Behavioural and Neuropharmacological Screening of Potential Serotonergic (5-HT_3) Ligands for Co-morbid Depression, Anxiety and Related Disorders

THESIS

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by

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CERTIFICATE

This is to certify that the thesis entitled **"Behavioural and Neuropharmacological Screening of Potential Serotonergic (5-HT_3) Ligands for Co-morbid Depression, Anxiety and Related Disorders"** and submitted by **Shvetank Bhatt, ID No. 2008PHXF432P**, for the award of Ph.D. Degree of the Institute, embodies the original work done by him under my supervision.

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Dedicated

to

My Affectionate Family

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Date: **Shvetank Bhatt**

List of Abbreviations/Symbols

5-HIAA	5-Hydroxy Indole Acetic Acid
3-HK	3-Hydroxy Kynurenine
5-HT	5-Hydroxy Tryptamine
5-HT_3	Serotonin Type-3
5-HTP	5-Hydroxy Tryptophan
5-HTP-HTR	5-Hydroxy Tryptophan Induced Head Twitch Response
5-HTT	5-Hydroxy Tryptamine Transporter
8-OH-DPAT	8-Hydroxy Dipropyl Amino Tetralin
AA	Anxious Arousal
Ach	Aetylcholine
ACMS	Anhedonic Chronic Mild Stress
ACTH	Adrenocorticotropic Hormone
AD	Anti-depressant
Ads	Anti-depressants
ALE	Advanced Lipoxidation End Product
AMPT	α-Methyl-Para-Tyrosine
ANOVA	Analysis of Variance
APA	American Psychiatric Association
ATP	Adenosine Triphosphate
BDNF	Brain Derived Neurotrophic Factor
BUP	Bupropion
CBT	Cognitive Behavioural Therapy
CAT	Catalase
CDNA	complementary DNA
CINV	Cancer Chemotherapy Induced Nausea and Vomiting

CMS	Chronic Mild Stress
CNS	Central Nervous System
CORT	Corticosterone
CREB	Cyclic AMP Response Elment Binding
CRF	Corticotrophin Releasing Factor
CSF	Cerebrospinal Fluid
CUMS	Chronic Unpredictable Mild Stress
DA	Dopamine
DBS	Deep Brain Stimulation
DMI	Desipramine
DSM	Diagnostic and Statistical Manual of Mental Disorder
DZM	Diazepam
ECT	Electroconvulsive Therapy
EDTA	Ethylene-diamine tetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EPM	Elevated Plus Maze
ESC	Escitalopram
FFT	Family Focused Therapy
FST	Forced Swim Test
FLX	Fluoxetine
GABA	γ- Aminobutyric Acid
GAD	Generalised Anxiety Disorder
GERD	Gastroesophageal Reflux Disease
GPCR	G-protein Coupled Receptor
GSH	Reduced Glutathione
HB	Hole Board
HPA	Hypothalamic Pituitary Adrenal Axis
Hr	Hour

HTR	Head Twitch Response
IAEC	Institutional Animal Ethics Committee
IBS	Irritable Bowel Syndrome
ICD	International Classification of Disorders
IDO	indoleamine 2,3 dioxygenase
IL	Interleukin
INF-γ	Interferon- γ
i.p.	Intra-peritoneal
IPT	Inter Personal Therapy
KA	Kynurenic Acid
KO	Knock Out
L/D	Light-Dark
LH	Learned Helplessness
LPS	Lipopolysaccharide
MAD	Mixed Anxiety Depression
MAO	Monoamine Oxidase
MAOI	Monoamine Oxidase Inhibitor
MCPBG	m-chlorophenylbiguanide
MDA	Malondialdehyde
MDD	Major Depressive Disorder
MHPG	3-methoxy 4-hydroxyphenylglycol
MLT	Melatonin
NaSSA	Nor-adrenergic and Specific Serotonergic Anti-Depressants
NCEs	New Chemical Entities
NCS	National Comorbidity Survey
NDRI	Nor-adrenaline and Dopamine Re-Uptake Inhibitor
NE	Nor-epinephrine

NET	Nor-epinephrine Transporter
NF-kB	Nuclear Factor kB
NMDA	N-methyl-D-aspartate
NRI	Nor-adrenaline Re-uptake Inhibitor
NS	Nitrosative Stress
NT	Neurotransmitter
OAE	Open arm entries
OB	Olfactory Bulb
OBX	Olfactory Bulbectomy
OCD	Obsessive Compulsive Disorder
OFT	Open Field Test
OND	Ondansetron
PA	Positive Affect
PAR	Paroxetine
PEG	Poly Ethylene Glycol
PKA	Protein Kinase-A
PKA	Cyclic AMP-dependent protein Kinase A
p.o.	Per-oral
PRG	Pargyline
PTL	Parthenolide
PTSD	Post Traumatic Stress Disorder
QA	Quinolic Acid
QOL	Quality of Life
RA	Receptor Antagonist
RIH	Reserpine-induced Hypothermia
ROS	Reactive Oxygen Species
RTMS	Repetitive Transcranial Magnetic Stimulation
SAD	Seasonal Affective Disorder

S	Seconds
SARI	Serotonin Antagonist and Re-uptake Inhibitors
SERT	Serotonin Transporter
SLA	Spontaneous Locomotor Activity
SNRI	Serotonin and Nor-adrenaline Re-uptake Inhibitors
SOD	Superoxide Dismutase
SSRE	Selective Serotonin Re-uptake Enhancers
SSRI	Selective Serotonin Re-uptake Inhibitor
TBARS	Thiobarbituric Acid
TBI	Traumatic Brain Injury
TCAs	Tricyclic Antidepressants
TDO	Tryptophan Dioxygenase
TMs	Trans Membranes
TNF-α	Tumor Necrosis Factor-α
TPH	Tryptophan
TRD	Treatment Resistant Depression
TSOA	Time Spent in Open Arm
TST	Tail Suspension Test
VLA	Venlafaxine
VNS	Vagus Nerve Stimulation
WHO	World Health Organisation

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Abstract

Depression and anxiety are the most prevalent psychiatric conditions in the community. Several animal studies have shown that 5-HT_3 receptor antagonists play a key role in the management of psychiatric disorders such as depression and anxiety. Moreover, several other preliminary studies have shown that the effect of 5-HT_3 antagonists in behavioural despair model of depression is attenuated by 5-HT_3 receptor agonists. The role of 5-HT_3 receptors in anxiety is confirmed by studies of 5-HT_{3A} knockout mice which revealed that 5-HT_{3A} receptor subtypes regulates depression- and anxiety-related behaviours. The positive outcomes from preliminary behavioural tests on 5-HT_3 receptor antagonists, their better safety profile and the complementary effectual regional distribution of 5-HT_3 receptors in the CNS have urged further research to establish their potential applications in a range of CNS disorders. Thus, the present study was designed to investigate thoroughly the anti-depressant and anxiolytic potential of in-house synthesized novel 5-HT_3 receptor antagonists (quinoxaline derivatives) namely **"6g","6n","6o"** and **"6p"** (details of which are given later with structure and IUPAC names) using rodent behavioural models.

Separate groups of mice received acute treatment of NCE's: **"6g","6n","6o"** and **"6p"**, were subjected to spontaneous locomotor activity test (SLA) and anti-depressant assays, namely, the forced swim test (FST), tail suspension test (TST), 5-hydroxytryptophan induced head twitch response (5-HTP-HTR) and reserpine-induced hypothermia (RIH). Acute treatment with all the tested compounds (1 and 2 mg/kg, i.p.), exhibited anti-depressant-like effect in FST and TST without influencing the baseline locomotion in actophotometer test. The tested compounds (1 and 2 mg/kg, i.p.) were observed to increase 5-HTP-HTR in mice and the compounds also antagonized RIH response in rats.

It was also observed that in addition to interaction studies, combined treatment of tested compounds and conventional anti-depressants had no significant effect on baseline locomotion. Interaction studies of compounds **"6g","6n","6o"** and **"6p"** (1 mg/kg, i.p.) were carried out with conventional anti-depressants like Fluoxetine (FLX), Venlafaxine (VLA) and Desipramine (DMI) in FST. All the tested compounds produced synergistic anti-depressant-

like effect with FLX (10 and 20 mg/kg, i.p.), DMI (10 and 20 mg/kg, i.p.) and VLA (4 and 8 mg/kg, i.p.) in FST.

All the tested compounds (1 and 2 mg/kg, i.p.) reversed the Parthenolide (PTL) (1 mg/kg, i.p.) induced increase immobility time in FST. Interaction studies with Bupropion (BUP) using TST, the tested compounds (**6g, 6n, 6o** and **6p**) at 1 mg/kg, i.p., produced synergistic anti-depressant-like effect. Besides the anti-depressant potential, in another set of study the preliminary anxiolytic effect of 5-HT_3 receptor antagonists were also investigated. Separate groups of mice received acute treatment of drugs (**6g, 6n, 6o** and **6p**) and subjected to experimental anxiety models [elevated plus maze (EPM), the light dark (L/D), hole board (HB) and open field test (OFT)]. Acute treatment with all the tested compounds (1 and 2 mg/kg, i.p.) exhibited anxiolytic-like effects in EPM, L/D, HB and OFT.

Further, to confirm the efficacy of 5-HT_3 receptor antagonists, the effects were also evaluated in chronic rodent behavioural test battery such as olfactory bulbectomy (OBX), traumatic brain injury (TBI), chronic unpredictable mild stress (CUMS) and LPS induced depression.

OBX was performed in anesthetized rats. After OBX surgery treatments [**6g** (1 and 2 mg/kg, p.o.), **6n** (1 and 2 mg/kg, p.o.), **6o** (1 and 2 mg/kg, p.o.) and **6p** (1 and 2 mg/kg, p.o.)] behavioural tests were carried out. OBX rats exhibited hyperactivity (increased ambulation, rearing and defecation) in open field test (OFT), phase aversion in EPM, decreased sucrose consumption which resembling anhedonia, increased hyper-emotionality behaviour (emotional anomalies to noxious stimuli). Chronic treatment (14 days) with above mentioned drugs significantly reversed the behavioural anomalies induced by OBX in rats in all the behavioural tests. Besides the behavioural tests biochemical and neurochemical estimations were also carried out to explore the possible biochemical mechanism.

The possible underlying mechanism(s) of **"6g"** and **"6n"** in OBX model were also investigated by measuring, neurotransmitter levels [serotonin (5HT), nor-epinephrine (NE) and dopamine (DA)]. OBX rats showed significantly

(P< 0.05) decreased the 5HT, NE and DA levels. Compounds **"6g"** and **"6n"** treatment significantly (P<0.05) reversed the OBX induced neurochemical signaling alterations (except DA) which could be possible mechanisms for anti-depressant-like effect in OBX model. Moreover, rats subjected to OBX showed a decrease in brain derived neurotrophic factor (BDNF) levels in brain which were reversed by chronic treatment with compounds (**6g, 6n**).

In another study, anesthetized rats were subjected to impact accelerated TBI. Post ten days of healing, chronic (14 days) **"6g"** (1 and 2 mg/kg, p.o.) and **"6n"** (1 and 2 mg/kg, p.o.) were administered. In addition to exploratory hyperactivity in OFT, TBI rats showed emotional anomalies to marbles in marble burying test and decreased sucrose consumption as compared to the sham control rats. These results demonstrated the complete neurological deficits following TBI and selective behavioural changes. Chronic treatment (14 days) with above mentioned drugs significantly reversed the behavioural anomalies induced by TBI in rats in modified open field exploration, sucrose consumption test and marble burying test.

The possible underlying mechanism(s) of **"6g"** and **"6n"** in TBI model were also investigated by measuring brain neurotransmitter levels. TBI rats have shown a marked decrease in the 5-HT, NE and DA levels. **"6g"** and **"6n"** treatment substantially reversed the TBI-induced neurochemical signaling alterations (except DA). Moreover rats subjected to TBI showed a decrease in BDNF levels in brain which were reversed by treatment with compound (**6g, 6n**).

The present study also included investigations to find out the involvement of 5-HT_3 receptor antagonists CUMS-induced depression and anxiety. Mice were subjected to unusual stress procedures daily for a period of 28 days to cause depressive-like behaviour. Drugs were administered during the last 21 days (8th -28th) of the CUMS paradigm. The results showed that 4-weeks CUMS produces significant depression-like behaviour (FST, TST and sucrose consumption test) and anxiogenic effect (EPM). Chronic treatment with **"6g"** (1 and 2 mg/kg., p.o.) and **"6n"** (1 and 2 mg/kg., p.o.) produced significant anti-depressant- and anxiolytic-like behaviour in FST (decreased duration of

immobility), TST (decreased duration of immobility), sucrose consumption test (increased preference to sweetened solution) and in EPM test [increased percentage open arm entries (OAE) and percentage time spent in open arm (TSOA)].

The underlying mechanism(s) in chronic unpredictable mild stress (CUMS) model was also adequately addressed. The possible underlying mechanism(s) of **"6g"** and **"6n"** in CUMS model was investigated by measuring hypothalamic-pituitary- adrenal (HPA) axis activity and oxidative stress/antioxidant markers levels. Stressed mice showed a significant high plasma corticosterone (CORT) levels. CUMS also significantly increased the oxidative stress markers and decreased the antioxidant enzymes activity. Treatment with compound **"6g"** and **"6n"** significantly ($P < 0.05$) reversed the CUMS induced biochemical alteration (oxidative stress parameters) which may be possible mechanism(s) for anti-depressant-like effect in CUMS model.

Exploratory investigation on the potential of 5-HT_3 receptor antagonist in treatment resistant depression (TRD), using LPS (lipopolysaccharide)-induced depression and anxiety model were studied. Mice were injected with LPS for one day followed by compound **"6g"** (1 and 2 mg/kg) treatment for next 7 days. The results showed that LPS injection produces significant depression-like behaviour in FST, TST, sucrose consumption test and anxiogenic effect in EPM and L/D test. Chronic treatment with compound **"6g"** (1 and 2 mg/kg., p.o.) produced significant anti-depressant-like behaviour in FST, TST and sucrose consumption test and anxiolytic-like effect of EPM and L/D test.

LPS treated mice also significantly increased the oxidative stress markers and decreased the antioxidant enzymes activity. Treatment with compound **"6g**" (1 and 2 mg/kg., p.o.) significantly ($P<0.05$) reversed the LPS induced biochemical alteration (oxidative stress parameters). Moreover, LPS treatment in mice significantly decreased the 5HT levels. Chronic treatment with compound **"6g"** significantly ($P<0.05$) reversed the transduction cascade alterations which may be responsible for anti-depressant-like effect in this model.

In conclusion, these findings strongly support that 5-HT_3 receptors play a fundamental role in the pathophysiology of depression and anxiety disorders. 5-HT_3 receptors antagonists produced anti-depressant and anxiolytic effect in various acute and chronic rodent models. In addition the possible mechanism for the 5-HT_3 receptors in depression and co-morbid anxiety mediated by modulating the HPA axis activity, neurotransmitter levels and oxidant/ antioxidant system.

1. Introduction

According to the World Health Organization (WHO), depression disorder is a serious issue and one of the primary causes of disability, globally. It is one of the most prevalent and expensive neurologic disorder of the developed world with a lifetime prevalence roughly around 7.5 to 17% (Kessler et al., 2003; Mahesh et al., 2013; http:// www.who.int/ mental_health / management/ depression/ definition/ en/).

The untreated, persistent or recurrent depression may disturb the normal physical, mental and social status of the depressives and in worst cases depressed person get the suicidal thought and hence, quick diagnosis and treatment is important to treat this disorder. Several standard therapies are available to treat depressive disorder. Regardless of the chemical structures, most of the clinically existing anti-depressant drugs exert their action by increasing the levels of monoamine(s) in synaptic cleft, either directly or indirectly through other mechanism(s) (Girish et al., 2010; Mahesh et al., 2013). The older anti-depressant drugs such as monoamine oxidase inhibitors (MAOIs) and tricyclic anti-depressants (TCAs) are unfortunately well known for their interaction with other drugs and food, anti-muscarinic, anti-histaminic and other adverse effects rather than their therapeutic potential (Mahesh et al., 2013). Introduction of ‘low side-effect anti-depressants’ such as SSRIs for the treatment of depression, improve the patient’s compliance towards pharmacotherapy of depression (Shelton, 2003). However, these molecules are less efficient than the older drug molecules and takes 4-6 weeks to produce their therapeutic effects (Ayuso-Gutierrez, 2002). Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV, 2000) has meticulously classified depression as a mood/personality disorder with various sub-types and underlying mechanisms (DSM-IV, 2000). Though diagnostic criteria (DSM-IV, 2000) has been continuously subjected to refinement, incidence of different overlapping symptoms and sub-types, co-morbidity with other psychiatric (Pollack, 2005; Evans et al., 2005) and /or terminal illnesses (Katon and Schulberg, 1992; Fisch, 2004) are the biggest hurdle in the anti-depressant drug development and therapy.

Anxiety is a condition of fear and apprehension. Similar to depression, anxiety disorders are common in the preliminary health care setups. In addition, co-morbid anxiety symptoms and anxiety disorders are common in patients with depression disorder (Kessler et al., 2005a,b). Their co-occurrence may directly affect the clinical treatment of depression. Lack of suitable preclinical model(s) for the evaluation of co-morbid depression is the bottleneck of its diagnosis and treatment. Further, a detail about co-morbid depression with anxiety disorder is elaborated in the review of literature section of this thesis.

1.1. Sub-types, Symptoms and Treatment of Depression Sub-types

Depressive illness comes in many forms as shown in Fig. 1. Depression can be classified in following subtypes:

1.1.1 Unipolar Depression (Major Depressive Disorder)

Unipolar depression may be described as feeling sad, rejected, sorrowful and miserable. True human depressive disorder is a psychiatric problem in which feelings of sadness, failure, anger or disappointment affect day-to-day life for weeks or more (Keller and Nesse, 2005). The exact cause of unipolar depression is not known. Many researchers believe it is caused by change in the neurotransmitter levels in the brain. Certain genetic or stressful events and sometimes may be combination of both may triggered this form of depression. The symptoms of unipolar depression have been shown in Table 2.

Diagnostic and Statistical Manual for Mental Disorder (DSM)

The DSM is the benchmark criteria for mental disorders by mental wellbeing experts in the U.S. It used by clinical specialists and research groups of different areas (e.g. biological, psychodynamic, cognitive, behavioural, interpersonal, family/systems). The DSM guidelines started with DSM-I (1968) and the latest is DSM-V (2013). The diagnostic criteria DSM-IV is structured into an axial system, which contain five parts. The Ist axis includes human disorders, whereas IInd axis covers personality problems and intellectual impairments and left over axes covers medical, psychosocial, environmental and childhood factors functionally essential to impart diagnostic feature for

evaluation of health care systems. The latest edition of the DSM (DSM-V) has incorporated many radical changes as well as introduced many new definitions and diagnostic criteria for the evaluation of previously unknown or poorly defined mental disorders. A detail of DSM-IV and DSM-V manuals have been summarized in as mentioned below (Table 1).

Table 1: The DSM-IV and DSM-V –Comparison (Richardson et al., 2005)

	DSM-IV	DSM-V
(A) Bipolar disorder	(1) Bipolar disorder comes under mood disorder category (2) Diagnosis of bipolar disorder, mixed episode, requiring that the individual simultaneously meet full criteria for both mania and major depressive disorder	(1) Bipolar disorder is now a separate category -It has been pulled out of the mood disorder category (2) For accurate diagnosis and earlier detection in clinical settings, **Criterion A** for manic and hypomanic episodes includes an emphasis on changes in activity and energy as well as mood. (3) A new specified, **"with mixed features**," has been added that can be applied to episodes of mania or hypomania when depressive features are present and to episodes of depression in the context of MDD or bipolar when features of mania/hypomania are present.
B) Other Specified Bipolar & Related Disorder)	(1) No specification of particular conditions	(1) This allows the specification of particular conditions including categorization for individuals with a past history of a MDD who meet all criteria for hypomania except the duration criterion (i.e., at least 4 consecutive days). (2) A second condition is that too few symptoms of hypomania are present to meet criteria for the full bipolar II syndrome, although the duration is sufficient at 4 or more days (http://www.dsm5.org)
(C) Depressive Disorders	(1) Dysthymic disorder is a separate category. (2) Disruptive mood dysregulation disorder is not addressed. **(3)** Pre-menstrual dysphoric disorder is not defined.	(1) Now falls under the category of persistent depressive disorder, which includes both chronic MDD & previous dysthymic disorder. (2) This is included to address concerns about potential over diagnosis and treatment of bipolar disorder in children up to age 18 years who exhibit constant irritability and frequent episodes of extreme behavioural change three or more times a week for more than a year.

	DSM-IV	DSM-V
		(3) Premenstrual dysphoric disorder is now has distinct diagnosis in the depressive disorders chapter (http://www.dsm5.org).
(D) Bereavement exclusion for Depression	(1) There was an exclusion criterion for a major depressive episode that was applied to depressive symptoms lasting less than 2 months following the death of a loved one (i.e., the bereavement exclusion). (2) No differentiation between Grief and Depression	(1) This exclusion is not there in DSM-V to remove the implication that bereavement typically lasts only 2 months when clinicians recognize that the duration is more commonly 1–2 years. (2) Several notes within the text delineating the differences between grief and depression (Richardson et al., 2005).
(E) Anxiety Disorders	(1) Social phobia was mentioned in DSM IV (2) OCD is included under anxiety. (3) Panic disorder and agoraphobia was linked. (4) Separation anxiety disorder and selective mutism fall under the chapter disorders of infancy, childhood or adolescence.	(1) Social phobia is now termed as social anxiety disorder (2) No longer includes OCD under anxiety. (3) Panic disorder and agoraphobia are unlinked, as several patients experience agoraphobia without panic symptoms (4) Separation anxiety disorder and selective mutism now fall under the anxiety disorders chapter
(F) Post-traumatic stress disorder (PTSD)	(1) PTSD was not present as a separate chapter, included under anxiety disorder.	(1) PTSD is no longer includes under anxiety disorder. Included in a new chapter in DSM-V on trauma- and stressor-related disorders. (2) DSM-V pays more attention to the behavioural symptoms that accompany PTSD and proposes four distinct diagnostic clusters

	DSM-IV	DSM-V
		instead of three. (3) PTSD will also be more sensitive for children and adolescents.
(G) Trauma- & Stressor-Related Disorders	(1) Criterion A2 regarding the subjective reaction to the traumatic event (e.g., "the person's response involved intense fear, helplessness or horror").	(1) This criterion has been removed.
(H) Panic Attack	(1) Situationally bound/cued, situationally predisposed and unexpected/uncued are the terms used for describing different type of panic attack.	(1) Expected and unexpected are the new terms to differentiate type of panic attack. (2) Panic attacks function as a prognostic factor for severity of diagnosis, course and co-morbidity across many anxiety and other disorders and thus can be listed as a specifier that is applicable to all DSM-V disorders.
(I) Agoraphobia, Specific Phobia & Social Anxiety	(1) There is 6 months of duration for diagnosis, only limited to individuals under age of 18.	(1) Changes include deletion of the requirement that individuals over age 18 years recognize that their anxiety is excessive or unreasonable. (2) 6 months of duration is extended for all ages to minimize over diagnosis of transient fear.

Apart from the DSM-IV/V criteria, the other common symptoms that are not essential in diagnosis include constipation, decreased salivation and diurnal variations in the symptoms (worsening in the morning). The exclusion criteria are occurrence of these symptoms in schizophrenia or other neurological disorders and no evidence of recent demise in the family or other stressful events. A group of disorders (Kandel, 2000) characterizes unipolar type of depression.

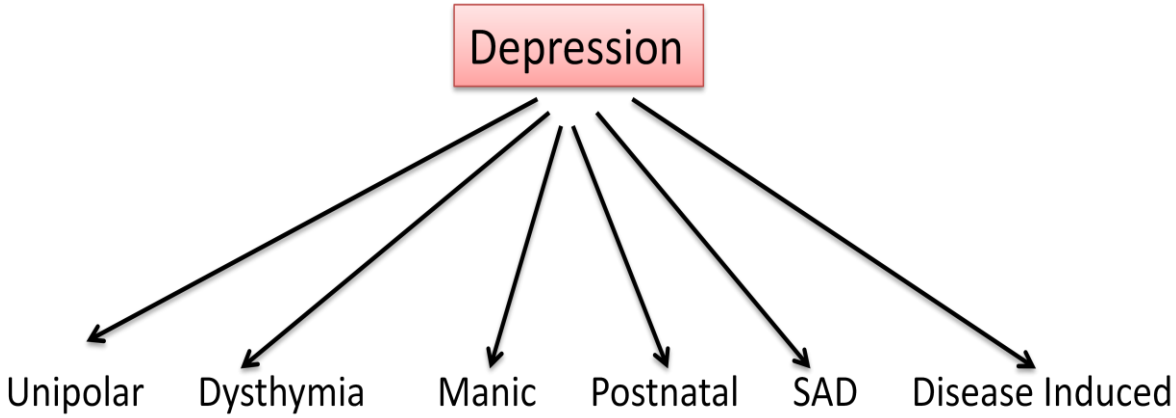


Fig.1. Types of depression (Potter and Hollister, 2007)

1.1.2. Dysthymia

Dysthymia is milder or less severe depression that persists for a long term typically more than two years. The reason of dysthymia is not well known. It is more predominant in women than in man. Many people suffering from dysthymia have persistent clinical problems such as insomnia, alcohol abuse or drug addiction (Stahl, 1998a). In dysthymic disorder, cognitive and psychotherapy are beneficial though treatment with newer SSRIs is most preferable (Koran et al., 2007). The symptoms of dysthymia have been shown in Table 2.

1.1.3. Bipolar Depressive (Manic-Depressive) Disorder

Mania is the other name of bipolar disorder. It is described as periodic episodes elevated mood followed by depressive symptoms (Stahl 1998a). The average age of onset of mania is approximately 20 years and it equally affects both men and women. Around 25% of the patients with major depression do experience a manic episode (http://www.medschool.pitt.edu/ somsa/ Depression. html). Mania often attacks thinking pattern, judgement and social behaviour. The

duration and intensity of bipolar disorder varies tremendously. After first incident of euphoria, later episodes of either euphoria or depression are likely to occur (Stahl, 1998a). Depressive episodes tend to become more pronounced with age (Kandel, 2000). The manic periods have to be controlled with anti-psychotics like olanzapine, mood-stabilizing agents such as lithium, valproate and electroconvulsive therapy. The depression component can be managed by conventional anti-depressants (ADs) like TCAs and SSRIs (Potter and Hollister, 2007). The symptoms of mania have been shown in Table 2.

1.1.4. Post-natal Depression

Postnatal depression is a type of depression experience by some women following a child birth. It generally develops in initial weeks after childbirth, although there are some instances where it may not appear for few months (Stahl, 1998a; Scrandis et al., 2007). Early detection of depression during pregnancy is important because depression can negatively affect health of newborn. The psycho-social and psychological approach is more effective in managing this type of depression than pharmacological treatment (Dennis and Hodnett, 2007). The most commonly used anti-depressants in the management of this type of depression are SSRIs (Fluoxetine) and the SNRIs (Venlafaxine) (Dennis and Hodnett, 2007). The symptoms of post-natal depression have been shown in Table 2.

1.1.5. Seasonal Affective Disorder (SAD)

SAD is a typical f depression type that generally occurs in the winter season. It is more common in females than in males, similar to other forms of depression (http://www.ncbi.nlm.nih.gov /pubmedhealth /PMH0002499/).

The treatment for this therapy is intense light. Light therapy works effectively for nearly everyone with SAD. Other treatment approaches are use of anti-depressants and behavioural therapy most useful in managing depressive symptoms (Lewy et al., 2006). The symptoms of post-natal depression have been shown in Table 2.

Table 2: Comparison of symptoms of various types of depression

Specific Symptoms							**Common Symptoms**
Types of Depression	**Unipolar Depression**	**Dysthymia**	**Bipolar Disorder**	**Post natal**	**Seasonal Affective**	**Disease Induced**	**Feelings of hopelessness**
	Agitation, Restlessness	Milder agitation, Restlessness than unipolar depression	Hyperactivity Increased talkativeness	Miserable specially morning and evening time	Increased sleep	Symptoms depend on the co-morbid disease such as cardiovascular disease or diabetes etc	Low energy or fatigue Poor appetite Poor sleep
	Irritability	Less irritability, than unipolar depression	Increased energy	Tearful	Increased appetite, particularly crave for carbohydrates	Restlessness Discomfort, heaviness near chest in case of cardiovascular disease	Poor concentration Thought of death and suicide
	Self hate and guilt	Mild loss of pleasure than unipolar depression	Flight of ideas	Strange behaviour towards baby	weight gain	Fatigue in case of hypothyroidism	Feeling of worthlessness Affects day to day life

1.1.6. Disease Induced Depression

Sometimes diseases such as hypertension, diabetes, obesity, parkinson's disease, heart disease, stroke, cancer and Cushing's syndrome can cause symptoms similar to that of depression (Regier et al., 1993). Another cause is hypothyroidism, where abnormality in the thyroid metabolism leads to impaired metabolism (Regier et al., 1993).

Cardiovascular and metabolic complications such as obesity and diabetes can increase the chances of depression, where the under-detection and inappropriate treatment is especially common (Mahesh et al., 2010b). Specific and common symptoms of different forms of depression have been shown in Table 2.

1.2. Pathophysiology of Depression

Multicomponent, cellular transduction cascade interacts at various levels thereby forming complex signaling networks that allow neural cells to receive, integrate and respond to stimulus as well as to modify the signals generated by multiple neuro-transmitter and neuro-peptide systems (Bhalla and Iyengar, 1999). These signaling pathways are undoubtedly involved in neuroplasticity that regulate complicated psychological and memory processes, as well as diverse physiological processes of the body such as appetite and wakefulness.

Consequently, considerable excitement has been generated in the clinical neuroscience community, by recent evidence that destructions of neuroplasticity and cellular resilience might trigger or will be involved in the pathology of depressive disorder. The anti-depressants and lithium exert their therapeutic effects on signalling cascades that regulate neuroplasticity, neuronal survival and neurogenesis. These findings are re-shaping views about the neurobiological under-pinnings of these disorders (Bhalla and Iyengar, 1999).

Abnormal concentrations of neuro-transmitters, disruption of the hypothalamic-pituitary-adrenal (HPA) axis, increased levels of corticotrophin-releasing factor (CRF), and abnormalities of second messenger transduction systems may also

implicated in the depression pathophysiology. In addition, stressful life events, personality changes and sexual disturbance may also play a role in progression of depression (Mann et al., 1996; Charney, 1998).

There is growing evidence concerning the role of specific neuro-transmitters and clinical manifestations of depression. Abnormalities in DA may be related to impaired inspiration and attention, low levels of NE and DA having a role in the weakness and hypersomnia and regulation of impaired NE and 5-HT may involve in physical symptoms. [http:// bestpractice.bmj.com/ best- practice/ monograph/ 55/ basics/ pathophysiology.html].

The pathophysiology of depression discussed in following major points

- **Psychological**
- **Social**
- **Genetic**
- **Neuro-chemical**

1.2.1. Psychological Factors

Various personality aspects and its progress emerge to be essential to the incidence of depression with destructive emotionality as a regular feature (Kanter et al., 2004). Although major depressive episodes are strongly linked with negative life incidents. Additionally, low self-worth and disorganised thinking are correlated with depression.

Depression is less predominant and more rapidly remit among those individuals, who are more spiritual. However, depressed patients who are capable to change their thought process often show elevated mood and guilt (Monroe et al., 2007). Fig. 2. describes complete pathophysiology of depression.

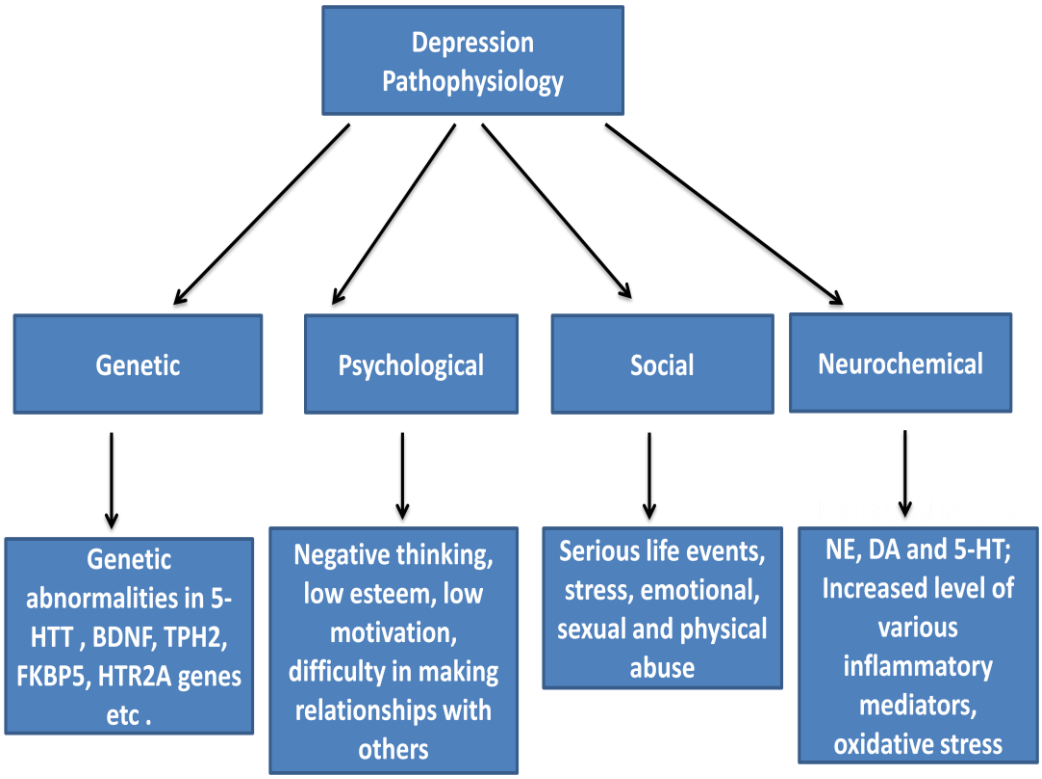


Fig. 2. Pathophysiology of depression

1.2.2. Social Factors

In general, increased risk of mental health problems are associated with social separation and poverty (Kanter et al., 2004). In addition, Child exploitation (physical/ psychological/ sexual) is increased the threat of developing depressive disorders afterwards in life (Slavich et al., 2009). Disturbances in family matters, such as parental separation, serious marital conflicts or divorce, death of near and dear ones are additional risk factors (Kanter et al., 2004). Onsets of major depressive incidents in adulthood are strongly associated with the stressful life events. First episode of depression is more likely to come after any traumatic life events than intermittent episodes are consistent with the hypothesis that patients may become susceptible to life related stress over consecutive recurrences of depression increasingly (Vilhjalmsson, 1993).

1.2.3. Genetic Factor and Depression

In major depression and bipolar disorder, genetic factors play a very important role. First-degree relative (a child) has roughly 25 % chance of affecting, if one parent is affected and 50 % chance, if both parents are manic. In major

depression, the familial history is less marked (Sullivan et al., 2000). Depression that results from a genetic inheritance is sometimes referred to as endogenous depression (Hamet and Tremblay, 2005). The major genes involved in depression have been shown in Fig. 2.

1.2.4. Neuro-chemical Factors (Monoamine theory)

Modifications in noradrenergic and serotonergic transmission in the central nervous system (CNS) have been involved in the progression of depression. In addition, most of the anti-depressant drugs also act by alteration in NE and 5-HT levels in synapse (Charney et al., 1998). The role of major neurotransmitters involved in depression is shown in Fig. 4.

A. Role of Serotonin (5-HT) in Depression

The role of monoamine 5-HT is well established as a neuro-transmitter in the pathophysiology and pharmacotherapy of depression (Nemeroff, 2008; Bhalla and Iyengar, 1999). A large number of studies confirm serotonergic system dysfunction in depression comes from showing recurrence of depression after tryptophan depletion. Reduced levels of the serotonergic metabolite, 5-hydroxyindolacetic acid (5-HTIAA), history of serious suicidal behaviour was observed in the brain of depressed patient. Sequence of abnormalities in 5-HT concentration in depression is shown in Fig. 3.

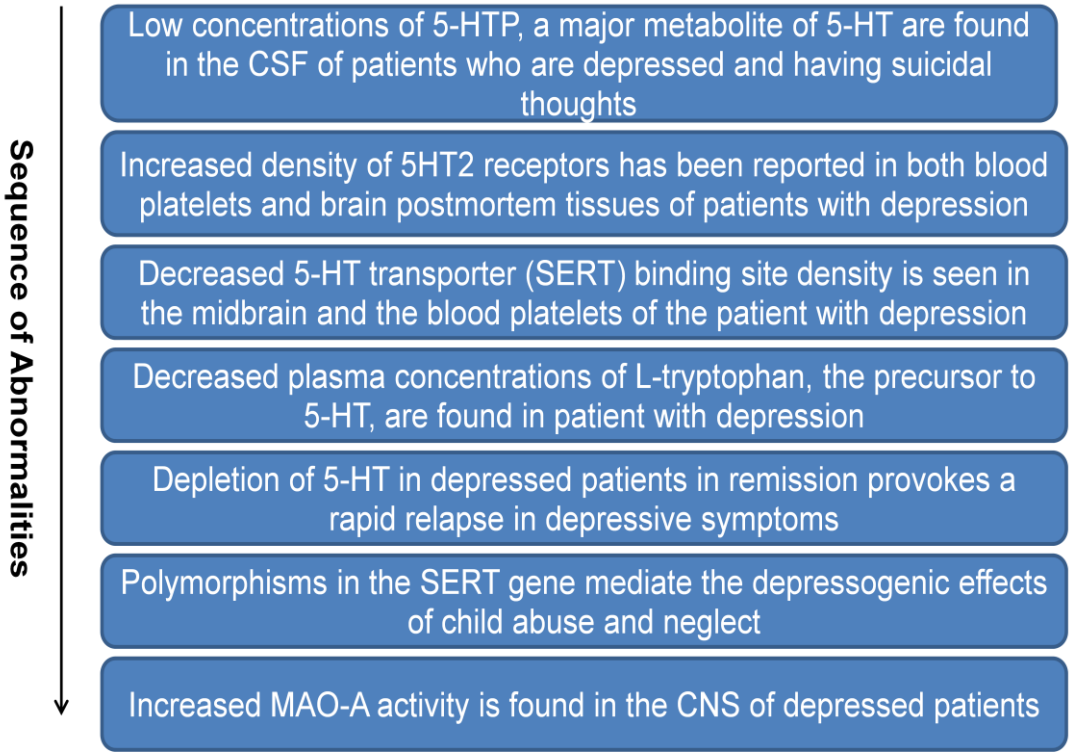


Fig. 3. Sequence of abnormalities in serotonin concentration in depression

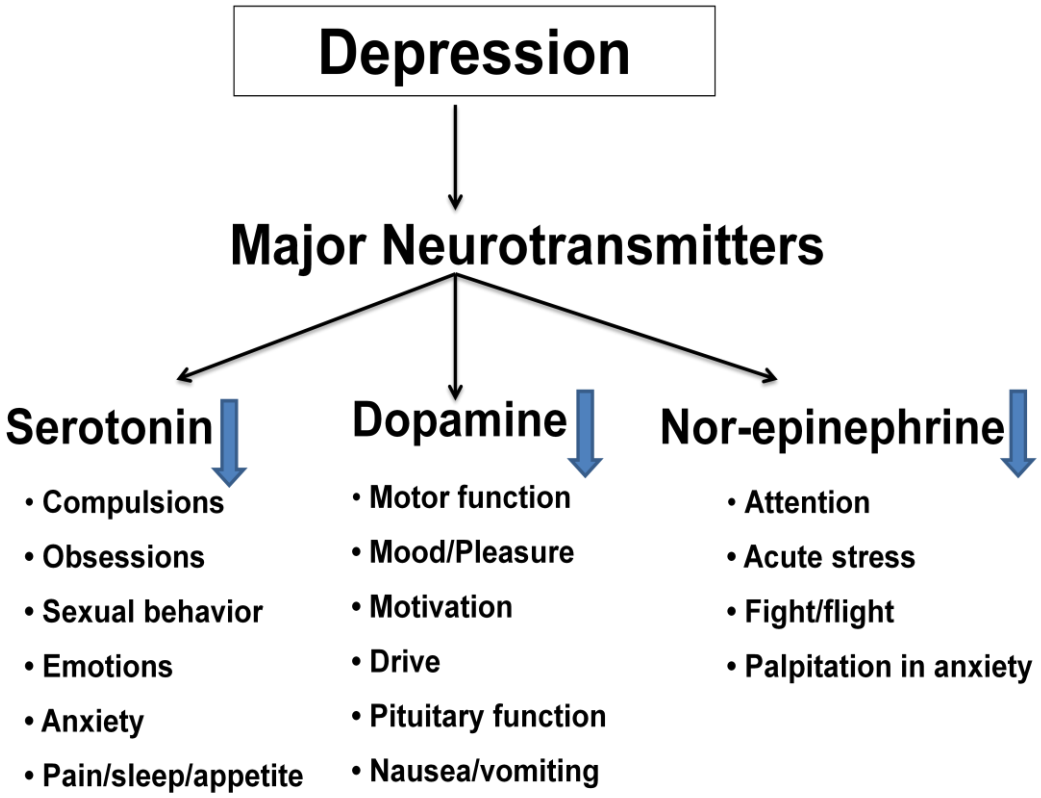


Fig. 4. Monoamine theory of depression

B. Role of Nor-epinephrine (NE) in Depression

Several neuro-chemical and neuro-endocrine studies in depressed patients and postmortem findings showed noradrenergic abnormalities in depression as described in Fig. 5. Alpha-methylparatyrosine (AMPT) inhibits the biosynthesis of brain catecholamines, has been used as a noradrenergic probe to investigate the catecholamine hypothesis that changes in neuro-transmission through the catecholamine system may be responsible the pharmacological effect to nor-epinephrine reuptake inhibitors (Nemeroff, 2008).

