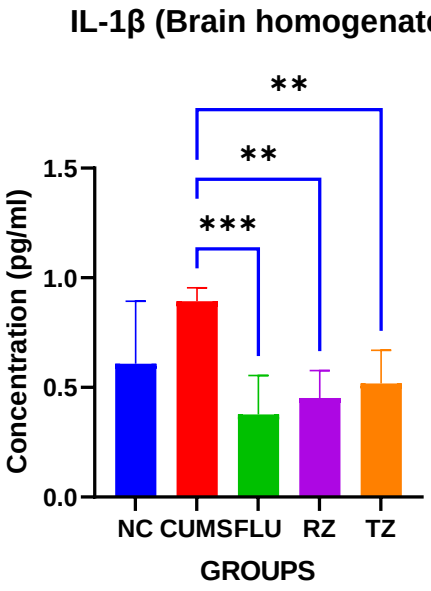
p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001;
Abbreviations: NC- Normal Control ; CUMS- Chronic Unpredictable Mild Stress ;FLU- Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant.

Graph 13: Effect of various treatments on Serum and Brain homogenate IL-1β



p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001; Abbreviations: NC- Normal Control ;CUMS- Chronic Unpredictable Mild Stress; FLU-Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Graph 14: Effect of various treatments on Serum and Brain homogenate IL-1β

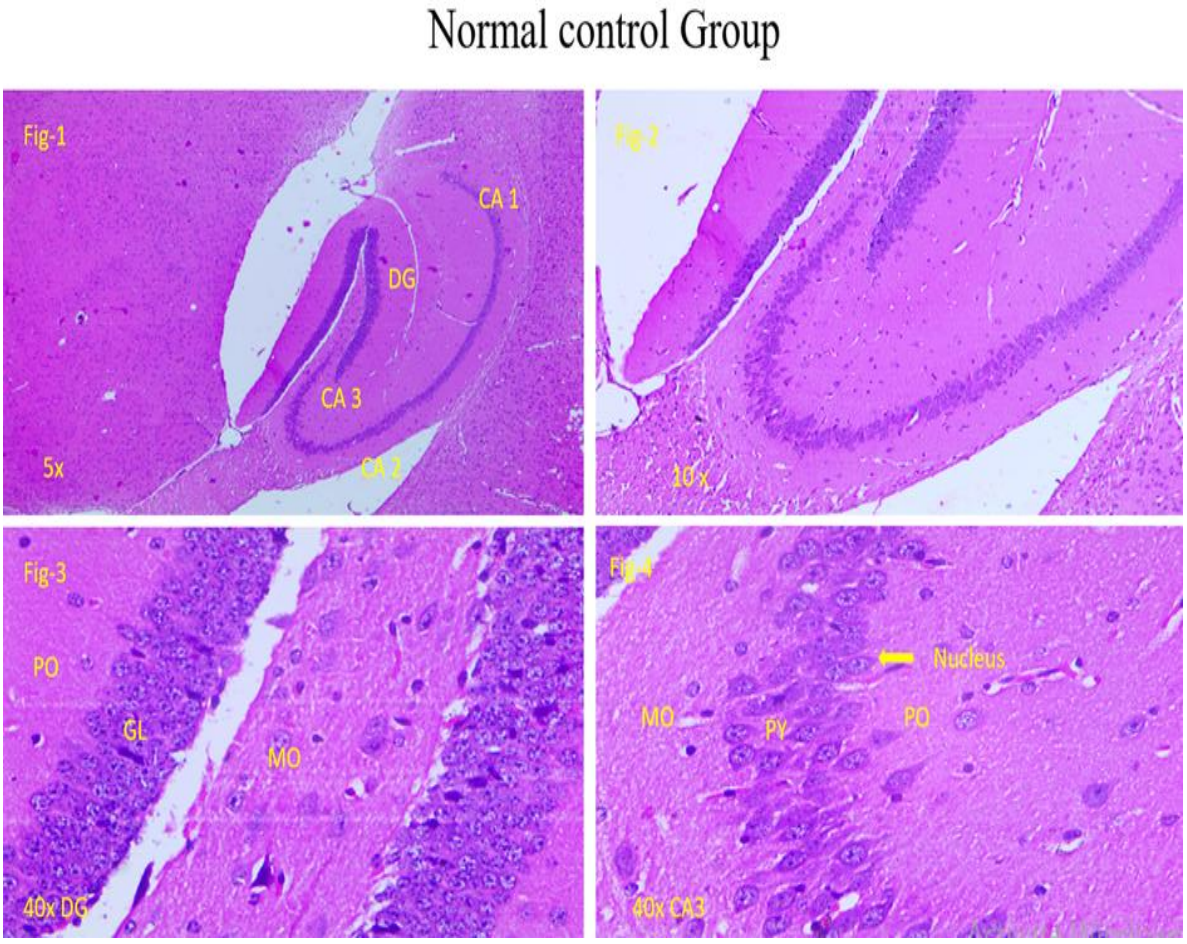


p< 0.05 : statistically significant; *p < 0.05;**p < 0.01;***p < 0.001;****p < 0.0001; Abbreviations: NC- Normal Control ;CUMS- Chronic Unpredictable Mild Stress ;FLU-Fluoxetine ; RZ- Ranolazine ; TZ- Trimetazidine ; CI- Confidence Interval; ns - not significant. ANOVA; Analysis of variance; Asterisks indicate p value generated by Tukey's test