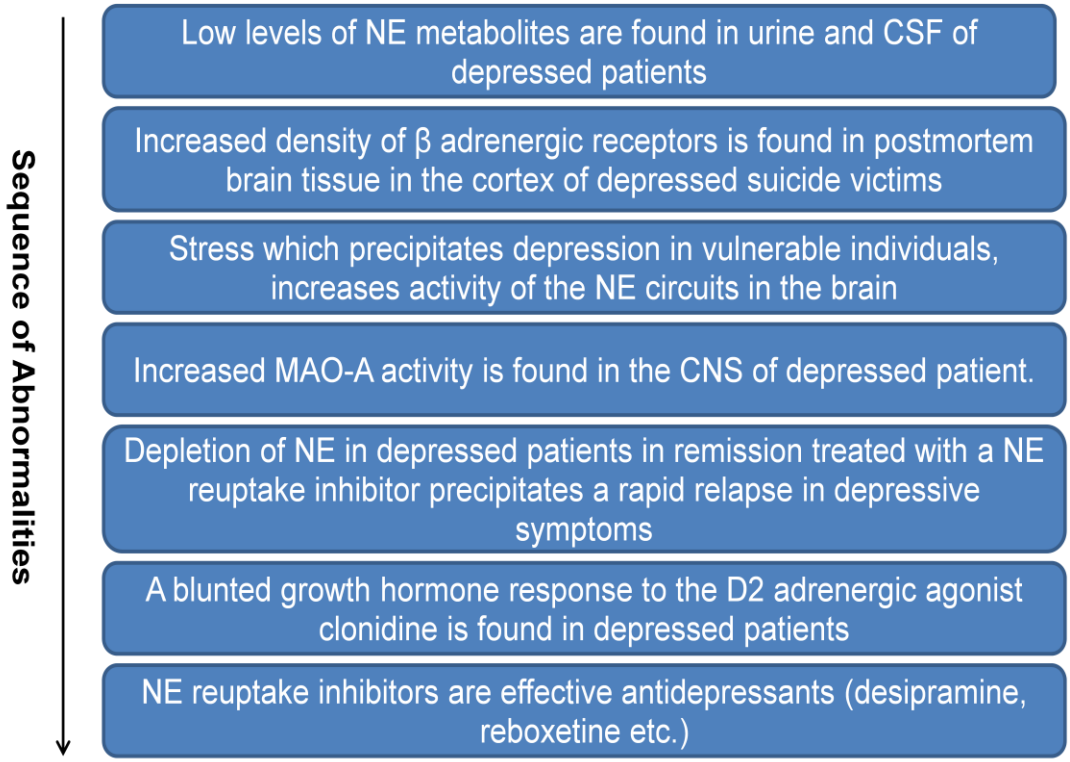


Fig.5. Sequence of abnormalities in nor-epinephrine concentration in depression

C. Role of Dopamine (DA) in Depression

DA is involved pleasure, motivation, drive, and anhedonia as per dopaminergic hypothesis. Fig.6. summarizes sequence of altered dopaminergic circuits in depression.

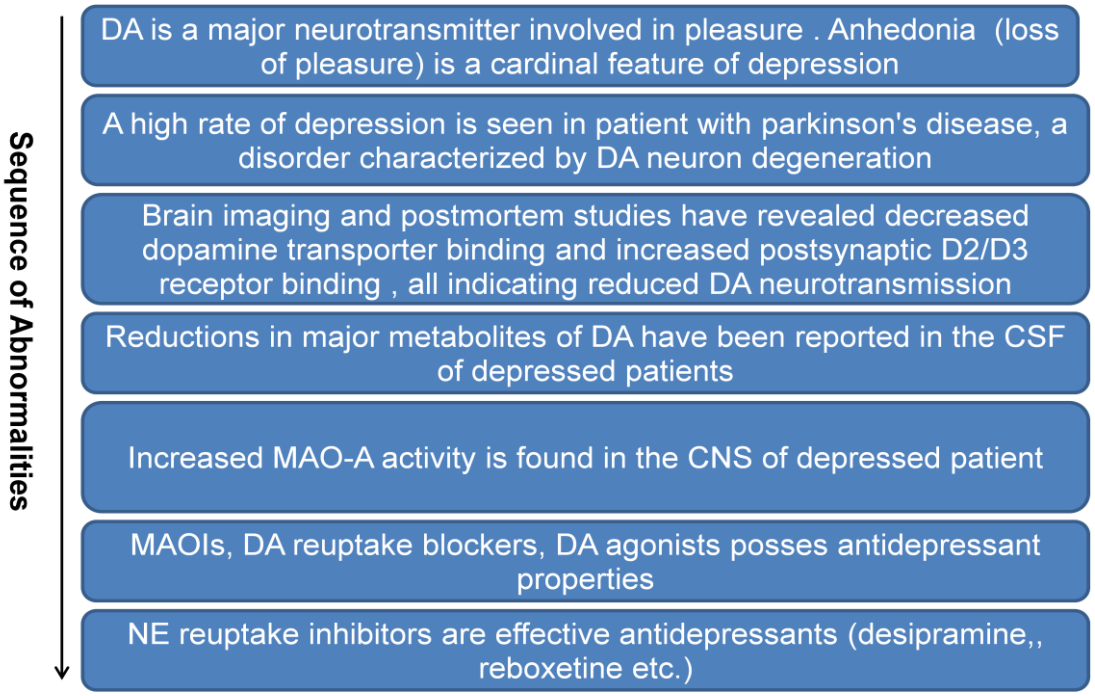


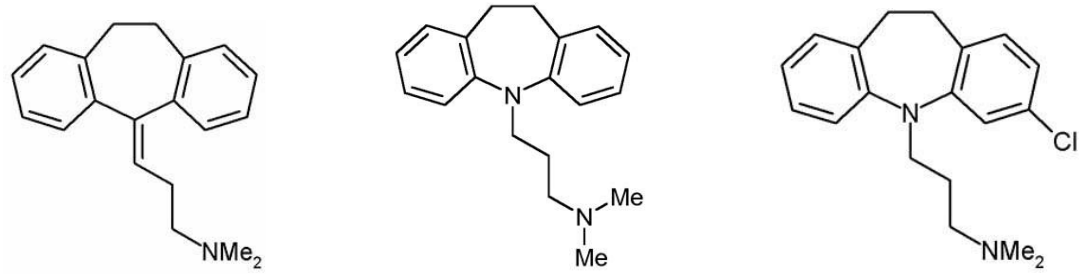
Fig.6. Role for altered dopaminergic circuits in depression

1.3. Treatment and Pharmacological Classification of Anti-depressants (ADs)

1.3.1. Treatment Based on Monoamine Theory of Depression

A. Tricyclic Anti-depressants (TCAs)

Drugs having five different pharmacological actions have been grouped into this class, collectively termed as TCAs (Stahl 1998b). 5-HT re-uptake inhibitor activity, NE re-uptake inhibitor activity, anti-cholinergic/ anti-muscarinic activity, α_1-adrenergic antagonistic activity, and anti-histaminic (H_1) activity (Richelson, 1982). At overdose they also inhibit sodium channels, causes potentially fatal atrial fibrillations and seizures. Pharmacological effect of the TCAs were due to 5-HT re-uptake inhibition as well as NE re-uptake inhibition. The extent of specificity of inhibition of the 5-HT over other NE transporters (NET) differ across the class of TCAs with clomipramine being more selective to 5-HT re-uptake transporter pump, whereas desipramine (DMI) and maprotiline being more selective to NE re-uptake pump. Side effects of the TCAs are due to their affinity towards H_1, M_1, and α_1 receptors. Chemical structure of some TCAs are shown in Fig. 7a.

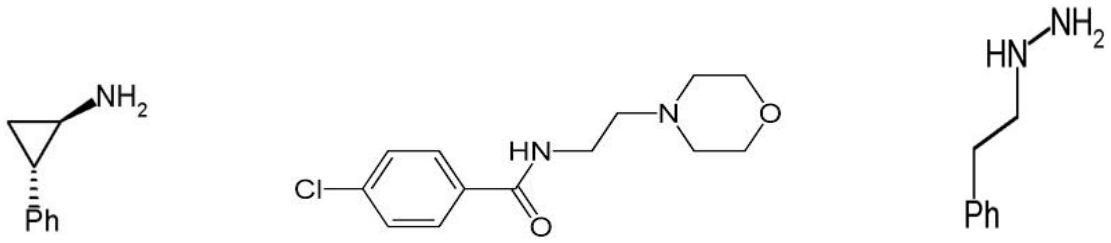


Amityptyline Imipramine Clomipramine

Fig.7a. Structure of various tricyclic anti-depressants

B. Monoamine Oxidase Inhibitors (MAOIs)

An intra-cellular enzyme located on the outer mitochondrial membrane is known as monoamine oxidase (MAO). It degrades monoamines in the cytoplasm viz. NE, 5-HT, DA, epinephrine and tyramine. There are two isoforms namely A and B out of which MAO-A predominantly metabolises NE, 5-HT and epinephrine. MAO-B is specific for phenethylamine. Both MAO-A and MAO-B metabolize DA. The mode of action of MAOI is to increase the levels of the mononergic amines by blocking their metabolic degradation. The conventional MAOIs (e.g. tranylcypromine) are non-selective and irreversible, whereas the recently developed MAOIs are specific for MAO-A or MAO-B as well as reversibly inhibit the MAO-A (Krishnan 2007). Chemical structures of few MAO inhibitors are shown in Fig. 7b.



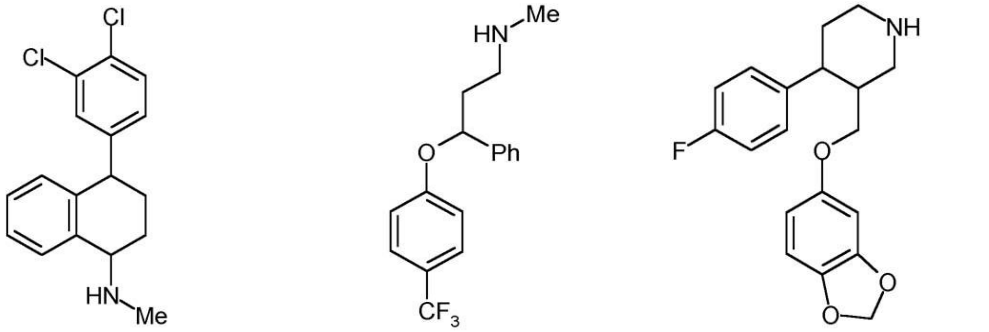
Tranylcypromine Moclobemide Phenelzine

Fig.7b. Structure of various monoamine oxidase inhibitors

C. Selective 5-HT Re-uptake Inhibitors (SSRIs)

SSRIs, selectively inhibit serotonergic transport. This action increase 5-HT concentration, markedly in the somatodendritic synaptic region of 5-HT neurons. With chronic administration, the constant increase of 5-HT can cause desensitization of the somatodendritic 5-HT_{1A} autoreceptors. Ultimately,

neuronal impulse flow is turned 'on' causing increased release of 5-HT from axon terminals (Blier and de Montigny, 1994). This further leads to desensitization of post-synaptic 5-HT receptors as a final step and down regulation of these receptors may be responsible for the pharmacological potential of SSRIs or could involve in the progression of tolerance to severe side effects of SSRIs (Carrasco and Sandner 2005; Vaswani et al., 2003). Chemical structures of some SSRIs are shown in Fig. 7c.

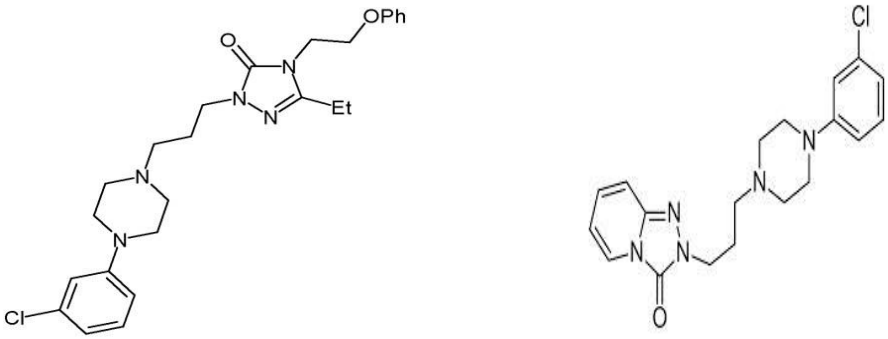


Sertraline Fluoxetine Paroxetine

Fig.7c. Structure of various selective serotonin reuptake inhibitors

D. 5-HT_2 Receptor Antagonism with 5-HT Re-uptake Blockers (SARIs)

The drugs, which belong to this class are Nefazodone and Trazodone and the only dissimilarity from SSRIs is the blockade of 5-HT_2 receptors. Owing to this difference, SARIs do not cause few side effects that SSRIs may cause such as, instant increase in anxiety or insomnia, akathisia, and sexual dysfunction (Stahl 1998b). Chemical structure of some 5-HT_2 receptor antagonist with 5-HT re-uptake blockers are shown in Fig. 7d.

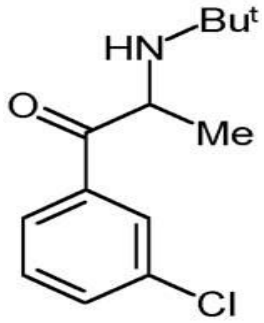


Nefazodone Trazodone

Fig.7d. Structure of various 5-HT_2 receptor antagonist with 5-HT re-uptake blockers

E. NE and DA Re-uptake Inhibition (Bupropion)

The only anti-depressant that does not interfere with serotonergic system and acts specifically on the NE and DA transduction pathways is BUP (Cooper et al. 1994). The pharmacological action of BUP suggests that clinical anti-depressant effects were due to increase of NE and DA levels in selected regions of the brain. The anti-depressant effect of bupropion could mediate through stimulation of dopaminergic transmission (Cryan et al., 2005). Ripoll and Colleagues (2003) have been performed the dose dependent study of bupropion. Chemical structure of bupropion is shown in Fig. 7c.

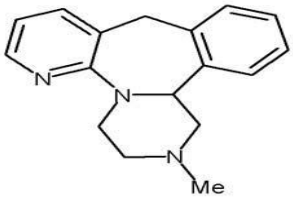


Bupropion

Fig. 7e. Structure of NE and DA Re-uptake Inhibitor

F. Noradrenergic and Specific Serotonergic Anti-depressant

Mirtazapine is the prototypical noradrenergic and specific serotonergic anti-depressant which blocks α_2, 5-HT_2 and 5-HT_3 receptors (Fawcett and Barkin, 1998). Its serotonergic actions are specifically regulated from the 5-HT_2 and 5-HT_3 to the 5-HT_{1A} receptor (Berendsen and Broekkamp 1997). Due to stimulation of 5-HT_2 receptors, mirtazapine does not produce the adverse effects of SSRIs, like nefazodone. Since it is also a 5-HT_3 receptor antagonist, it doesn't have the effect of SSRIs that leads to the modulation of 5-HT_3 receptors, such as disturbances of gastrointestinal tract. Besides this, mirtazapine has the adverse effects of weight gain and hypresomnia due to its strong anti-histaminic properties (Anttila and Leinonen 2001). Chemical structure of mirtazapine is shown in Fig. 7f.

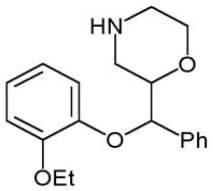


Mirtazapine

Fig. 7f. Structure of noradrenergic and specific serotonergic AD

G. NE Specific Re-uptake Inhibitor

Reboxetine is selective NE re-uptake inhibitor. Pharmacologically and chemically distinct to TCA or SSRIs, reboxetine has predominant binding capacity for the NET, and poor affinity for 5-HT, DA, histamine, muscarinergic and α adrenergic receptors. NE depletion studies suggest that while NE re-uptake inhibition may improve the symptoms of depressive disorder, NE regulation may be more specifically related with patient's improvements in power, attention, curiosity, disturbance, helplessness, and hopelessness (Wong et al., 2000). Chemical structure of reboxetine is shown in Fig. 7g.



Reboxetine

Fig. 7g. Structure of NE specific re-uptake inhibitor

As mentioned above, several strategies have been adopted to control this disorder and its subtypes. However, the intriguing neural circuitry and molecular mechanisms behind this disorder is still remaining elusive

1.3.2. Non Pharmacological Treatment

i. Psychotherapy

Psychotherapy or "talk therapy", is a therapeutic approach, to treat people with depression and other CNS disorders by creating the awareness and knowledge about the disease. It literates people about the approaches of psychotherapy and gives them a thought to fight with daily life stress, unconstructive thoughts and behaviours (http://www.nimh.nih.gov/health/topics/psychotherapies).

Types of Psychotherapy

Depending on the patient's requirement, he or she receives the type of psychotherapy (http://www.nimh.nih.gov/health/topics/psychotherapies). The most frequently used psychotherapies are:

(A) Cognitive Behavioural Therapy (CBT)

CBT is a combination of cognitive therapy (CT) and behavioural therapy (BT). CT focuses on the change in the person's thought process from negative to positive direction and accordingly changes in the action plan. Several clinical trials showed effectiveness of CBT (Proudfoot et al., 2004). CBT is successfully used in following disorders as shown in Table 3.

(B) Interpersonal Therapy (IPT)

IPT is commonly used to treat various depressive disorders on individual basis. IPT improves communication skills between people to treat depression effectively. IPT helps recognize how a depressed patient interacts with other people. Sometimes IPT is used along with anti-depressant medications (De Mello., 2005).

(C) Family Focused Therapy (FFT)

FFT was introduced with an intention to improve interfamily relationships of patient with bipolar disorder. The treatment plan also involve family members who are expected to support the treatment rendered to the patient and facilitate better outcomes (Miklowitz et al., 2003). Expert clinician in FFT works to find out the issues among family members, which may deteriorate the patient's condition. The clinician literates' family members about their relatives problem, its severity and treatment plan (Rea et al., 2003). Several studies indicated FFT as an effective treatment approach for patients with relapsed depressive condition (Miklowitz et al., 2003; Morris et al., 2007). The therapy showed positive effect in recognizing and learning to cope with traumatic life actions that activate recurrences mania as well as re-establishing physiological associations after a mood episode (Morris et al., 2007).

Table 3: Cognitive behavioural therapy for the treatment of various disorders

CBT				
Depression	**Anxiety**	**Bipolar Disorder**	**Eating Disorder**	**Dialectical behaviour therapy (DBT)**
Major neuro-transmitters involved- 5-HT, NE, DA,	Major neuro-transmitters involved- GABA, 5-HT, NE	Major neuro-transmitters involved- NE, 5-HT	Major neuro-transmitters involved- 5-HT, NE, DA,	Major neuro-transmitters involved- 5-HT, NE
Some people with depression are effectively treated with CBT and medicines. It improves the negative thought pattern of a person.	The therapy is useful in phobias and OCD treatment, Based on the change of environment from fear to support.	The therapy is useful in mood stabilization. It also used to treat bipolar disorder	CBT helps to reduce the chances of relapse in adults with anorexia who have weight gain problem	This therapy used to serve people with suicidal thoughts. DBT involves both individual and group therapy

(D) Light Therapy

Light therapy is useful for the treatment of seasonal affective disorder (SAD), which generally affects the population during winter season, when the intensity of normal sunlight is less. SAD occurs in some population due to disturbance in their daily body rhythms by short days and long nights. The concentration of 5-HT, the brain neuro-transmitter, associated with mood elevation, rises with sunrise (exposure to sun light) and decreases with sunset (Eagles, 2004). In addition, previous studies have reported that the seasonal change affects the level of melatonin hormone. Melatonin regulates the body's rhythms and responses towards light and dark. A patient takes a seat in front of a "light box" during therapy, for some time, usually in the morning hours. The box emits a full spectrum light, help to reset the body's daily rhythms of the patient (Koopman et al., 2005).

ii. Brain Stimulation Therapies

(A) Electroconvulsive Therapy (ECT)

ECT is usually given only to those patients whose condition has not improved after other treatments, such as SSRIs, TCA and CBT. ECT is preferred therapeutic treatment approach for the treatment of severe treatment-resistant depression (Fig. 8). However it is rarely used to treat bipolar disorder or schizophrenia (Payne and Prudic, 2009)

Fig. 8. Electroconvulsive therapy (http://www.nimh.nih.gov/health/topics/brain-stimulation-therapies/brain-stimulation-therapies.shtml)

Just before ECT is given, patient is sedated with general anesthetic along with a muscle relaxant to avoid any movement during the surgery. An anesthesiologist measures inspiration rate, pulse rate, heart beat and blood pressure during whole ECT surgical process, which is supervised by a skilled clinician. Electrodes are properly positioned at specific locations on the cranium. An electric stream passes through the brain via electrodes, causing a seizure that lasts generally from minutes to hour. The patient can resume normal activities after the ECT (Ujkaj et al., 2012). A typical treatment plan of ECT is about 3-4 times in a week until the patient's condition improves, which take six to twelve treatment schedules. The common unwanted effects linked with ECT are headache, stomach upset, memory problems and muscle aches.(http:// www.nimh.nih.gov/ health/ publications/depression).

(B) Vagus Nerve Stimulation (VNS)

VNS was initially developed for the treatment for epilepsy (Fig. 9). In addition, it had effects on mood, specially depressive symptoms. The pulses used for VNS therapy also modify some neuro-transmitters related with mood, including 5-HT, NE, γ-amino butyric acid (GABA) and glutamate (George et al., 2000). In VNS a device inserted under the skin that programmed to send electrical

signals through left side vagus nerve (http:// www.medscape.com/ viewarticle/ 804311).

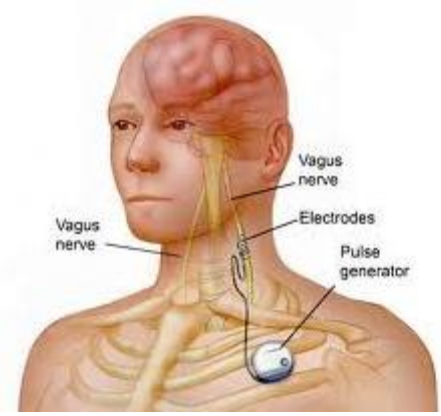


Fig. 9. Vagal nerve stimulation (http://www.sparrow.org/HealthLibrary/Content)

In 2005, the United States, Food and Drug Administration (FDA) approved VNS as a therapy of depressive disorder in particular situations—patient's condition has not improved after other treatment strategies, such as SSRIs, TCA and CBT. In spite of FDA approval, Outcomes of the previous studies using VNS make it controversial for the treatment of depression (http:// www.nimh.nih.gov/ health/ topics/ brain-stimulation-therapies/ brain-stimulation-therapies.shtml, Thase et al.,1995)

(C) Repetitive Transcranial Magnetic Stimulation (rTMS)

rTMS therapy is a non-systemic and non-invasive form of neuro-stimulation which modulates signal transduction through nerve cells in a specific location of the brain that has a role in depression, by supplying highly specific MRI-strength magnetic pulses (Fig. 10).

rTMS is more effective for treatment of major depression (http:// www. nimh.nih.gov/ health/ topics/brain-stimulation-therapies/brain-stimulation-therapies.shtml; Nemeroff, 2007).

For those patients who have not responded to minimum one anti-depressant, rTMS was allowed by the FDA in 2008, as a treatment for depression. rTMS targeted specific sites of brain (Schutter, 2009) in comparison with ECT which is generalized.

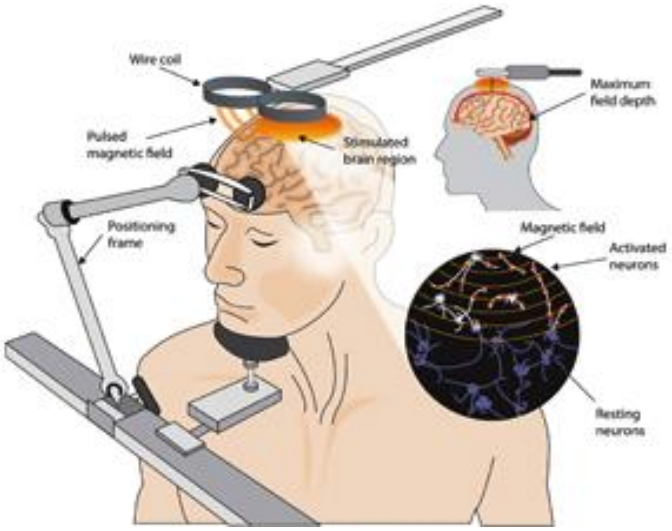


Fig. 10. Repetitive transcranial magnetic stimulation (http://tmssandiego.com/what-is-tms/)

A typical rTMS does not require anesthesia and session lasts 30 to 60 minutes. An electromagnetic coil is held in front of forehead closer to a region of brain involved in alteration of mood. Then, short electromagnetic beats are passed via coil. The electromagnetic pulses passes through the skull and small electrical currents excite neurons in the specified regions of the brain (Padberg and George, 2009). rTMS has unwanted side effects such as headaches with mild or moderate or no seizures (Paus and Barrett, 2004).

(D) Deep Brain Stimulation (DBS)

DBS was first developed as a treatment strategy to reduce symptoms of parkinson's disease (Fig. 11).

Speculated Mechanism for deep brain stimulation

Depolarization blockade, synaptic inhibition and depression, stimulation induced disruption of pathologic network activity (Schlaepfer et al., 2013).

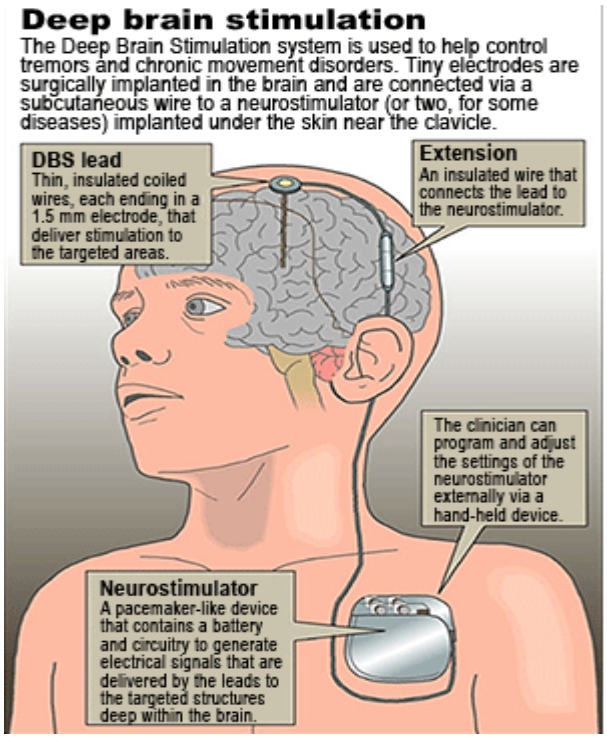


Fig. 11. Deep brain stimulation (http://speakcampaigns.org/deep-brain-stimulation/)

In DBS, when the patient is ready for surgery, two holes are made into his head. Then electrodes are placed on either side of a particular part of the brain. The electrodes are then connected to wires that run along with the body from the head to the chest, where a pair of generators (battery-operated) are fixed. Stimulation can be dosed, like a medication and the dose can be changed as often as is necessary (Lipsman et al., 2013). This technique is commonly used as alternatives to long-term medications. The frequency of the therapy depends on the severity of depression (http://www.nimh.nih.gov/health /topics/brain-stimulation-therapies/brain-stimulation-therapies.shtml).

1.4. Challenges in Depression Therapy

There are many anti-depressant drugs available in the market. The problem of relapse, treatment resistance and late onset, etc. leads to the failure of anti-depressant therapy. According to the WHO, depression is affecting approximately 121 million people all over the world. Moreover, depression is a complex disorder in terms of symptoms, co-morbidities and other complications. Despite the steady increase in the number of available anti-depressants (Papakostas and Fava, 2005), many patients still do not respond

to available anti-depressant medications. Unfortunately, nearly 50 years after the introduction of the first class of anti-depressants and despite the prevalence and consequences of MDD, no full proof therapy or treatment approach among specialists have emerged regarding the proper clinical management of patients with MDD, who do not experience satisfactory improvement in the symptoms following adequate treatment. The major problems encountered during the depression therapy are discussed below.

1.4.1. Delayed Onset

One challenge to the designing of an anti-depressant with faster onset of action is the very small number of animal models that are available to screen the NCEs in a pre-clinical setting. Conceptually, any drug that has a late onset of action would be expected to show weak/poor early efficacy but superior medium-term efficacy. It is observed that antidepressants take several weeks to show their effect in patients. However, newer potential anti-depressant therapies, which target 5-HT and NE neurotransmitter systems such as the SNRI (VLA) and the NaSSA (mirtazapine) have been introduced with an understanding that, they will produce faster onset of action. Evidence from preclinical studies are expected to substantiate the same (Rojo et al., 2005).

1.4.2. Poor Diagnosis

Numerous reasons may be responsible for missed or delayed diagnosis of depression. A proper diagnosis of depression disorder may be failed because depressive symptoms can be closely linked to many other co-morbid conditions. At the same time, depression can be associated with other conditions, i.e., severe pain and tiredness which may be due to fibromyalgia and or may not accompany depression. Simple psychological situations such as grief/sorrow may be mis-judged as depression. Such situations lead to the wrong diagnosis and treatment of depression (Edwards and Clarke, 2004).

1.4.3. Treatment Resistance

Previous reports have confirmed that about 50% of patients with depression fail to properly respond to standard anti-depressant therapy and more than 30% of these patients are resistant to a combination of treatments (Hirschfeld et al., 2002). People with treatment resistant depression (TRD) are more prone to have CNS disorders, which suggest that strong genetic basis responsible for this condition. They are also more susceptible to the psychological (e.g., death of a family members or loved ones, divorce, unemployment and financial issues) changes during their life time, which suggests that day-to-day stress plays a major role in this illness. People who are chronic drinker and using abusing substances are having more chances to develop TRD, as drinking alcohol can impair a person's mood as well as modulate the effectiveness of psychiatric medications (Koch et al., 2004; http://www.nami.org; Thase et al., 2007).

2. Review of Literature

Disorders of mood have been described since 4th century BC. In spite of this early acknowledgment, their aetiology is still a foundation of debate. Depression is now known as a complex disorder involving the whole body and the diagnosis of depression is based on a diverse syndrome. The criteria have gradually developed as documented by both the American Psychiatric Association (DSM-IV, 1994; DSM-V, 2013) and the WHO, Geneva (International Classification of Diseases, ICD-10, 1993), providing necessary guidance for both clinicians and researchers.

According to DSM-IV (APA), a depression manifests with symptoms at the psychological, behavioural and physiological stages. An episode necessitates the presence for at least 2 weeks of one or two core symptoms of depression: dysphoric mood and anhedonia (a loss of pleasure or interest that are normally enjoyable). In addition, the following four symptoms are commonly associated (three, if above mentioned core symptoms are present): disturbances of sleep, feelings of worthlessness or guilt, lack of concentration, increased or decreased psychomotor activity, sexual anhedonia, appetite disturbance or weight change and suicidal thoughts.

In the last decades, huge progress in the treatment strategies of depression has been achieved in terms of refractory cases, late responses and side effects related with standard anti-depressants are among the most important clinical problems and challenges. Similarly, therapeutic methodologies in treatment are often complicated in case of co-morbid depression with some other disorder(s). Thus, understanding the biological phenomenon that could involve such co-morbidity may offer a potential therapeutic approach.

2.1. Co-morbid Depression

The term “co-morbidity” has often been puzzling and is a bottleneck in the anti-depressant drug developments. Co-morbidity is generally referred as the co-existence of two diseases in one person, regardless of the fact that disorders are co-incidentally or casually linked (Feinstein, 1970; Krishnan et al., 2002). The phenomenon of co-morbidity is important concept because it has a

substantial impact on outcomes related to health, overall 'quality of life' (QOL), severe disability and health care use (Gijsen et al., 2001). Co-morbid CNS disorders occur with long lasting medical problems in many patients, causing marked destruction, work loss and work cutback (Kessler and Wang, 2008). Affecting around 10.3% of the U.S. adult population groups in a single year (Kessler et al., 1994), depression has become the predominant cause of disability in adults (Murray and Lopez, 1996). Depression increases symptomatic burden, functional impairment and worsens prognosis for co-morbid disorders such as heart disease, stroke, diabetes mellitus etc. (Mahesh et al., 2010b). Some of the previous studies have demonstrated an association between depression and some neurological problems (Fig.12).

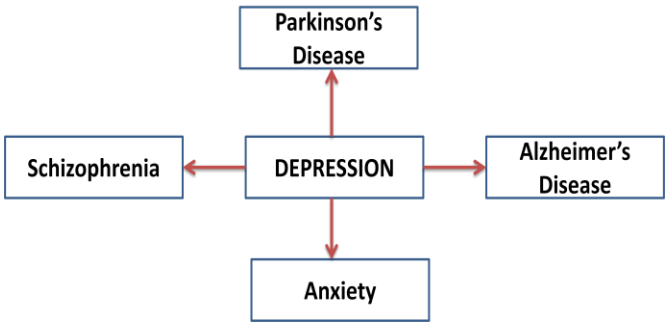


Fig.12. Co-morbid psychiatric disorders with depression (based on Kessler et al., 1994).

2.2. Co-morbid Symptoms

There are many symptoms that are general between anxiety and depression. Common symptoms include insomnia, loss of appetite and libido. On the other hand, symptoms that are not common between two conditions include social impairment, hyper-vigilance, which is typical symptom of anxiety and hopelessness, anhedonia or low mood symptoms, which are present in patients with depressive disorder.

According to DSM-IV guidelines for MAD disorder, an individual experiences at least four of the symptoms as given in the following table to be categorised as MAD disorder (Table. 4) (Gorman, 1996).

Table 4: DSM-IV/V Diagnostic Criteria for MAD Disorder

A. Long term or frequent dysphoric mood lasting at least 1 month

B.The dysphoric mood is followed by at least 1 month of 4 (or more) of the following 10 symptoms:

- Difficulty in concentrating on something
- Improper sleep
- Apprehension
- Emotional instability
- Natural aversion
- Exhaustion or tiredness
- Irritability and worry
- Desperation
- Low sense of worth
- Social isolation

C. The symptoms cause impairment in humans at social, occupational or other essential areas of life.

2.3. Pathophysiology of Co-morbid Depression/ Anxiety

The main feature of anxiety is uneasiness, apprehension and phobia, with associated insomnia mostly in the evening. It often leads to fatigue due to involvement of high energy. On the other side depression is a low-energy state, with loss of drive and enthusiasm. These differences in states of depression in anxiety and depression is more closely related to nor-epinephrine levels as there is increase in nor-epinephrine level takes place in anxiety and decrease in depression. It is also frequently linked with non-specific symptoms of pain (Liebowitz, 2004). The physiological role of neuro-transmitters involved in depression has been shown in Fig. 13.

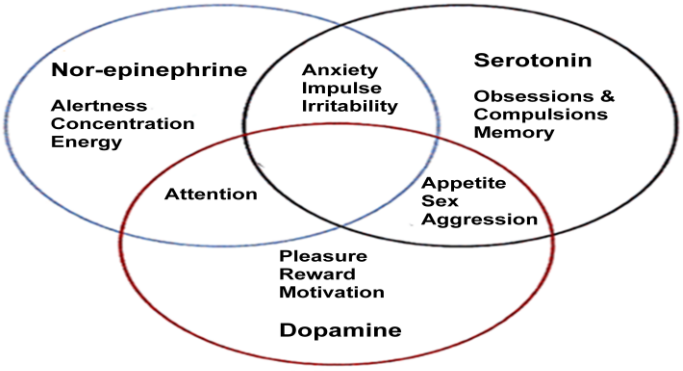


Fig.13. Physiological role of neuro-transmitters involved in depression (Blows, 2000; Garraway and Hochman, 2001)

Cagampang and Shin-chi (1994) demonstrated that in pineal gland of rats, 5-HT is released in three phases, namely: phase I; high and steady levels during a day, phase II; quick increase in synthesis, release of 5-HT, starting late evening or night, phase III; reduction in 5-HT levels beyond mid night. The reason for decline in 5-HT levels during night is that 5-HT gets converted to melatonin by pineal gland as night progresses (Snyder et al., 1965). Change in the levels of serotonin during the day has been shown in Table 5.

Table 5: Change in the level of serotonin during the day (24 hr)

Light intensity/ Neuro-transmitter	Morning (8 am)	Afternoon (12 pm)	Evening (6 pm)	Night (12 am)
Light intensity	***	*****	**	*
Serotonin (ng/g) brain tissue	+++	++++	++	+

* shows comparative light intensity during the day.
+ sign in table shows qualitative level of serotonin during the day.

2.4. Validation Criteria for Animal Models

The development of appropriate animal models for CNS disorders represents a major challenge. The problem in all animal model(s) and in particular, models for psychiatric conditions that are in part defined through subjective experience, is to define with clarity in the criteria, which allows assertion in the validity of the model.

a. Construct validity: theoretical rationale of the model Addresses by this validity. However, this evaluation largely relies on the proper knowledge of the pathophysiology of depression.

*b. Face validity: R*efers to the similarity between the behaviour, modelled in the animal and the symptoms of depression. A model which parallels multiple symptoms of human depression is considered valuable.

c. Predictive validity: Concerns the extent to which the model responds appropriately to anti-depressant effect as in humans (Willner, 1984).

Thus, understanding the possible advantages and disadvantages of the existing animal models is crucial for obtaining valid animal data to parallel and/or complement the available clinical outcomes. Different animal models/tests and paradigm with validity criteria and major neuro-transmitters involved have been shown in Table. 6 with their advantages and disadvantages.

2.5. Co-morbidity of Depression/Anxiety in CINV and Involvement of Serotonergic System

Depression and anxiety are neuropsychiatric disorders, which are generally coexisting with each other (Wittchen et al., 1998; Hirschfeld, 2002) Similarly, these disorders are also co-morbid with several chronic diseases and disorders such as cancer, cardiovascular diseases, diabetes, coronary disease, obesity, etc (Aina and Susman, 2006). Patients with chronic illness who exhibited co-morbidity of depression and anxiety showed higher severity of illness, higher chronicity, impaired work function that affected the 'Quality of Life' (QOL) than the patients not suffering from co-morbid depression and anxiety (Katon and Sullivan, 1990; Weitzner et al., 1997; Aass et al., 1997). The undiagnosed co-morbidity condition leads to an increased rate of hospitalization and also enhanced the suicide risk of the patient. The patients who are associated with co-morbid depression and anxiety have higher incidence of suicide attempt rates than the patients with an uncomplicated disorder (Hirschfeld, 2002; Brintzenhofe-Szoc et al., 2009).

Due to the presence of symptom similarities between the depression and anxiety, the presence of depression masks the presence of anxiety and vice-versa i.e. loss of appetite and insomnia are common symptoms of both depression and anxiety (Hirschfeld, 2002). In severe disease conditions, in particular chronic illnesses, the risk rate of co-morbid depression and anxiety is high. Up to 50% of the patients with severe medical illness will develop depression; this rate is also common for the development of anxiety disorder in chronic illnesses (Hirschfeld, 2002). The undiagnosed co-morbid depression and anxiety will leads to three fold increased likelihood of non-patient

compliance. The suicidal risk of uncomplicated anxiety disorder is 7%, whereas in association with depression, the risk rate is raised to 23.6%. Likewise, suicide rate in major depressive disorder without anxiety is 7.9% and in association with anxiety, the rate is raised to 19.8% (Hirschfeld, 2002).

The depression and anxiety are not only co-morbid with severe disease and disorder conditions; unfortunately these disorders are also associated with adverse effects of the treatments of chronic illnesses, such as cancer (Cankurtaran et al., 2008). The serotonergic system, specifically serotonin type-3 receptor is a common linker for the co-morbidity of depression and anxiety in adverse effects (nausea and vomiting) of cancer treatments.