Histopathological Evaluation

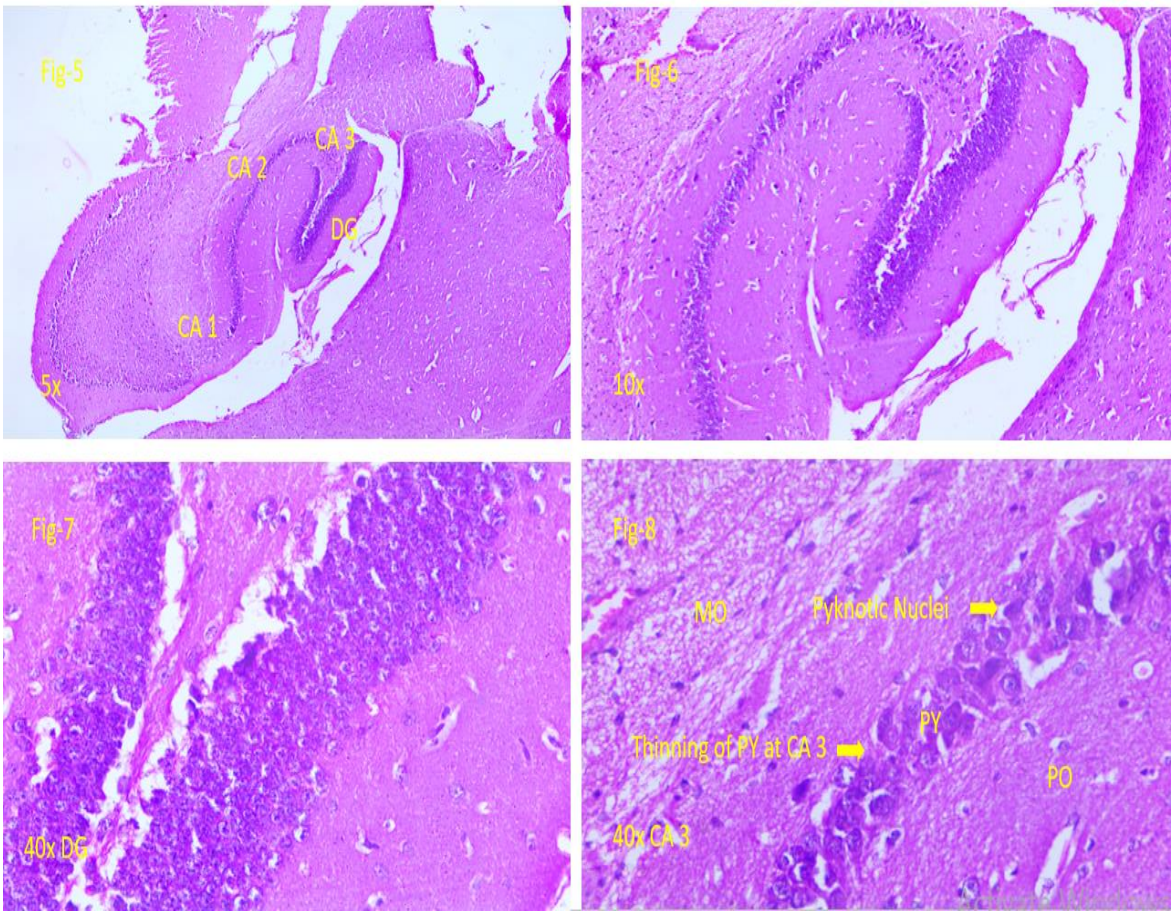
Various treatments have an effect on the hippocampus of mice's brains. Intracellular micro-vacuolisation, eosinophilia, nuclear pyknosis, and karyorrhexis were used to determine apoptosis. Figures 1, 2, 3, and 4 show normal hippocampus anatomy. Figure 4 shows a normal stratum pyramidale. Cell death was most evident in the disease control group ((Fig 5, 6, 7 and 8 showed reduction in the pyramidal layer thickness at CA 3 Layer) In contrast to disease control, standard pharmacological therapy (Fig 9, 10, 11 and 12) showed reduced evidence of cell death. The Ranolazine (Fig 13 to Fig 16) and Trimetazidine (Fig 17 to Fig 20) groups showed decreased signals of cell death, similar to normal medication therapy.

Photographs have a resolution of 5x, 10x and 40x.



Abbreviations: CA- Cornu Ammonis; DG- Dentate Gyrus; PO- Polymorphic Layer; PY- Pyramidal Layer; GL - Granular cell Layer; MO- Molecular Cell Layer

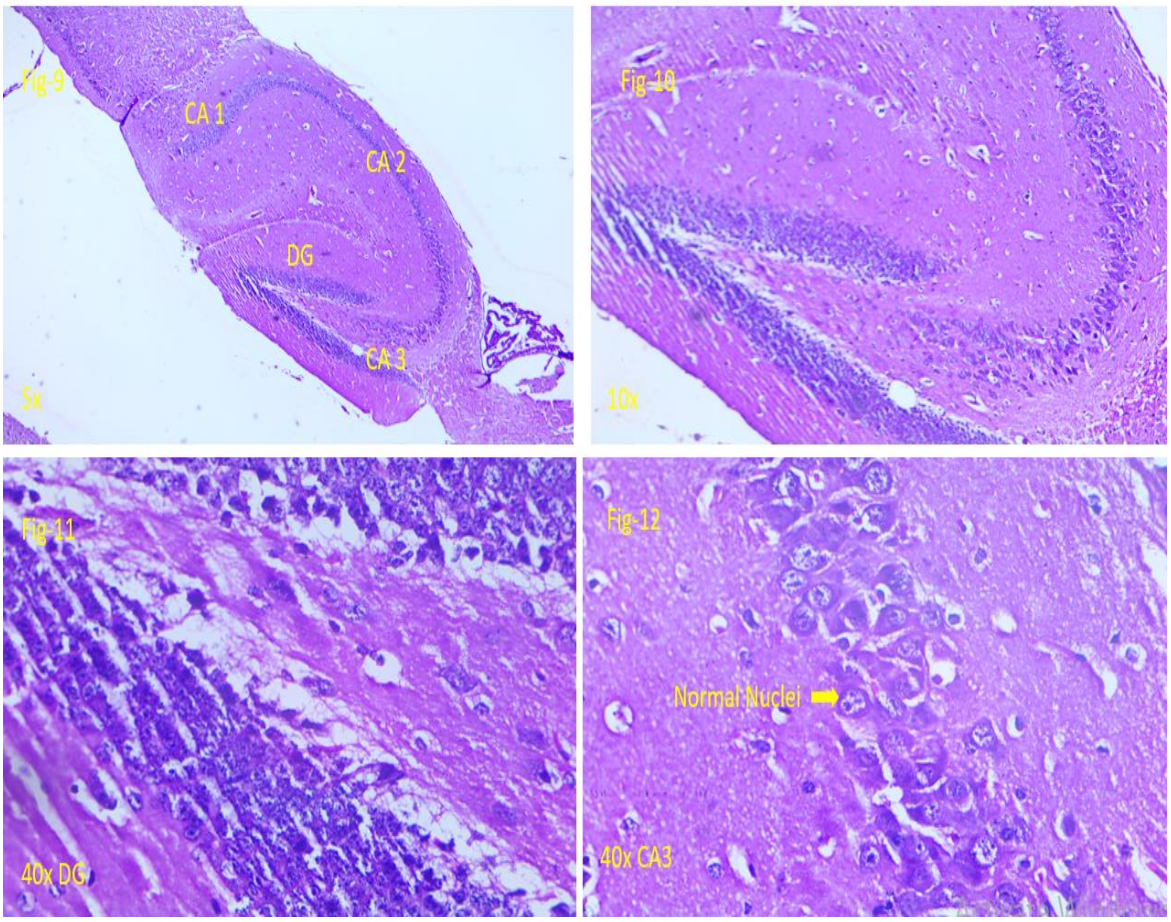
Chronic Unpredictable Mild Stress Group



Abbreviations: CA- Cornu Ammonis; DG- Dentate Gyrus; PO- Polymorphic Layer; PY- Pyramidal Layer; GL - Granular cell Layer; MO- Molecular Cell Layer; .