Table 6: Animal models/tests and paradigm with validity criteria and major neuro-transmitters involved

Model	Species	Neuro-transmitter	Validity Criteria	Advantages	Disadvantages	Concept	Reference
FST (Despair-based acute model of depression)	Mice, Rat	5HT, DA & NE	Face and Predictive	(1) Sensitive to AD treatment (2) Easy to perform (3) Can be used for mice and rats	(1) Does not reliably detect SSRIs (2) Chances of hypothermia (3) Training is required	Exposure to unavoidable & inescapable stress, develop hopelessness	Porsolt et al. 1977
TST (Despair-based acute model of depression)	Mice	DA & NE	Face and Predictive	(1) More sensitive to AD treatments than FST (2) Training of animal is not required (3) Avoids problems of hypothermia	(2) Rats can not be used due to weight issue	Exposure to unavoidable & inescapable stress, develop hopelessness	Steru et al., 1985
CUMS (Stress based chronic model of depression)	Rat and Mice	(5HT, NE and corticosterone)	Face, Constructive and Predictive	(1) Better reflects the human situation cha- racterized (long term stress) (2) Effects evident after chronic treatment (3) Shows clear involvement of HPA axis	(1) Reproducibility is poor in behavioural abnormalities and their response to ADs within and between labs	Exposure to stressors leads disruption of neuroendocrine hormonal pathways	Ducottet et al., 2003

Model	Species	Neuro-transmitter	Validity Criteria	Advantages	Disadvantages	Concept	Reference
LPS induced depression	Mice, Rat	5-HT	Face, Constructive and Predictive	(1) Sensitive to chronic anti-depressants (2) Can be used for co-morbid depression and anxiety	(1) Also affects the other physiological functions of animal	Alteration of the neuro-inflammatory mediators	O'Connor et al., 2009
TBI (Lesion based chronic model of depression)	Rat	(5HT, NE, DA, GABA and Ach)	Face & Predictive	(1) Used to explore the role of different brain region in specific disorder (2) Model for co-morbid disorders	(1) mechanism of action poorly understood (2) Not specific for ADs	Weight drop on the brain regions leads the neural circuitry alteration	Heath and Vink, 1999
EPM (exploration based acute model of anxiety)	Mice, Rat	GABA, 5-HT	Predictive	(1) Permits a rapid screening of anxiety-modulating drugs (2) Investigate the psychological and neuro-chemical basis of anxiety	(1) Performance on exploration-based locomotor activity can produce a false positive increase or decrease in anxiety-like behaviour	Natural aversion of rodents for heighted/ open areas	Biala and Kurk, 2008
L/D (natural aversion to **illumination** based model of anxiety	Mice, Rat	GABA, 5-HT, NE	Predictive	(1) Permits a rapid screening of anxiety-modulating drugs (2) Easy to use, without the prior	(1) Different measures and procedures used by different laboratories (2)	Innate aversion of rodents to brightly illuminated areas	Crawley, 2000

Model	Species	Neuro-transmitter	Validity Criteria	Advantages	Disadvantages	Concept	Reference
				training of animals	contribute to a number of false positive results.		
5HTP-induced HTR (Pharmacology based model of depression	Mice, Rat	5-HT	Constructive and Predictive	(1) Sensitive to acute ADs treatment (2) Direct assess the effects of a compound on neuro-transmitter levels. (3) Specific test for 5-HT neuro-transmitter	(1) Limited in their face validity	Increase neuro-transmitter (5-HT) in synapse	Bhatt et al., 2013a
RIH (Pharmacology based model of depression	Mice, Rat	5-HT, NE and DA	Constructive and Predictive	(1) Sensitive to acute ADs treatment	(1) Limited in their face validity (2) Not a specific test for particular neuro-transmitter	Increase neuro-transmitter relief from depressive symptoms	Devadoss et al., 2010

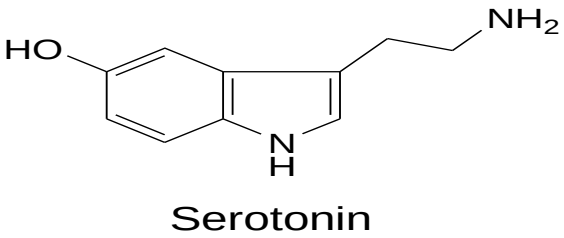
2.6. Evidence for the Involvement of 5-HT_3 Receptors in Depression/Anxiety and Cancer Chemotherapy Induced Nausea and Vomiting (CINV)

5-HT_3 receptor antagonists are gold standard for the treatment of CINV. The involvement of 5-HT_3 receptors in CINV is well known for several years. These receptors are located in median raphe, hypothalamus, hippocampus and amygdala, which are neural correlates of depression and anxiety (Kidd et al., 1993; Kilpatrick et al., 1987). Several animal studies demonstrated the involvement of 5-HT_3 receptors in depression and anxiety (Rajkumar and Mahesh, 2010); serotonin type-3 receptors antagonists reverse the escape deficits in rat learned helpless test, a sensitive anti-depressant screening protocol (Martinez-Turrillas et al., 2005). The anti-depressants, such as fluoxetine, imipramine, desipramine, phenelzine, iproniazid, mirtazapine and reboxetine inhibit the 5-HT current mediated by 5-HT_3 receptor in rat (Fan, 1994a,b; Eisensamer et al., 2003).

A standard 5-HT_3 receptor antagonist, ondansetron potentiates the anti-depressant (increase the anti-immobility) effect of SSRIs, indicating the role of serotonin type-3 receptor in depression (Redrobe and Bourin, 1997). Similarly, several pre-clinical studies demonstrated the anxiolytic effect of 5-HT_3 receptor antagonist, for example: tropisetron proved good as an anxiolytic, ondansetron abolished the emotion-potentiated startle response (Harmer et al., 2006. Ondansetron also reduced the anxiety and depression scores in patients with OCD (Hewlett et al., 2003). The aforementioned discussion clearly evidences the involvement of 5-HT_3 receptors in depression, anxiety and CINV.

2.7. Pharmacology of Serotonin

Serotonin (Fig. 14), a major neuro-transmitter identified first from serum and chemically known as 5-hydroxytryptamine, 5-HT. Serotonin was extensively studied for its several biological effects in the last few decades following its detection in the year 1948 (Rapport et al., 1948a,b). Serotonin was named based on the Latin word serum and Greek word tonic, the term serum represents isolation from serum and tonic refers to vasoconstrictor nature.



Serotonin

Fig. 14: Structure of serotonin

2.7.1. Biosynthesis

Serotonin is biosynthesized from the essential amino acid, L-tryptophan in 2 steps as represented schematically in Fig. 15 (Nichols and Nichols, 2008). It is largely synthesized in enterochromaffin cells followed by brain, heart, kidney and to some extent in the platelets (Tyce, 1990).

2.7.2. Storage and Release

The synthesized serotonin is stored in vesicles along with macromolecules known as serotonin binding proteins (Standford, 2001). In central nervous system the synthesized serotonin is stored in the pre-synaptic neurons (Mohammad-Zadeh et al., 2008). The release of serotonin occurs through exocytosis and controlled by the auto and heteroreceptor of serotonin, 5-HT1A and 5-HT1B/1D, respectively (Standford, 2001). Serotonergic neuro-transmission in synapse has been shown in Fig. 16.

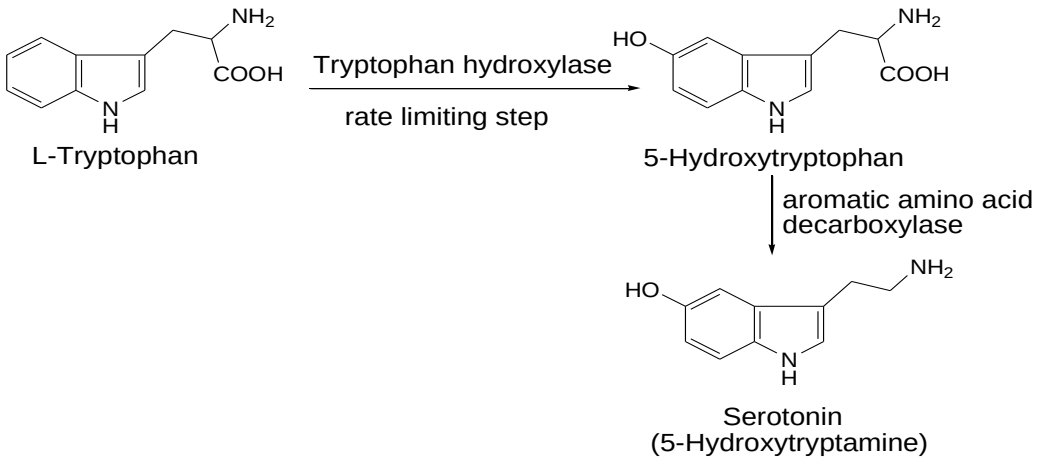


Fig. 15. Serotonin biosynthesis

2.7.3. Distribution

More than 90% of the body serotonin is located in the peripheral system such as enterochromaffin cells, platelets, cardiovascular system and kidney. The 99% of the body serotonin is located intracelluarly; the availability of serotonin in tissues is mainly concerned with synthesis and metabolic rate of serotonin (Tyce, 1990), where only 5% of tryptophan is converted into serotonin due to limited availability of tryptophan hydroxylase enzyme. The 90% of the body serotonin is located in the gastro-intestinal tract (GIT) (Tyce, 1990); a small percent present in platelets and all the regions of brain (Settembrini and Villar, 2004).

2.7.4. Metabolism

5-hydroxyindoleacetic acid (5-HIAA) is a major metabolite of serotonin, which is pharmacologically inert and hence is completely excreted in urine unchanged (Tyce, 1990; Standford, 2001). In the pineal gland, serotonin is metabolized into melatonin. Besides these pathways, glucuronidation, sulfation, and N-methylation also occur as minor metabolic pathways of serotonin (Tyce, 1990).

2.7.5. Pharmacological Actions

Gastro-Intestinal Tract: Serotonin may enhance or decrease the gastric motility via their receptors. In esophagus, it causes either relaxation or contraction, which is based on the species. Serotonin receptor on vagal and other afferent neurons and on enterochromaffin cells plays a major role in vomiting (Grunberg and Heslath, 1993).

Platelets: The presence of serotonin in platelet helps the aggregation of platelets, which in turn helps the blood clotting processes (Hilton and Cumings, 1971).

Central Nervous System: Serotonin present, in rostral nuclei, of this system helps in temperature maintenance, appetite, sleep cycles, emesis and behaviour. Projections from caudal nuclei involve in nociception and motor tone (Mohammad-Zadeh et al., 2008).

Blood vessels: Depending on the strain, type of capillary and status of the endothelial cell membrane, exogenous 5-HT can be a constrictor or dilator of a blood vessel (Mohammad-Zadeh et al., 2008).

Cardiovascular System: In the heart, 5-HT is produced as a positive chronotrope, positive ionotrope and mediator of mitogenesis of the cardiac myocyte (Nebigil and Maroteaux, 2001; Brattelid et al., 2004).

Kidney: Serotonin functions as a mesangial cell mitogen, increases renal perfusion pressure when given exogenously and it also promotes sodium retention and phosphate loss (Moran et al., 1997; Berndt et al., 2001).

2.7.6. Serotonin Receptors

Targeting 5-HT is interesting for the development of newer anti-depressants. 5-HT and its receptors are distributed in CNS, peripheral nervous system (PNS), as well as in a number of non-neuronal tissues in the gut, cardiovascular system and blood. 5-HT has been involved in the pathophysiology of many disorders, including depression, anxiety, social phobia, psychosis and OCD; in addition to headache, high blood pressure, eating disorders, nausea-vomiting and irritable bowel syndrome are known to modulate mood, emotion, sleep, and appetite (Hoyer et al., 2002).

Based on the signal transduction and amino acid sequence, now the serotonin receptors are classified into seven major types ($5\text{-}HT_{1\text{-}7}$) (Nichols and Nichols, 2008; Lanfumey et al., 2004). All the serotonin receptors belong to the super family of G-protein coupled receptor (GPCR), except $5\text{-}HT_3$ receptor which is a superfamily of ligand gated cation channel receptor. Table 7. illustrates the type, location and function of serotonin receptors (Nicholas and Nicholas, 2008; Rajkumar and Mahesh, 2010). In serotonin receptors, $5\text{-}HT_3$ receptor is unique, not only with respect to signal transduction and amino acid sequence but it's also distinct with respect to the involvement in various physiological and patho-physiological conditions. Since, antagonism of this ligand gated ion channel

receptor in various pre-clinical studies expressed beneficial effects in depression, anxiety, schizophrenia, cognition, pain, etc (Walstab et al., 2010).

Table 7: Serotonin receptor types, location and their functions

Receptor subtype	Location: Function
5-HT_{1A}	CNS: neuronal inhibition, behavioural effects (sleep, depression, anxiety and thermoregulation)
5-HT_{1B}	CNS: behavioural effects Vascular: pulmonary vasoconstriction
5-HT_{1D}	CNS: behavioural effects Vascular: cardiac function and movement
5-HT_{1E}	CNS: cognition, memory process
5-HT_{1F}	CNS: regulates the cerebrovascular functions and dural inflammation
5-HT_{2A}	CNS: neuronal excitation, behavioural effects Smooth muscle: contraction, vasoconstriction/dilatation Platelet: aggregation
5-HT_{2B}	CNS: post-synaptic inhibition, behavioural effect Vascular: pulmonary vasoconstriction Heart: regulate the cardiac functions and structure
5-HT_{2C}	CNS: choroid plexus, CSF secretion, behavioural effects
5-HT_3	CNS: behavioural effects, emesis PNS: neuronal excitation, emesis, GIT motility
5-HT_4	CNS: behavioural effects PNS: GIT motility Heart: regulate the ion of cardiac functions and structure
5-HT_5	CNS: behavioural effects; motor control, anxiety, depression, learning, memory consolidation, adaptive behaviour.
5-HT_6	CNS: behavioural effects; cognition, mood,
5-HT_7	CNS: behavioural effects; sleep, circadian rhythms, mood

2.7.7. An Overview of 5-HT_3 Receptor

More than a fifty years before, 'the 5-HT_3 receptor' was demonstrated as the so-called 'M receptor' in the ileum of guinea-pig (Gaddum & Picarelli, 1957). The high degree of receptor variability emphasised the functional role of 5-HT and pointed to an amazing variety of function. Specifically targeting 5-HT receptor sub-types at different sites might have allowed a better therapeutic approach on individual basis. Some recent studies in cellular genetics give direction towards person specific therapeutic strategies treating complicated disorders such as CNS and gastrointestinal disorders as well as unravelling pharmacogenetic s using person specific drug response.

a) Basic Pharmacology of 5-HT_3 Receptors

Serotonin type-3 (5-HT_3) receptors are ligand gated ion channels exhibits pentameric structure and belong to superfamily of Cys-loop receptors. Activation of receptor causes fast excitatory modulation and release of neuro-transmitter depending on their localisation in specific area of neuron. 5-HT_3 receptors are expressed in the CNS in regions participated in the nausea, vomiting reflex, perception of pain, reward centre, memory and anxiety control. They are present on a wide range of nerve and immunological cells, in the periphery (Gaddum and Picarelli, 1957; Davies et al., 1999). 5-HT_3 receptors are known to be involved in vomiting, pain, drug abuse, CNS and gastrointestinal disorders (Walstab et al., 2010). The structure of typical 5-HT_3 receptor has been shown in Fig. 17.

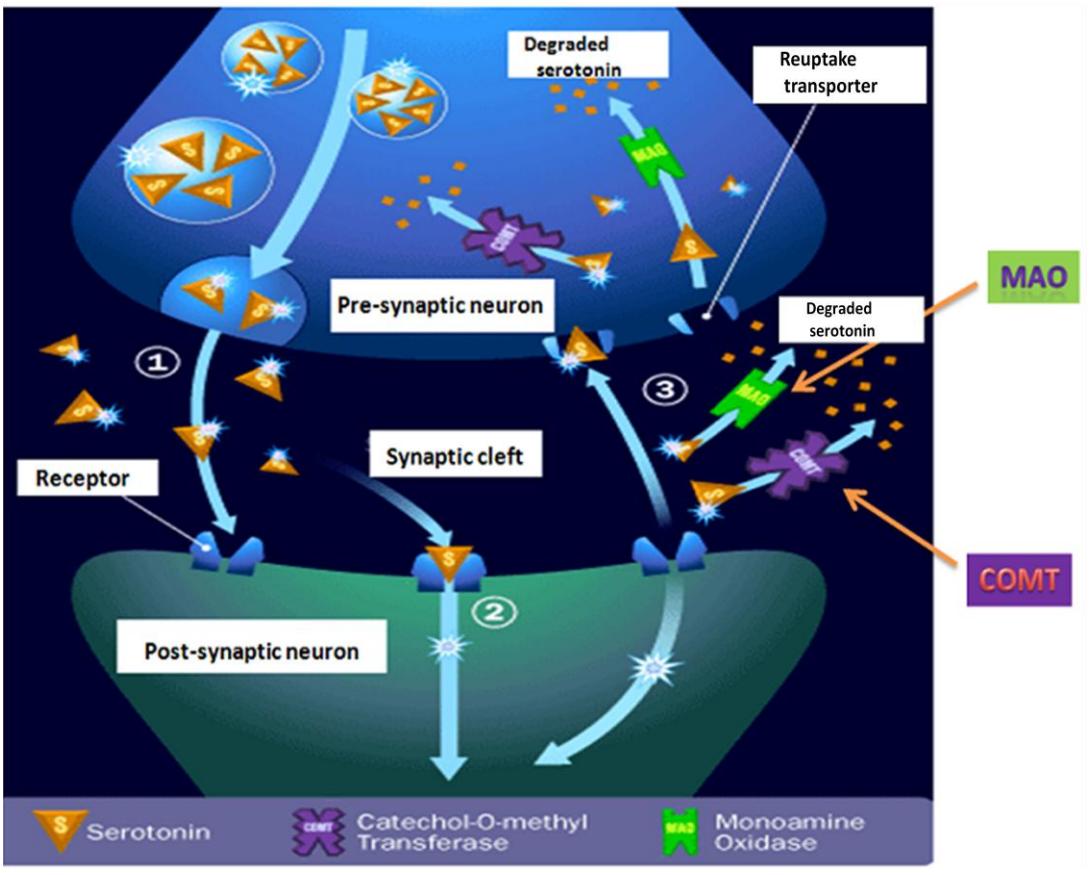


Fig 16. Serotonergic neurotransmission (http://health.howstuffworks.com/human-body/systems/nervous-system)

c) Expression

Binding studies using the serotonin type-3 receptor antagonist [3H] GR65630 has given preliminary proof of a 5-HT_3 receptor presence in the rat brain (Kilpatrick et al., 1987). Clinical studies using specific 5-HT_3 receptor binding ligands showed heterogeneous expression of these receptors throughout the brain within the brainstem, e.g. nucleus tractus solitarius, area postrema, spinal trigeminal nucleus as well as some specific parts of forebrain such as hippocampus, amygdala, nucleus accumbens, putamen, caudate (Parker et al., 1996).

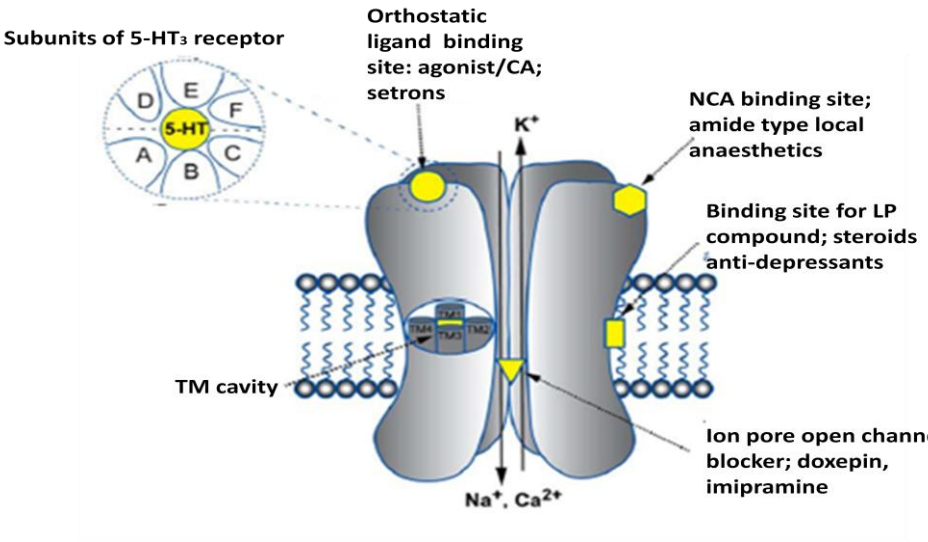


Fig.17. Structure of 5-HT_3 receptors; CA-competitive antagonist; NCA- noncompetitive antagonist; LP-lipophilic drugs; TM-trans membrane (Walstab et al., 2010)

Preclinical research proved that approximately 80% of the 5-HT_3 receptors located pre-synaptically associated with axons and nerve terminals except for the hippocampus part of the brain, where they locate mainly post-synaptically in somato-dendritic regions (Miquel et al., 2002). The 5-HT_3 receptors on neuronal endings modulate the release of other chemical messangers such as DA, cholecystokinin, glutamate, Ach and GABA (Hannon and Hoyer, 2008). Serotonin type-3 receptors expressed in the peripheral sites that include vagal innervations from the heart and GI tract are also of functional importance (Malinowska et al., 1995).

A recent research using 5-HT_{3A}- and 5-HT_{3B}-selective antibodies evidently exhibited presence of both receptor subunits within the hippocampus region of

human brain (Brady et al., 2007). Later on, the expression of 5-HT_{3A} was validated in myenteric plexus ganglia of the GI tract of human. Moreover, radioligand binding studies have authenticated the presence of serotonin type-3 receptor binding sites in the GI tract myenteric plexus (Bottner et al., 2010). In GI tract of human within the submucosal plexus the presence of the 5-HT_{3A} and 5-HT_{3B} subunits have already been recognized (Michel et al., 2005; Gershon, 2005).

Expression of the 5-HT_{3A} subunit has also been found peripherally in immunological cells such as macrophages and thrombocytes (Stratz et al., 2008). This emphasizes the importance of 5-HT_3 receptors in immunological reactions and inflammation processes and indicates that they may possibly be involved in disorders like atherosclerosis, tendomyopathies and fibromyalgia.

d) Preclinical Investigation

The preliminary investigations (i.e. behavioural, neuro-chemical and electrophysiological) on 5-HT_3 receptor antagonists in the early 1990s marked the inception of research into the role of 5-HT_3 receptors in CNS disorders including depression.

- Systemic administration of tropisetron prevented restraint stress-induced dopamine release in the nucleus accumbens and prefrontal cortex in rats, which indicating that 5-HT_3 receptors mediate stress-dependent activation of dopaminergic neurotransmission (Imperato et al., 1990).
- Acute administration of ondansetron, significantly reduced glucose utilization in the limbic regions of the rat brain, especially the median raphe nucleus, which is associated with depression (Mitchell and Prat, 1991).
- In rat FST, tropisetron exhibited anti-depressant-like effects and pretreatment with mCPBG, a potent high affinity 5-HT_3 agonist (Kilpatrick et al., 1990), attenuated the anti-depressant- like effects of tropisetron, imipramine, desipramine and mianserin (Nakagawa et al., 1998).
- Pindolol exhibited an additive anti-depressant effect when combined with a low dose of ondansetron indicating a link between 5-HT_{1B} and 5-HT_3 receptor function in mouse FST (Bourin et al., 1998).

- The clinical anti-depressant effects of N-methyl D-aspartate (NMDA) receptor antagonists are attributed to their noncompetitive 5-HT_3 receptor antagonistic property (Rammes et al., 2001).
- The hypothesis of the anti-depressant effects of 5-HT_3 receptor antagonists and the role of 5-HT_3 receptors in the neurobiology of depression is strengthened by recent preclinical reports. MDL 72222 (bemesetron), a selective 5-HT_3 receptor antagonist, has been shown to reduce the duration of immobility in the mouse tail suspension test (TST), and the anti-depressant-like effects are augmented by ketamine (Kos et al., 2006).

e) Probable Mechanism of Action: Preclinical Investigation

The putative mechanism of anti-depressant action of 5-HT_3 receptor antagonists is based on the behavioural and neuro-pharmacological investigations that have been conducted in rodents. To propose a mechanism of action, it is vital to conceptualize the effect of 5-HT_3 receptor antagonism on various neuro-transmitter systems. In accordance with the monoamine hypothesis of depression, enhancement in serotonergic neurotransmission is deemed to be a requisite for a candidate anti-depressant drug.

The results from the rodent anti-depressant assays indicate that the 5-HT_3 receptor antagonists:

(i) decreased the duration of immobility in FST and TST.

(ii) increased the swimming behaviour in FST (Mahesh et al., 2007; Ramamoorthy et al., 2008).

(iii) potentiated anti-depressant- like effects of serotonin and nor-epinephrine reuptake inhibitors and SSRIs in FST (Mahesh et al., 2007; Ramamoorthy et al., 2008).

(iv) reversed olfactory bulbectomy-induced hyperactivity (Ramamoorthy et al., 2008).

(v) alleviated reserpine-induced hypothermia; and (vi) potentiate 5-hydroxytryptophan- induced head twitch responses (Mahesh et al., 2012).

At low concentrations, 5-HT_3 receptor antagonists inhibit the postsynaptic 5-HT_3 receptors, which mediate a fast excitatory potential in the limbic brain regions (Sugita et al., 1992). Although the cascade of events following the fast transmission blockade remains elusive, an overall anti-depressant-like behaviour is conceivable. Postsynaptic 5-HT_3 receptor antagonism in serotonergic neurons can facilitate specific binding of 5-HT to other postsynaptic receptors such as 5-HT_{1B} (Bourin et al., 1998), 5-HT_{2A} and 5-HT_{2C}, thereby aiding in serotonergic transmission (Fig. 18) as observed with the novel anti-depressant, mirtazapine (Anttila and Leinonen, 2001). At higher dose levels, the presynaptic and somatodendritic 5-HT_3 receptor blockade inhibits 5-HT release, eventually reducing the synaptic 5-HT levels that predispose to depression- like effects (Ramamoorthy et al., 2008).

Besides 5-HT, the 5-HT_3 (hetero) receptor located on nerve terminals, alters the release of other neuro-transmitters, namely NE, DA, GABA and acetylcholine (ACh). The accumulated evidence suggests that inhibition of this receptor has a variable impact on synaptic levels of these neuro-transmitters, consequently affecting behaviour. For example, in the rat hippocampus, stimulation of 5-HT_3 receptors in the neuron terminal field facilitates 5-HT release (Martin et al., 1992) and mediates the inhibitory effect of 5-HT on potassium-evoked NE release (Matsumoto et al., 1995). In rat hypothalamus, tropisetron has been shown to prevent the inhibitory effect of 5-HT on NE release. Since increases in synaptic NE levels in the aforementioned regions has been related to anti-depressant-like effects (Delgado and Moreno, 2000), the involvement of 5-HT_3 receptor is anticipated in such an effect. However, there are reports on neuro-chemical effects of 5-HT_3 receptor antagonists that may be inconsistent with anti-depressant effects.

Several studies have indicated that 5-HT_3 antagonists modulate evoked DA release in three separate dopaminergic pathways: the mesolimbic, mesocortical and nigrostriatal (Porras et al., 2003) receptors facilitate 5-HT neurotransmission. At low concentrations, 5-HT_3 receptor antagonists inhibit the postsynaptic 5-HT_3 receptors, which mediate a fast excitatory potential in

the limbic brain regions (Sugita et al., 1992). Although the cascade of events following the fast transmission blockade remains elusive, an overall anti-depressant- like behaviour is conceivable. Postsynaptic 5-HT_3 receptor antagonism in serotonergic neurons can facilitate specific binding of 5-HT to other postsynaptic receptors such as 5-HT_{1B} (Bourin et al., 1998), 5-HT_{2A} and 5-HT_{2C}, thereby aiding serotonergic transmission as shown in Fig. 18.

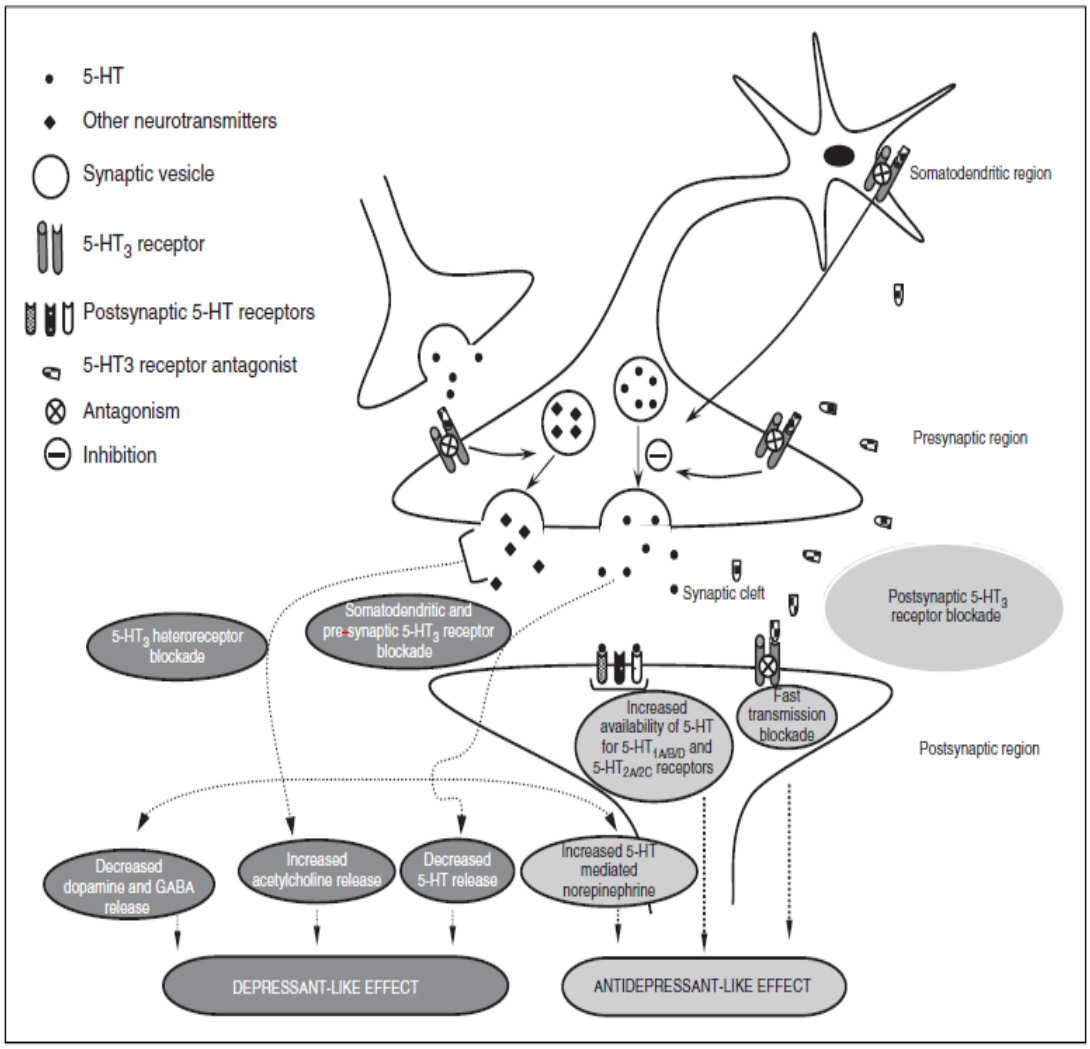


Fig. 18. Schematic representation of the depression related neuronal events (at the synapse in response to the 5-HT_3 antagonism (Rajkumar and Mahesh, 2010).

f) Therapeutic Potential of 5-HT_3 Receptor Antagonists

Several preclinical rodent studies using 5-HT_3 antagonists demonstrated that 5-HT_3 receptors are participated in the physiology of emotion, cognition and memory, pain perception and GI tract. Thus, they may possibly be involved in the pathophysiology of CNS and gastrointestinal disorders. Besides their importance in the treatment of CINV, promising research on the therapeutic

usefulness of 5-HT_3 antagonists has been reported for therapy of CNS disorders such as insomnia, fear, depressive disorder, psychosis, IBS, memory dysfunction, drug addiction (Rajkumar and Mahesh, 2010). The therapeutic potential of 5-HT_3 antagonists has been reviewed extensively most recently in Rajkumar and Mahesh (2010).

g) Potential Role of 5-HT_3 Receptor Antagonists in Anxiety and Depression

Several preclinical studies demonstrated that 5-HT_3 antagonists have anxiolytic potential by hindering limbic hyperactivity response (Rajkumar and Mahesh, 2010). Since 5-HT_3 receptors are expressed in areas of brain involved in the regulation of anxiety and mood. 5-HT_3 antagonists can cross the blood–brain barrier effectively (Wolf, 2000). They act as excellent therapeutic candidates. In spite of the huge pharmacological significance of these compounds, no pharmacodynamic strategy has been successful till date (Thompson & Lummis, 2007). Several researches on human reported the positive effects of 5-HT_3 antagonists in the treatment of anxiety: tropisetron has shown anxiolytic effects by blockade of 5-HT_3 receptor (Lecrubier et al., 1993). The participation of 5-HT_3 receptors in anxiety is exhibited by research studies of 5-HT_{3A}-knock out mice, which exposed that 5-HT_{3A} regulates depression- and anxiety-like behaviours (Kelley et al., 2003). It is quite good to conclude that 5-HT_3 receptors are mediated the modulation of anxiety-like behaviour and that pharmacological treatment targeting 5-HT_3 receptors could be another choice for the treatment of anxiety disorders. Evidence for the importance of 5-HT_3 antagonists in the pharmacotherapy of depression stems from human trials in which patients affecting from complicated disorders such as fibromyalgia and bulimia showed improvement of the co-morbid depression (Faris et al., 2006; Mahesh et al., 2013). In conclusion, the small studies examining 5-HT_3 receptor antagonists in the treatment of anxiety and depression were hopeful, but further large scale human trials would be required to undoubtedly prove their effectiveness as anxiolytic and anti-depressive pharmaceutical agents in the clinical setups.

2.8. Gap in Research

Depression is a serious mental disorder that will affect most of our lives at some point of their lifetime. Significant advances have been made to evaluate the mechanism(s), pathophysiology, intracellular signaling pathways of depression and anxiety disorders. More advanced diagnostic features have offered more precise differences between the discrete depressive, anxiety and mixed anxiety-depressive disorder seen in humans. Studies of anti-depressants and anxiolytic drugs are giving more substantial outcomes about the underlying neurobiological pathologies responsible for these CNS disorders. In the past, researchers explored to the study the treatment of depression, which mainly focused on the fast acting anti-depressants. Treatment with anti-depressants takes several weeks to demonstrate improvement in symptoms in humans though the medications prescribed alter brain biochemistry beginning with first dose (Hamet and Tremblay, 2005). In addition, some studies indicate that anti-depressant effects result from slow-onset adaptive alterations within the brain cells or neurons (Fig.19 and 20).

Despite the steady increase in the number of available medications, many patients do not respond properly to available treatments. Major drawbacks of available therapy are:

- Delayed onset of action with the available drugs, poses several challenges in the treatment of depression.
- More side effects of existing anti-depressant therapy such as SSRI and TCA
- Further, the high chances of co-morbid depressive disorder with anxiety can be responsible for changes in diagnosis during the therapy of disorder, further confounding the identification of candidate drug molecule.

Efforts to enhance the effectiveness of anti-depressant therapies have focused on decreasing the lag phase of the response and on finding approaches for treatment-resistant cases. Some selective studies are required on this basis on more specific targets that could bring in efficiency of the treatment and ultimately lead to improve and faster activity. Till date in the present clinical

setups the anti-depressants take more than two weeks. The design of animal model(s) and development of faster acting therapeutic regimens in a scientific and a methodical process requires a clear understanding of the problems and complexities of depression as well as its standardization with valid animal models. Effect of stress and anti-depressant therapy on neuronal differentiation have been shown in Fig. 20.

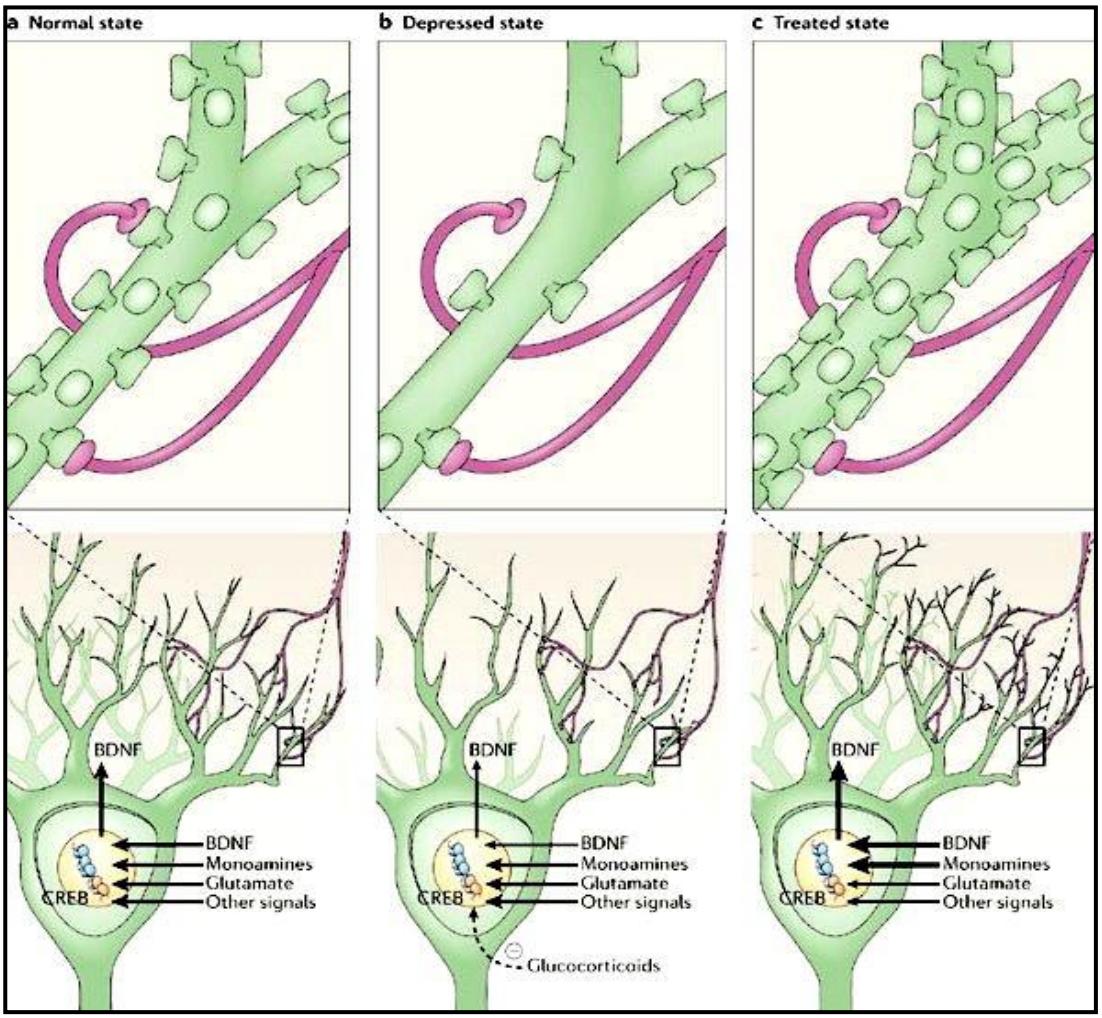


Fig.19. Intracellular neurotrophic mechanism beyond the receptor level indicating the mechanism of depression and anti-depressant action (Olivier and Nestler, 2006). (a). Shows a normal hippocampal pyramidal neuron and its innervation by glutamatergic, monoaminergic and other types of neuron. Its regulation by BDNF, which is derived from the hippocampus or other brain areas, is also shown. (b) Severe stress causes several changes in these neurons, including a reduction in their dendritic arborization, and a reduction in BDNF expression (which could be one of the factors mediating the dendritic effects). The reduction in BDNF is mediated partly by excessive glucocorticoids, which could interfere with the normal transcriptional mechanisms (for example, through CREB that control BDNF expression. (c) Anti-depressants produce the opposite effects to those seen in b: they increase dendritic arborization and BDNF expression of these hippocampal neurons.

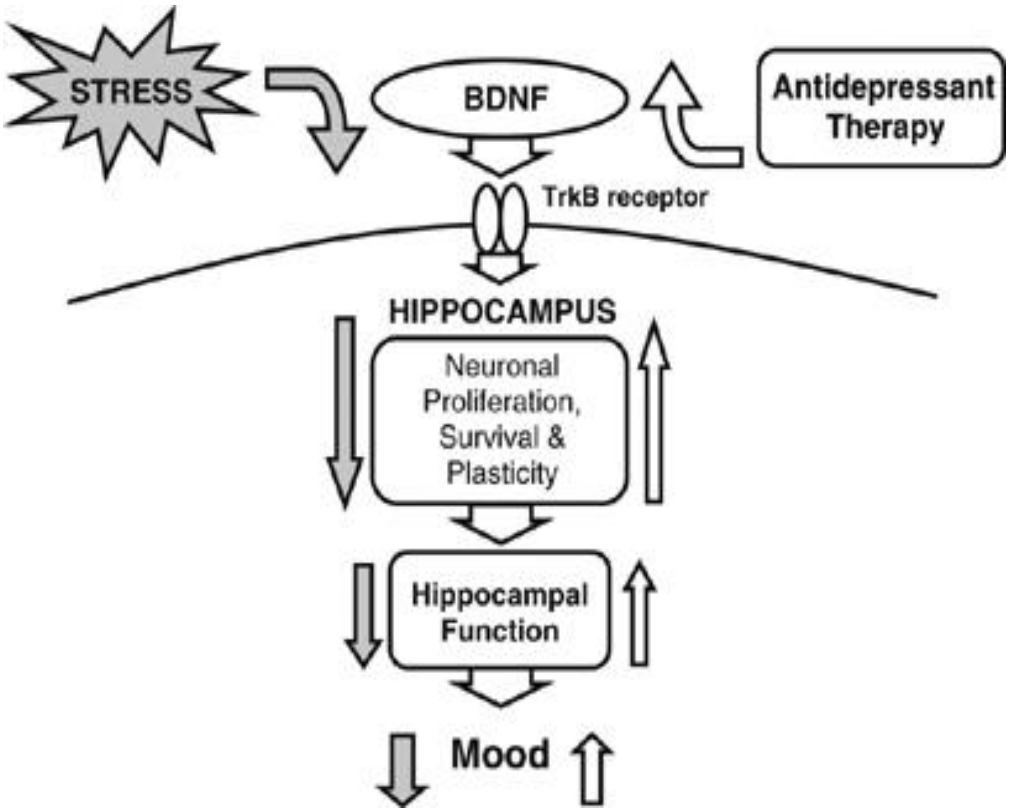


Fig. 20. Effect of Stress and Anti-depressant Therapy on neuronal growth (Groves, 2007)

Several human studies demonstrated earlier depicting favourable pharmacological actions of 5-HT_3 receptor (ligand gated ion channel-fast synaptic transmission) antagonists in the treatment of anxiety and depression; have also other effects such as i) schizophrenia; ii) IBS; iii) cognitive dysfunction; iv) substance abuse and addiction; v) nausea and vomiting. Blockade of 5-HT_3 receptor by tropisetron has shown reduction in anxiety symptoms (Lecrubier et al., 1993). It is rational to summarize that 5-HT_3 receptors are responsible for anxiety-like behaviour and that pharmacotherapy targeting 5-HT_3 receptors could be an another option for the treatment of depression and anxiety disorders.