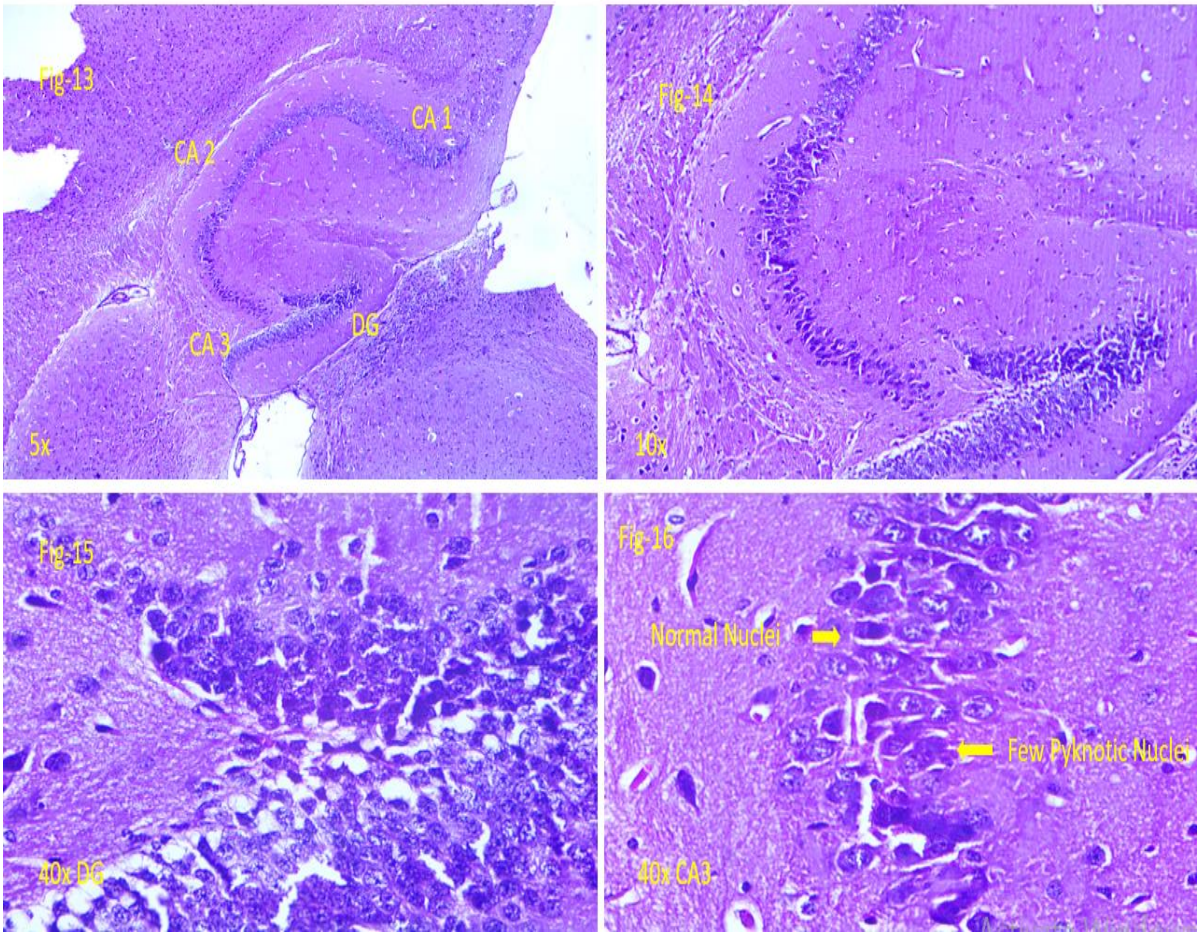
Note: the reduction in the pyramidal layer thickness at CA 3 Layer.

Fluoxetine Group



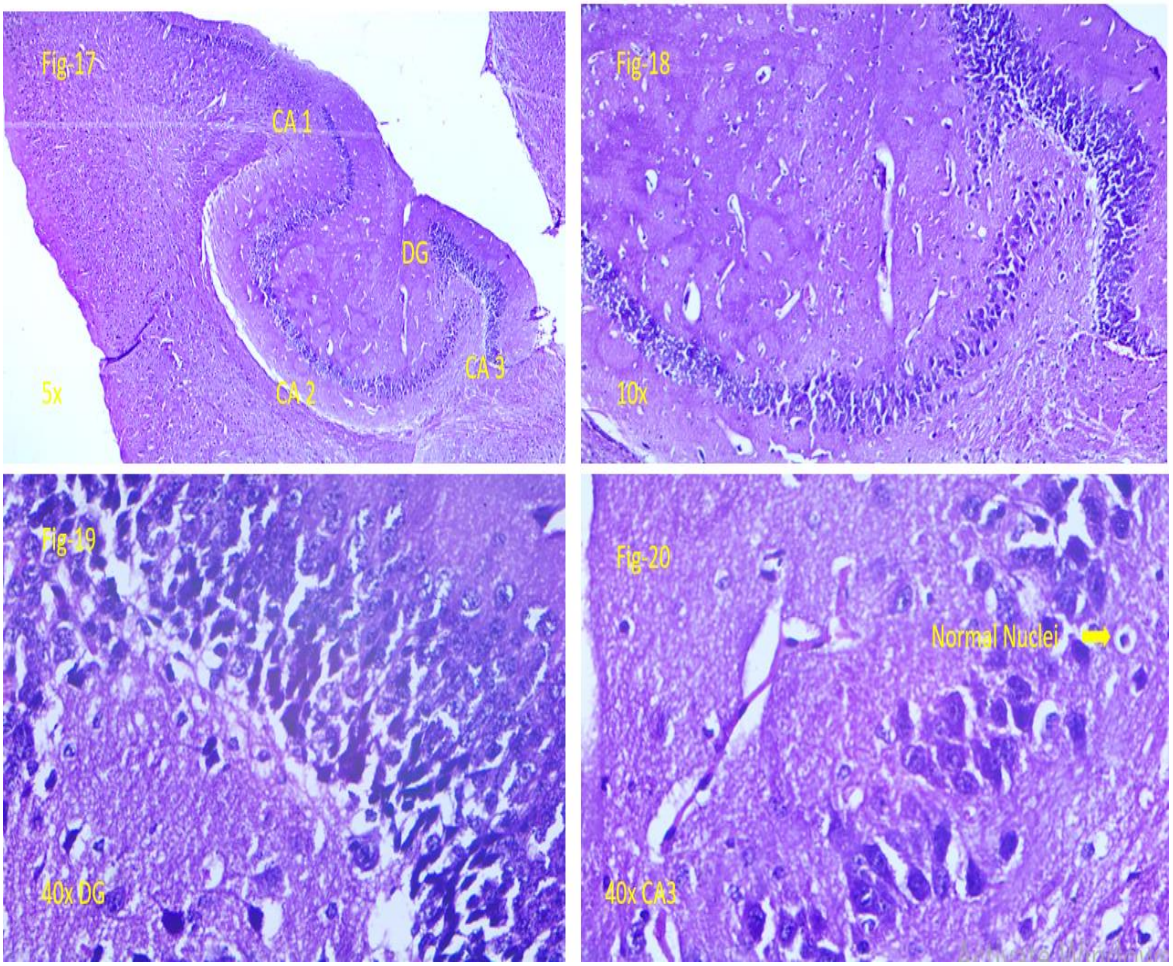
Abbreviations: CA- Cornu Ammonis; DG- Dentate Gyrus; PO- Polymorphic Layer; PY- Pyramidal Layer; GL - Granular cell Layer; MO- Molecular Cell Layer