3. Broad Objectives

One of the important reasons to design and screen for co-morbidity is that unrecognised depression/anxiety co-morbidity is associated with an increased rate of hospitalization due to psychiatric condition and increased rate of suicide (Kuzel, 1996; Gorman, 1996). Several factors have led to the suggestion that depression and anxiety are actually similar kind of diseases; generally they coexist with each other; there are common symptoms between the two pathological conditions; Similar type of therapeutic strategies to treat both mental states; the similar neuro-transmitters are involved in both conditions and stress plays a significant role in pathophysiology of both disorders. The aim of the current research work was to establish a rodent model of co-morbid depression linked with anxiety in the laboratory setting and to study pathophysiological and neurobiological aspects related to the co-morbid model. In particular, it was intended to investigate behaviour linked to co-morbid depression and anxiety. Many studies have examined psychopharmacological treatment strategies for depressed person co-morbid with anxiety, which consider the administration of anti-depressants, anxiolytics and other potential and novel compounds. While some results of the studies advocate that standard evidence-based treatments are effective in reducing the severity of both the conditions, other studies advocate some modification in older therapeutic approcahes to reduce the treatment resistance (Kuzel, 1996; Gorman, 1996).

To date, though, no psycho-pharmacologic or psycho-therapeutic treatment has been confirmed for the treatment of depression with co-morbid anxiety. In order to better realize what therapies are most beneficial in treating depressed patients with co-morbid anxiety disorders, it is first critical to understand the various symptoms of co-morbid disorders. Therefore, there is a requirement for screening tests that assess similar pathophysiological mechanism(s), risk factor, symptoms and co-morbidity associated with these disorders. SSRIs, in example are effective in the treatment of the depression and anxiety, difference in dose, time of onset of action and in some cases, mechanism may be different. For comparative study, four major class of anti-depressants like SSRI

(Fluoxetine), SNRI (Venlafaxine), NDRI (Bupropion) and TCA (Desipramine) were used in the current study. The tested compounds (quinoxaline derivatives), are novel 5-HT_3 receptor antagonist (Rajkumar and Mahesh, 2010, Mahesh et al., 2012, Bhatt et al., 2013a) with higher pA_2 value were selected for co-morbid studies. The effect of the 5-HT_3 receptor antagonists (**"6g", "6n","6o", "6p"**) were evaluated for their co-morbid potential as a separate study. As 5-HT_3 receptors antagonists freely pass the blood–brain barrier, these compounds have been found to be ideal therapeutic candidates for neuro-behavioural studies (Greenshaw, 1993). Further, the effects of the novel compounds in chronic unpredictable mild stress (**"6g", "6n"**), LPS induced depression (**"6g"**) model was also evaluated find out change in various behavioural and biochemical parameters. Moreover the effect of the tested compounds on neuro-transmitter (serotonin, dopamine and nor-epinephrine) and neurotrophic factor (BDNF) levels were evaluated in animal models of co-morbid depression and anxiety (OBX, TBI) as well as in LPS induced depression (serotonin levels were measured) model.

3.1. Specific Objectives of the Proposed Research

- To best address the nature and scope of the problems of treatment resistant and co-morbid depression.
- To design behavioural models to reflect the patho-physiological and neuro-behavioural and mechanistic aspects in the depression and anxiety model.
- To develop and standardize *in-vivo* animal models for depression and anxiety and co morbidity.
- To evaluate a anti-depressant and anxiolytic potential of in house synthesized novel 5-HT_3 receptor antagonists (**"6g", "6n", "6o", "6p"**) following acute and chronic administration in the battery of standardized rodent *in vivo* anti-depressant and anxiolytic assays.
- To evaluate the effect of synthesized novel 5-HT_3 receptor antagonists (**"6g", "6n", "6o", "6p"**) on the endocrine factor (corticosterone), neurotrophic factor (BDNF), neuro-transmitter (5HT, NE and DA) and oxidative (TBARS and nitrite)/antioxidant (SOD, CAT and GSH) enzyme concentration to find out/postulate the possible mechanism of action.

3.2. Plan of Work

In 2009, when the study was planned, the overall aim of the study was to investigate the correlation of anxiety with depression. To do so, it was decided to first induce various brain lesions by injury or surgery followed by behavioural tests simulating the human co-morbid symptoms. In the course of the study, several factors have influenced how the co-morbid models, behavioural test and the drugs were selected to be tested in these models. Meanwhile comprehensive literature search on depression affecting anxiety treatment and anxiety induced vulnerability to depression were also included. The quantity of research literature was so great that it provided the opportunity to generate and explore new hypotheses and to develop a theoretical model to explain recurrent depression. Based on the literature review, the following steps were outlined to achieve the objectives.

- Designing of animal model(s) that can be simulated as model(s) for screening of the anti-depressant and anxiolytic potential. Animal models of depression and co-morbid anxiety has been shown in Table 8.

Table 8: Animal model of depression and co-morbid anxiety

SN.	Animal Model/Compound	Treatment/Tests sequence
1.	Olfactory bulbectomy (OBX) (**6g, 6n, 6o, 6p**)	(i) Surgery (ii) Drug Treatment (iii) Behavioural tests (iv) Biochemical assays
2.	Traumatic Brain Injury (TBI) (**6g, 6n**)	(i) Surgery (ii) Drug Treatment (iii) Behavioural tests (iv) Biochemical assays
3.	Chronic Unpredictable Mild Stress (CUMS) (**6g, 6n**)	(i) Chronic stress (ii) Drug Treatment (iii) Behavioural test (iv) Biochemical assays
4.	Lipopolysaccharide (LPS) induced depression and anxiety (**6g**)	(i) Admin. LPS for 1days (ii) Drug Treatment (7 days) (iii) Behavioural tests (iv) Biochemical assays

- Evaluation of neuro-behavioural aspects of depression and anxiety using symptomatological approaches in rodents
- Evaluation of novel 5-HT_3 receptor antagonists such as **"6g", "6n", "6o"** and **"6p"** in various depression and anxiety model(s)

- Biochemical and neuro-chemical estimation in brain and blood sample (for different models) (1). Estimation of 5HT, NE and DA in brain sample (2).Estimation of corticosterone in plasma sample (3) Estimation of BDNF brain sample (4) Estimation of oxidative stress and antioxidant markers in brain sample

3.3. Preclinical Studies

3.3.1. Preliminary Work

Selection of **"6g", "6n", "6o", "6p"** as a test substance: In our laboratory, several 5-HT_3 receptor antagonists (quinoxaline derivatives) were designed and synthesized based on log P, pA_2 value, three point pharmacophore model the above compounds were selected for pharmacological evaluation. The compounds having optimum log P and higher pA_2 (more than 7) than ondansetron (pA_2 6.9) were selected for pharmacological testing.

The test substances and interacting agents (standard anti-depressant or anxiolytic drugs; mentioned later) were subjected to the mice spontaneous locomotor activity test in order to identify their influence on locomotion. Those substances (tested at specific dose levels and schedule) which exhibited insignificant influence on locomotion were short listed for behavioural AD assays. Preliminary work involved the DRC of the selected compound in FST and TST.

3.3.2. Dose Response Studies

The dose response curves following acute administration of test substances were constructed using validated animal models of depression viz. FST, TST, potentiation of 5-HTP induced head twitch in mice and reserpine induced hypothermia (RIH) in rats.

3.3.3. Preliminary Anxiolytic studies

The above mentioned drugs were also tested for their anxiolytic potential in experimental models of anxiety such as EPM, HB, L/D, and OFT.

3.3.4. Interaction Studies

Interaction studies with conventional anti-depressant and research compounds viz. Fluoxetine (FLX), Venlafaxine (VLA), Desipramine (DMI), Parthenolide (PTL) and Bupropion (BUP) were carried out using mice FST and TST.

3.3.5. Screening in Chronic Models

Depression requires chronic treatment to relapse the symptoms clinically. Hence to explore the efficacy for drug treatment selected doses were selected on the dose response studies and screened in chronic models such as OBX, CUMS, TBI and LPS injection for their anti-depressant and anxiolytic-like effect.

A. Behavioural Test Procedures and Parameters Measured in Chronic Model (s)

It is important to differentiate between the specific features of an rodent model and how we assess them (animal tests). The procedure for testing cannot be considered as model. Instead, the animal exposed to trauma and chronic mild stress may be believed as an animal model of behavioural disorder.

B. Biochemical Test Procedures and Parameters Measured in Chronic Model (s)

Finally the neuro-transmitter(s) (5HT, NE and DA), oxidative stress markers and antioxidant enzymes were measured in brain areas. The corticosterone levels in plasma and BDNF levels in brain were estimated (Behavioural observations post OBX and TBI surgery have been shown in Table 9; Behavioural observations post CUMS and LPS induced depression and anxiety have been shown in Table 10).

Table 9: Behavioural observations post OBX and TBI

SN	Test	Observation
1	Open Field Test (OFT)	Ambulation, rearing and number of faecal pellets
2	Elevated Plus Maze (EPM) Test	% Open arm entries (OAE) and % time spent in open arm (TSOA)
3	Sucrose Consumption Test	Volume of sucrose consumed
4	Hyper-emotionality Test (In OBX only)	Struggle, startle and fight response
5	Marble burying Test (in TBI only)	No. of marbles buried.

Table 10: Behavioural observations post CUMS and LPS induced depression and anxiety

SN	Test	Observation
1	Forced Swim Test (FST)	Duration of immobility
2	Tail Suspension Test (TST)	Duration of immobility
3	Sucrose Consumption Test	Volume of sucrose consumed
4	Elevated Plus Maze (EPM)	% Open arm entries (OAE) and % time spent in open arm (TSOA)
5	Light and Dark (L/D) model	Time spent in lit area, latency time and number of transitions

4. Experimental Methodology

4.1. Animals

Male Swiss albino mice (22-30 g) and male Wistar rats (180-300g) were obtained from Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. All animals were maintained under standard laboratory conditions [12 hour light/dark cycle (lights on at 7:00 AM); temperature 23±2ºC; relative humidity; 60±5%] in the Central Animal Facility. Both rats and mice were given sterilized food (standard pellet chow feed) and filtered water *ad libitum*. Following a quarantine period of two weeks the animals were randomly assigned to different experimental groups.

4.2. Ethical Approval

The experiments on animal were performed in accordance with the protocol approved by the Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/4/1, IAEC/RES/14/04, IAEC/RES/17/2).

4.3. Drugs and Chemicals

4.3.1. (4-benzylpiperazin-1-yl)(3-methoxyquinoxalin-2-yl)methanone (**6g**) novel 5-HT_3 receptor antagonist (Fig.21a), was synthesized in Medicinal Chemistry Laboratory, Birla Institute of Technology & Science, Pilani.

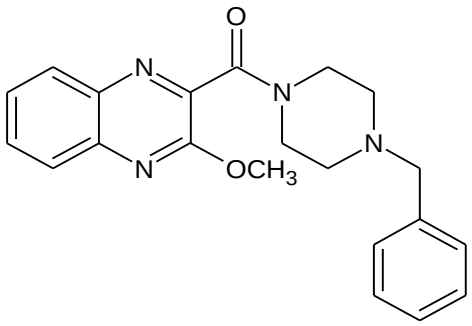


Fig. 21a. Structure of **"6g"**

4.3.2. *N*-n-propyl-3-ethoxyquinoxaline-2-carboxamide (**6n**) novel 5-HT_3 receptor antagonist (Fig.21b), was synthesized in Medicinal Chemistry Laboratory, Birla Institute of Technology & Science, Pilani.

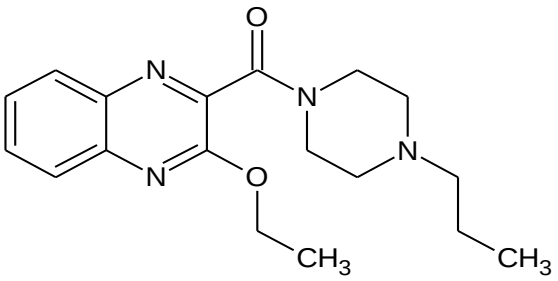


Fig. 21b. Structure of **"6n"**

4.2.3. *N*-n-butyl-3-methoxyquinoxaline-2-carboxamide (**6o**), novel 5-HT_3 receptor antagonist (Fig.21c), was synthesized in Medicinal Chemistry Laboratory, Birla Institute of Technology & Science, Pilani.

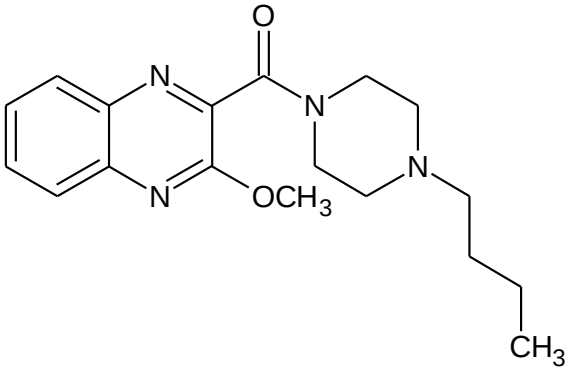


Fig.21c. Structure of **"6o"**

4.3.4. (3-Methoxyquinoxalin-2-yl)(4-phenylpiperazin-1-yl)-methanone (**6p**), novel 5-HT_3 receptor antagonist (Fig.21d), was synthesized in Medicinal Chemistry Laboratory, Birla Institute of Technology & Science, Pilani.

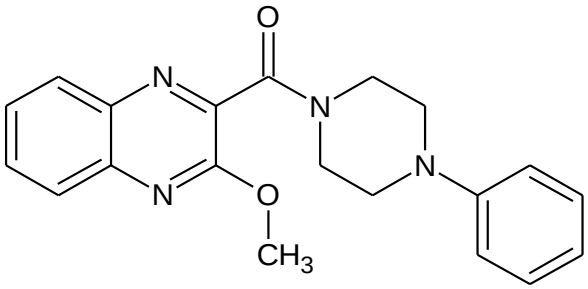


Fig. 21d. Structure of **"6p"**

4.3.5. Standard Drugs and Chemicals

Fluoxetine (FLX), Paroxetine (PAR) Venlafaxine (VLA), Bupropion (BUP), Desipramine (DMI) and Diazepam (DZM) were procured from IPCA and Ranbaxy Research laboratories, respectively as gift samples. Parthenolide

(PTL) was purchased from Tocris Chemicals (UK). Pargyline (PRG) was purchased from Sigma-Aldrich. Ketamine and xylazine were purchased from Indian Immunologicals, India. All the drugs were freshly prepared and administered intraperitoneal (i.p.) and per-orally (p.o.). The compound **"6g"** and **"6n"** were dissolved distilled water and compound **"6o"** and **"6p"** were prepared by triturating with PEG/distilled water and final volume make with distilled water. Water and mixture of water and PEG was used for the vehicle treatment for the respective studies. The doses of all drugs were selected on the basis of preliminary testing.

4.3.6. Chemical for Biochemical Parameters

Thiobarbituric acid, trichloroacetic acid, N-(1-naphthyl) ethylenediamine dihydrochloride, sulphanilamide, sulfosalicylic acid, 5-dithio-bis (2-nitrobenzoic acid), (−)-epinephrine, potassium dichromate, acetic acid, phosphoric acid EDTA and hydrochloric acid were purchased from different companies such as SD Fine, Hi-Media, Spectrochem Chemicals, India.

4.3.7. ELISA Kits

The ELISA kits for Serotonin, noradrenalin and dopamine neuro-transmitters were procured from DLD, Diagnostika, GMBH, Germany. ELISA kit for corticosterone and BDNF were procured from Immuno- Biological Laboratories, Inc (IBL), America and Boster Biological Technology Co., LTD, USA, respectively.

4.3.8. Neuro-chemical Estimation

Cysteine hydrochloride and n-heptane were purchased from SD Fine Chemicals, India. Serotonin hydrochloride and corticosterone were purchased from Sigma Chemicals, USA.

4.4. Surgicals

4.4.1. Haemostatic Sponge: AbGel, Absorbable gelatin sponge USP, Srikrishna Laboratories, Mumbai, India.

4.4.2. Sterile Sutures: Ethicon 4-0, Non-absorbable surgical sutures USP, Ethicon 4-0, Absorbable surgical sutures, USP (Catgut), Johnson and Johnson, India and Mersilk (Braided silk black).

4.4.3. Surgical Needle: Curved surgical needles were obtained from Pricon Surgicals, New Delhi, India.

4.5. Equipments

- ✓ Automated animal tracking system: Panlab Co., USA
- ✓ Digital EPM: SN Scientific, India
- ✓ Stereotaxic Frame: Inco Ambala, India
- ✓ Centrifuge: Eppendorf refrigerated centrifuge, 5702-R, Eppendorf AG, Germany
- ✓ Elisa Pate reader and washer: Ark Diagnostic, India
- ✓ Autoanalyzer: Ark Diagnostic, India
- ✓ Tissue Homogeniser: Kinematica™ Polytron™ Homogenizers, Germany
- ✓ Spectrophotometer: UV-1800 Shimadzu, Japan
- ✓ Digital Microscope: Optika, Microscopes, Italy
- ✓ Deep freeze (-70ºC): OPR-DFC-300CE, Operon Co. Ltd., Korea.

4.6. Pharmacological Procedures

General Considerations for Behavioural Studies

Separate sets of animals were used for each experiment to avoid habituation effects with experimental situations. The drugs solutions were freshly prepared in distilled water and administered p.o. / i.p. (as specified) in a constant volume of 10 ml/kg before experiment. The drug administrations and behavioural screenings were performed between 0900 and 1500 hrs, as animals showed their maximum activity during this time. The drug/vehicle treated animals were acclimatized to the experimentation room for one hour before testing. Proper care was taken before and after surgical procedure. Different animal models used to evaluate anti-depressant and anxiolytic-like effect of tested compounds has been shown in Table 11.

4.7. Preliminary Behavioural Assay for Antidepressant-like Effect

4.7.1. Spontaneous Locomotor Activity (SLA)

The SLA was assessed using an actophotometer (Boissier and Simon, 1965). This test is used as a preliminary test for dose selection. The animals were individually placed in a square arena (30 cm×30 cm) with walls painted black and fitted with photocells just above the floor level. The photocells were checked before the beginning of the experiment. After an initial 2 min familiarization period, the digital locomotor scores were recorded for the next 10 min in a dimly lit room. The arena was cleaned with dilute alcohol and dried between trails (Mahesh et al., 2012).

Purpose: To exclude false positive and false negative effect of the NCE and for selection of the dose.

4.7.2. Evaluation of Novel 5-HT_3 Receptor Antagonist Activity of NCE Using Mice in Forced Swim Test (FST)

The procedure reported elsewhere (Porsolt et al., 1977; Bourin et al., 1996) was adopted with slight modifications done in the diameter and height of the glass cylinder (Devadoss et al., 2010; Mahesh et al., 2012. Mice were dropped individually into glass cylinder (height: 30 cm, diameter: 22.5 cm) containing a depth of 15 cm of water maintained at 23–25°C. A mouse was judged immobile if it floated on water in an upright position and exhibited only small movements to keep its head above water or just made other passive movements. The duration of immobility was recorded during the last 4 min. of the 6-min. observation period. All the mice were subjected to a 15 min. training session on the day before testing. Water was changed between each trial. The changes in behaviour in FST like swimming and climbing reflect the role of neuro-transmitters serotonin and nor-adrenaline, respectively. This test is based on the hypothesis that depression is also caused by stress. The state of immobility has been named behavioural despair on the assumption that the animal has given up hope of ‘escaping’ a symptom which mirrors clinical observations of depressive disorders.

Purpose: To evaluate the effect of NCE on behavioural despair caused by forced swimming.

Table 11: Different animal models used to evaluate anti-depressant and anxiolytic-like effect of tested compounds

Model of depression (D) & Anxiety (A)	Species/No. of animals used in each group	Validity Criteria	Remarks / Purpose (Indicate the phenotypes of depression or anxiety)
SLA (Dose selection)	Mouse/8 mice	Face, Predictive	To avoid false positive and false negative results
FST (D)	Mouse/8 mice	Face, Predictive	Increase duration of immobility and decrease swimming episode
TST (D)	Mouse/8 mice	Face, Predictive	Increase duration of immobility
5HTP-induced HTR (D)	Mouse/8 mice	Constructive, Predictive	Potentiation of head twitches
RIH (D)	Rat/ 8 rats	Constructive, Predictive	Mean decrease in temperature
OBX (D, A)	Rat /6 rats	Face, Constructive Predictive	Hyperactivity in novel, brightly-lit open field, Increase hyper-emotionality behaviour and decrease intake of sweetened solution
CUMS (D, A)	Mouse/8 mice	Face, Constructive, Predictive	Increase immobility in despair tests, decrease intake of sweetened solution & decreased %TSOA and OAE .
LPS Injection (D, A)	Mouse/8 mice	Face, Constructive, Predictive	Increased immobility on despair tests, & Altered locomotor activity, decreased %TSOA and OAE and decreased time spent in light chamber and transition between chambers
TBI (D & A)	Rat/6 rats	Face, Predictive	Hyperactivity in novel, brightly-lit open field arena, increase Hyper-emotionality behaviour and Less intake of sweetened solution
EPM (A)	Mouse/8 mice	Predictive	Decrease % of both time spent on and number of entries in open arm
L/D (A)	Mouse/8 mice	Predictive	Decrease latency to leave and time spent in light box
HB (A)	Mouse/8 mice	Predictive	Decrease latency to leave and time spent in light box
OFT (A)	Mouse/8 mice	Predictive	Decrease ambulation, rearing scores, Increase defecation score

Experimental Design for Mice FST

A) In the preliminary investigation (FST), the animals were divided into five matched groups namely;

1. Normal control = 8
2. "6g" (0.5) = 8
3. "6g" (1) = 8
4. "6g" (2) = 8
5. ESC (10) = 8

B) In the preliminary investigation (FST), the animals were divided into five matched groups namely;

1. Normal control = 8
2. "6n" (1) = 8
3. "6n" (2) = 8
4. "6n" (4) = 8
5. ESC (10) = 8

C) In the preliminary investigation (FST), the animals were divided into five matched groups namely;

1. Normal control = 8
2. "6o" (0.5) = 8
3. "6o" (1) = 8
4. "6o" (2) = 8
5. ESC (10) = 8

D) In the preliminary investigation (FST), the animals were divided into five matched groups namely;

1. Normal control = 8
2. "6p" (1) = 8
3. "6p" (2) = 8
4. "6p" (4) = 8
5. ESC (10) = 8

4.7.3. Evaluation of Novel 5-HT_3 Receptor Antagonist Activity of NCE Using Mice in Tail Suspension Test (TST)

This is yet another test to confirm the depression-like symptoms by evaluating duration of immobility. This is more sensitive test to check behavioural pattern

than FST. Mice were individually suspended by the tail to a horizontal bar (distance from floor was 50 cm) using scotch tape (distance from tip of tail was approximately 1 cm). Typically, mice exhibited several escape-oriented behaviour interspersed with temporally increasing bouts of immobility (Steru et al., 1985; Devadoss et al., 2010). The duration of immobility (in seconds) during the 6-min test session was recorded. The changes in behaviour in TST like climbing reflect the role of neuro-transmitters DA and NE.

Purpose: To evaluate the effect of NCE on behavioural despair by suspension of the animal via tail.

A) In the preliminary investigation (TST), the animals were divided into five matched groups namely;

1. Normal control = 8
2. "6g" (0.5) = 8
3. "6g" (1) = 8
4. "6g" (2) = 8
5. BUP (20) = 8

B) In the preliminary investigation (TST), the animals were divided into five matched groups namely;

1. Normal control = 8
2. "6n" (1) = 8
3. "6n" (2) = 8
4. "6n" (4) = 8
5. BUP (20) = 8

C) In the preliminary investigation (TST), the animals were divided into five matched groups namely;

1. Normal control = 8
2. "6o" (0.5) = 8
3. "6o" (1) = 8
4. "6o" (2) = 8
5. BUP (20) = 8

D) In the preliminary investigation (TST), the animals were divided into five matched groups namely;

1. Normal control = 8
2. "6p" (1) = 8
3. "6p" (2) = 8
4. "6p" (4) = 8
5. BUP (20) = 8

4.7.4. Mechanistic Models

A. Reserpine induced Hypothermia (RIH) in Rats

Reserpine inhibits the uptake of neuro-transmitters (monoamines) in to the synaptic vesicles. The normal levels of NE, DA and 5-HT involved in temperature regulation (Englert et al., 1973). In this, test rats were gently hand-restrained and the glycerol lubricated thermometer probe was inserted into the rectum. The rectal temperature of the rats treated with reserpine (1 mg/kg, i.p.) was recorded at 30, 60, 90 and 120 min after the drug administration. The difference in the rectal temperature between the baseline and 60th min values were tabulated. On the day preceding to experimentation, the rectal temperature of the rats were assessed in a similar manner in order to habituate the animals to the experimental procedures (Bhatt et al., 2013a; Mahesh et al., 2012).

Purpose: This test is nonspecific and gives idea about the involvement of monoaminergic system (NE, DA, 5-HT).

A) In the preliminary investigation (RIH), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6g" (1) = 8
3. "6g" (2) = 8
4. ESC (10) = 8

B) In the preliminary investigation (RIH), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6n" (1) = 8
3. "6n" (2) = 8
4. ESC (10) = 8

C) In the preliminary investigation (RIH), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6o" (1) = 8
3. "6o" (2) = 8
4. ESC (10) = 8

D) In the preliminary investigation (RIH), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6p" (1) = 8
3. "6p" (2) = 8
4. ESC (10) = 8

B. 5-Hydroxytryptophan Induced Head Twitch Response (5-HTP-HTR)

The 5-HT_3 receptor antagonists increases the availability of serotonin at 5-HT_{2A} receptors and hence responsible for characteristic head twitch response. The vehicle/drug treated mice were injected with PRG (75 mg/kg, i.p) and 5-HTP (5 mg/kg), 30 and 15 min prior to drug administration, respectively and gently placed in separate, clear plexi-glass cages (12×12×10 cm). The total number of head twitches (characterized by abrupt lateral movements) episodes was recorded for the next 15 min (Martin et al., 1989, Bhatt et al., 2013a).

Purpose: This test demonstrated a characteristic head twitch response in mice due to increase in the the 5-HT concentration of in synapse.

A) In the preliminary investigation (5-HTP-HTR), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6g" (1) = 8
3. "6g" (2) = 8
4. FLX (20) = 8

B) In the preliminary investigation (5-HTP-HTR), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6n" (1) = 8
3. "6n" (2) = 8
4. FLX (20) = 8

C) In the preliminary investigation (5-HTP-HTR), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6o" (1) = 8
3. "6o" (2) = 8
4. FLX (20) = 8

D) In the preliminary investigation (5-HTP-HTR), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6p" (1) = 8
3. "6p" (2) = 8
4. FLX (20) = 8

4.7.5. Interaction Studies Using FST and TST in Mice

The animals were treated either with vehicle or with one of the following test compounds namely, FLX (10 and 20 mg/kg, i.p.), a selective serotonin re-uptake inhibitor, DMI (10 and 20 mg/kg, i.p.), a nor-epinephrine reuptake inhibitor, VLA (4 and 8 mg/kg, i.p.), a serotonin nor-epinephrine reuptake

inhibitor, PTL (1 mg/kg i.p.), a serotonin release inhibitor. Interaction of BUP (10 and 20 mg/kg, i.p), a nor-epinephrine and dopamine re-uptake inhibitor was carried out in TST since it is more sensitive than that of FST. All the standard drug doses were adopted from the previous work and standard responses recorded (Ripoll et al., 2003; Bourin et al., 2005). For each interaction 8 animals were taken in each group.

Purpose: Interaction study helps to find out the mechanistic properties of the drug as well as it also helps to reduce the dose of standard if the NCE potentiate the effect of standard.

4.8. Preliminary Behavioural Evaluation for Anti-anxiety Effect

4.8.1. Elevated Plus Maze (EPM)

The EPM test was first evaluated for rats and later adapted for mice (Biala and Kurk 2008). In brief, the apparatus consisted of a wooden maze with two enclosed arm (30 × 5 × 15 cm) and two open arms (30 × 5 × 0.25 cm) that extended from a central platform (5 × 5 cm) to form a plus sign. The plus-maze apparatus was elevated to a height of 45 cm and placed inside a sound-attenuated room. The trial was started by placing a mouse on the central platform of the maze facing its head towards an open arm. The behavioural performances recorded during a 5 minute test period were; percentage open arm entries (OAE) and percentage time spent in open arm (TSOA) (Klodzinska et al., 2004). Entry into an arm was considered valid only when all four paws of the mouse were inside that arm. The apparatus was thoroughly cleaned with 70% ethanol after each trial.

Purpose: This test is performed to evaluate the effect of anxiolytic drugs by change in the exploratory behaviour of mice at a particular height and open environment.

A) In the preliminary investigation (EPM), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6g" (1) = 8
3. "6g" (2) = 8
4. DZM (2) = 8

B) In the preliminary investigation (EPM), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6n" (1) = 8
3. "6n" (2) = 8
4. DZM (2) = 8

C) In the preliminary investigation (EPM), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6o" (1) = 8
3. "6o" (2) = 8
4. DZM (2) = 8

D) In the preliminary investigation (EPM), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6p" (1) = 8
3. "6p" (2) = 8
4. DZM (2) = 8

4.8.2. Light/Dark (L/D) Aversion Test

The L&D apparatus comprised of a box divided into two separate compartments, occupying two-thirds and one-third of the total size, respectively. The larger compartment (light compartment) was illuminated by a 60-watt bulb, while the smaller (dark compartment) was entirely black and enclosed under a dark cover. The L/D compartments were separated by a partition with a tunnel to allow passage from one compartment to the other (Mi et al., 2005). At the beginning of the test, the mouse was placed individually at the center of the light compartment facing towards the tunnel and was allowed to explore the entire apparatus for 5 min. The behavioural parameters such as latency time for the first crossing to the light compartment, total time spent in the light compartment and number of transitions between the L/D compartments were recorded. A compartment entry was considered valid when the animal's all four paws were inside that chamber. The apparatus was thoroughly cleaned with 70% ethanol after each trial (Bhatt et al, 2013b).

Purpose: To demonstrate the anti-anxiety effect of drugs via reduction in the natural aversion towards the light

A) In the preliminary investigation (L/D), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6g" (1) = 8
3. "6g" (2) = 8
4. DZM (2) = 8

B) In the preliminary investigation (L/D), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6n" (1) = 8
3. "6n" (2) = 8
4. DZM (2) = 8

C) In the preliminary investigation (L/D), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6o" (1) = 8
3. "6o" (2) = 8
4. DZM (2) = 8

D) In the preliminary investigation (L/D), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6p" (1) = 8
3. "6p" (2) = 8
4. DZM (2) = 8

4.8.3. Hole Board (HB) Test

The HB apparatus consisted of a grey Plexiglas platform (40 × 40 cm) raised to a height of 15 cm from the floor of a gray wooden box (40 × 40 × 40 cm). The grey Plexiglas platform consisted of 16 equivalent square compartments (12 peripheral and 4 central), each featuring a central circular hole (3 cm diameter). Test session was started by placing each animal in the center of the HB and allowed to freely explore on the apparatus for 5 min. The behavioural performances such as number of head dipping, total time spent in head dipping and latency to the first head dipping (Silva et al., 2007) were recorded.

Purpose: This test demonstrated that, the anti-anxiety drugs increases the curiosity and exploratory behaviour of mice

A) In the preliminary investigation (HB), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6g" (1) = 8
3. "6g" (2) = 8
4. DZM (2) = 8

B) In the preliminary investigation (HB), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6n" (1) = 8
3. "6n" (2) = 8
4. DZM (2) = 8

C) In the preliminary investigation (HB), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6o" (1) = 8
3. "6o" (2) = 8
4. DZM (2) = 8

D) In the preliminary investigation (HB), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6p" (1) = 8
3. "6p" (2) = 8
4. DZM (2) = 8

4.8.4. Open Field Test (OFT)

The apparatus consisted of a wooden box (60 x 60 x 30 cm) with the floor divided into 16 squares (15 x 15 cm) squares by black parallel and intersecting lines. The apparatus was illuminated with 60 watt bulb suspended 100 cm above. At the beginning of the test, the mouse was placed individually at the center of the square arena. The ambulation scores (number of square crossed) and rearing number (standing upright on hind legs) were recorded for 5 min period. After each individual test session the floor was thoroughly cleaned with 70% ethanol (Yadav et al., 2008).

Purpose: To demonstrate the anti-anxiety effect of drugs via reduction in the natural aversion towards the light as well as increase in exploratory behaviour.

A) In the preliminary investigation (OFT), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6g" (1) = 8
3. "6g" (2) = 8
4. DZM (2) = 8

B) In the preliminary investigation (OFT), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6n" (1) = 8
3. "6n" (2) = 8
4. DZM (2) = 8

C) In the preliminary investigation (OFT), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6o" (1) = 8
3. "6o" (2) = 8
4. DZM (2) = 8

D) In the preliminary investigation (OFT), the animals were divided into four matched groups namely;

1. Normal control = 8
2. "6p" (1) = 8
3. "6p" (2) = 8
4. DZM (2) = 8

4.9. Olfactory Bulbectomy (OBX) Induced Co-morbid Depression Associated with Anxiety

Removal of olfactory bulb(s) in rats is an approach to damage the neuronal circuit leading to neuro-behavioural disorder. Bilateral olfactory bulb ablation was performed as described elsewhere (Van Riezen and Leonard, 1990; Kelly et al., 1997), with slight modifications. Male Wistar rats were anesthetized with combination of ketamine and xylazine (75 and 5mg/kg) before the surgical procedure. All the surgical equipments were sterilized before use. The head was shaved and cranium exposed by a mid-line sagittal incision. Two burr holes (2mm in diameter) were drilled, 8 mm anterior to bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the orbit of the eye (Fig.22). The entire procedure was carried out with adequate precautions in the animal operation theatre of the Central Animal Facility. The olfactory bulbs were ablated by suction, avoiding damage to the frontal cortex and the dead space was filled with haemostatic sponge in order to prevent excessive bleeding. The scalp was sutured with absorbable catgut (4-0) to prevent infection. Step wise procedure is shown in Fig.23.The animals were given Sulprim injection (each ml containing sulphadiazine 200 mg and trimethoprim 40 mg), intra-muscularly (0.2 ml/300g) once a day for 4 days, post-surgery. The sham operation was performed in a similar manner with the bulbs left intact. During the 14 days recovery period, the animals were handled regularly to avoid aggressive behaviour, which might have developed otherwise. All drug treatments were started on the 15^{th} day of surgery and were continued once a day, for 14 days (28^{th} day of surgery, Table 12).

Co-morbid tests of anxiety and depression were performed in single sets of rat's post-OBX. An alternate depression and anxiety test was scheduled in the study. In a day, one depression and one anxiety test were performed in OBX rats. PAR and test compounds (**6g**, **6n**, **6o**, **6p**) was evaluated at different doses for the first time in OBX induced anxiety associated depression at different doses.

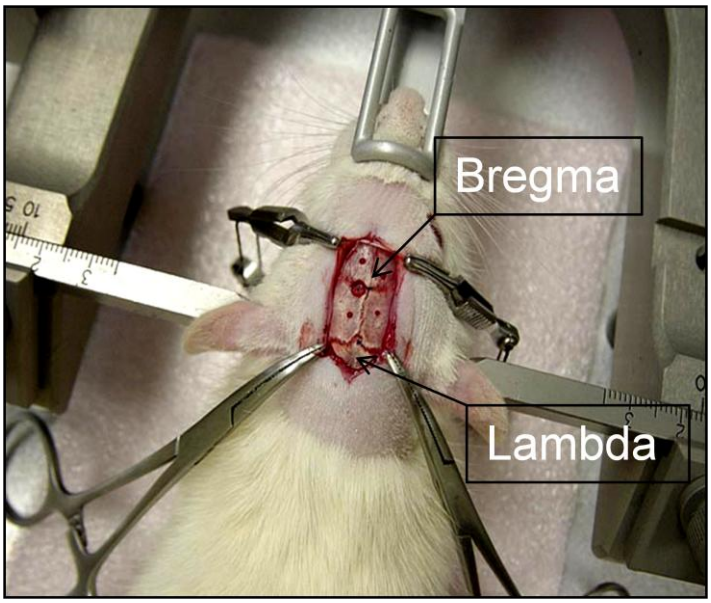


Fig.22. Bregma region for OBX surgery in rats

A) Experimental Design for "6g" in OBX Rats

In the OBX the animals were divided into eight matched groups namely;

Sham Control	= 6	OBX Control	= 6
Sham + PAR (10)	= 6	OBX + PAR (10)	= 6
Sham + "6g" (1)	= 6	OBX + "6g" (1)	= 6
Sham + "6g" (2)	= 6	OBX + "6g" (2)	= 6

B) Experimental Design for "6n" in OBX Rats

In the OBX the animals were divided into eight matched groups namely;

Sham Control	= 6	OBX Control	= 6
Sham + PAR (10)	= 6	OBX + PAR (10)	= 6
Sham + "6n" (1)	= 6	OBX + "6n" (1)	= 6
Sham + "6n" (2)	= 6	OBX + "6n" (2)	= 6

C) Experimental Design for "6o" in OBX Rats

In the OBX the animals were divided into eight matched groups namely;

Sham Control	= 6	OBX Control	= 6
Sham + PAR (10)	= 6	OBX + PAR (10)	= 6
Sham + "6o" (1)	= 6	OBX + "6o" (1)	= 6
Sham + "6o" (2)	= 6	OBX + "6o" (2)	= 6

D) Experimental Design for "6p" in OBX Rats

In the OBX the animals were divided into eight matched groups namely;

Sham Control	= 6	OBX Control	= 6
Sham + PAR (10)	= 6	OBX + PAR (10)	= 6
Sham + "6p" (1)	= 6	OBX + "6p" (1)	= 6
Sham + "6p" (2)	= 6	OBX + "6p" (2)	= 6

Rationale: OBX is based on the assumption that depression is caused by neuronal regulatory deficits. Bilateral olfactory bulbectomy results in changes in behaviour and neuro-transmitter systems that simulate many of those seen in patients with major depression. A disconnection of the olfactory bulb(s) has shown to produce abnormalities in emotional behaviour (termed the bulbectomy syndrome) due to a disruption in the homeostatic regulation of impulse traffic in the limbic system. Hence it was hypothesized that OBX can be the model of depression associated with anxiety and therefore post OBX, set of alternative behavioural tests of depression and anxiety were performed. Two tests were performed in a day, depression followed by anxiety and next day anxiety (A) test followed by depression (D). The alternative tests were carried out to avoid the carry-over effect of one test to another. Olfactory bulb(s) were removed in one or two attempts to avoid severe injury to the brain. Test compounds and standard were administered once a day for 14 days post-OBX. Treatment was started 14 days post-surgery to ensure that the animals completely recovered following surgery. Behavioural tests were started after 20 hr of last dosing to avoid acute effects of drugs. Animals were shifted to the observation room one hour before the behavioural tests maintained at similar environmental conditions (temp., RH, lighting).

Table 12: Schedule for OBX, treatment and behavioural tests

Day 0	**0th -1st day**	**1st -14th day**	**15th -28th**	**Day 29th -31st Behavioural assessments**		
				29th	**30th**	**31st**
Surgery	Recovery from surgery (conti-nuous care)	Re-habilitation period (Daily handling and observation)	Drug/ vehicle treatment (Once a day p.o for 14 days	Modified Open field behaviour (D) Elevated plus maze test (A)	Sucrose consumption test (24 hr) (D)	Hyper-emotionality test (D)

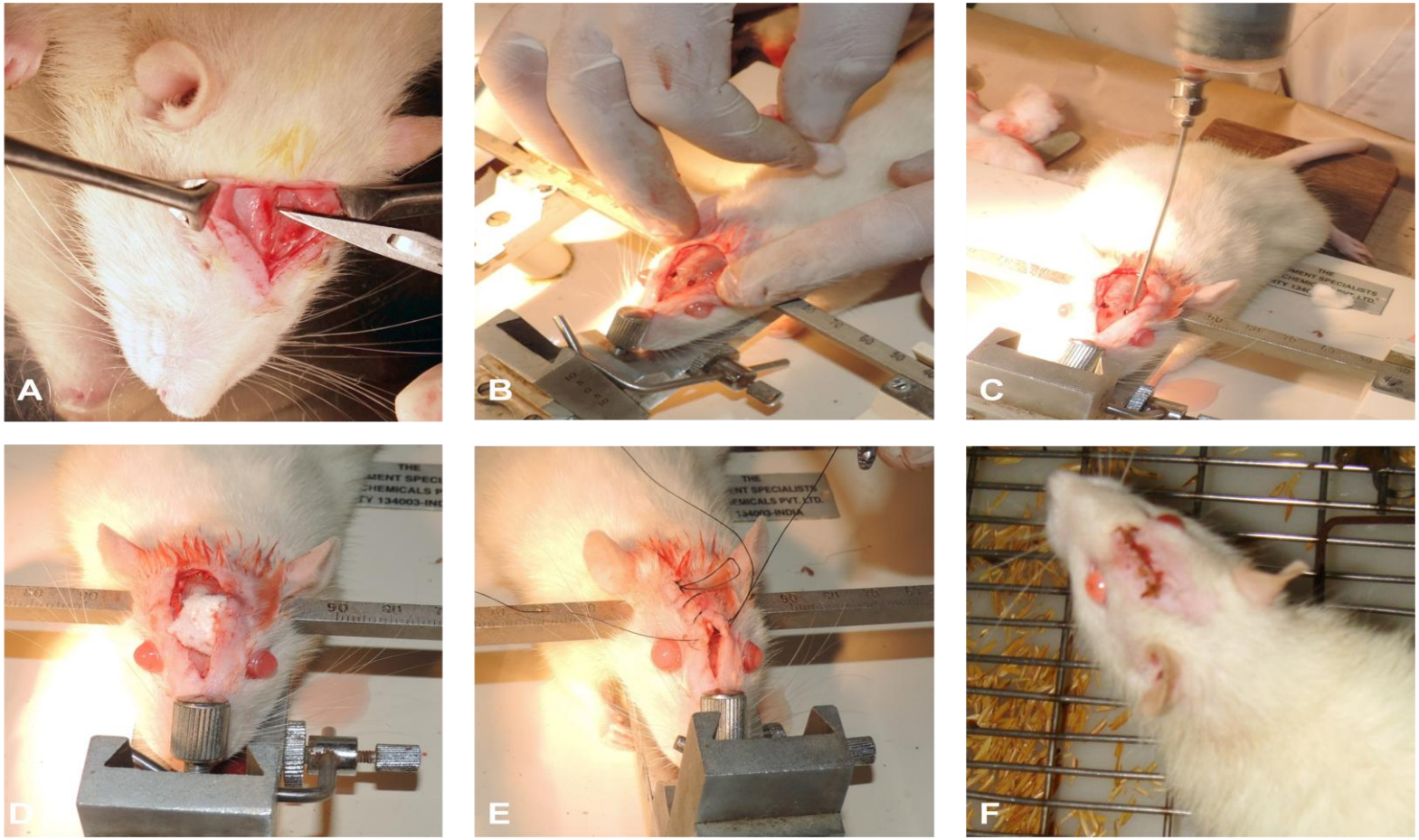


Fig.23: Preliminary steps of OBX surgical procedure. (A) Positioning of rats in sterotaxic system, (B) Midline sagittal incision, (C) Burr holes on either side of midline, (D) hemostatic sponge, (E) Suturing of incision site and (F) Healing of the surgical wound on 7th day.