Ranolazine Group



Abbreviations: CA- Cornu Ammonis; DG- Dentate Gyrus; PO- Polymorphic Layer; PY- Pyramidal Layer; GL - Granular cell Layer; MO- Molecular Cell Layer

Trimetazidine Group



Abbreviations: CA- Cornu Ammonis; DG- Dentate Gyrus; PO- Polymorphic Layer; PY- Pyramidal Layer; GL - Granular cell Layer; MO- Molecular Cell Layer

DISCUSSION

In the present study, evaluation of Ranolazine and Trimetazidine for their antidepressant's activity using experimental model of depression was carried out. CUMS as an animal model to study the effect of drugs in the depression is quite well established.[75] The Result of this study show that CUMS was able to induced anhedonia in the animals and is quite reliable and effective model for evaluation of antidepressant activity. The disease control group stands in contrast to the normal group in all aspect of effect measurement namely Forced swim test, Tail suspension test, Elevated plus maze , Histopathology and of Serum and Brain homogenate (TNF-α, IL-1β) inflammatory markers Levels indicating that CUMS can evoke Pathological processes at different levels inside the body, contributing to a generalized state of depression .These effects of CUMS has been reported and reviewed previously by Willner P et al. and Konkle AT et al.[75,76]

Today's depression models are often evaluated by fulfilling three main criteria (a) Face validity (the requirement for a reasonable degree of symptomatic homology),(b) Construct (or etiological) validity (the requirement for similar causative factors),and(c)pharmacological validity (which requires the reversal of depressive symptoms by available antidepressants). These criteria serve as guides to compare models against each other but each criteria suffers basic flaws.[77] unfortunately, current animal models have substantial limitations, ranging from weak validation to poor predictive power for drug efficacy in human disease.[78] In addition, highly penetrant genetic variants that cause depression have not yet been identified. These considerations highlight the challenge in constructing and validating animal models of depression.

The current theories about major depression do not provide sufficient explanations for the exact cause and nature of depression despite extensive research in this area.[79,80,81] Now in

the current scenario, inflammation and neurodegeneration appear to play an important role in the pathogenesis of depression.[5,6]

In the present study Ranolazine and Trimetazidine were taken as test drugs and Behavioural test was performed at 2 weeks to know the establishment of depression by CUMS and at the end of study that was after 6 weeks.

Forced Swim Test: Duration of Immobility time at the end of the experiment CUMS group demonstrated the highest immobility time in comparison to others groups indicating that these mice were under chronic depression. Fluoxetine significantly improved immobility time compared to the untreated depressed mice. These findings in line with study by David L. Arndt et al and Ding L et al.[86]

Additionally, Ranolazine significantly reduced the immobility time compared to the CUMS mice. Trimetazidine also showed similar reduction but it was not statistically significant.

FST is a standard test used for accessing the antidepressants actions of test drugs the improvement un FST parameters shown by Ranolazine indicates its possible antidepressant action on the other hand Trimetazidine did show a probable antidepressant action however it was no as efficacious as Ranolazine.

Tail Suspension Test: Mean of immobility time

No Significant Result were observed at the end of 2 weeks. But at the end of 6 weeks Statistically significant Increased in immobility was seen with CUMS Group when compared with Fluoxetine treated group and Normal control Group. Result of the present study showed that the selected Ranolazine and Trimetazidine was able to significantly decreased the immobility time when compared with CUMS Group After 6 weeks of treatment with Test drugs.

TST is another well-established model for the screening of antidepressant drugs. in our study the standard drug fluoxetine and test drugs ranolazine and Trimetazidine significantly reduced the immobility time compared to CUMS group. PREVIOUSLY researchers have proven the anti-depressant activity test drugs using TST as one of the parameter (Lijuan Sun et al. and David L. Arndt et al).[85,87] The finding of our study strongly suggest possible use of Ranolazine and Trimetazidine in depressions however this needs confirmation from further studies.

Elevated Plus Maze:

a) In our study two parameters were assessed in EPM namely mean of time spent in open arms, Number of entries in both open arms both ranolazine and trimetazidine significantly improved these parameters in comparison with CUMS group. Moreover, this improvement was comparable with the beneficial effects of fluoxetine.

Overall, in our study both Ranolazine and Trimetazidine were affecting in improving the CUMS Induced depression.

Effect of various treatments on Locomotor activity

The locomotor movement of the Disease control (CUMS) mice was reduced in comparison with normal mice however the difference was not statistically significant.

Fluoxetine significantly improved the locomotor activity in compare to the disease control these findings are similar to previous study done by Ginpreet Kaur et al and David L. Arndt further more Ranolazine and Trimetazidine improved the locomotor activity of mice in compare to CUMS groups, infect Trimetazidine mice showed higher movements in comparison with normal mice. These findings suggest that both Ranolazine and Trimetazidine have a potential to improve the locomotor function.

The locomotor movement of the Disease control (CUMS) mice was reduced in comparison with normal mice however the difference was not statistically significant. Moreover, Ranolazine and Trimetazidine improved the locomotor activity of mice in compare to CUMS groups, Infact Trimetazidine mice showed higher movements in comparison with normal mice. But none of these changes were statistically significant.

Inflammatory markers

In Our study it was observed that both Ranolazine and trimetazidine significantly reduced TNF-α, and IL 1 β in comparison with untreated depressed mice.

The anti-inflammatory properties of ranolazine and trimetazidine are well established et al. and Sanaz Ramezany Yasuj et al in a recent study concluded that duloxetine may have reduced anxiety- and depression-like behaviour in methamphetamine-dependent rats, by inhibiting TNF-α, and IL 1 β and subsequently supressing possibly through the modulation of cAMP/CREB/BDNF pathway.

(REF)

In contrast Ranolazine and Trimetazidine was significantly able to reduce inflammatory markers and improve in mice behaviour in FST, TST, and EPM.

Histopathological evaluation

Histopathological Evaluation Rajkowska G et.al and Zakzanis KK et.al in Their studies stated that depression is associated with cognitive deficits and post-mortem analyses show hippocampal atrophy.[82,83] Study by Reagan LP et. al states that data from preclinical and post-mortem studies suggest that prolonged stress can induce hippocampal neuronal loss.[84] In parallel with the above-mentioned studies microphotograph of disease showed that on analysis of cell nuclei, signs of nuclear pathology i.e. nuclear control shrinkage or

fragmentation was found to be present. Marked nuclear damage with widespread micro vacuolisation, pyknotic nuclei, karyorrhexis was observed. Nuclei staining hazy and inconsistent indicating apoptosis. Microphotograph of Fluoxetine showed that treatment with standard was able to protect against the stress-induced neurodamage, most probably indicating that the Fluoxetine was able to restore the apoptotic changes in mice hippocampus compared to the control group. Microphotograph of Ranolazine and Trimetazidine showed that the analysis of cell nuclei showed signs of nuclear pathology i.e. Pyknosis and karyorrhexis. However, the apoptotic changes observed were less compared to the control group.

CONCLUSION

Our study showed that Ranolazine and Trimetazidine have antidepressant properties as evidenced by their effects at various levels in the mice , right from the behavioural parameters (forced swim test, tail suspension test, elevated plus maze and locomotor activity), through Serum and Brain homogenate inflammatory markers (IL-1BETA and TNF-ALPHA) and hippocampal cell viability (histopathology). Since, Trimetazidine and Ranolazine are already being utilized safely in patients with angina, we propose the idea of exploring these drugs as safe and effective, potential candidates for the treatment of depression in humans. Ranolazine and Trimetazidine can also serve as adjuvants to conventional antidepressant pharmacotherapy for the management of depression. Choosing antianginal to treat patients with both depression and Cardiovascular Diseases (angina) might help reduce the therapeutic doses of antidepressant drugs and their adverse effects.

Funding and Declaration of interest: The study was self-funded and we declare no conflict of interest.

SUMMARY

Depression is the biggest threat to public health worldwide today. It is rapidly getting worse and affects all age groups. It is rampant especially in developed countries and is on the rise in developing countries. Depression not only causes increased morbidity affecting life quality, but also causes significant mortality in the form of suicide and homicide as well. Depression is associated with and to a large extent is considered as one of the important etiological factors for the development of non-communicable diseases, including but not limited to cardiovascular diseases. These findings are indeed supported by multiple clinical observations.

The present study evaluated the effects of two drugs namely Ranolazine and Trimetazidine currently employed in the management of cardiovascular diseases angina. These drugs showed their antidepressant action in Swiss albino mice, by limiting the hippocampal inflammation and the oxidative stress induced by CUMS inside the body.

Human clinical equivalent mice doses of both the drugs showed therapeutic activity in all the measured parameters on anhedonia and hippocampal microstructure compared to the disease control (vehicle treated).

Various mechanisms have been propounded to explain the neuroprotective activity of Ranolazine and Trimetazidine namely reduction in inflammatory markers and modulation of NO pathway etc. Undeniably these are the same mechanisms to which development of depression has been tied to all these years by researchers. Hence, Ranolazine and Trimetazidine seem to be promising and can be further evaluated as potential antidepressant drug in humans.