4.10. Traumatic Brain Injury (TBI) Induced Co-morbid Depression Associated with Anxiety

TBI was performed based on Foda and Marmarou, (1994); Heath and Vink, (1999). Rats weighing (250-300g) were anesthetized using a mixture of ketamine (75mg/kg i.p.) and xylazine (5 mg/kg i.p.). A 1 cm midline scalp incision was made and the muscles were retracted to expose the skull. A stainless steel disc (10 mm in dia. and 3 mm in depth) was placed centrally between the lambda and bregma sutures (Fig.22). Injury was then induced using impact acceleration model of TBI. A 400 g metal weight was dropped from a height of 1 m guided through straight pipe (Length: 1 m; Diameter: 35 mm), onto the metal disc placed over the rat's skull (Fig. 24). A 10 cm foam bed underneath the animal helped to absorb the impact. After the impact, the metal disc was removed and skin was sutured and Povidone-iodine (10% w/v, Betadine) was applied. During a 10 day–rehabilitation period following injury, the animals were housed (two per cage) in standard laboratory cages and separated from the others rats in the housing unit. Wounds were daily inspected to ensure complete healing. Fig.25 shows the various steps of TBI. Impact accelerated (weight drop) method was adopted here to minimize the chances of mortality. Sham operated rats were treated in the same way, including mid-line incision except TBI. Neurological outcome was assessed using different functional tests. This procedure was standardized in house with modifications in procedure described by Foda and Marmarou, (1994); Heath and Vink, (1999). Schedule for TBI surgery, treatment and behavioural tests have been shown in Table 13.

Force Applied for Injury was Calculated by Newton's Law (eq.1)

eq. 1 **F= m x a** F= force, m= mass, a= acceleration

Here, m=400g & a = 9.8 (acceleration due to gravity)

F= 400 x 9.8

F= 3920 Newton

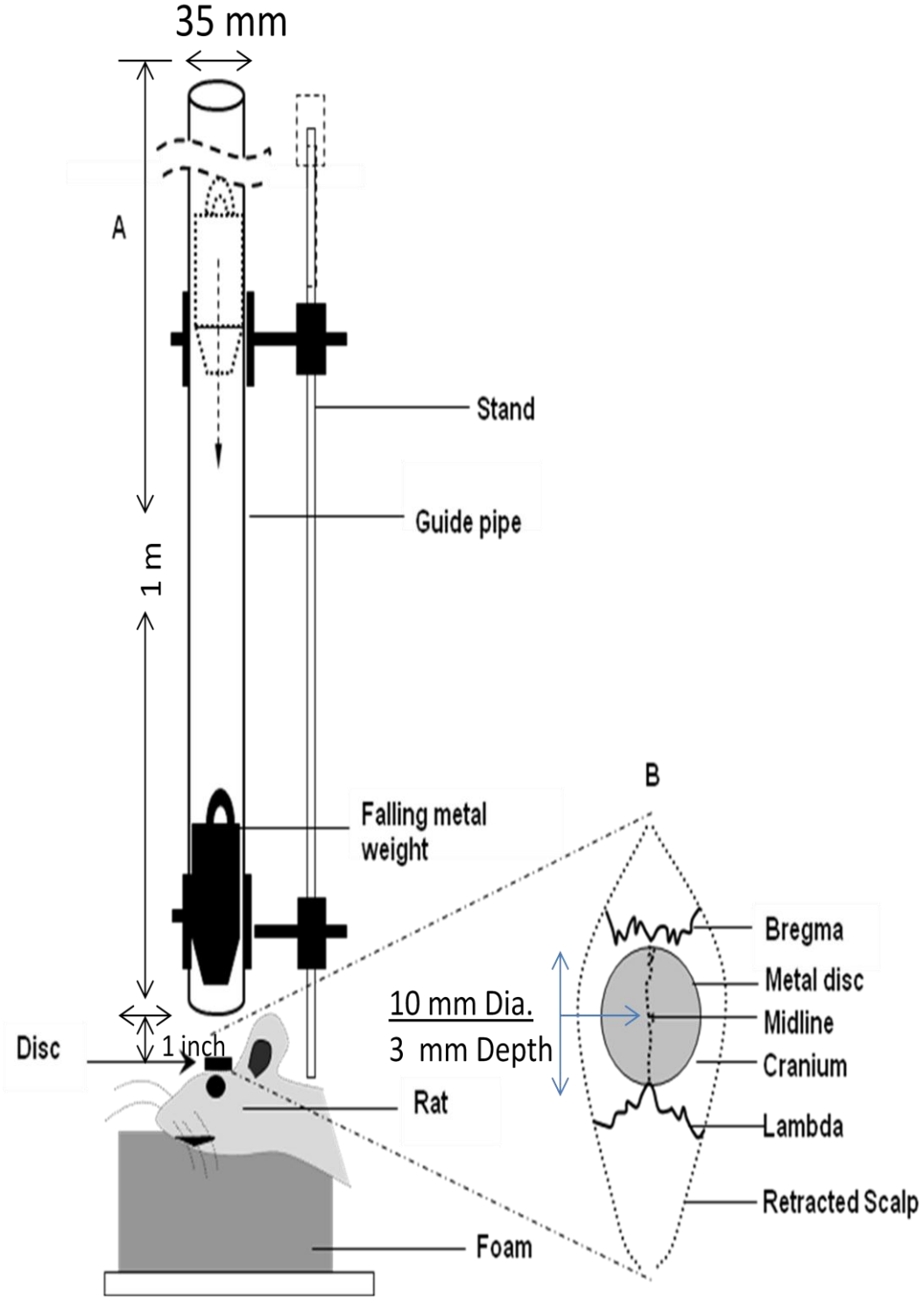


Fig.24. Schematic representation of the method employed to induce traumatic brain injury in rats. B. Dorsal view of the rat cranium showing the positioning of the metallic disc (Mahesh et al., 2010a).

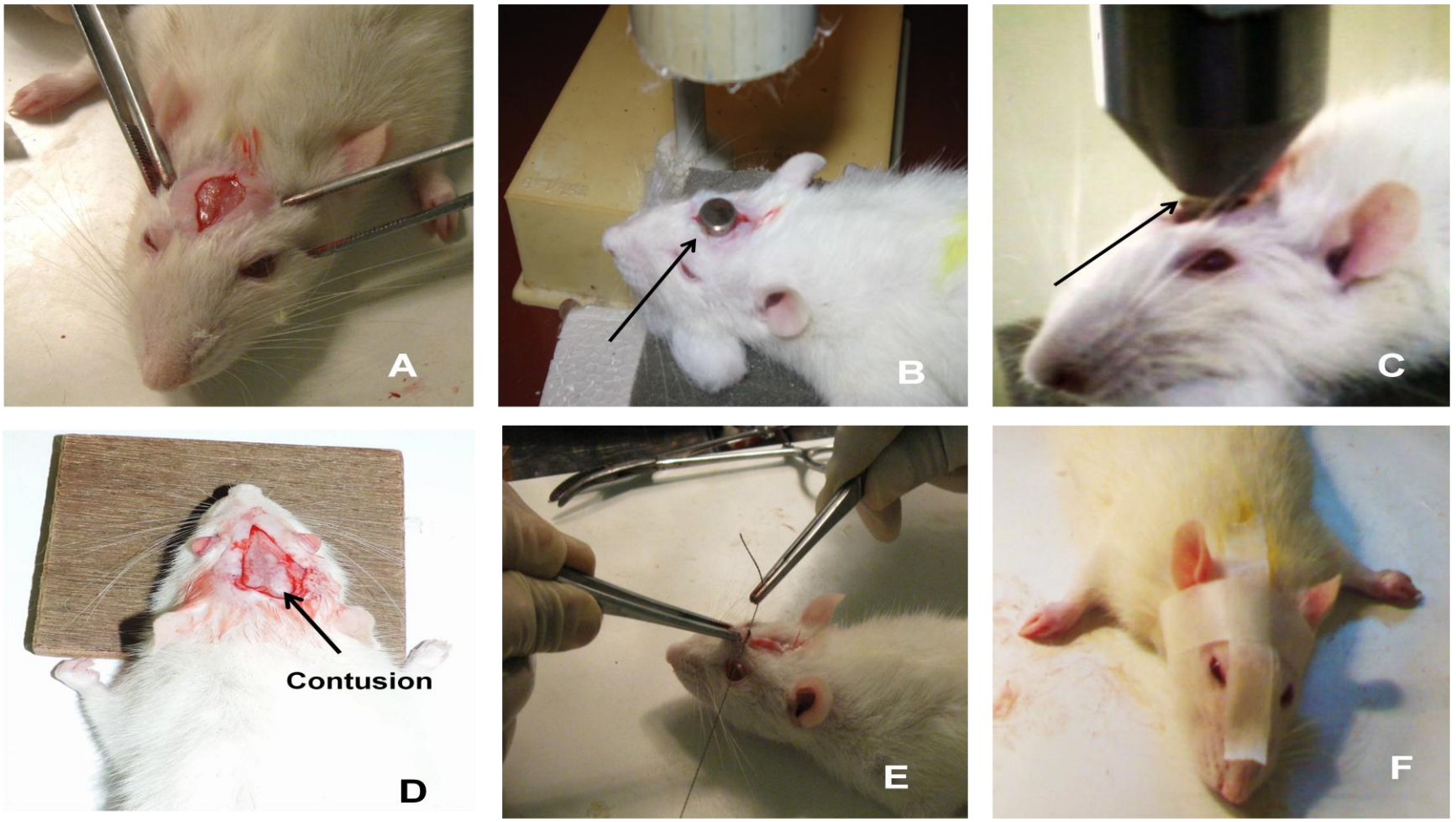


Fig.25. Various steps of traumatic brain injury (TBI) in rats. (A) Mid-line incision, (B) Positioning of metal disc (C) Falling of metal bob over rat head, (D) Contusion post injury (E) Suturing of incision site (F) Rat after surgery

Experimental Design (Two NCE's)

TBI model was standardized after different attempts with modifications in the height of pipe, weight of the metal bob and dimension of the disc. Extra care was taken to prevent skull fracture and bleeding during TBI in rats.

A) Experimental Design for "6g" in TBI Rats

In the TBI the animals were divided into eight matched groups namely;

Sham Control	= 6	TBI Control	= 6
Sham + PAR (10)	= 6	TBI + PAR (10)	= 6
Sham + "6g" (1)	= 6	TBI + "6g" (1)	= 6
Sham + "6g" (2)	= 6	TBI + "6g" (2)	= 6

B) Experimental Design for "6n" in TBI Rats

In the TBI the animals were divided into eight matched groups namely;

Sham Control	= 6	TBI Control	= 6
Sham + PAR (10)	= 6	TBI + PAR (10)	= 6
Sham + "6n" (1)	= 6	TBI + "6n" (1)	= 6
Sham + "6n" (2)	= 6	TBI + "6n" (2)	= 6

Animals with abnormal behaviour and/ or diseased condition (1-2 %) were not included in the study. Dosing schedule and behavioural tests were decided based on the time taken for recovery of TBI rats. At the end of the study, rats were sacrificed and brain samples were collected and stored at -70 ºC until the estimation of neuro-transmitter.

Table 13: Schedule for TBI, treatment and behavioural tests

0 Day	**0^{th} -1^{st} Day**	**1^{st} -10^{th} Day**	**11^{th} -24^{th} Day**	**25^{th}-27^{th} day Behavioural assessments**		
				25^{th}	**26^{th}**	**27^{th}**
Surgery	Recovery from surgery	Rehabilitation period (Daily handling and observation)	Drug/ vehicle treatment (Once a day p.o for 14 days	Open field behaviour (D) Elevated Plus maze (A)	Sucrose Consumption test (D)	Marble burying behaviour (A)

4.11. Behavioural Assays Performed Post- Olfactory bulbectomy (OBX) and Post-Traumatic Brain Injury (TBI) in Rats

Once the TBI, OBX treatment gets over, behavioural tests were carried out. A set of behavioural tests were performed and parameters associated with the tests were designed in such a manner, so as to simulate the behavioural co-morbid depression and anxiety symptoms in rats.

A. Open Field Test (OFT)

The OFT was conducted as described by Kelly et al. (1997) with slight modifications. The apparatus consisted of a circular (90-cm diameter) arena with 75-cm high polished metal walls and floor equally divided into 10 cm squares (Fig. 26). A 60 W light bulb was positioned 90 cm above the base of the arena which was the only source of illumination in the testing room. Each animal was individually placed in the center of the open field apparatus and the following parameters were observed for 5 min.

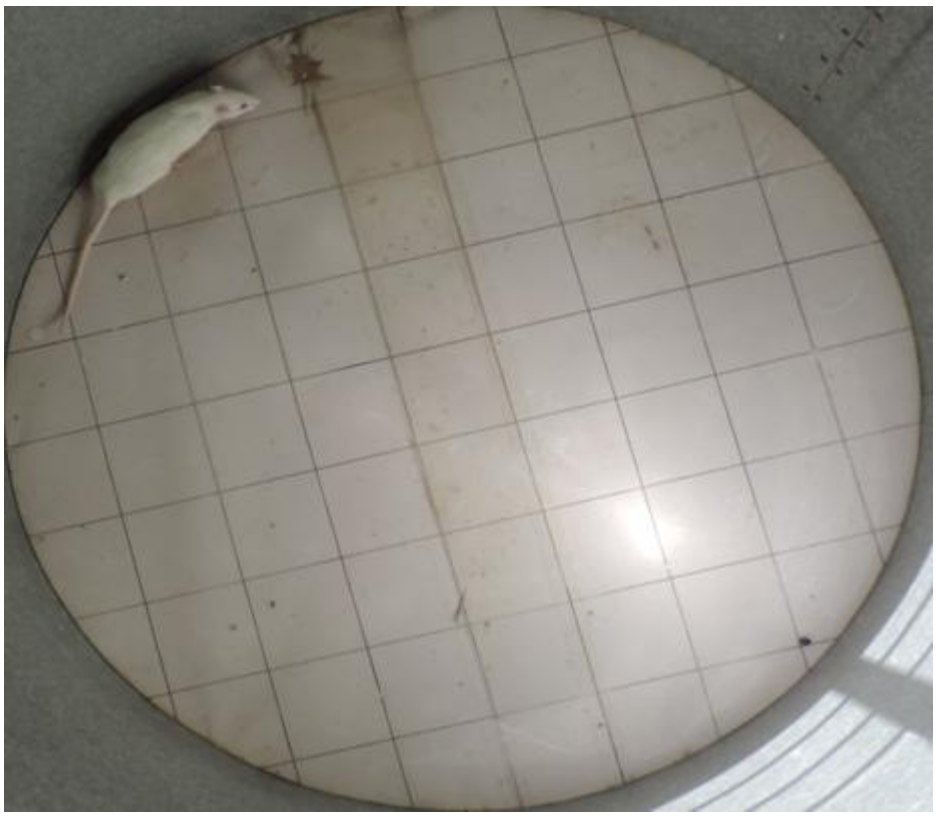


Fig.26. An OBX rat in modified open-field exploration apparatus

Ambulation scores (number of squares crossed) and number of rearing episodes were noted as horizontal and vertical activity, respectively. Crossing of a square was considered only when the hind limbs of the animal moves to the next square. Other parameter observed was defecation. The number of fecal pellets was counted at the end of each 5 min. trial. The apparatus was cleaned with ethyl alcohol and dried between trials to remove any residual odour.

Purpose: Measurement of hyperactivity reflects psychomotor effect.

B. Elevated Plus Maze (EPM)

The procedure was adopted same as described (Yamada et al., 2000). The plus-maze consisted of two open (50 cmx10 cm) arms and two enclosed (50 cm x10 cm) arms surrounded by 30-cm high walls. The four arms were joined by a central platform (10 cm x 10 cm) open to all the arms, to form a plus shape.

Fig.27. A rat exploring the open arm of EPM

The entire apparatus was elevated to a height of 60 cm above the floor. The apparatus was indirectly illuminated with a ceiling mounted lamp (60 W) which was placed 90 cm above the apparatus. At the beginning of the test, the animal was placed in the central platform facing an open arm. The number of OAE

and TSOA were recorded for 5 min. (Fig.27). After each test, the apparatus was cleaned with dilute alcohol and wipe down properly to remove any odour.

Purpose: Natural aversion to open area, preference of protected and unprotected area.

C. Hyper-emotionality Tests

Hyper-emotionality was measured by adopting the procedure described by Brady and Nauta (1955) and Shibata et al. (1982) with slight modification. Briefly, hyper-emotionality in rats was measured by scoring the responses to the following stimuli, *Startle response*: startle response to a stream of air (using 10-ml syringe) directed at the dorsum was scored. *Struggle response*: struggle response was scored by handling the animal with a gloved hand. *Fight response*: fight response was scored by pinching the tail with forceps.

These responses were graded as follows:

0- No reaction :

1- Slight

2- Moderate

3- Marked

4- Extreme response.

All animals in each group were observed on the same day. The score for each animal in emotional response was given within 5 min. Total of all the hyper-emotionality scores of each rat is summed in the test**.** The observers were blind with respect to the drug treatment.

Purpose: loss of emotional component was measured.

D. Marble Burying Behaviour

A method of Broekkamp et al. (1986) was adopted with modification. The test apparatus was a 40 cm x 25 cm x 12 cm plastic rat cage similar to the animal's home cage. The floor of the cage was evenly covered with 5 cm of bedding material. Each rat was assigned to a particular test cage for the duration of the experiment. The animal was placed singly in the

test cage with 25 polished glass marbles (20mm diameter) placed in a triangular formation in the cage (Fig.28). The number of marbles buried by rats in a 30 minute period of observation was recorded.

Purpose: measurement of repetitive behaviour (obsession) in neophobic condition.

Fig.28. Displaying marble burying behaviour

E. Sucrose Consumption Test

The procedure was performed with modification as described by Willner et al. (1992). In the present study, sucrose solution (1%) was placed in pre-weighed bottles and rats were allowed to consume the fluid for 24 hr. Next day, bottles were removed and volume intake was estimated by weighing bottles.

Purpose: measurement of anhedonia.

4.12. Biochemical Analysis

Biochemical estimations for OBX, TBI rats were carried out 24 h after completion of the all behavioural assessments.

A. Neurochemistry: Estimation of Rat Brain Serotonin

Neuro-chemical estimation was performed after the behavioural studies on TBI and OBX rats.

B. Harvest

The TBI/OBX/Sham rats were decapitated and the brain was harvested quickly as per procedure mentioned elsewhere (Glowinski and Iversen, 1966) and placed on a petridish in an ice bath.

C. Dissection

The rat brain was carefully removed, blotted and chilled. Dissections were performed on an ice-cooled glass plate. All the brain samples were collected and stored in deep freezer at -70°C.

D. Extraction of 5-HT, DA, and NE

Procedure for Extraction of 5-HT, DA, and NE is shown in section no 4.8.9.5 and 4.8.9.6.

E. Estimation of 5-HT, DA, and NE: Standard Curve

The standard curve for the corticosterone assay was prepared by using the instruction on kit.

4.13. Evaluation of "6g" and "6n" in Chronic Unpredictable Mild Stress (CUMS) Induced Depression and Anxiety

Chronic stress is closely related to various neurological disorders. The excessive stress leads to alteration in the neuronal activity, immune response, cardiovascular, neuroendocrine and sympathetic nervous system via activation of the hypothalamus-pituitary-adrenal (HPA) axis (Dayas et al., 2001; Reyes et al., 2003). In normal physiological conditions, oxygen derived species are biotransformed to less toxic compounds with participation of the most significant antioxidant enzymes like SOD, CAT and GSH (Maes et al., 2011). Stress causes deregulation of antioxidant activity of enzymes such as GSH, SOD, CAT and increases lipid peroxide and nitrite levels in brain structure of rodents (Liu et al; 1996; Kumari et al., 2007). Fig. 29 demonstrates the protective and destructive mechanisms involved in oxidative stress

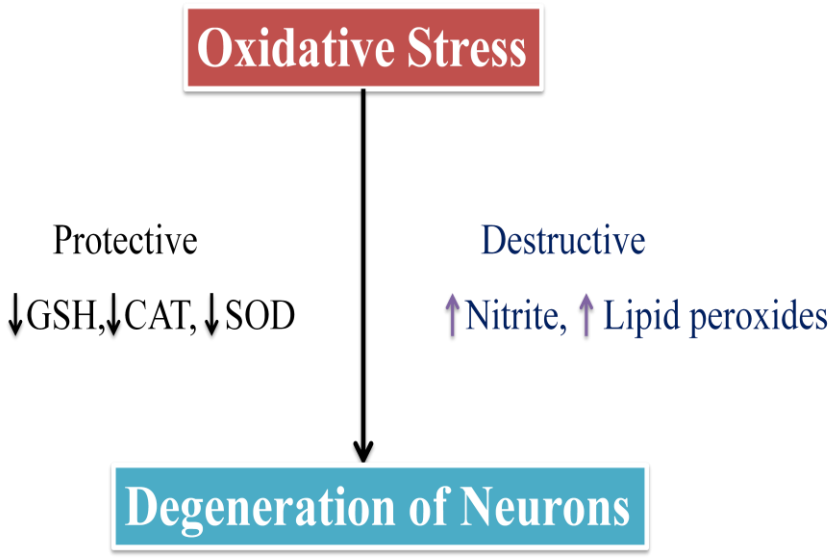


Fig. 29. Protective and destructive mechanism involved in oxidative stress

CUMS model of depression is widely used in screening of anti-depressants for investigating the etiology and pathophysiology of depression and the associated therapeutic interventions (Katz et al., 1981; Garcia, 2009). This model was developed in an attempt to mimic/resemble a variety of behavioural, neuro-chemical, neuroendocrine and neuroimmune alterations observed in human depressive disorders (Holsboer, 2000; Sapolsky, 2003; McEwen, 2005).

Postsynaptic 5-HT_3 receptor antagonism in serotonergic neurons facilitated the specific binding of 5-HT to other postsynaptic receptors such as 5-HT_{1B} (Bourin et al., 1998), 5-HT_{2A} and 5-HT_{2C}, thereby aided in serotonergic transmission as observed with the novel anti-depressant, mirtazapine (Rajkumar and Mahesh, 2010). Moreover, blockade of 5-HT_3 postsynaptic receptors also increased the 5-HT mediated nor-epinephrine release (Rajkumar and Mahesh, 2010). Further several preclinical studies performed in our lab also showed the anti-depressant like effect of novel 5-HT_3 receptor antagonists (Devadoss et al., 2010). The hypothesis of the anti-depressant effects of 5-HT_3 receptor antagonists and the potential role of 5-HT_3 receptors in the neurobiology of depression is proved by recent preclinical study results (Mahesh et al., 2012). MDL 72222 (bemesetron), a selective 5-HT_3 receptor antagonist, which shown to reduce the duration of immobility in mouse tail suspension test (TST) and the anti-depressant-like effects are augmented by ketamine. Similarly, female

Fischer rats treated with tropisetron spent less time immobile in the FST (Rajkumar and Mahesh, 2010). One of the probable mechanism for anti-depressant effect of NCE's in CUMS model is that it may influence the serotonergic transmission affected by increased level of inflammatory mediators and highly activated indoleamine-2, 3-dioxigenase (IDO) enzyme responsible for degradation of tryptophan (O'Connor et al., 2009).

Purpose: Models based on chronic, social or early life stress appear to have greater etiologic validity compared to those, which rely on lesions or monoamine depletion, which is a not a common etiologic factor in human depression. Therefore, CUMS model can be considered as an animal model of depression implicating stress as the etiological cause of depression. The model also has predictive validity since the reversal of abnormal behaviour requires 2-3 weeks of treatment.

Groups for Evaluation of "6g" were Divided as Follows:

1. Normal Control = 8
2. Stress Control =8
3. Stress+ "6g" (1) =8
4. Stress+ "6g" (2) =8
5. Stress+ FLX (20) =8

Groups for Evaluation of "6n" were Divided as Follows:

1. Normal Control = 8
2. Stress Control =8
3. Stress+ "6n" (1) =8
4. Stress+ "6n" (2) =8
5. Stress+ FLX (20) =8

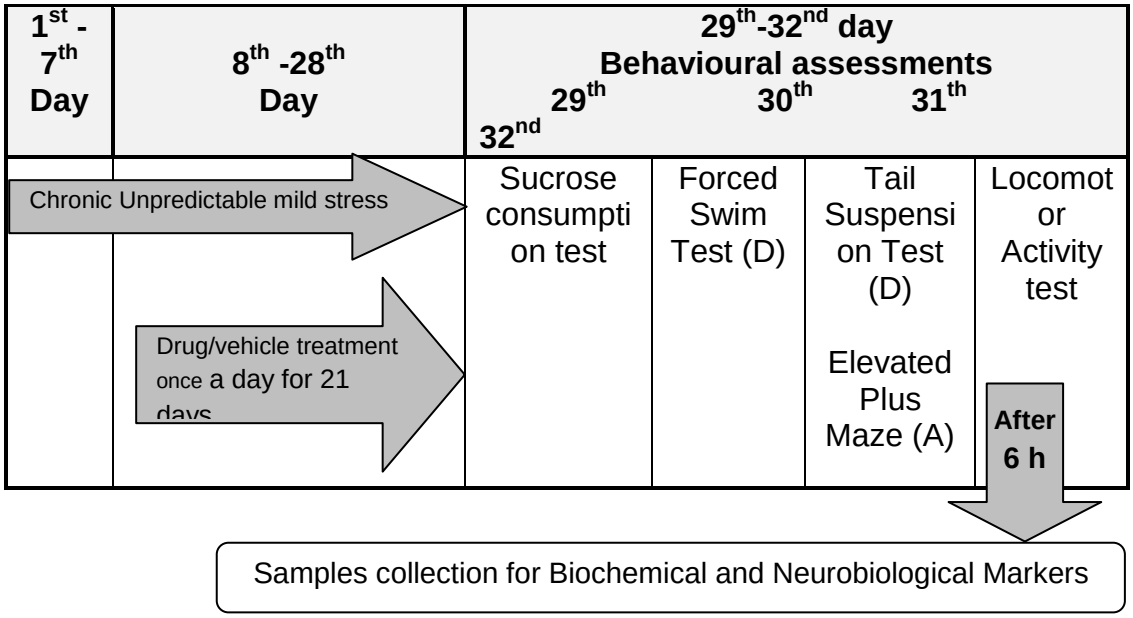
4.14. Chronic Unpredictable Mild Stress (CUMS) Procedure

The CUMS procedure was performed as described by Ducottet et al., (2003), with slight modifications (foreign object and water temperature in forced swimming) (Jindal et al., 2012, 2013b). Briefly, CUMS consisted of exposure to a variety of unpredictable stressors (randomly); namely: (1) 24 h food deprivation (FD), (2) 24 h water deprivation (WD), (3) 1 h exposure to a empty bottle (EB), (4) 7 h cage tilt (CT) (45°), (5) overnight illumination (OI), (6) 24 h soiled cage (SC) (200 ml water in 100 g sawdust bedding), (7) 6 minutes forced swimming (FS) at 12 °C, (8) 2 h physically restraint (PR), and (9) 24 h

exposure to a foreign object (FO) (e.g., glass marbles). Schedule of CUMS, drugs treatment, behavioural and biochemical tests has been shown in Table 14.

These stressors were randomly scheduled over a 1-week period and repeated throughout the 4-week experiment. Control animals were undisturbed except for necessary housekeeping procedures.

Table 14: Schedule for CUMS, drugs treatment, behavioural and biochemical tests



4.15. Behavioural Assessments

A. Spontaneous Locomotor Activity (SLA)

In order to identify the association of immobility in the FST with changes in motor activity, the spontaneous locomotor activity of mice was assessed using the actophotometer (Boissier & Simon, 1965) which contains a square arena (30 × 30 cm) with walls that are fitted with photocells just above the floor level. The photocells were checked prior to the commencement of the experiment. The mice were then individually placed in the arena. After two minutes acclimatization period, the digital locomotor scores were recorded for the next 8 minutes in a dimly lit room.

B. Forced Swim Test (FST)

The test was performed as described earlier (Porsolt et al., 1977) with slight modifications (Mahesh et al., 2007). In brief, each mouse was placed individually in a glass cylinder (diameter: 22.5 cm, height: 30 cm) containing 15 cm of water at 23 ± 2 °C. The mice were placed in the water and forced to swim for 6 minutes. The duration of immobility was recorded during the last 4 minutes of the 6 minutes test. A mouse was considered to be immobile when it stopped struggling & passively moved to remain floating & keep its head above water. Water was changed between trials and temperature was maintained at 23 ± 2 °C.

C. Tail Suspension Test (TST)

This is yet another test to confirm the depression-like symptoms by evaluating duration of immobility. This is more sensitive test to check the preliminary behavioural pattern than FST. Mice were individually suspended by the tail to a horizontal bar (distance from floor was 50 cm) using scotch tape (distance from tip of tail was approximately 1 cm). typically, mice exhibited several escape-oriented behaviour interspersed with temporally increasing bouts of immobility (Steru et al., 1985; Thierry et al., 1986) .The duration of immobility (in seconds) during the 6-min test session was recorded. The changes in behaviour in TST like climbing reflect the role of neuro-transmitters dopamine and nor-adrenaline in this test.

D. Elevated Plus Maze (EPM)

The EPM apparatus consisted of a wooden maze with two enclosed arm (30 × 5 × 15 cm) and two open arms (30 × 5 × 0.25 cm) that extended from a central platform (5 × 5 cm) to form a plus sign. The plus-maze apparatus was elevated to a height of 45 cm and placed inside a sound-attenuated room. Each trial was started by placing a mouse on the central platform of the maze facing its head towards an open arm. The behavioural performances recorded during a 5 minute test period were; percentage open arm entries (OAE), percentage time spent in open arm (TSOA) (Biala and Kurk, 2008). Entry into an arm was

considered valid only when all four paws of the mouse were inside that arm. The apparatus was thoroughly cleaned with 70% ethanol after each trial.

E. Sucrose Preference Test

Sucrose preference test was carried out at the end of 4 weeks of CUMS exposure. The test was performed as described earlier (Casarotto and Andreatini, 2007; Luo et al., 2008) with slight modifications described by (Jindal et al., 2013; Willner et al., 1997). In brief, before the test, mice were trained to adapt to sucrose solution (1%, w/v) by placing two bottles of sucrose solution in each cage for a period of 24 h; then one bottle of sucrose solution was replaced with water for 24 h. After adaptation, mice were deprived of water and food for 24 h. The mice were housed in individual cages and were free to access to two bottles containing 100 ml of sucrose solution (1% w/v) and 100 ml of water, respectively. After 24 h, the volume consumption of sucrose solution and water were recorded. Then percentage of sucrose consumption was calculated as ratio of the amount of sucrose solution to that of total solution (sucrose & water) ingested within 24 h.

4.16. Biochemical Analysis

All the biochemical, neurochemical and neurobiological estimations were carried out 6 hrs after completion of all the behavioural assessments.

A. Tissue Extraction

The rats and mice were decapitated and brain was harvested quickly as per procedure mentioned elsewhere (Glowinski and Iversen, 1966) and placed on a petri dish in an ice bath. The homogenate for different estimations were performed as per the ELISA kits instruction or the reported literatures.

B. Oxidants and Anti-oxidants Assays

i. Brain Homogenate Preparation

Brains were quickly removed and washed with ice-cold sterile saline (0.9%). The whole brain samples were then homogenized with ice-cold 0.1 M

phosphate buffer (pH 7.4) 10 times (w/v). The homogenate was centrifuged at 4000 rpm (4 °C) for 20 min to remove cellular debris and aliquots of supernatant were separated and stored at -80 ºC until the oxidative and anti-oxidant assays were carried out.

ii. Preparation of Reagents for Oxidant/Anti-oxidant Markers Estimation

a. Greiss reagent preparation:

This was prepared by adding 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid.

b. Dichromate-acetic acid reagent:

This was prepared by adding 5% potassium dichromate and glacial acetic acid in ratio of 1:3.

iii. Estimation of Lipid Peroxidation Level

The malondialdehyde (MDA) content, a quantitative measurement of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARS) by the method of Wills, (1966). In this 0.1 ml of supernatant was incubated with 0.5 ml tris HCL (0.1 M, pH 7.4) for 2 h. To this, 1 ml of trichloroacetic acid (10% w/v) was added and centrifuged at 1000 × g for 10 min. To 1 ml supernatant, 1 ml (0.67% w/v) thiobarbituric acid was added and kept in the boiling water bath for 10 min, cooled and then 1 ml distilled water was added. The amount of lipid peroxidation products was measured by reaction with thiobarbituric acid at 532 nm using the spectrophotometer (UV-1800 Shimadzu, Japan). The values were expressed as nanomole of MDA per milligram of protein.

iv. Estimation of Nitrite/Nitrate Level

The accumulation of nitrite in brain supernatant, an indicator of the production of nitric oxide was determined by a colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al., (1982). Equal volumes of

supernatant and Greiss reagent were mixed, the mixture incubated for 10 min at room temperature in the dark and the absorbance determined at 540 nm using Spectrophotometer (UV-1800 Shimadzu, Japan). The concentration of nitrite in supernatant was determined from sodium nitrite standard curve and expressed as micromole per milligram of protein.

v. Estimation of Reduced Glutathione Level

The test procedure was adopted as indicated elsewhere (Ellman, 1959) with slight modifications (Jindal et al., 2013). In brief, the procedure is as follows. 1 ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4 °C for 1 h. The samples were centrifuged at 1200×g for 15 min at 4 °C. To 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1 mol/l, pH 8) and 0.2 ml of 5, 5-dithio-bis (2-nitrobenzoic acid) were added. The colour developed was measured immediately at 412 nm using Spectrophotometer (UV-1800, Shimadzu, Japan). Results were expressed as micromole per milligram of protein.

vi. Estimation of Superoxide Dismutase Activity

SOD activity was measured by the method of Misra and Fridovich, (1972). Auto-oxidation of epinephrine at pH 10.4 was measured. In this method, supernatant of the tissue was mixed with 0.8 ml of 50 mM glycine buffer, pH 10.4 and the reaction was started by addition of 0.02 ml (−)-epinephrine. After 5 min the absorbance was measured at 480 nm using spectrophotometer (UV-1800, Shimadzu, Japan). The activity of SOD was expressed as % activity of sham control group.

vii. Estimation of Catalase Activity

Brain CAT activity was assayed by the method described earlier (Sinha, 1972). The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 mol/l phosphate buffer (pH 7), 0.1 ml of brain homogenate supernatant and 0.4 ml of 2 mol/l hydrogen peroxide. The reaction was stopped by the addition of 2 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio). The absorbance was measured at 620 nm using Spectrophotometer

(UV-1800, Shimadzu, Japan) and expressed as micromoles of hydrogen peroxide decomposed/min/milligram protein.

viii. Protein Estimation

The protein content was measured in all brain samples by the biuret method using bovine serum albumin as standard (Koller, 1984).

4.17. Hormonal Parameter

A. Estimation of Endocrinology Hormone (Corticosterone)

To determine the alterations in the HPA axis serum corticosterone level was measured. Measurement of serum CORT was performed using a commercially available ELISA kit (IBL, USA) according to the manufacturers' instructions.

B. Serum Sample Separation

The blood samples were collected by decapitation of rats or mice after 6 h of the last behavioural assessments as depicted in the schedule of drug administration and behavioural assessment sections (Page No. 85). Blood samples were collected and allowed to coagulate at room temperature for 30 min and were subsequently centrifuged at 3500g for 15 min. Serum was separated and all the samples were stored at -80 ºC until the corticosterone or other biochemical estimations were carried out.

C. Standard Curve

The standard curve for the corticosterone assay was prepared by using the kit instruction. Already prepared standards, provided in the ELISA kit were used to plot a standard curve. A standard curve was constructed by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis. Procedure for corticosterone measurement has been shown in Fig. 30 as a flow chart.

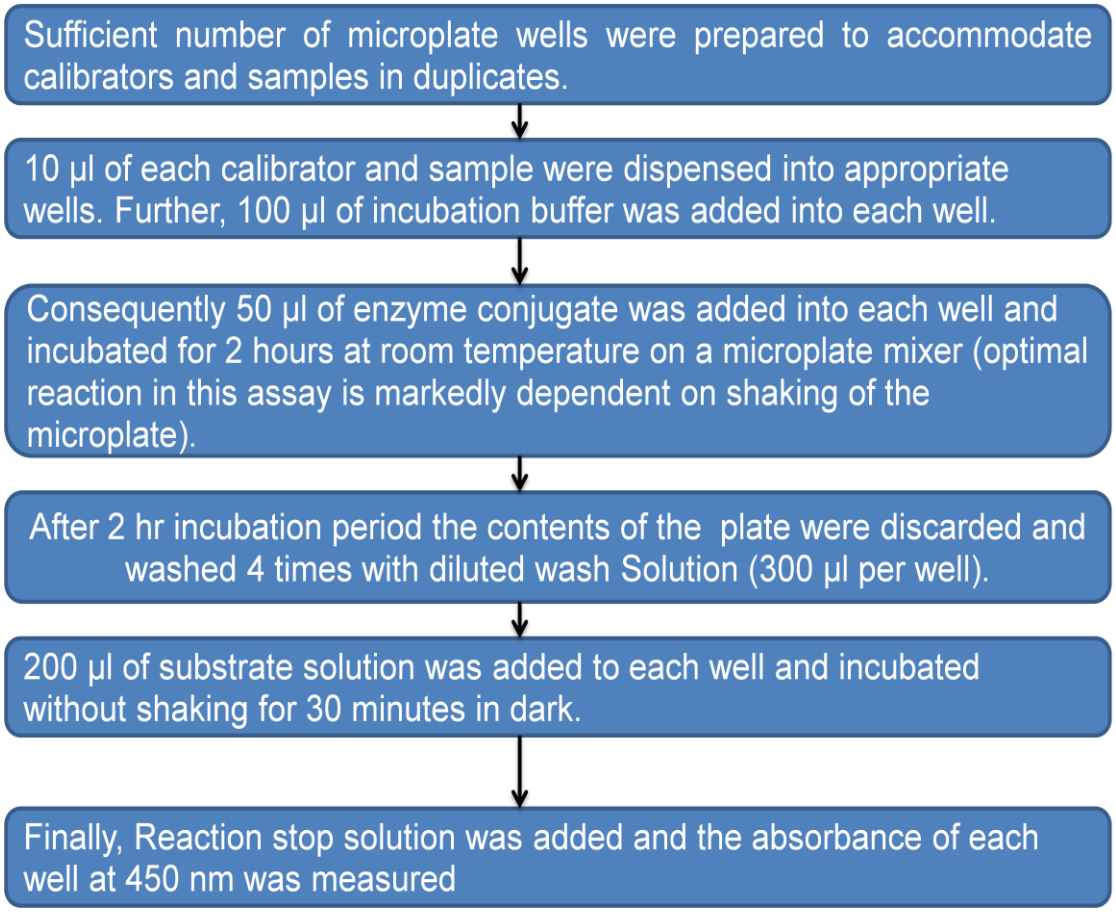


Fig. 30. Estimation of corticosterone levels in mice and rats plasma

4.18. Neuro-chemical Estimation

A. Serotonin (5-HT), Nor-epinephrine (NE) and Dopamine (DA) Estimation

The measurement of neuro-transmitters level was performed using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (DLD, Diagnostika, GMBH, Germany), according to the manufacturer instructions.

B. Tissue Homogenate Preparation

The brain was homogenized in 10 ml of cold acidified n-butanol using a homogenizer. After centrifugation for 5 min at 3000 rpm the supernatant was pipette out.

C. Preparation of Reagents for Neuro-transmitter Estimation

Acid Butanol: This was prepared by adding 0.85 ml of conc. hydrochloric acid (HCl) to n-butanol (volumes make up to 1 litre) (This solution was used to prepare the brain tissue homogenate).

D. Standard Curve Preparation

The standard curves for serotonin, adrenalin, nor-adrenaline and dopamine were plotted according the instruction of the ELISA kit. Procedure for serotonin estimation has been shown in Fig. 31 and procedure for nor-epinephrine and dopamine estimation has been shown in Fig. 32 as a flow chart.