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ANNEXURE – I - IAEC APPROVAL CERTIFICATE

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

Chairperson, IAEC.	Main Nominee - CPCSEA	Member - Secretary IAEC
Prof & Head Physiology,	Prof & Head of Pharmacology,	Asso Prof of Pharmacology
J.N.Medical College, Belagavi	USM-KLE, IMP, Belagavi	J.N.Medical College, Belagavi

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

MEMBERS:

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

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

Dr. Sudha Devareddy.
Hon.Veternarian,
Belagavi.

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

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

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

CERTIFICATE

This is to certify that the M.D/ M.D.S/ Ph.D/ Research project Entitled "Effect of Ranolazine and Trimetazidine on Depression Paradigm in Male Swiss Albino Mice"

Submitted by- , PG Pharmacology, JNMC.

Has been approved by the Institutional Animal Ethical Committee Meeting held on 22-2-20 vide Resolution No. 12/4

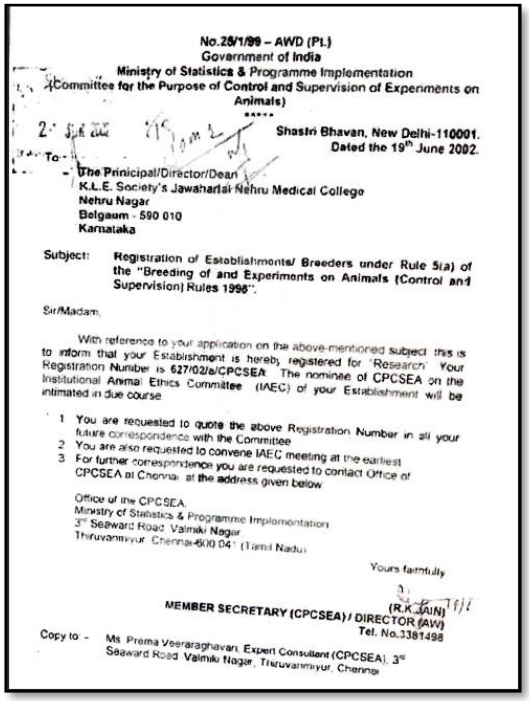
For sanction of 40 Male Swiss Albino Mice

Dr. P A Patil

Signature and Name:
CPCSEA-Main Nominee

Signature and Name:
Chairman/Mem.Secretary

ANNEXURE - II - CPCSEA REGISTRATION & RENEWAL



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

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

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

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

Sir/Madam,

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

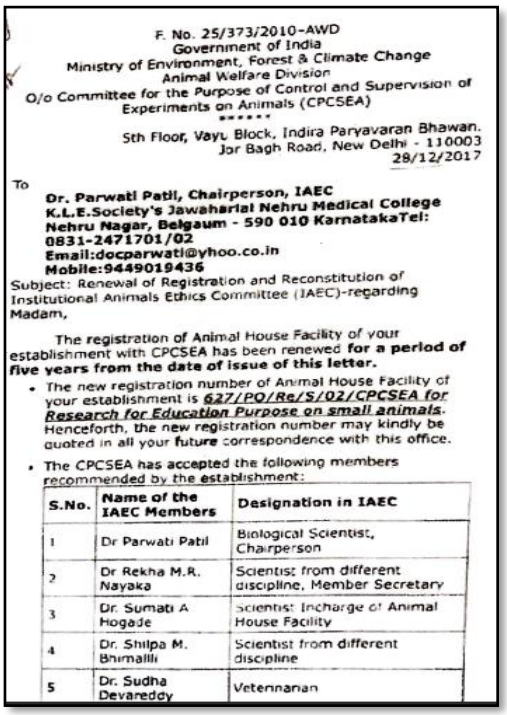
1 You are requested to quote the above Registration Number in all your future correspondence with the Committee
2 You are also requested to convene IAEC meeting at the earliest
3 For further correspondence you are requested to contact Office of CPCSEA at Chennai at the address given below

Office of the CPCSEA,
Ministry of Statistics & Programme Implementation
3rd Seaward Road, Valmiki Nagar
Thiruvanmiyur, Chennai-600 04 (Tamil Nadu)

Yours faithfully,

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

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



F. No. 25/373/2010-AWD
Government of India
Ministry of Environment, Forest & Climate Change
Animal Welfare Division
O/o Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)
******
5th Floor, Vayu Block, Indira Paryavaran Bhawan,
Jor Bagh Road, New Delhi - 110003
28/12/2017

To
Dr. Parwati Patil, Chairperson, IAEC
K.L.E.Society's Jawaharlal Nehru Medical College
Nehru Nagar, Belgaum - 590 010 KarnatakaTel:
0831-2471701/02
Email:docparwati@yhoo.co.in
Mobile:9449019436

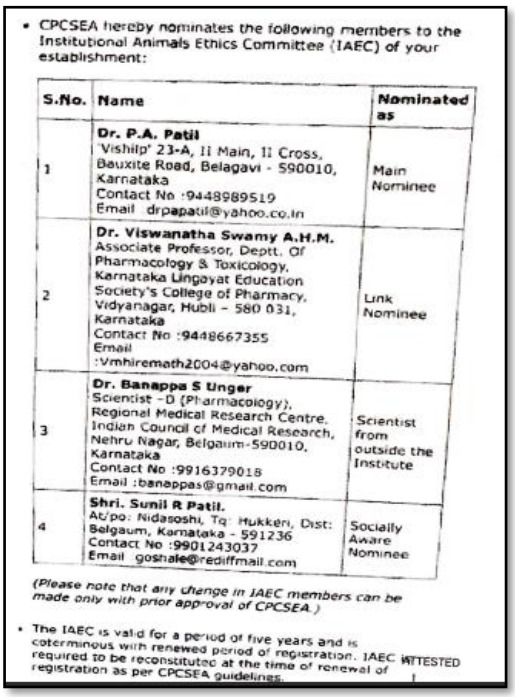
Subject: Renewal of Registration and Reconstitution of Institutional Animals Ethics Committee (IAEC)-regarding

Madam,

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

- The new registration number of Animal House Facility of your establishment is ***627/PO/Re/S/02/CPCSEA for Research for Education Purpose on small animals***. Henceforth, the new registration number may kindly be quoted in all your future correspondence with this office.
- The CPCSEA has accepted the following members recommended by the establishment:

S.No.	Name of the IAEC Members	Designation in IAEC
1	Dr Parwati Patil	Biological Scientist, Chairperson
2	Dr Rekha M.R. Nayaka	Scientist from different discipline, Member Secretary
3	Dr. Sumati A Hogade	Scientist Incharge of Animal House Facility
4	Dr. Shilpa M. Bhimalli	Scientist from different discipline
5	Dr. Sudha Devareddy	Veterinarian

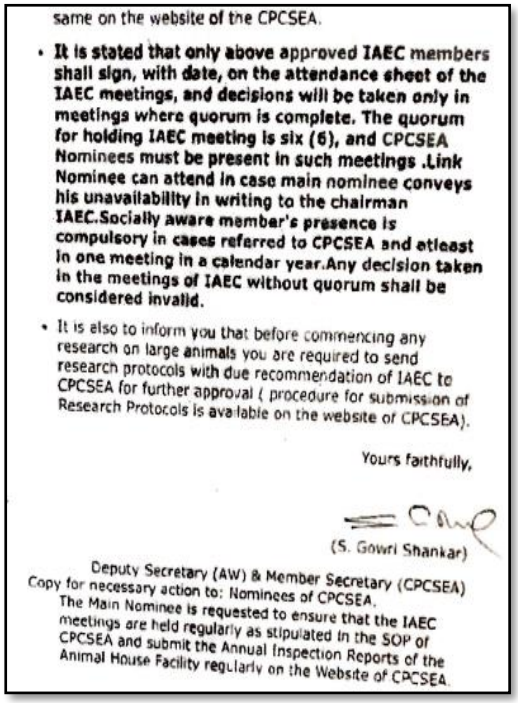


- CPCSEA hereby nominates the following members to the Institutional Animals Ethics Committee (IAEC) of your establishment:

S.No.	Name	Nominated as
1	**Dr. P.A. Patil** 'Vishilp' 23-A, II Main, II Cross, Bauxite Road, Belagavi - 590010, Karnataka Contact No :9448989519 Email :drpapatil@yahoo.co.in	Main Nominee
2	**Dr. Viswanatha Swamy A.H.M.** Associate Professor, Deptt. Of Pharmacology & Toxicology, Karnataka Lingayat Education Society's College of Pharmacy, Vidyanagar, Hubli - 580 031, Karnataka Contact No :9448667355 Email :Vmhiremath2004@yahoo.com	Link Nominee
3	**Dr. Banappa S Unger** Scientist -D (Pharmacology), Regional Medical Research Centre, Indian Council of Medical Research, Nehru Nagar, Belgaum-590010, Karnataka Contact No :9916379018 Email :banappas@gmail.com	Scientist from outside the Institute
4	**Shri. Sunil R Patil.** At/po: Nidasoshi, Tq: Hukkeri, Dist: Belgaum, Karnataka - 591236 Contact No :9901243037 Email :goshale@rediffmail.com	Socially Aware Nominee

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

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



same on the website of the CPCSEA.

- **It is stated that only above approved IAEC members shall sign, with date, on the attendance sheet of the IAEC meetings, and decisions will be taken only in meetings where quorum is complete. The quorum for holding IAEC meeting is six (6), and CPCSEA Nominees must be present in such meetings .Link Nominee can attend in case main nominee conveys his unavailability in writing to the chairman IAEC.Socially aware member's presence is compulsory in cases referred to CPCSEA and atleast in one meeting in a calendar year.Any decision taken in the meetings of IAEC without quorum shall be considered invalid.**
- It is also to inform you that before commencing any research on large animals you are required to send research protocols with due recommendation of IAEC to CPCSEA for further approval (procedure for submission of Research Protocols is available on the website of CPCSEA).

Yours faithfully,

(S. Gowri Shankar)
Deputy Secretary (AW) & Member Secretary (CPCSEA)

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