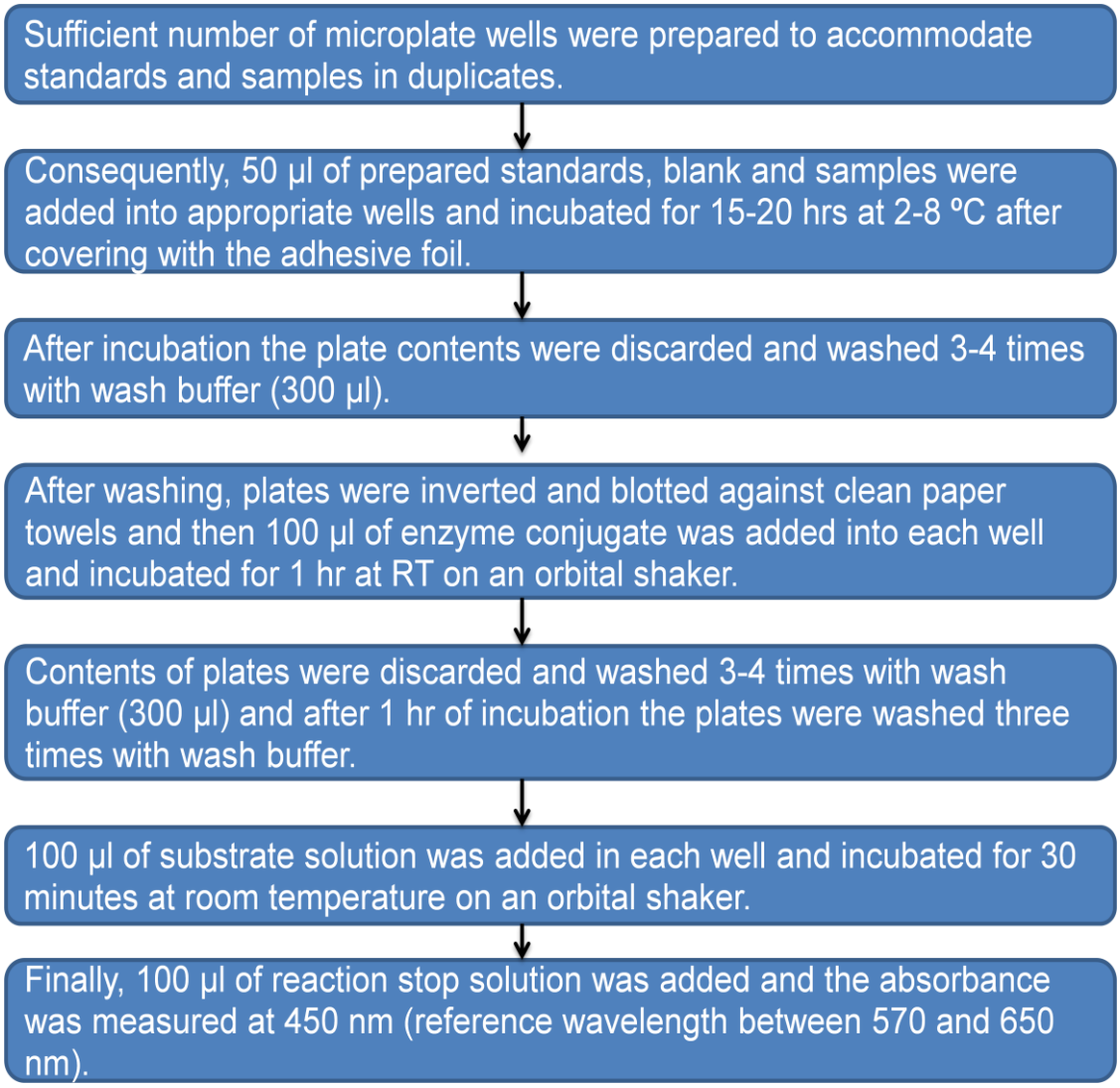


Fig.31. Estimation of serotonin levels in rats brain tissue homogenate

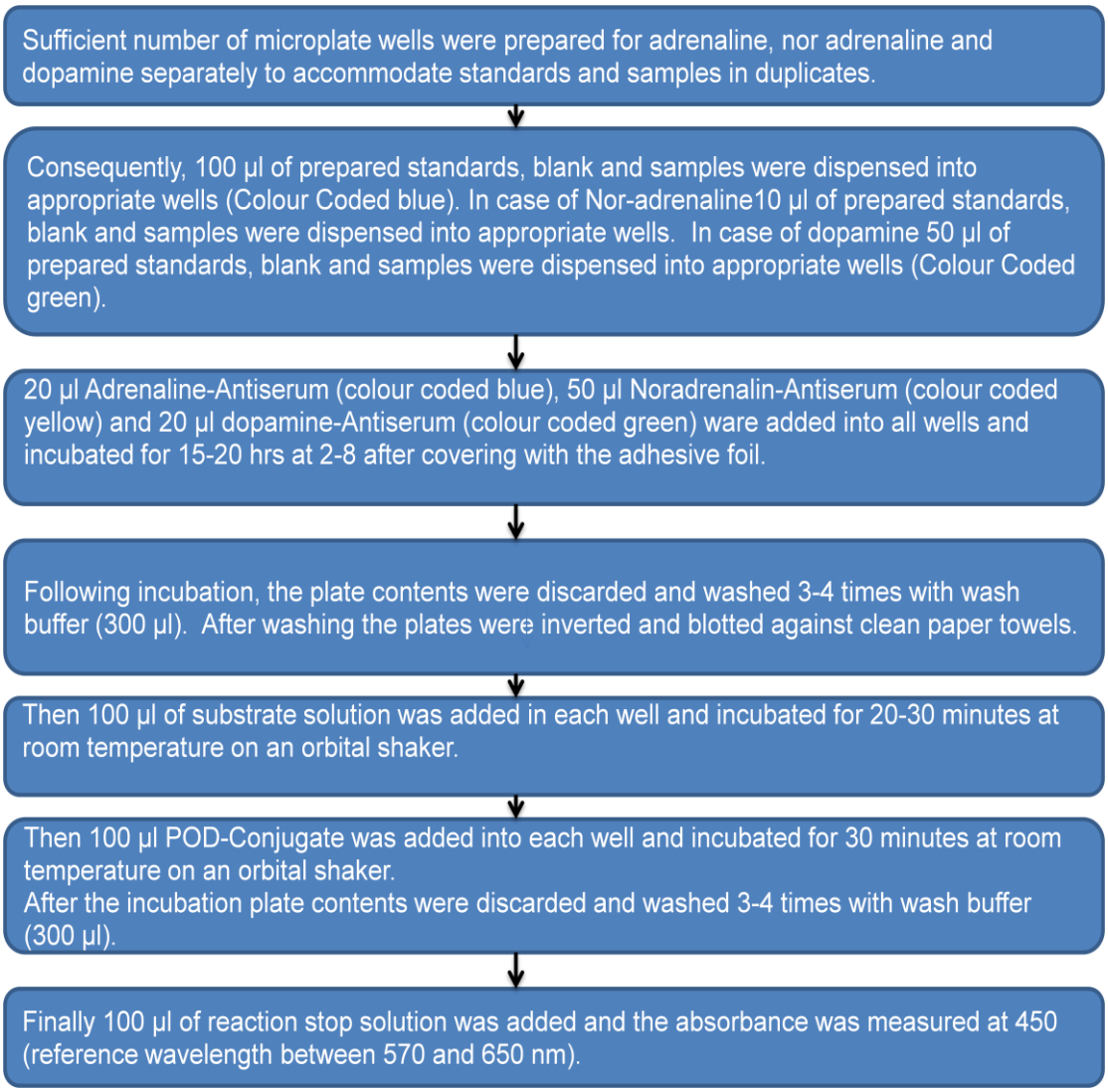


Fig.32. Estimation, Nor-adrenaline and Dopamine levels in rat brain tissue homogenate

4.19. Estimation of Brain Derived Nerotrophic Factor (BDNF)

The measurement of BDNF level was performed using commercially available ELISA kit (Boster Biological Technology Co., LTD, CA, USA), according to the manufacturer instructions.

A. Sample Separation

The brain tissues sample preparation was carried out as per the ELISA kit instruction. The brain tissues were homogenated in the kit calibration buffer and centrifuged. The supernatant was separated out and stored at ≤ -20 ºC.

B. Standard Curve Preparation

The standard curve was plotted according the instruction of the ELISA kit. The different standards (Concentrations: 1000 – 500 – 250 – 125 - 62.5 - 31.2 pg/ml) provided in the ELISA kit were used to plot a standard curve. A standard curve was plotted as the relative absorbance value of each standard solution (Y) vs. the respective concentration of the standard solution (X). The rat BDNF concentration of the samples can be interpolated from the standard curve. Procedure for nor-epinephrine and dopamine estimation has been shown in Fig. 33 as a flow chart.

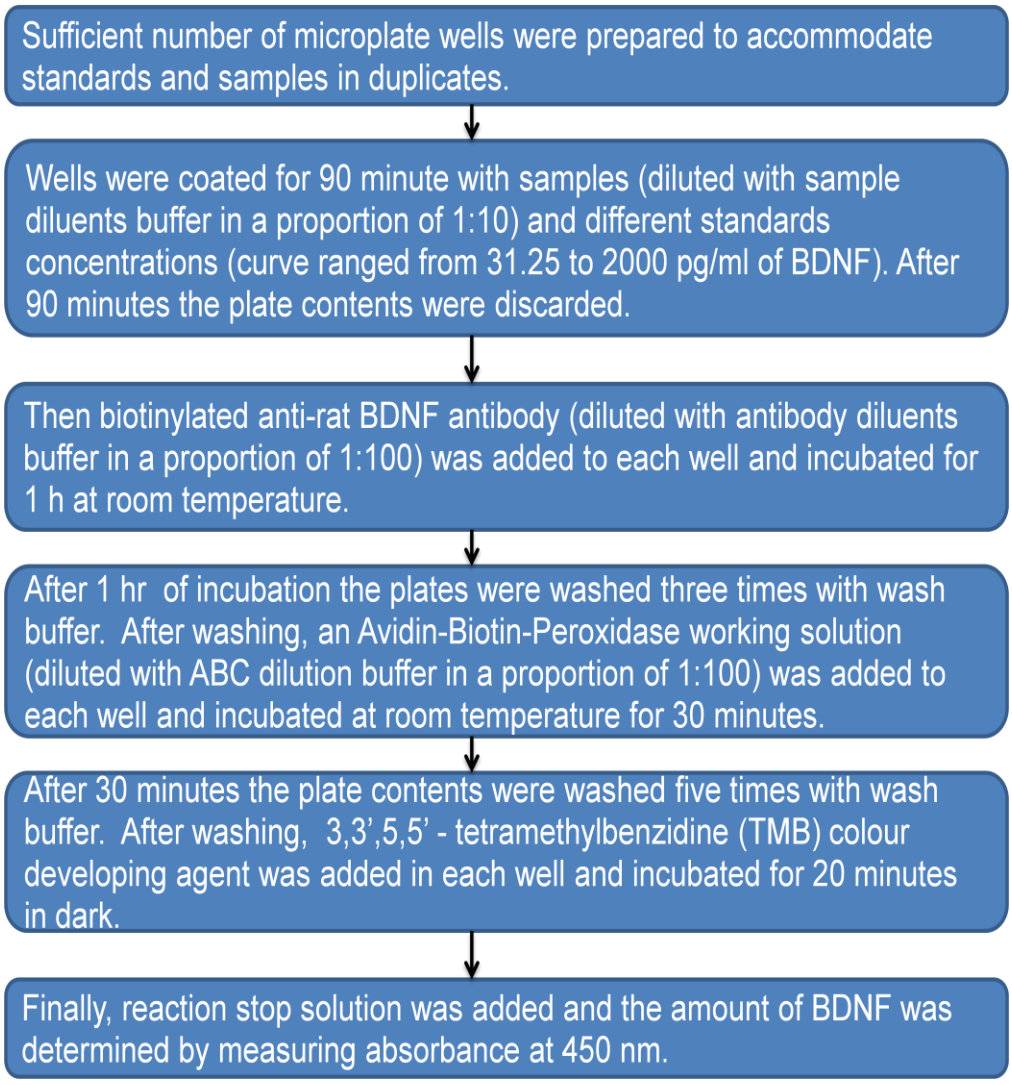


Fig.33. Estimation of BDNF levels in rats samples

4.20. Lipopolysaccharide (LPS) Induced Depression and Anxiety

Experimental and clinical evidence indicates that activation of the immune system contributes to the pathogenesis of mood disorders (Maes et al., 1995, 2011). Patients with major depression have frequently been observed to present with elevated levels of proinflammatory cytokines in blood plasma and cerebrospinal fluid (Yirmia et al., 2009). There is extensive co-morbidity of major depression with medical conditions involving inflammation and an increased expression of cytokines, and the therapeutic use of cytokines such as interferons is known to induce a depression-like and anxiety-like syndrome in a sizeable proportion of patients (Raison et al., 2006). These lines of clinical evidence are complemented by a plethora of animal studies.

Rationale: Oxidation of Tryptophan is generally catalyzed by tryptophan dioxygenase (TDO), and negligibly by indoleamine 2,3 dioxygenase (IDO). However, IDO is greatly by pro-inflammatory mediators such as cytokines, including interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) (Fig. 34). Degradation of tryptophan through the kynurenine pathway has important neuropsychiatric implications (Dantzer et al., 2008). During inflammation the kynurenine pathway predominates and level of serotonin in the synapse decreased.

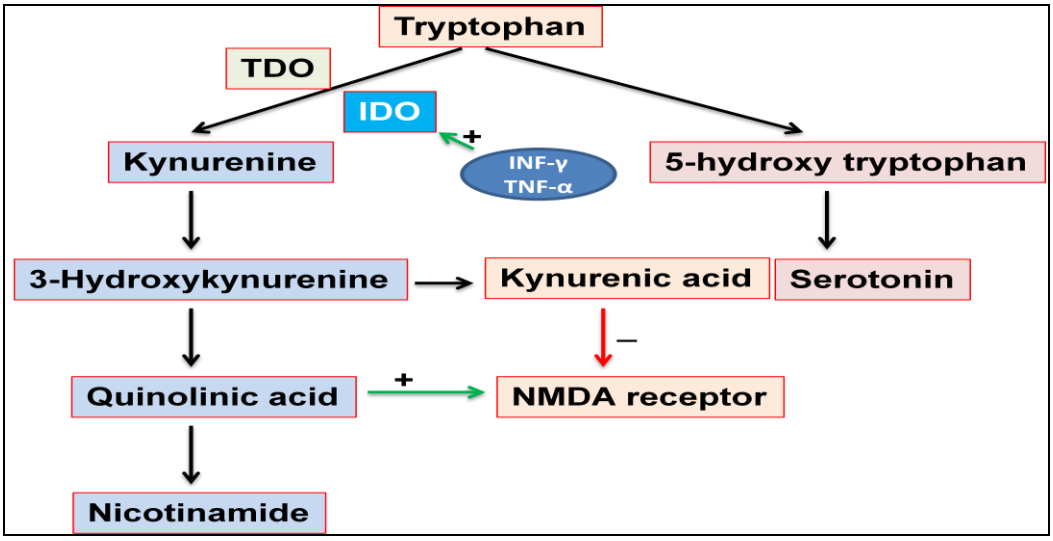


Fig.34. Tryptophan degradation by induction of inflammatory pathway (Dantzer et al., 2008). TDO= tryptophan dioxygenase; IDO= indoleamine 2,3 dioxygenase; INF-γ=Interferon-γ; TNF-α=Tumor Necrosis Factor- α

A. Depression and Anxiety Induction Procedure

LPS was injected to mice in all the groups (except normal control) on Day-0. Behavioural tests (Actophotometer, FST, TST, EPM and L&D test were performed on day-1 to check the induction of depression and NCE and standard was administered at "6g" (1 and 2 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.) daily, for seven days (started at Day-1). On Day-8 and Day-9 all the behavioural tests (SLA, FST, TST, EPM, L/D) were performed (Table 15).

Table 15: Study Plan of LPS Induced Depression and Anxiety Model

	Days									
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Treat-ment	**LPS Injection** →	→	→	**NCE**	**Standard**	→	→	→		
Strain / tests	Swiss albino mouse	Behaviour al analysis (Depressi on confirmati on)							Behaviour -al tests	Behavioural tests, Brain samples were collected for biochemical analysis

B. Biochemical Analysis

Biochemical estimations were carried out with in 6 h after completion of the all behavioural assessments. Brain homogenates were prepared as described in earlier part of the thesis for determination of oxidative stress parameters (similar procedure was adopted as shown in CUMS induced depression and anxiety model) and serotonin estimation (ELISA kit). The groups were divided as follows:

1. Normal control = 8
2. LPS Control = 8
3. "6g" (1) = 8
4. "6g" (2) = 8
5. FLX (20 = 8

4.21. Statistical Analysis

All the results are expressed as mean ± S.E.M. The data obtained in studies from various groups were statistically analyzed using one way analysis of variance (ANOVA) followed by the post-hoc Tukey's test in Graph pad prism 3 software. The value of $P < 0.05$ was considered as statistically significant. The data from interaction and co-morbid studies were analyzed using two-way ANOVA followed by Bonferroni test.

5.0. Results

5.1. Evaluation of "6g" in Rodent Models of Depression

5.1.1. Effect of "6g" on SLA of mice

Compound **"6g"** (0.5, 1, 2 mg/kg, i.p.) did not show any significant [F (4, 28)=5.05, P>0.05] effect on base line locomotion as compared to control (Fig. 35).

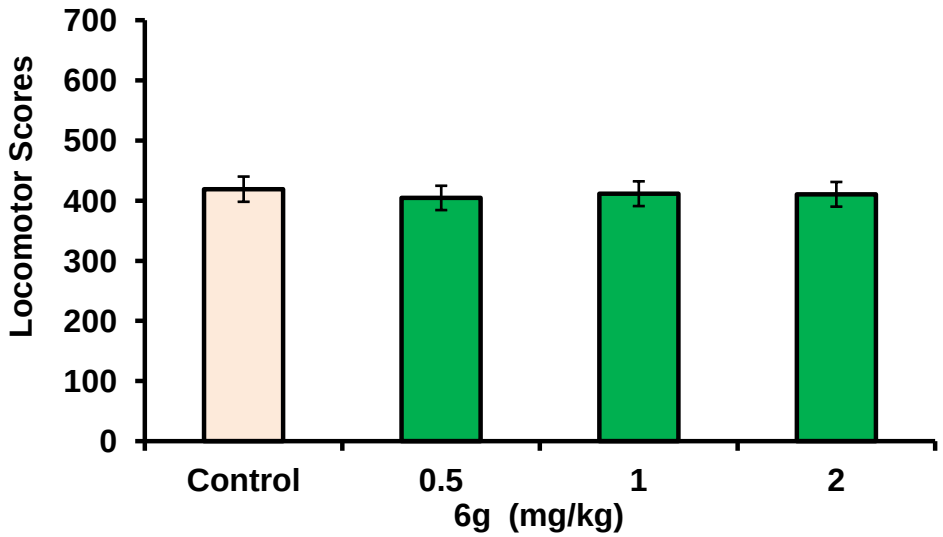


Fig. 35. The columns represent mean locomotor scores and error bars indicate S.E.M. n=8/group.

5.1.2. Effect of "6g" on duration of immobility in mice using FST

In FST, the acute treatment with **"6g"** (1 and 2 mg/kg, i.p.) and ESC (10 mg/kg, i.p.) showed prominent [F (4, 35)=9.91, P<0.05] decrease in the duration of immobility as compared to vehicle treatment (Fig. 36), whereas at lower dose, **"6g"** (0.5 mg/kg, i.p.) was not able to produced any significant effect on duration of immobility.

5.1.3. Effect of "6g" on duration of immobility in mice using TST

In TST, the acute treatment with **"6g"** (1 and 2 mg/kg, i.p.) and BUP (20 mg/kg, i.p.) markedly at [F (4, 35)=7.38, P<0.05] decreased the duration of immobility as compared to vehicle treatment (Fig. 37), while at lower dose **"6g"** (0.5 mg/kg, i.p.) was not able to produce any significant effect on duration of immobility.

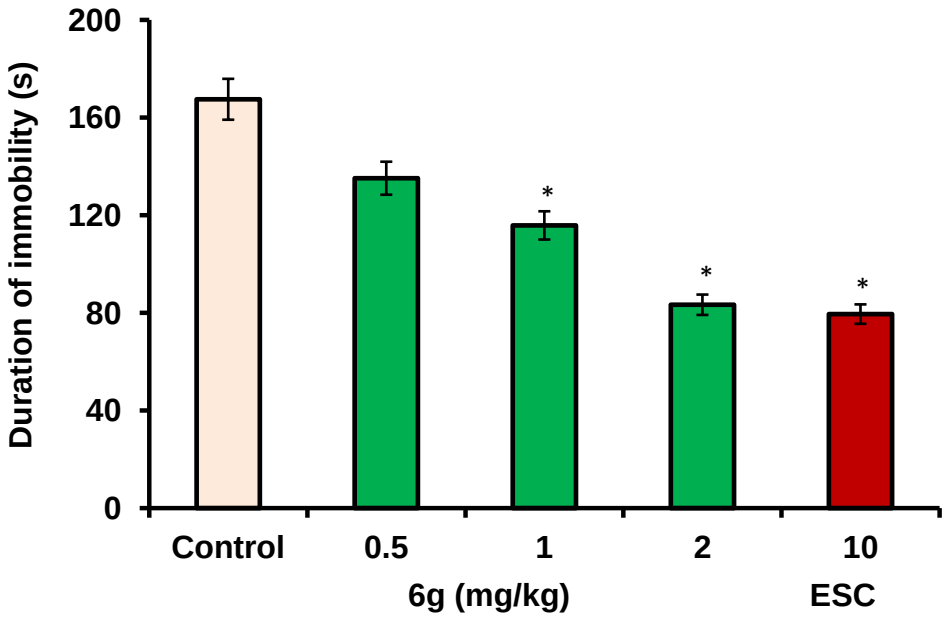


Fig. 36. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8/group. *P < 0.05 compared with vehicle treated group. ESC = scitalopram.

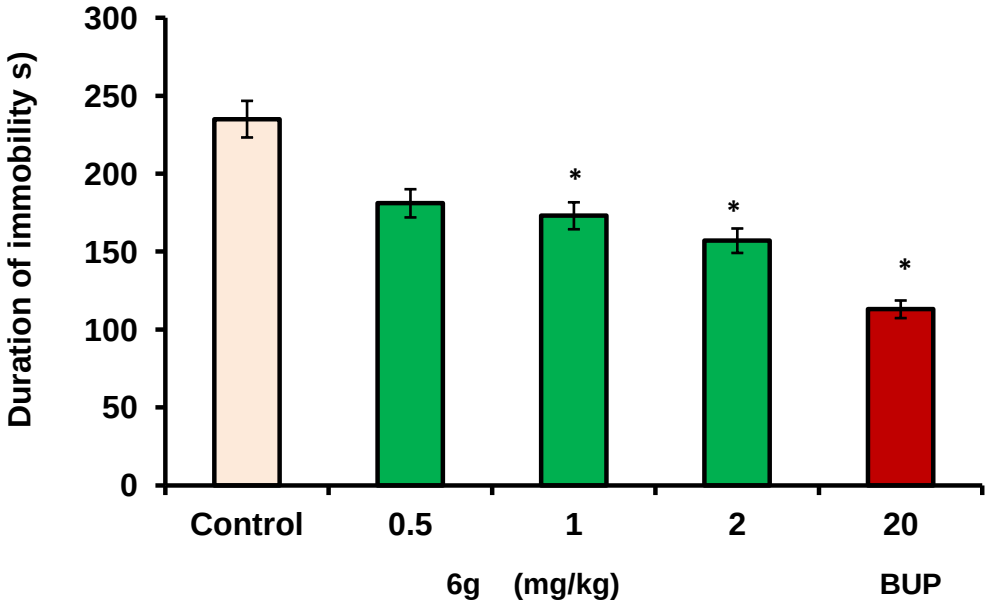


Fig. 37. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle treated group. BUP = Bupropion.

5.1.4. Effect of "6g" on RIH in mice

Reserpine (1 mg/kg i.p) elicited a pronounced decrease in core body temperature in rats. This effect was significantly [F (3, 28)=51.65, P<0.05] reversed with **"6g"** (1 and 2 mg/kg) and ESC (10 mg/kg) treatments (Fig. 38).

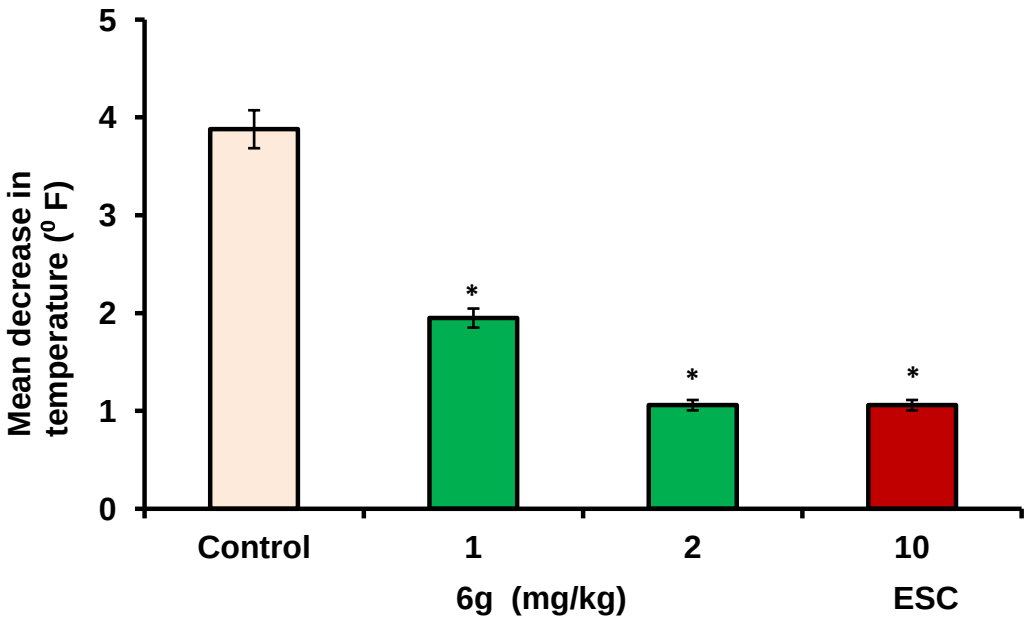


Fig. 38. The columns represent mean decrease in temperature (^{0}F) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle treated group. ESC = Escitalopram.

5.1.5. Effect of "6g" on 5-HTP-HTR in mice

"6g" (1 and 2 mg/kg, i.p.) and FLX (20 mg/kg, i.p.) predominantly [F (3, 28)=21.06, P<0.05] potentiated the 5-HTP/PRG induced head twitches in mice (Fig. 39).

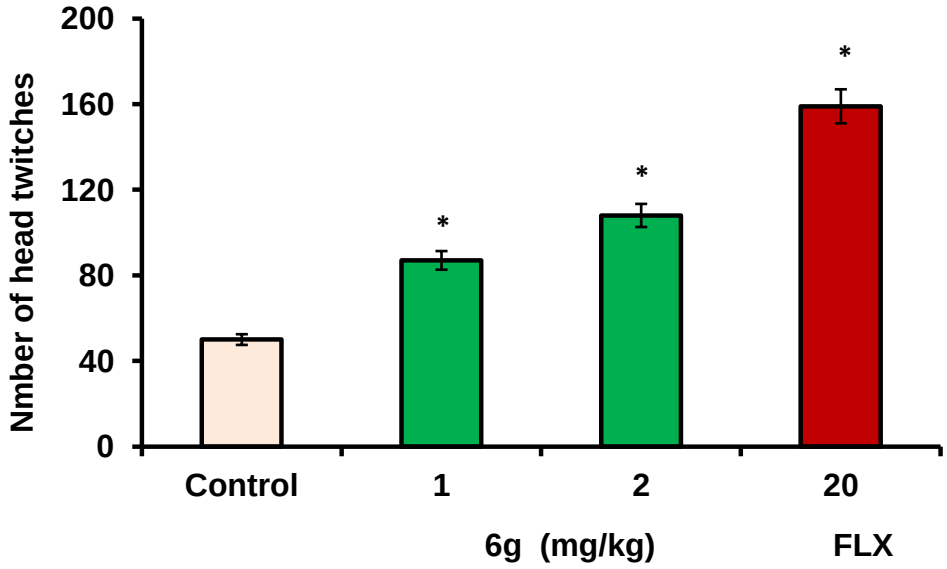


Fig. 39. The columns represent mean number of head twitches and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle-treated group. FLX = Fluoxetine.

5.1.6. Interaction studies of "6g" with standard drugs

Interaction studies with standard anti-depressants were carried out for a conclusive evaluation of anti-depressant potential of 5-HT_3 receptor antagonists. **"6g"** pre-treatment (1mg /kg, i.p.) was found to enhance anti-depressant-like effects of FLX (10 and 20 mg/kg, i.p.) [F (1, 42)=11.18, P<0.05], DMI (10 and 20 mg/kg, i.p.) F (1, 42)=7.098, P<0.05] and VLA (4 and 8 mg/kg, i.p.) [F (1, 42)=12.45, P<0.05] (Fig. 40-42). Moreover, **"6g"** predominantly reversed the depressant-like effect of PTL (1 mg/kg, i.p.) at [F (1, 42)=9.323, P<0.05] as shown (Fig. 43). Further, **"6g"** (1 mg/kg, i.p.) markedly enhanced the anti-depressant activity of BUP (10 and 20 mg/kg, i.p.) [F (1, 42)=11.41, P<0.05] in mice TST (Fig. 44).

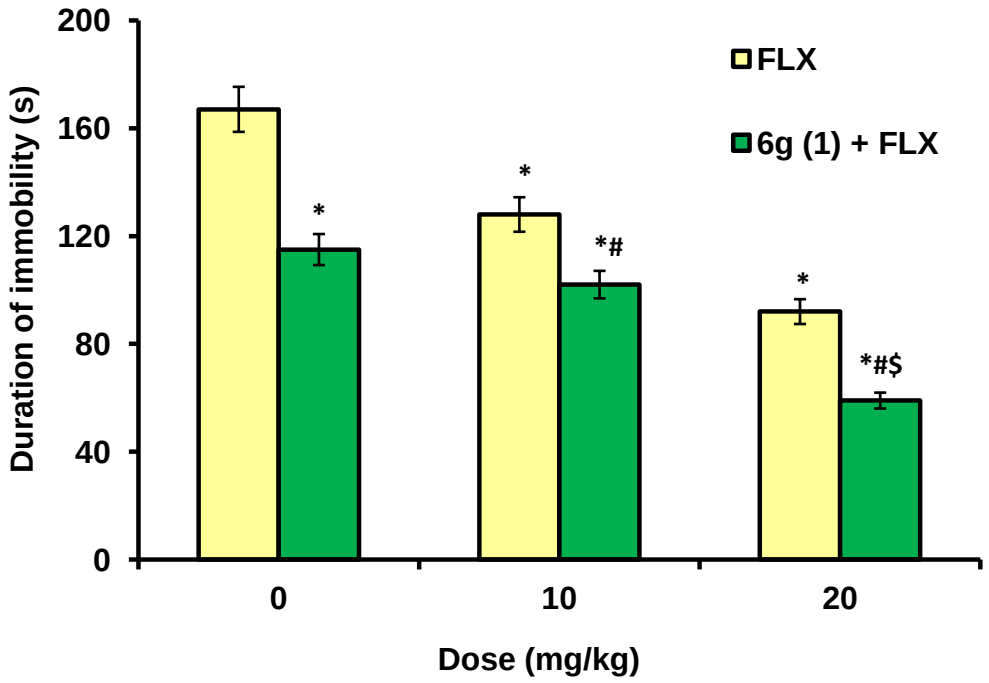


Fig. 40. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with FLX (10 and 20 mg/kg) treated group and $P < 0.05 compared with alone **"6g"** treated group. FLX = Fluoxetine.

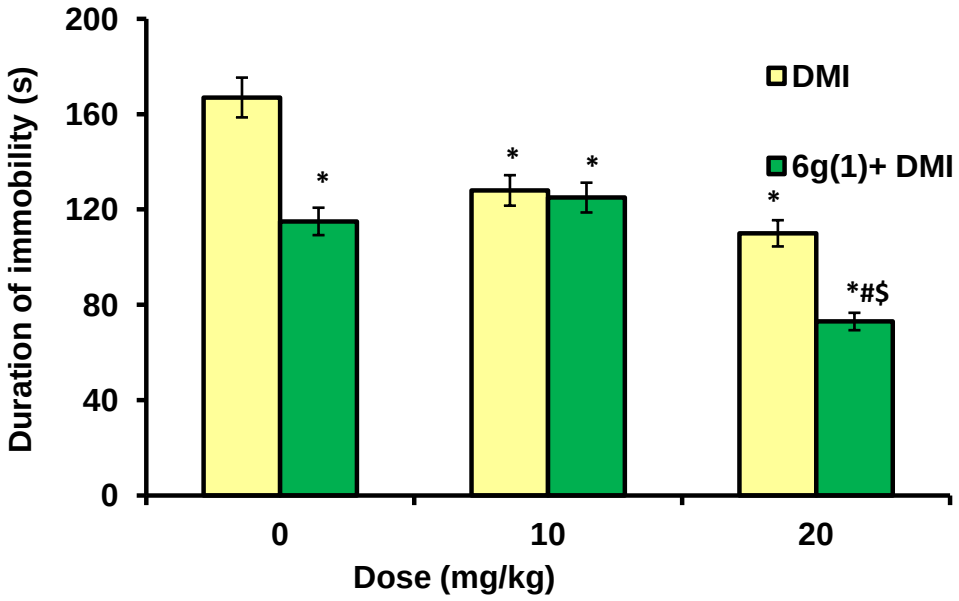


Fig. 41. The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n=8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with DMI (10 and 20 mg/kg) treated group alone and $P < 0.05 compared with alone **"6g"** treated group. DMI=Desipramine

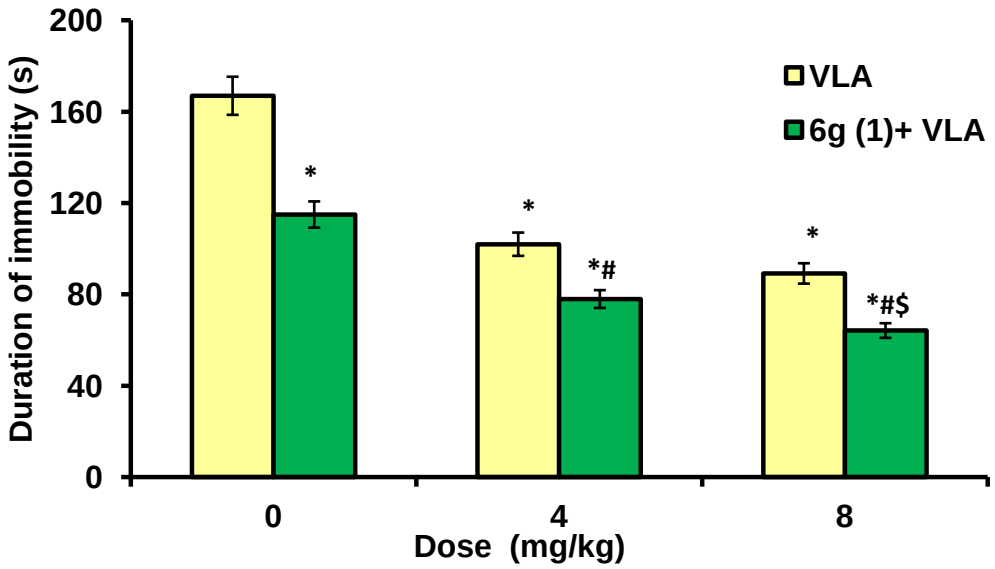


Fig. 42. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n=8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with VLA (4 and 8 mg/kg) treated group and $P < 0.05 compared with alone **"6g"** treated group. VLA=Venlafaxine

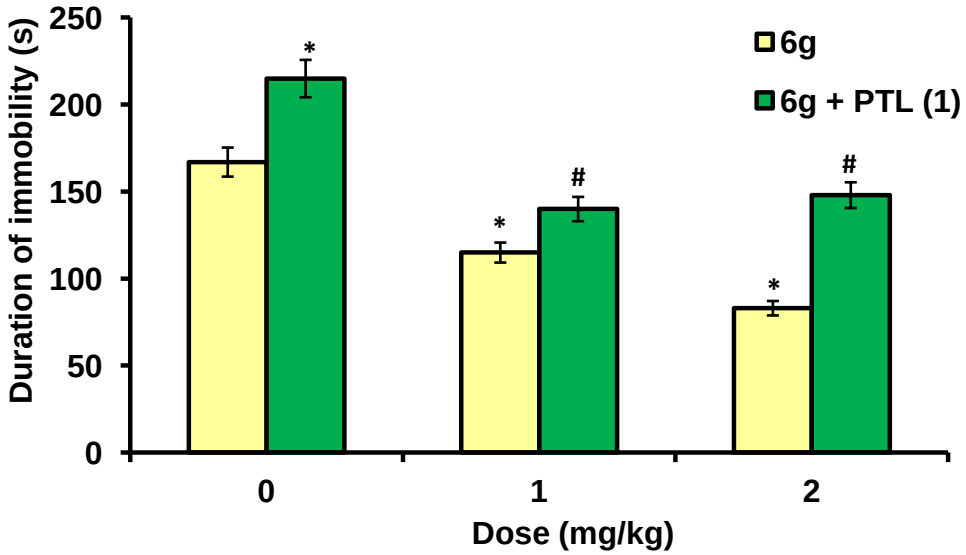


Fig. 43. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 vs. vehicle treated group and # P < 0.05 compared with PTL(1 mg/kg) treated group. PTL=Parthenolide.

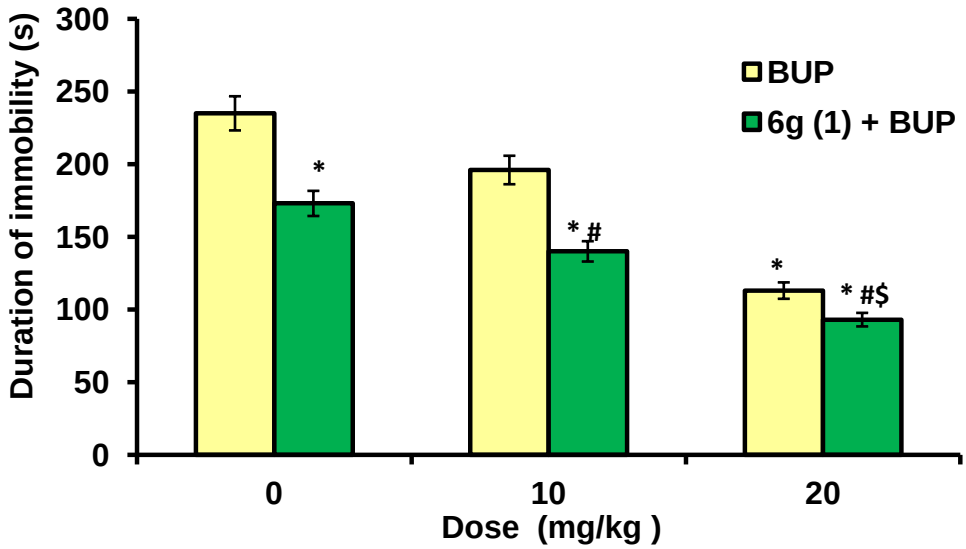


Fig. 44. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n=8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with BUP (10 and 20 mg/kg) treated group and $P < 0.05 compared with alone **"6g"** treated group. BUP=Bupropion.

5.2. Evaluation of "6g" in Animal Models of Anxiety

5.2.1. Effect of "6g" on behaviour of mice in EPM test

Acute treatment with **"6g"** (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) showed pronounce increase in the percentage in both OAE [F (3, 28)=19.54, P<0.05] and TSOA [F (3, 28)=6.685, P<0.05] as compared to vehicle control group (Table 16). **"6g"** (1 mg/kg, i.p.) was not able to produce any significant change on both the parameters as compared to vehicle control group.

Table 16: Effect of "6g" on behaviour of mice in EPM test

Treatment (mg/kg)	% TSOA	% OAE
Vehicle Control	2.00 ± 0.68	11.17 ± 1.97
DZM (2)	11.17 ± 1.47*	37.70 ± 4.73*
"6g" (1)	1.67 ± 0.67	9.75 ± 0.68
"6g" (2)	10.61 ± 3.65*	35.35 ± 4.46*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group ; n = 8/group. DZM=Diazepam.

5.2.2. Effect of "6g" on behaviour of mice in L/D test

Compound **"6g"** (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment predominantly [F (3, 28)=4.541, P<0.05] increased the number of entries from one compartment to other as well as markedly [F (3, 28)=23.62, P<0.05] increase in the total time spent in lit area (Table 17). Lower dose of **"6g"** (1 mg/kg, i.p.) did not produce significant change in any of the parameters as compared to vehicle control group.

Table 17: Effect of "6g" on behaviour of mice in L/D test

Treatment (mg/kg)	Time spent in Lit area (s)	No. of transitions
Vehicle Control	49.67 ± 4.90	19.83 ± 1.25
DZM (2)	101.83 ± 5.08*	31.33 ± 1.91*
"6g" (1)	47.00 ± 4.80	23.83 ± 2.41
"6g" (2)	70.00 ± 5.97*	27.93 ± 3.30*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.2.3. Effect of "6g" on behaviour of mice in HB test

The results of HB test are shown in Table 18. Compound **"6g"** (1 and 2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment showed prominent increase in the number of head dips [F (3, 28)=8.000, P<0.05] and number of square crossed [F (3, 28)=14.96, P<0.05], where as decrease in latency time of head dips was observed [F (3, 28)=29.83, P<0.05] as compared to vehicle control group.

Table 18: Effect of "6g" on behaviour of mice in HB test

Treatment (mg/kg)	No. of head dips	No. of square crossed	Latency Time (s)
Vehicle Control	8.50 ± 1.06	2.83 ± 0.87	9.67 ± 0.67
DZM (2)	26.50 ± 4.03*	18.50 ± 2.01*	2.33 ± 0.42*
"6g" (1)	18.50 ± 1.84*	12.82 ± 1.49*	5.17 ± 0.76*
"6g" (2)	23.17 ± 3.15*	15.50 ± 2.31*	3.00 ± 0.52*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam

5.2.4. Effect of "6g" on behaviour of mice in OFT

Compound **"6g"** (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment significantly increased the number of square crossed [F (3, 28)=37.03, P<0.05] as compared to vehicle treatment group. At lower dose, **"6g"** (1 mg/kg, i.p.) did not produce any notable (P<0.05) change in ambulation score. None of the tested dose of DZM and **"6g"** affect the rearing score as compared to vehicle control (Table 19).

Table 19: Effect of "6g" on behaviour of mice in OFT

Treatment (mg/kg)	Ambulation scores	Rearing
Vehicle Control	145.67 ± 4.08	9.40 ± 0.64
DZM (2)	205.23 ± 5.09*	10.20 ± 0.45
"6g" (1)	172.25 ± 3.84	7.40 ± 0.56
"6g" (2)	190.76 ± 3.75*	8.00 ± 0.62

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.3. Co-morbid Evaluation in OBX Rats Using Behavioural Test Battery

5.3.1. Effect of OBX on body weight of rats

Table 20. displays the effect of OBX on body weight. Body weight of sham and OBX rats was continuously observed till (28 days) the behavioural tests were started. Decrease in body weight was observed in OBX rats for few days post surgery. Statistical analysis revealed that weight gained in OBX rats was noticeably (P<0.05) less as compared to sham rats.

Table 20: Effect of OBX on change in body weight of rats.

Groups	Dose (mg/kg)	Initial weight	Final weight
Sham control	0	255.50 ± 2.50	294.00 ± 8.00
Sham + **"6g"**	1	261.50 ± 7.50	292.00 ± 9.00
Sham + **"6g"**	2	253.00 ± 7.00	283.50 ± 4.50
Sham+ PAR	10	251.50 ± 3.50	280.50 ± 10.50
OBX control	**0**	**259.60 ± 11.55**	**263.00 ± 13.47***
OBX + **"6g"**	1	258.67 ± 5.30	281.00 ± 4.67
OBX + **"6g"**	2	254.00 ± 4.28	270.33 ± 4.40
OBX + PAR	10	260.67 ± 8.43	280.67 ± 7.76

Each value represents mean ± S.E.M. *P < 0.05 represent the mean change in body weight. **"6g"**/PAR/vehicle (mg/kg) were administered p.o. once a day for 14 days. *P<0.05 vs sham control. n= 6/group

5.3.2. Open field test (OFT)

OFT was the first behavioural study to be performed in OBX rats, post 14 days of treatment (Table 21). The effects of **"6g"** on the behaviour of OBX/sham rats were analyzed in different circumstances as shown in (Table 10). Chronic (14 days, p.o) treatment with **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) predominantly reduced the number of ambulation [$F(7, 40)=44.48$, $P<0.05$], rearing [$F(7, 40)=49.38$, $P<0.05$] in OBX treated rats as compared to the vehicle treated OBX rats. While PAR (10 mg/kg, p.o.) only showed significant [$F(7, 40)=23.99$, $P<0.05$] reduction in number of fecal pellets and no marked reduction in number of fecal pellets takes place in case of **"6g"** (1 and 2 mg/kg, p.o.) as compared to OBX control.

5.3.3. Sucrose consumption test

Sucrose (1%) consumption test was performed to measure anhedonia (loss of pleasure) in rodents. Sucrose consumption was tested in sham and OBX rats. Substantial decrease in sucrose consumption ($P<0.05$) was observed in OBX rats as compared to sham rats. Chronic **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment markedly [$F(7, 40)=5.698$, $P<0.05$] increased the sucrose consumption in OBX treated rats as compared to vehicle treated OBX rats (Table 22).

Table 21: Effect of "6g" on open field behaviour in sham and OBX rats

Treatment (mg/kg)	No. of Ambulation	No. of Rearing	No. of Fecal Pellets
Sham Control	85.00 ± 7.65	10.00 ± 1.25	2.00 ± 0.25
Sham+**"6g"** (1)	90.50 ± 8.82	8.25 ± 1.00	2.33 ± 0.15
Sham+**"6g"** (2)	88.25 ± 8.45	7.50 ± 1.00	2.00 ± 0.35
Sham+ PAR (10)	84.25 ± 4.75	8.10 ± 0.75	2.00 ± 0.30
OBX Control	225.00 ± 9.12*	35.00 ± 2.65*	7.00 ± 0.69*
OBX+**"6g"** (1)	120.00 ± 5.82#	14.00 ± 1.01#	5.85 ± 0.62
OBX+**"6g"** (2)	115.00 ± 6.25#	9.50 ± 1.12#	5.00 ± 0.45
OBX+ PAR (10)	90.56 ± 4.50#	9.00 ± 0.65#	2.10 ± 0.27#

Each value represents mean ± S.E.M.,* $P < 0.05$ when compared to the sham operated rats, #$P < 0.05$ when compared to the vehicle-treated OBX rats; n = 6/group). PAR=Paroxetine

5.3.4. Elevated plus maze (EPM)

EPM was employed for the anxiolytic test in laboratory set-up. Percentage OAE and TSOA were measured in EPM test. OBX rats exhibited increased in percentage of both open arms entries [P<0.05] and time spent in open arm [P<0.05] of the maze in comparison with sham-operated rats (opposite to that observed in anxiety). Chronic **"6g"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment prominently decreased percentage OAE [F (7, 40)=11.94, P<0.05] and percentage TSOA [F (7, 40)=24.66, P<0.05] in EPM as compared to vehicle treated OBX rats (Table 23). Chronic **"6g"** (1 mg/kg, p.o.) treatment did not reverse OBX behaviour significantly (P<0.05) in EPM as compared to vehicle treated OBX rats.

Table 22: Effect of "6g" on sucrose consumption in sham and OBX rats

Treatment /Groups (mg/kg)	% Sucrose Consumption
Sham Control	66.75 ± 5.49
Sham+**"6g"** (1)	60.25 ± 5.68
Sham+**"6g"** (2)	62.25 ± 6.03
Sham+ PAR (10)	66.75 ± 3.47
OBX Control	31.50 ± 2.97*
OBX+**"6g"** (1)	52.50 ± 4.51#
OBX+**"6g"** (2)	56.25 ± 3.92#
OBX+ PAR (10)	58.50 ± 4.60#

Tabulated results are expressed as mean ± S.E.M. *P < 0.05 vs sham control, #P<0.05 vs OBX control. n = 6/group. PAR= Paroxetine

Table 23: Effect of "6g" on % OAE and % TSOA in sham and OBX rats

Treatment (mg/kg)	% OAE	% TSOA
Sham Control	32.25 ± 3.49	19.00 ± 2.49
Sham+**"6g"** (1)	30.00 ± 4.58	20.25 ± 3.41
Sham+**"6g"** (2)	36.76 ± 3.63	17.55 ± 2.82
Sham+ PAR (10)	40.25 ± 3.47	20.58 ± 2.21
OBX Control	85.50 ± 9.52*	82.24 ± 8.84*
OBX+**"6g"** (1)	76.23 ± 8.51	74.00 ± 8.01
OBX+**"6g"** (2)	65.56 ± 6.92#	61.25 ± 5.72#
OBX+ PAR (10)	50.00 ± 5.60#	55.38 ± 6.04#

The value represents mean percentage OAE and percentage TSOA. Results were expressed in mean ± S.E.M. *P < 0.05 vs sham control , #P < 0.05 vs OBX control. n = 6 /group. PAR=Paroxetine

5.3.5. Hyper-emotionality test

Fig.45. displays mean scores for hyper-emotionality in sham and OBX rats. OBX rats showed substantial increase in hyper-emotional behaviour such as startle, struggle, and fight response, as compared to sham rats. Hyper-emotional behaviour exhibited by OBX rats was predominantly [F (7, 40)=55.01, P<0.05] observed to be reversed by chronic treatment with **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) as compared to OBX control group.

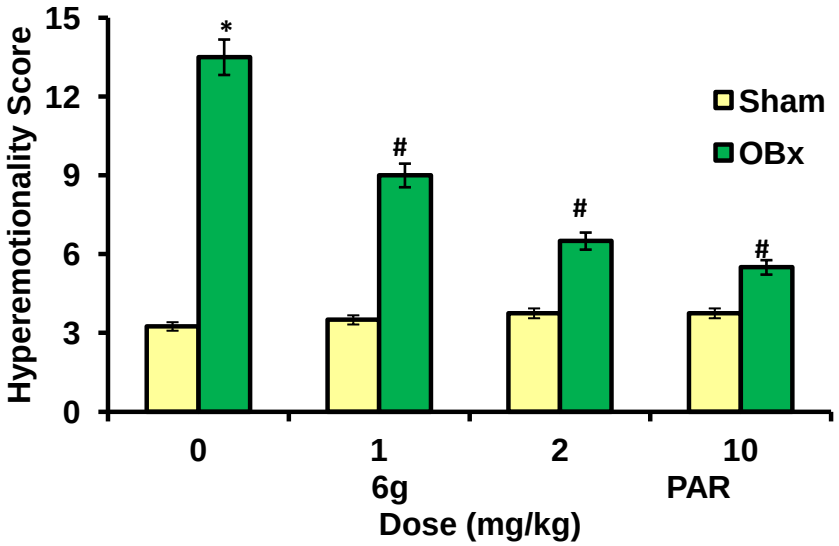


Fig. 45. Effect of **"6g"** (1 and 2 mg/kg) and PAR (10 mg/kg) on hyperemotionality scores of OBX and sham rats. Results are expressed as mean hyper-emotionality scores. Error bars represent mean S.E.M. *P <0.05 vs sham control, # P < 0.05 vs OBX control. n =6 /group. PAR=Paroxetine.

5.3.6. Effect of "6g" on the level of 5-HT, NE and DA in sham and OBX Rats

OBX significantly (P<0.05) reduced the 5-HT (ng/g wet brain tissue), NE (pg/g wet brain tissue) and DA (pg/g wet brain tissue) levels in rat brain as compared to the sham operated controls. Treatment with **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) markedly increased the 5-HT levels [F (7, 40)=11.18, P<0.05] as compared to OBX control (Table 24). Moreover notable [F (7, 40)=63.82, P<0.05] effect on NE levels were observed after treatment with **"6g"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) as compared to OBX control. However, **"6g"** (1 mg/kg) was not able to produce any significant effect on NE

levels as compared to OBX control rats. (Table 24). Furthermore treatment with **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) did not produce any substantial [F (7, 40)=9.937, P<0.05] effect on DA levels as compared to OBX control rats (Table 24).

Table 24: Effect of "6g" on the level of 5-HT, NE and DA in sham and OBX rats

Treatment groups	5-HT	NE	DA
Sham control	540.33 ± 21.67	425.56 ± 11.67	381.38 ± 21.55
Sham + **"6g"** (1)	531.90 ± 17.33	414.38± 21.04	392.45 ±15.21
Sham + **"6g"** (2)	540.67 ± 17.21	438.23 ± 16.33	403.32 ± 22.04
Sham + PAR (10)	545.45 ± 13.65	435.14 ± 15.98	372.65 ±17.19
OBX+ Vehicle	**393.36 ± 18.32***	**145.33 ± 18.48***	**307.93 ± 14.16***
OBX+ **"6g"** (1)	419.25 ± 10.45#	164.50 ± 12.46	289.67 ± 12.55
OBX+ **"6g"** (2)	478.64 ± 22.18#	196.00 ± 17.35#	281.65 ± 19.75
OBX + PAR (10)	499.37 ± 17.45#	272.13 ± 15.27#	301.00± 16.74

Tabulated data represent the mean number of neuro-transmitter level [5-HT (ng/g), DA (pg/g) and NE (pg/g)]. Results are expressed as means ± S.E.M. **"6g"**/PAR/vehicle were administered once a day p.o. for 14 days. *P < 0.05 vs sham control, #P < 0.05 vs OBX control. n = 6/group. PAR=Paroxetine.

5.3.7. Effect of "6g" on the level of BDNF in sham and OBX Rats

OBX significantly (P<0.05) reduced the BDNF levels in rat brain as compared to the sham operated controls. Treatment with **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) markedly increased the brain BDNF levels [F (7, 40)=11.18, P<0.05] as compared to OBX control (Table 25).

Table 25: Effect of "6g" treatment on BDNF levels in sham and OBX rats

Groups	Dose (mg/kg)	BDNF(ng/mg protein)
Sham control	0	82.95 ± 2.37
Sham + **"6g"**	1	89.09 ± 8.50
Sham + **"6g"**	2	87.05 ± 4.85
Sham + PAR	10	85.77 ± 6.21
OBX Control	0	31.60 ± 2.68*
OBX + **"6g"**	1	61.35 ± 4.65#
OBX + **"6g"**	2	75.70 ± 2.11#
OBX + PAR	10	65.00 ± 7.25#

The values are expressed as mean ± S.E.M. The drug/vehicle treatments were carried out once a day for 14 days. *P < 0.05 compared with sham control, #P<0.05 compared with vehicle treated OBX group; n = 6/ group. PAR=Paroxetine.

5.4. Co-morbid Evaluation in TBI Rats Using Behavioural Test Battery

5.4.1. Effect of TBI on body weight of rats

Body weight of sham and TBI rats was continuously observed till the behavioural tests were started. Decrease in body weight was observed post TBI initially. Statistical analysis revealed that TBI rats gained lesser weight than sham group (Table 26).

Table 26: Effect of TBI on body weight.

	Dose (mg/kg)	Initial weight	Final weight
Sham control	0	258.50 ± 3.50	298.00 ± 5.00
Sham + **"6g"**	1	262.50 ± 4.50	296.00 ± 9.00
Sham + **"6g"**	2	259.00 ± 4.00	299.50 ± 4.50
Sham+ PAR	10	265.50 ± 3.50	288.50 ± 10.5
TBI control	**0**	**271.60 ± 4.55**	**275.0 ± 4.47***
TBI + **"6g"**	1	255.67 ± 5.30	284.0 ± 4.25
TBI + **"6g"**	2	254.00 ± 3.50	292.33 ± 4.4
TBI + PAR	10	262.67 ± 4.55	292.67 ± 7.76

Each values represents the mean change in body weight. **"6g"**/PAR/vehicle (mg/kg) were administered p.o. once a day for 14 days. *P < 0.05 vs sham control. n=6/group. PAR=Paroxetine

5.4.2. Open field test (OFT)

The open field test is the behavioural test used to evaluate the hyperactivity (characterized by ambulation, rearing and fecal pellets) in TBI rats as summarized in Table 27. TBI rats exhibited increase in ambulation [F (7, 40)=73.36, P<0.05], rearing [F (7, 40)=11.76, P<0.05] and number of fecal pellets [F (7, 40)=7.209, P<0.05], behaviour for 5 min after being exposed to open field arena. Increased frequencies of ambulation, rearing and fecal pellets were ameliorated by chronic **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment.

5.4.3. Elevated plus maze (EPM)

Table 28. indicates the percent time spent and entries in open arms cumulated over a 5-min test period in EPM. TBI and **"6g"** treated rats demonstrated variable responses in EPM task. TBI rats exhibited a significant (P<0.05) increase in percent OAE and TSOA as compared to sham rats. Chronic treatment with compound **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.)

markedly decreased the number of OAE [F (7, 40)=11.39, P<0.05] and TSOA [F (7, 40)=9.386, P<0.05] as compared to vehicle treated TBI rats in EPM test.

5.4.4. Marble burying behaviour

The effect of TBI and **"6g"** treatment on marble burying behaviour, as one of many anxiety related sequelae is shown in Fig. 46. TBI significantly (P<0.05) increased the marble burying behaviour as compared to sham rats. The chronic **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment significantly [F (7, 40)=5.76, P<0.05] suppressed the aversive marble burying behaviour as compared to vehicle treated TBI rats.

Table 27: Effect of "6g" on the ambulation, rearing and fecal pellets of sham and TBI rats in OFT

Treatment (mg/kg)	No. of Ambulation	No. of Rearing	No. of Fecal pellets
Sham control	105.25 ± 0.85	10.00 ± 1.24	3.00 ± 0.40
Sham + **"6g"** (1)	110.00 ± 1.22	10.00 ± 0.85	2.33 ± 0.71
Sham + **"6g"** (2)	109.25 ± 1.65	8.33 ± 1.15	2.16 ± 0.64
Sham + PAR (10)	99.67 ± 9.37	8.67 ± 0.71	2.00 ± 0.58
TBI Control	231.00 ± 7.92*	31.00 ± 3.5*	8.75 ± 0.85*
TBI+ **"6g"** (1)	152.75 ± 5.218$^{\#}$	17.00 ± 4.41$^{\#}$	4.25 ± 1.70$^{\#}$
TBI+ **"6g"** (2)	158.00 ± 5.49$^{\#}$	13.25 ± 1.70$^{\#}$	3.50 ± 0.64$^{\#}$
TBI+ PAR (10)	109.75 ± 2.65$^{\#}$	9.50±1.040$^{\#}$	2.50 ± 0.28$^{\#}$

Tabulated data represent the mean number of ambulation, rearing and fecal pellets. Results are expressed as means ± S.E.M. **"6g"**/PAR/vehicle were administered once a day p.o. for 14 days. *P < 0.05 vs sham control, $^{\#}$ P < 0.05 vs TBI control. n = 6/group. PAR=Paroxetine

Table 28: Effect of "6g" on the % OAE and % TSOA of sham and TBI rats in EPM

Groups	% OAE	% TSOA
Sham control	25.21 ± 1.63	31.75 ± 3.85
Sham + **"6g"** (1)	22.75 ± 2.05	34.25 ± 4.25
Sham + **"6g"** (2)	25.62 ± 2.46	29.25 ± 2.05
Sham + PAR (10)	21.25 ± 2.46	28.01 ± 3.55
TBI Control	58.25 ± 6.82*	70.00 ± 6.82*
TBI+ **"6g"** (1)	32.61 ± 4.02#	39.25 ± 3.55#
TBI+ **"6g"** (2)	34.50 ± 3.77#	41.50 ± 4.64#
TBI+ PAR (10)	25.00 ± 2.51#	36.65 ± 4.85#

Tabulated results are expressed as mean percentage OAE and TSOA. Error bars represent mean S.E.M. *P < 0.05 vs sham control, # P < 0.05 vs TBI control. n =6 /group. PAR=Paroxetine

5.4.5. Effect of 6g on sucrose consumption of sham and TBI rats

Sucrose (1%) consumption test was performed to measure anhedonia (loss of pleasure) in rodents. Sucrose consumption was tested in sham and TBI rats. Significant decrease in sucrose consumption (P<0.05) was observed in TBI rats as compared to sham rats. Chronic **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatments [F (7, 40)=3.998, P<0.05] increased the sucrose consumption in TBI treated rats as compared to vehicle treated OBX rats (Table 29).

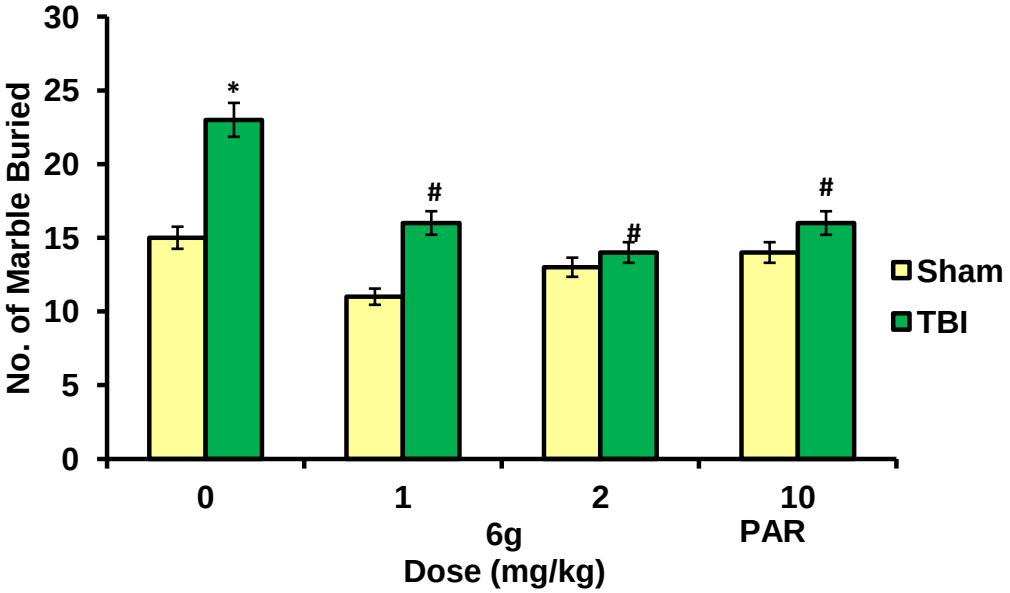


Fig.46. Effect of **"6g"** (1 and 2 mg/kg) and PAR (10 mg/kg) on the number of marbles buried by TBI and sham rats in marble burying test. Results are expressed as mean number of marbles buried. Error bars represent mean S.E.M. * P <0.05 vs sham control, # P < 0.05 vs TBI control. n =6 /group. PAR=Paroxetine

Table 29: Effect of "6g" on the % sucrose consumption in sham and TBI rats

% Sucrose Consumption	
Sham control	76.75 ± 9.25
Sham + **"6g"** (1)	75.25 ± 7.03
Sham + **"6g"** (2)	78.00 ± 8.06
Sham + PAR (10)	79.45 ± 8.35
TBI Control	34.00 ± 3.75*
TBI+ **"6g"** (1)	63.71 ± 6.92$^{\#}$
TBI+ **"6g"** (2)	67.67 ± 6.26$^{\#}$
TBI+ PAR (10)	68.00 ± 8.24$^{\#}$

Tabulated results are expressed as mean ± S.E.M. *P < 0.05 vs sham control , $^{\#}$P < 0.05 vs OBX control. n = 6/group. PAR= Paroxetine

5.4.6. Effect of "6g" on the levels of 5-HT, NE and DA in sham and TBI Rats

In TBI rats, (P<0.05) reduced the 5-HT (ng/g wet brain tissue), NE (pg/g wet brain tissue) and DA (pg/g wet brain tissue) levels in rat brain were observed as compared to the sham controls. Treatment with **"6g"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) significantly increased the 5-HT levels [F (7, 40)=49.30, P<0.05] as compared to OBX control. While **"6g"** (1 mg/kg, p.o.) was not able to produce any major change effect on 5-HT levels, as compared to OBX control rats (Table 30). Moreover, the pronounced [F (7, 40)=14.27, P<0.05] effect on NE level was observed following treatment with **"6g"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) as compared to OBX control. **"6g"** (1 mg/kg, p.o.) was not able to produce any marked change in NE levels as compared to OBX control rats. (Table 30). Furthermore treatment with **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) were not able reduced the DA levels [F (7, 40)=108.00, P<0.05] as compared to OBX control rats (Table 30).

Table 30: Effect of "6g" on the levels of 5-HT, NE and DA in sham and TBI Rats

Treatment groups	5-HT	NE	DA
Sham control	470.22 ± 12.63	476.56 ± 10.52	580.25 ± 20.55
Sham + **"6g"** (1)	475.90 ± 17.32	470.38 ± 12.04	591.45 ±13.27
Sham + **"6g"** (2)	482.00 ± 12.78	485.23 ± 15.62	595.25 ± 10.35
Sham + PAR (10)	484.23 ± 10.32	481.14 ± 15.78	578.90 ±12.98
TBI + Vehicle	**270.00 ± 12.26***	361.33 ± 12.48*	**497.33 ± 15.33***
TBI + **"6g"** (1)	287.25 ± 11.45	361.00 ± 12.45	483.00 ± 18.52
TBI + **"6g"** (2)	332.64 ± 15.00#	410.50 ± 14.35#	492.23 ± 17.75
TBI + PAR (10)	389.22 ± 11.22#	442.23 ± 16.33#	495.21 ± 10.25

Tabulated data represent the mean number of neuro-transmitter level [5-HT (ng/g), DA (pg/g) and NE (pg/g)]. Results are expressed as means ± S.E.M. **"6g"/**PAR/vehicle were administered once a day p.o. for 14 days. *P < 0.05 vs sham control, # P < 0.05 vs TBI control. n = 6/group. PAR=Paroxetine.

5.4.7. Effect of "6g" on the level of BDNF in sham and TBI Rats

TBI significantly (P<0.05) reduced the BDNF levels in rat brain as compared to the sham operated controls. Treatment with **"6g"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) markedly increased the brain BDNF levels [F (7, 40)=11.18, P<0.05] as compared to TBI control (Table 31).

Table 31. Effect of "6g" treatment on BDNF levels of sham and TBI rats

Groups	Dose (mg/kg)	BDNF (ng/mg protein)
Sham control	0	74.65 ± 5.23
Sham + PAR	10	85.00 ± 4.50
Sham + **"6g"**	1	69.33 ± 4.00
Sham + **"6g"**	2	82.00 ± 5.00
TBI control	0	20.72 ± 4.87*
TBI + PAR	10	48.25 ± 3.15#
TBI + **"6g"**	1	39.65 ± 2.75#
TBI + **"6g"**	2	43.52 ± 3.45#

The values are expressed as mean ± S.E.M. The drug/vehicle treatments were carried out once a day for 14 days. *P<0.05 compared with sham control, #P<0.05 compared with vehicle treated TBI group; n = 6/ group. PAR= Paroxetine.

5.5 Co-morbid Evaluation of Mice Under CUMS Using Behavioural Test Battery of Depression and Anxiety

5.5.1. Behavioural Observations

A. Effect of "6g" on SLA

The locomotor activity was evaluated after 28 days of CUMS procedure using actophotometer. There was no marked [F (4, 35)=1.964, P>0.05] difference observed in the locomotor scores in mice of different groups (Fig. 47)**.**

B. Effect of "6g" on immobility time in FST

The mice subjected to CUMS showed significant increase in duration of immobility as compared to control mice. Chronic treatment with **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) substantially [F (4, 35)=35.43, P<0.05] decreased the duration of immobility in stressed mice as compared to stress control group (Fig. 48).

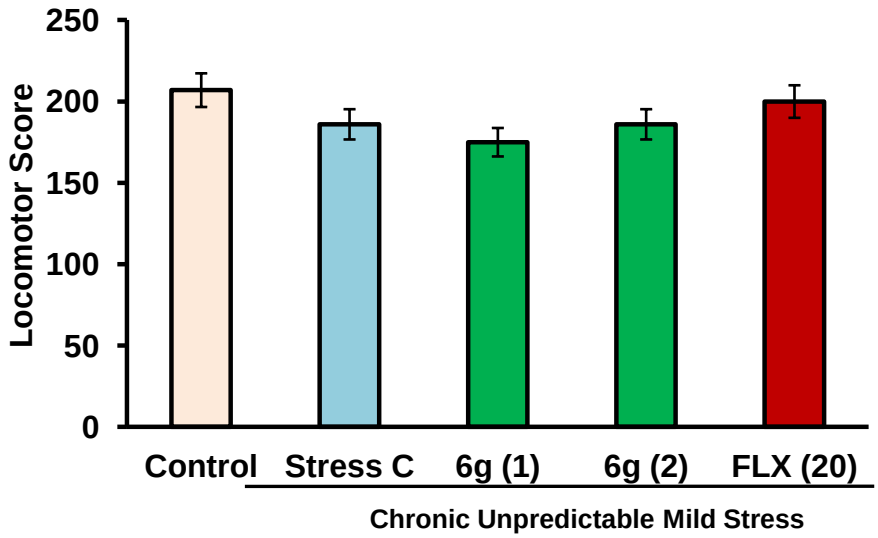


Fig.47. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on locomotor scores in stressed mice. Each column represents mean locomotor scores recorded in 8 min observation period. The error bars indicate S.E.M.; n = 8/group. FLX= Fluoxetine.

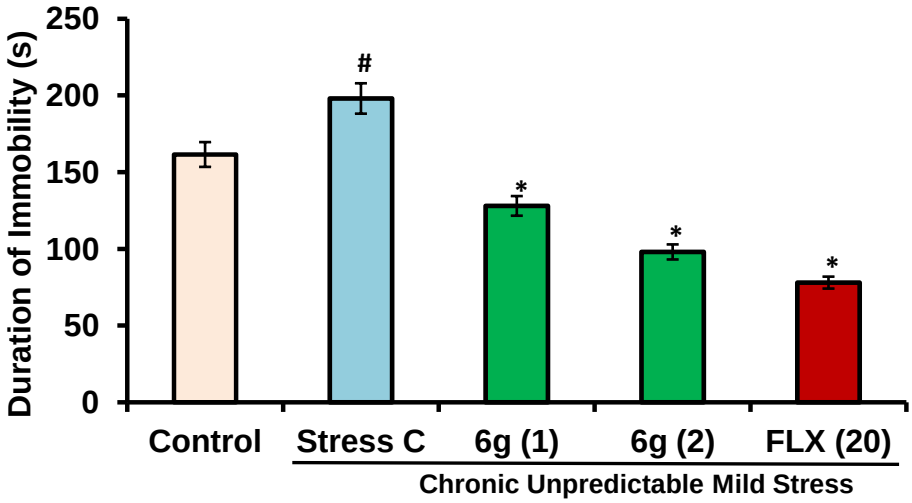


Fig.48. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on duration of immobility in stressed mice. Each column represents mean duration of immobility (s). The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress vehicle treated group; n = 8/group. FLX= Fluoxetine.

C. Effect of "6g" on the percentage of sucrose consumption

Stressed mice showed a significant reduction in the percentage of sucrose consumption as compared to control mice. The chronic administration of **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) significantly [F (4, 35)=31.45, P<0.05] restored the percentage of sucrose consumption in stressed mice as compared to stress control group (Fig. 49).

D. Effect of "6g" on immobility time in TST

The mice subjected to CUMS showed marked increase in duration of immobility as compared to control mice. Chronic treatment with **"6g"** (2 mg/kg, p.o.) and FLX(20 mg/kg, p.o.) substantially [F (4, 35)=28.50, P<0.05] decreased the immobility duration of stressed mice as compared to stress control group . While **"6g"** (1 mg/kg, p.o.) was not able to show any significant effect on duration of immobility in stressed mice as compared to stress control group (Fig. 50).

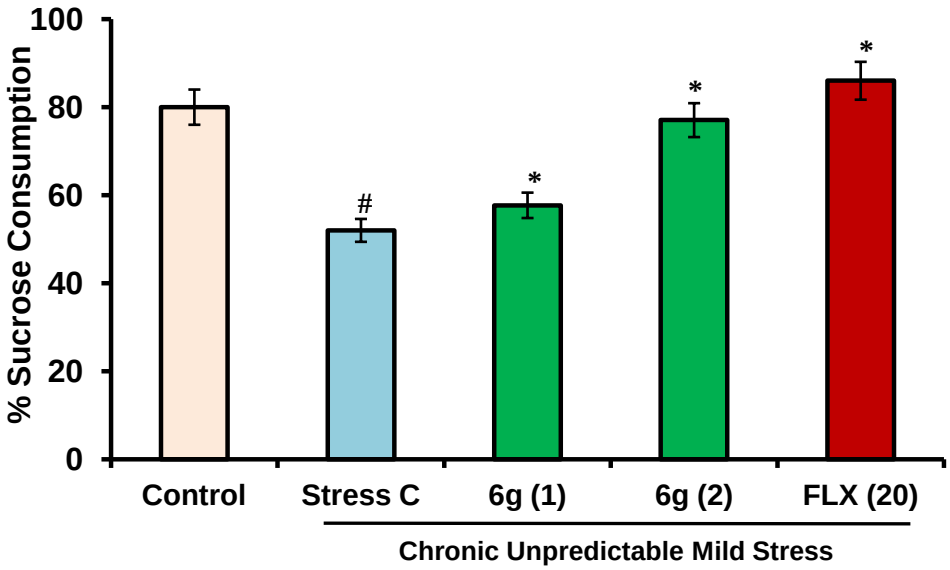


Fig.49. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on sucrose preference (%) in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress vehicle treated group; n = 8/group. FLX= Fluoxetine.

E. Effect of "6g" on percentage TSOA and percentage OAE in EPM test

Mice subjected to CUMS showed significant decrease in percentage of TSOA and OAE as compared to control mice. Chronic treatment with **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) predominantly increased percentage TSOA [F (4, 35)=35.25, P<0.05] and percentage OAE [F (4, 35)=15.45, P<0.05] of stressed mice as compared to stress control group (Table 32).

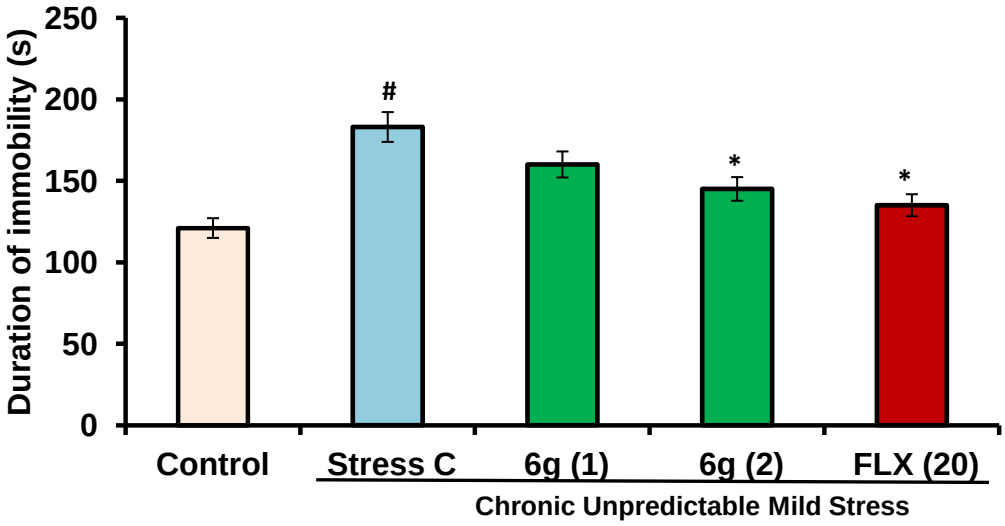


Fig.50. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on duration of immobility in stressed mice. Each column represents mean duration of immobility (s). The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with control group; n = 8/group. FLX= Fluoxetine.

Table 32: Effect of "6g" on % TSOA and % OAE in EPM test

Treatment	Dose (mg/kg)	% TSOA	% OAE
Control	0	31.24 ± 3.21	39.17 ± 1.97
Stress C	1	9.93 ± 0.95*	5.27 ± 2.34*
FLX	20	24.72 ± 1.47#	37.70 ± 4.73#
"6g"	1	30.33 ± 3.39#	25.55 ± 3.50#
"6g"	2	25.53 ± 1.50#	22.17 ± 2.85#

Tabulated results are expressed as mean percentage TSOA and OAE. Error bars represent mean S.E.M. *P <0.05 vs normal control, # P < 0.05 vs Stress control. n =8/group. Fluoxetine=FLX.

5.5.2. Biochemical Analysis

A. Effect of "6g" treatment on brain lipid peroxidation levels

The Thiobarbituric acid reactive substances (TBARS) level was significantly increased in brain of stressed mice as compared to control mice (Fig. 51). Repeated treatment with **"6g"** (1 and 2 mg/kg, p.o.) produced a marked [F (4, 35)=35.90, P<0.05] reduction in TBARS levels in the brain of stressed mice as compared to stress control group. Besides, FLX (20 mg/kg, p.o.) treatment also significantly (P<0.05) reduced TBARS levels in the brain of stressed mice.

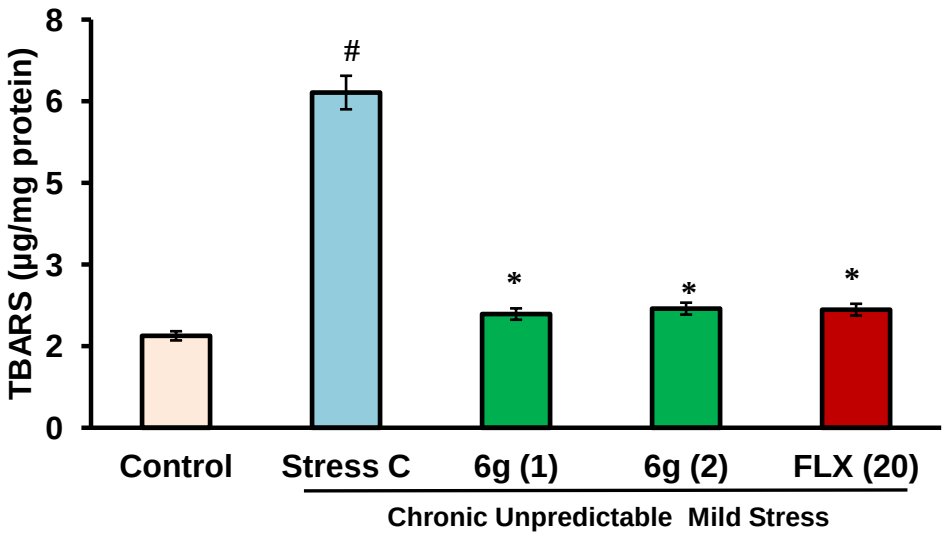


Fig.51. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain lipid peroxidation levels in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress vehicle treated group; n = 8/group. FLX= Fluoxetine.

B. Effect of "6g" treatment on brain GSH levels

The brain GSH levels were found to be significantly depleted in brain of stressed mice compared to control mice (Fig. 52). Chronic treatment with **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) showed a pronounced [F (4, 35)=30.25, P<0.05] increase in brain GSH level as compared to stress control group.

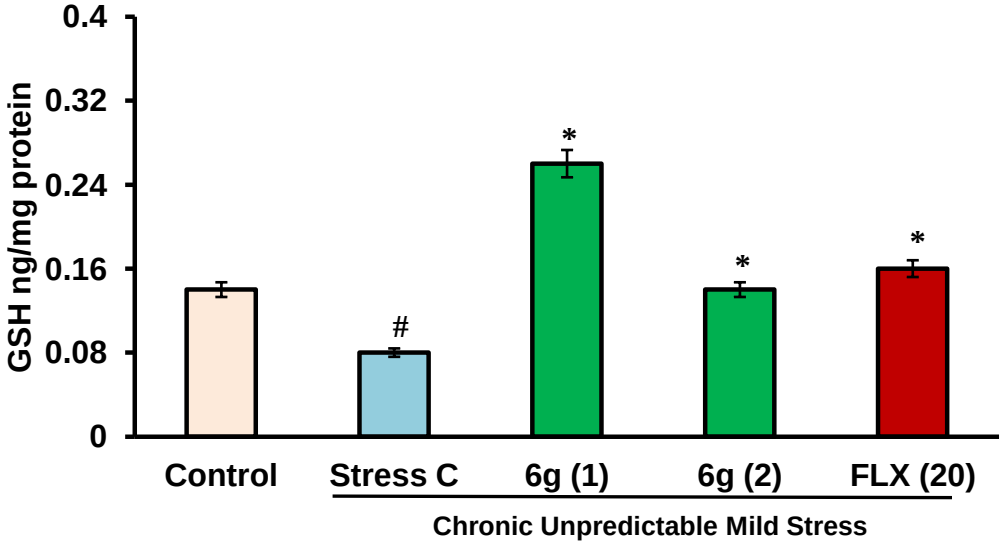


Fig.52. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain reduced GSH levels in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress vehicle treated group; n = 8/group. FLX= Fluoxetine.

C. Effect of "6g" treatment on brain CAT levels

CUMS subjected mice showed a noticeable (p < 0.05) reduction in brain CAT activity as compared to control mice. Chronic treatment with **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) significantly [F (4, 35)=21.45, P<0.05] restored CAT activity in the brain as compared to stress control group substantially as shown in Fig. 53.

D. Effect of "6g" treatment on brain SOD levels

The effect of **"6g"** on brain SOD activity is shown in Fig.54. The enzymatic activity of SOD was significantly decreased in brain of stressed mice as compared to control mice. Repeated treatment with **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) significantly [F (4, 35)=22.45, P<0.05] improved the SOD activity in stressed mice brain as compared to stress control group.

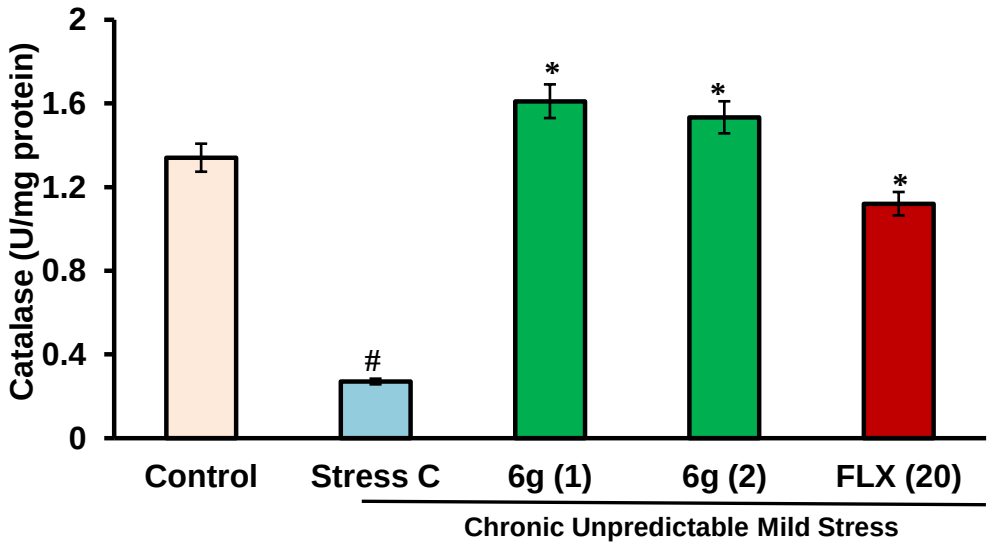


Fig.53. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain CAT levels in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group n = 8/group. FLX= Fluoxetine.

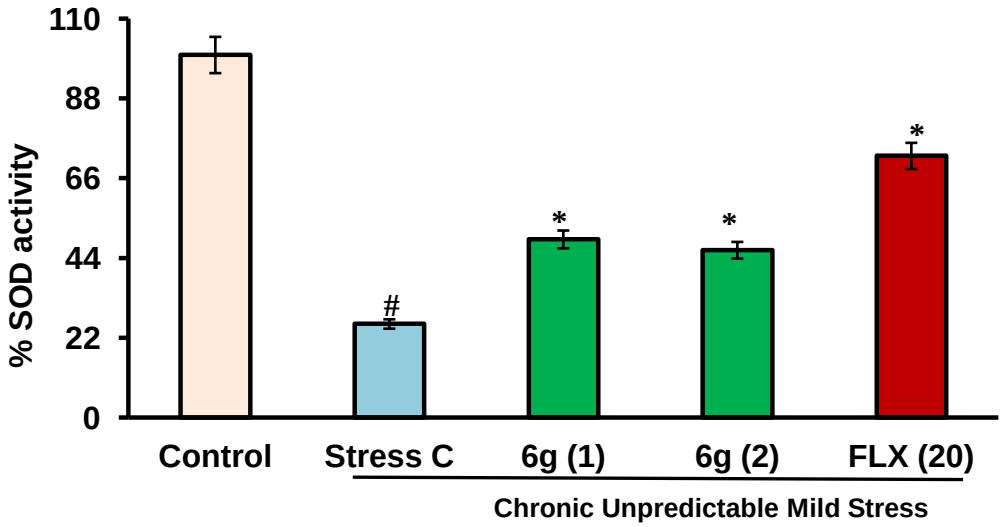


Fig.54. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain percentage SOD activity in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group; n = 8/group. FLX= Fluoxetine.

E. Effect of "6g" treatment on brain nitrite level

Stressed mice expressed elevated brain nitrite levels as compared to control group mice (Fig. 55). The chronic administration of **"6g"** (1 and 2 mg/kg, p.o.) did not produce [F (4, 35)=20.00, P<0.05] much reduction in elevated brain nitrite levels in stressed mice, as compared to stress control group (Fig. 55). The positive control FLX (20 mg/kg, p.o.) showed a significant (P<0.05) decrease in brain nitrite levels.

F. Effect of CUMS on the level of corticosterone

CUMS mice showed marked [P<0.05] increase in the plasma corticosterone levels as compared to normal control mice (Fig. 56). The chronic treatment with compound **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) predominantly [F (4, 35)=35.25, P<0.05] reversed the increased corticosterone levels as compared to stress control group.

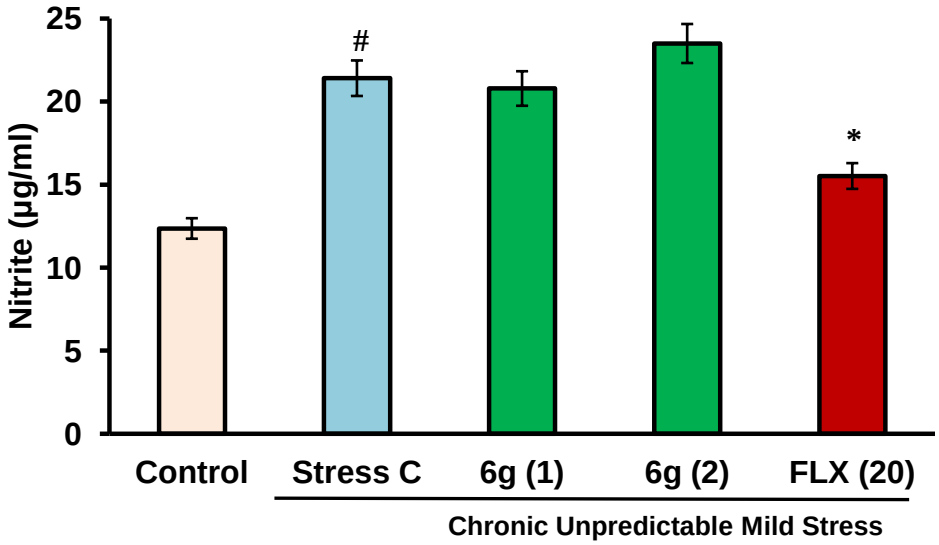


Fig.55. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain nitrite levels in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group n = 8/group. FLX= Fluoxetine.

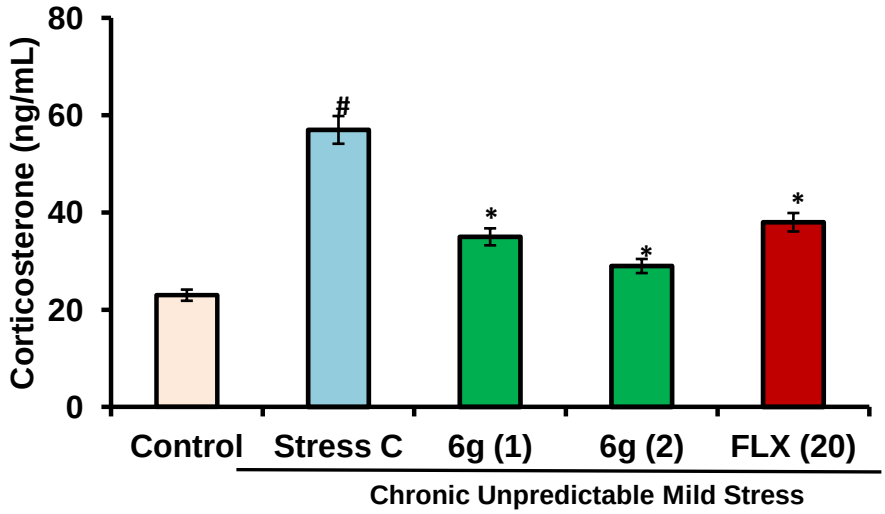


Fig.56. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on the plasma corticosterone levels in normal and CUMS mice. Each column represent mean corticosterone levels. Error bars represent mean S.E.M. #P < 0.05 vs normal control, *P < 0.05 vs CUMS control. n=8/group. FLX=Fluoxetine.

5.6. Effect of "6g" in LPS Induced Co-morbid Depression and Anxiety Behavioural and Biochemical Parameters

5.6.1. Behavioural Observations

A. Effect of "6g" on SLA of mice

Significant (P<0.05) decrease in the locomotor activity observed in LPS (0.83 mg/kg, i.p.) treated mice as compared to normal control (Fig 57). Whereas no significant [F (4, 35)=22.56, P>0.05] difference in locomotor activity was observed with LPS+**"6g"** (1), LPS+**"6g"** (2), LPS+FLX (20) treatment as compared to normal control.

B. Effect of "6g" on duration of immobility in mice using FST

In FST, Compound **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) predominantly [F (4, 35)=22.56, P<0.05] decreased the duration of immobility in LPS treated mice as compared to LPS control group (Fig. 58).

C. Effect of "6g" on duration of immobility in mice using TST

In TST, Compound **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) notably [F (4, 35)=21.25, P<0.05] decreased the duration of immobility in LPS treated mice as compared to LPS control group (Fig. 59).

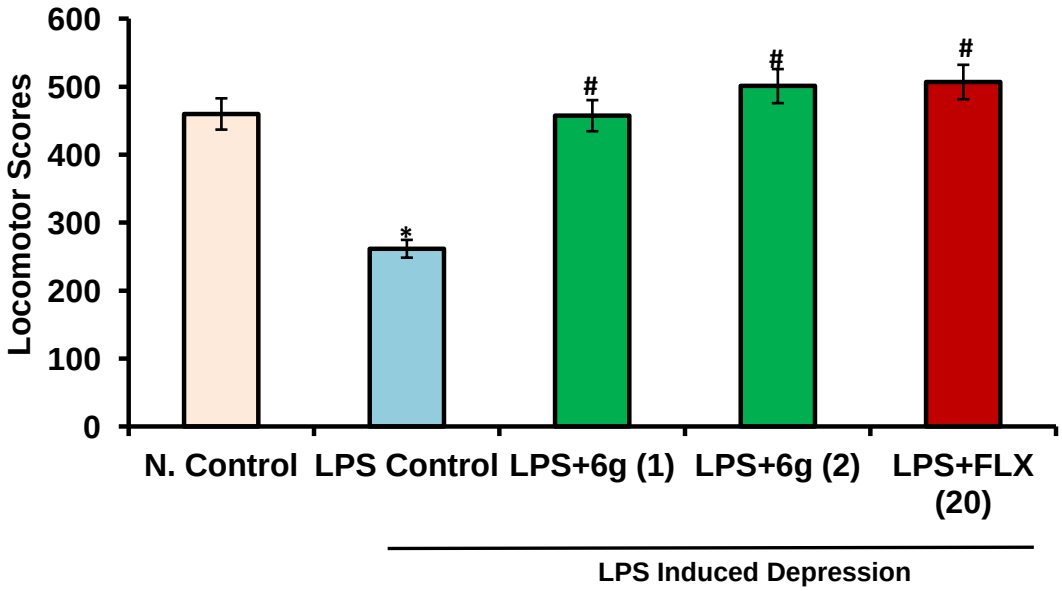


Fig.57. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on locomotor scores in LPS injected mice. Each column represents mean locomotor scores recorded in 8 min observation period. The error bar indicates S.E.M., *P < 0.05 when compared with normal control, #P < 0.05 when compared with LPS control group; n = 8/group; FLX= Fluoxetine.

D. Effect of "6g" on percentage OAE and percentage TSOA using EPM in mice

In EPM, Compound **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) significantly increased the percentage of both OAE [F (4, 35)=7.518, P<0.05] and TSOA [F (4, 35)=12.81, P<0.05] as compared to LPS control group (Table 33).

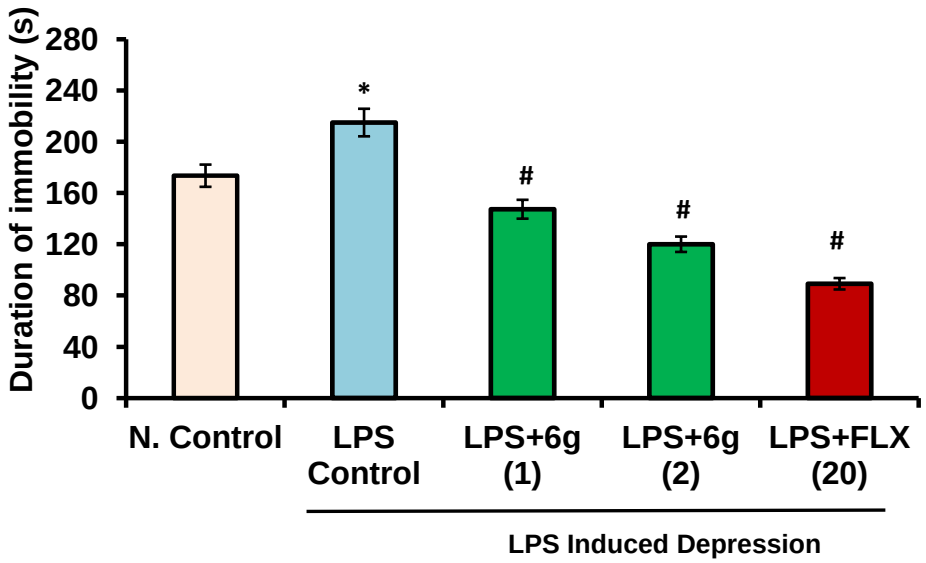


Fig.58. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on duration of immobility in LPS injected mice. Each column represents mean duration of immobility (s). The error bar indicates S.E.M.,*P < 0.05 when compared with normal control; #P < 0.05 when compared with LPS control group; n = 8/group; FLX= Fluoxetine.

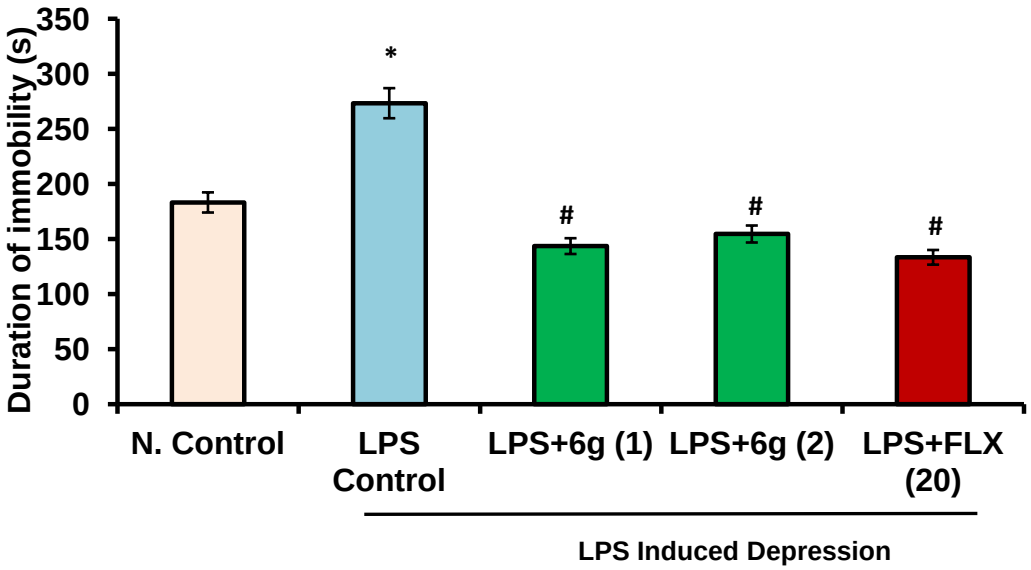


Fig.59. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on duration of immobility in LPS injected mice. Each column represents mean duration of immobility (s). The error bar indicates S.E.M.,*P < 0.05 when compared with normal control; #P < 0.05 when compared with LPS control group; n = 8/group.; FLX= Fluoxetine.

Table 33: Effect of "6g" on % TSOA and % OAE in EPM test

Treatment	% TSOA	% OAE
N. Control	80.00 ± 3.54	51.50 ± 3.57
LPS Control	55.16 ± 3.97*	28.16 ± 2.92*
LPS +**"6g"** (1)	71.16 ± 3.06#	42.00 ± 3.19#
LPS +**"6g"** (2)	72.00 ± 3.96#	43.33 ± 2.53#
LPS+FLX (20)	89.00 ± 2.79#	44.16 ± 3.17#

Tabulated results are expressed as mean percentage TSOA and OAE. Error bars represent mean S.E.M. *P <0.05 vs normal control, #P < 0.05 when compared with LPS control group, n =8 /group. FLX= Fluoxetine.

E. Effect of "6g" on time spent in lit area, no. of transitions and latency period in L/D test

"6g" (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) treatment predominantly [F (4, 35)=21.25, P<0.05] increased the number of entries from one compartment to other as well as decreased the latency time markedly [F (4, 35)=15.25, P<0.05] to leave compartment (Table 34). Moreover **"6g"** (2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) treatment significantly [F (4, 35)=19.55, P<0.05] increased the time spent in light chamber, while lower dose of **"6g"** (1 mg/kg, p.o.) did not produce significant change in time spent in light chamber (Table 34).

Table 34: Effect of "6g" on time spent in lit area, no. of transitions and latency period in L/D test

Treatment	Time spent in Lit area (s)	No. of transitions	Latency (s)
N. Control	132.16 ± 8.90	16.50 ± 1.47	11.16 ± 1.60
LPS Control	53.00 ± 9.33*	6.66 ± 0.76*	56.80 ± 3.05*
LPS+**"6g"** (1)	68.00 ± 9.88	15.16 ± 1.30#	36.16 ± 2.18#
LPS+**"6g"** (2)	86.60 ± 6.00#	15.00 ± 1.80#	26.00 ± 2.86#
LPS+FLX (20)	73.00 ± 5.30#	21.16 ± 3.37#	31.16 ± 2.91#

Tabulated results are expressed as mean time spent in lit area, no. of transitions and latency period. Error bars represent mean±S.E.M. *P < 0.05 vs normal control, #P < 0.05 vs LPS control group; n =8/group. FLX= Fluoxetine.

5.6.2. Biochemical Parameters

A. Effect of "6g" treatment on brain lipid peroxidation levels

The TBARS level was noticeably increased in brain of LPS treated mice as compared to the normal control mice (Fig. 60). Repeated treatment with **"6g"** (2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) produced a marked [F (4, 35)=10.76, P<0.05] reduction in TBARS levels in the brain of LPS treated mice as compared to stress LPS vehicle treated control group. **"6g"** (1 mg/kg, p.o.) treatment was not able to produce any significant effect on TBARS levels as compared to LPS control group.

B. Effect of "6g" treatment on brain GSH levels

The brain GSH levels were found to be significantly depleted in brain of stressed mice compared to control mice (Fig. 61). Chronic treatment with **"6g"** (1 and 2 mg/kg, p.o.) did not produce a pronounced increase in GSH levels in the brain of LPS treated mice as compared to stress LPS vehicle treated control group. While FLX (20 mg/kg, p.o.) showed a predonminant [F (4, 35)=20.18, P<0.05] increased in brain GSH level as compared to the LPS control group.

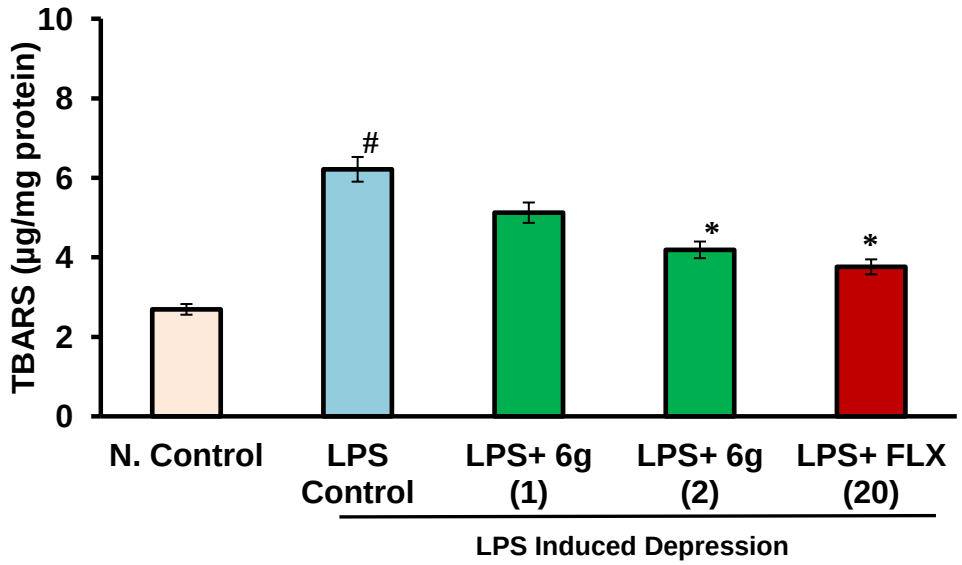


Fig.60. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain lipid peroxidation levels in LPS injected mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with LPS control group; n = 8/group. FLX= Fluoxetine.

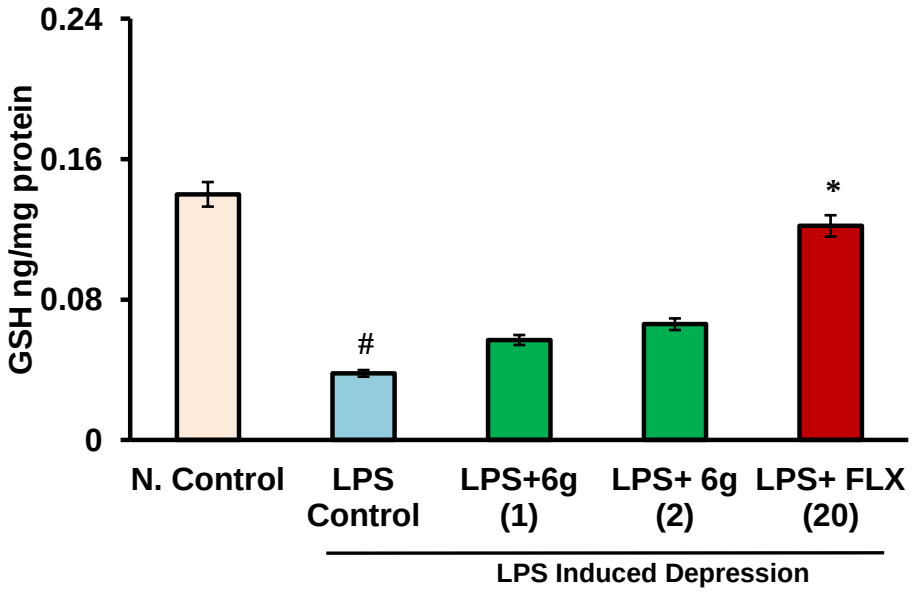


Fig.61. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain GSH levels in LPS injected mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with LPS control group; n = 8/group.; FLX= Fluoxetine.

C. Effect of "6g" treatment on brain CAT levels

The CAT level was significantly increased in brain of LPS vehicle treated control mice as compared to normal control mice. Repeated treatment with **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) produced a marked [F (4, 35)=21.13, P<0.05] reduction in CAT levels in the brain of LPS treated mice as compared to LPS control group (Fig. 62).

D. Effect of "6g" treatment on brain SOD activity

The effect of **"6g"** on the brain SOD activity is shown in Fig.63. The enzymatic activity of SOD was significantly decreased in brain of LPS vehicle control mice as compared to normal control mice. Repeated treatment with **"6g"** (2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) significantly [F (4, 35)=22.56, P<0.05] improved the SOD activity in LPS treated mice brain as compared to LPS control group. **"6g"** (1 mg/kg, p.o.) treatment was not able to produce any marked effect on SOD activity as compared to LPS control group.

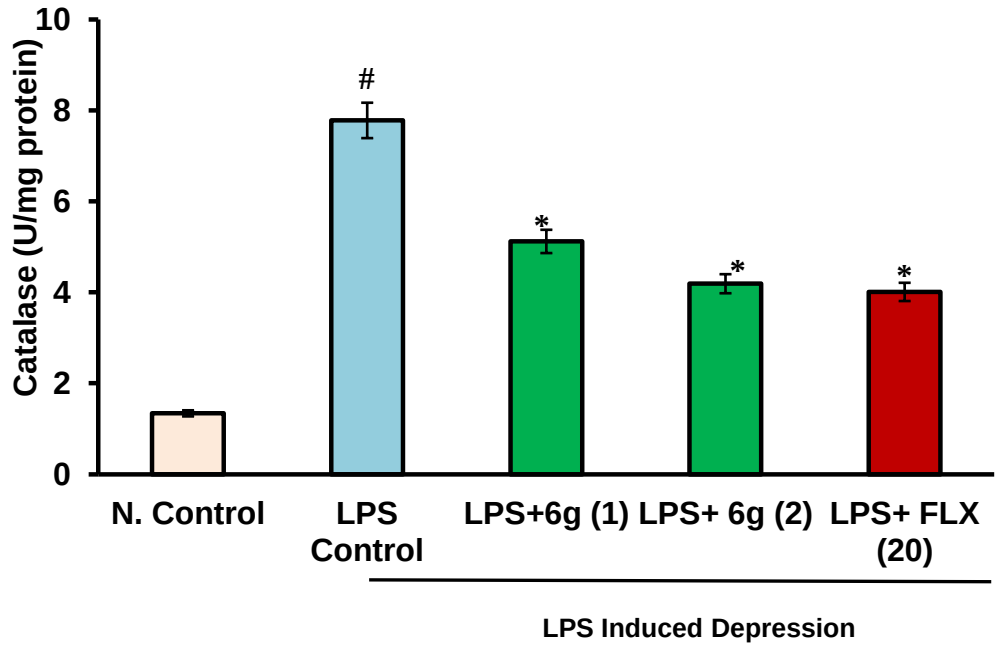


Fig.62. Effect of "6g" (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain CAT levels in LPS injected mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with LPS control group n = 8/group; FLX= Fluoxetine.

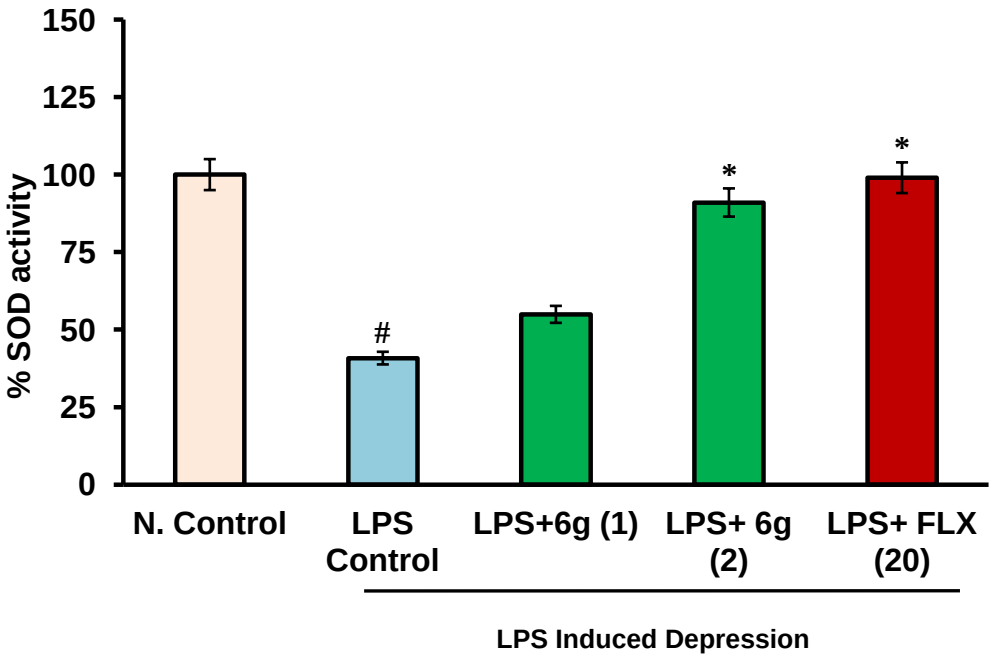


Fig.63. Effect of **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain SOD activity in LPS mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with LPS control group; n = 8/group.; FLX= Fluoxetine.

E. Effect of "6g" treatment on brain nitrite levels

LPS vehicle treated control mice showed elevated brain nitrite levels as compared to normal control mice (Fig. 64). The chronic administration of **"6g"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) did not produce any significant [F (4, 35)=2.161, P>0.05] reduction in elevated brain nitrite levels when compared with LPS control group (Fig. 64).

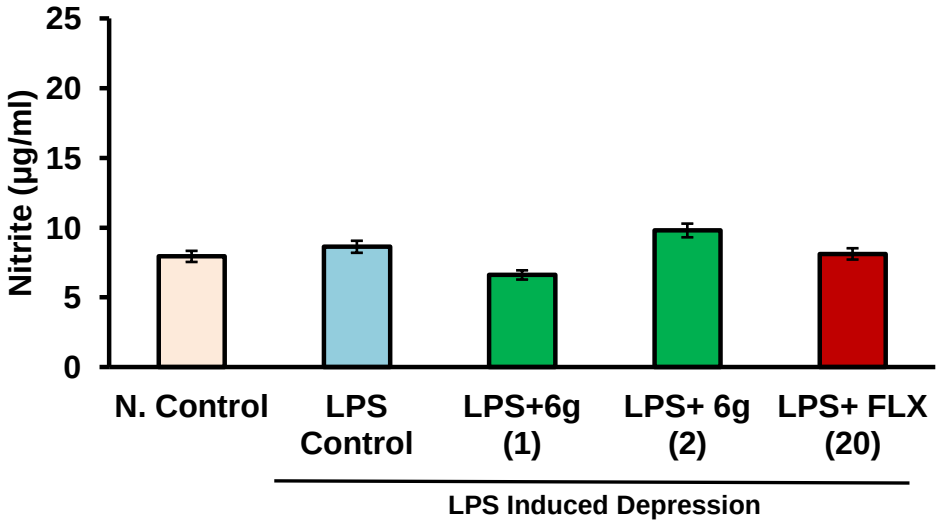


Fig.64. Effect of "6g" (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain nitrite levels in LPS injected mice. The error bar indicates S.E.M. n = 8/group; FLX= Fluoxetine.

F. Effect of "6g" on the level of 5-HT in LPS treated Rats

LPS treatment significantly (P<0.05) reduced the 5-HT levels (ng/g wet brain tissue) in rat brain as compared to the normal vehicle treated animals. **"6g"** (1 and 2 mg/kg) and FLX (20 mg/kg) predominantly [F (4,35)=12.14, P<0.05 enhanced the 5-HT levels as compared to LPS control rats (Table 35).

Table 35: Effect of "6g" on the level of 5-HT in LPS treated rats

Treatment groups (mg/kg)	5-HT (ng/g)
Normal control	506.99 ± 15.12
LPS Control	371.22 ± 17.22*
LPS+ **"6g"** (1)	421.00 ± 15.78$^{\#}$
LPS+ **"6g"** (2)	445.23 ± 10.32$^{\#}$
LPS+ FLX (20)	457.00 ± 11.75$^{\#}$

Tabulated data represent the mean number of neuro-transmitter level [5-HT (ng/g]. Results are expressed as means ± S.E.M. *P < 0.05 vs normal control, $^{\#}$P < 0.05 vs LPS control rats; n = 8/group. FLX= Fluoxetine.

5.7. Evaluation of "6n" in Rodent Models of Depression

5.7.1. Effect of "6n" on SLA of mice

Compound **"6n"** (0.5, 1, 2, 4 mg/kg, i.p.) did not show any significant [F (4, 35)=1.863, P>0.05] effect on base line locomotions as compared to control (Fig. 65).

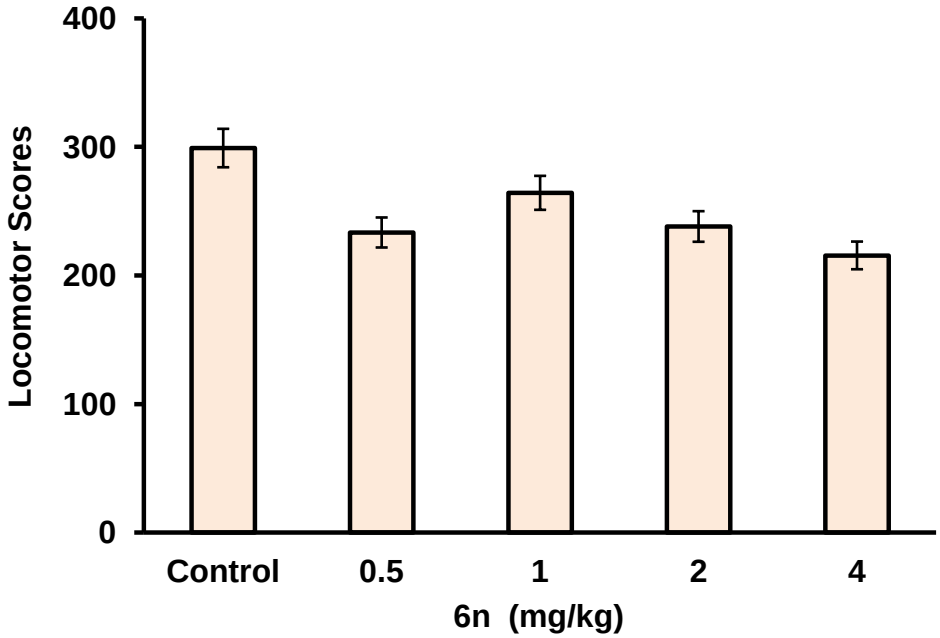


Fig. 65. The columns represent mean locomotor scores and error bars indicate S.E.M. n = 8/group.

5.7.2. Effect of "6n" on duration of immobility in mice using FST

In FST, the acute treatment with **"6n"** (1, 2 and 4 mg/kg, i.p.) and ESC (10 mg/kg, i.p.) showed prominent [F (4, 35)=11.88, P<0.05] decrease in the duration of immobility as compared to vehicle treatment (Fig. 66).

5.7.3. Effect of "6n" on duration of immobility in mice using TST

In TST, the acute treatment with **"6n"** (1, 2 and 4 mg/kg, i.p.) and BUP (20 mg/kg, i.p.) markedly [F (4, 35)=6.40, P<0.05] decreased the duration of immobility as compared to vehicle treatment (Fig. 67).

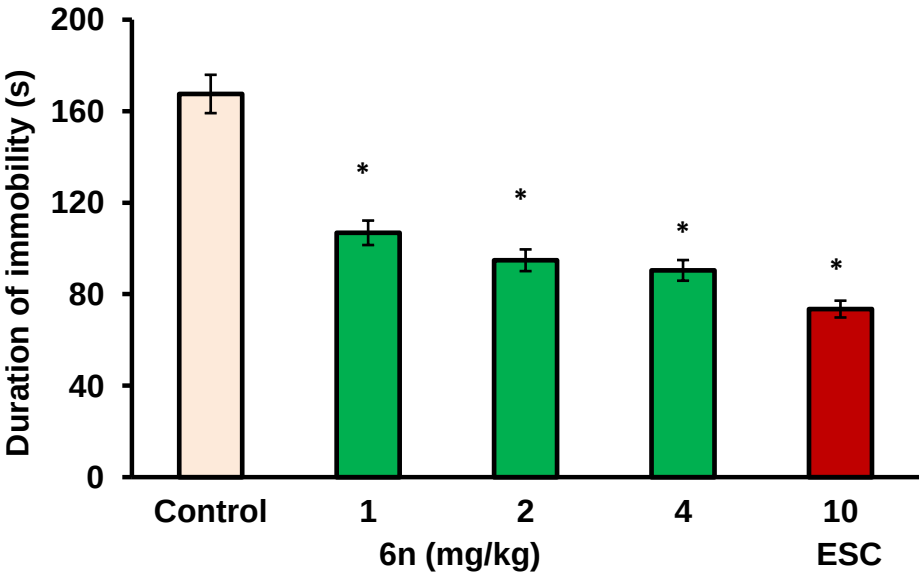


Fig 66. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8/group. *P < 0.05 compared with vehicle treated group. ESC = Escitalopram.

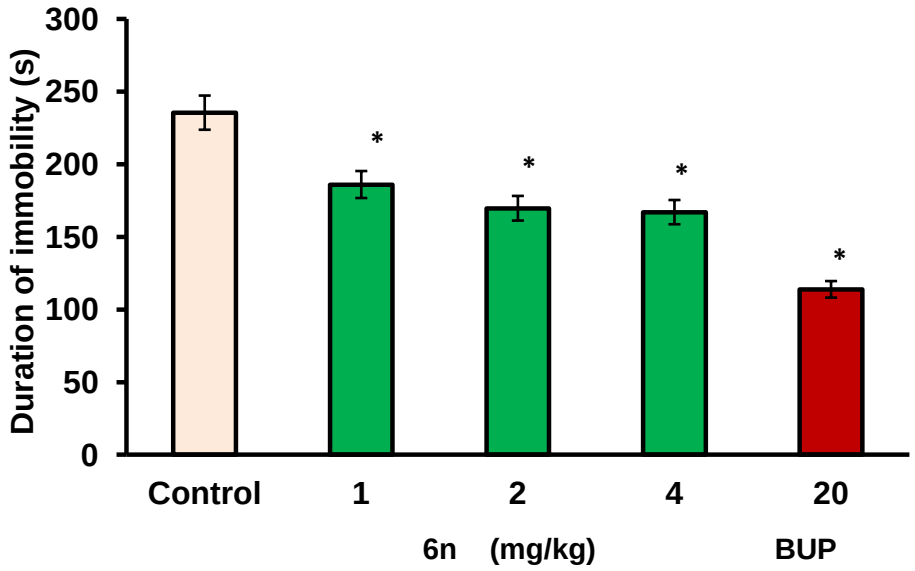


Fig. 67. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8/group. *P < 0.05 compared with vehicle treated group. BUP = Bupropion.

5.7.4. Effect of "6n" on RIH in mice

Reserpine (1 mg/kg i.p) elicited a pronounced decrease in core body temperature of rats. This effect was predominantly [F (3, 28)=12.98, P<0.05] reversed by **"6n",** (1 and 2 mg/kg) and ESC (10 mg/kg) treatments(Fig.68).

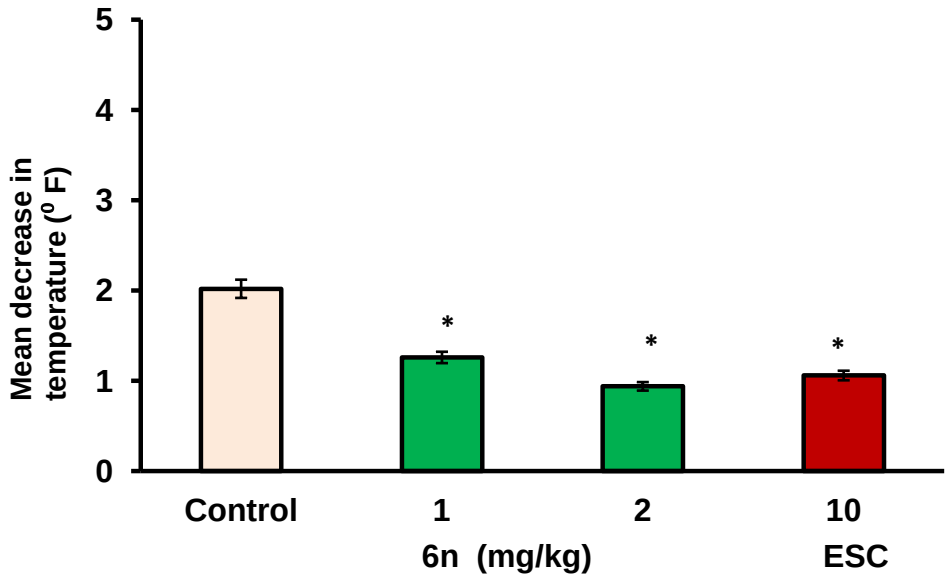


Fig. 68. The columns represent mean decrease in temperature ($^{\circ}$F) and error bars indicate S.E.M. n = 8/group. *P < 0.05 compared with vehicle treated group. ESC = Escitalopram.

5.7.5. Effect of "6n" on 5-HTP -HTR in mice

"6n" (2 mg/kg, i.p.) and FLX (20 mg/kg, i.p.) significantly [F (3, 28)=37.92, P<0.05] potentiated the 5-HTP/PRG induced head twitches in mice (Fig.69). While **"6n"** at 1 mg/kg, i.p. dose was not able to produce any significant effect.

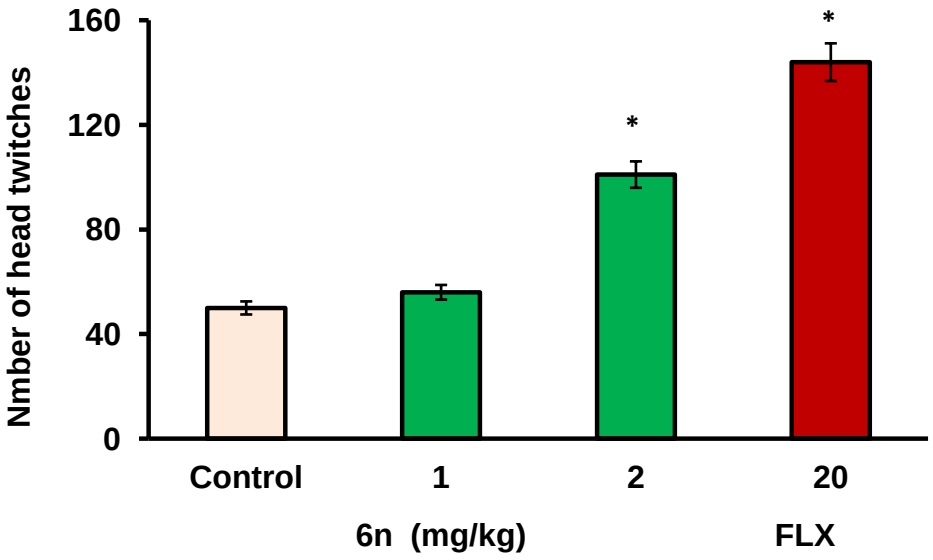


Fig. 69. The columns represent mean number of head twitches and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle-treated group. FLX = Fluoxetine

5.7.6. Interaction studies of "6n" with standard drugs

For a conclusive evaluation of anti-depressant potential of 5-HT_3 receptor antagonists, interaction studies with standard anti-depressants were carried out. **"6n"** pre-treatment (1mg /kg, i.p.) was found to significantly enhance anti-depressant-like effects of FLX (10 and 20 mg/kg, i.p.) [F (1, 42)=12.42, P<0.05], DMI (10 and 20 mg/kg, i.p.) [F (1, 42)=19.48, P<0.05] and VLA (4 and 8 mg/kg, i.p.) [F (1, 42)=17.09, P<0.05] as shown in Fig. 70-72 respectively. Moreover, **"6n"** predominantly reversed the depressant-like effect of PTL (1 mg/kg, i.p.) [F (1, 42)=12.39, P<0.05]as shown (Fig. 73). Further **"6n"** (1 mg/kg, i.p.) substantially [F (1, 42)=13.21, P<0.05] enhanced the anti-depressant activity of BUP (10 and 20 mg/kg, i.p.) in mice TST (Fig. 74).

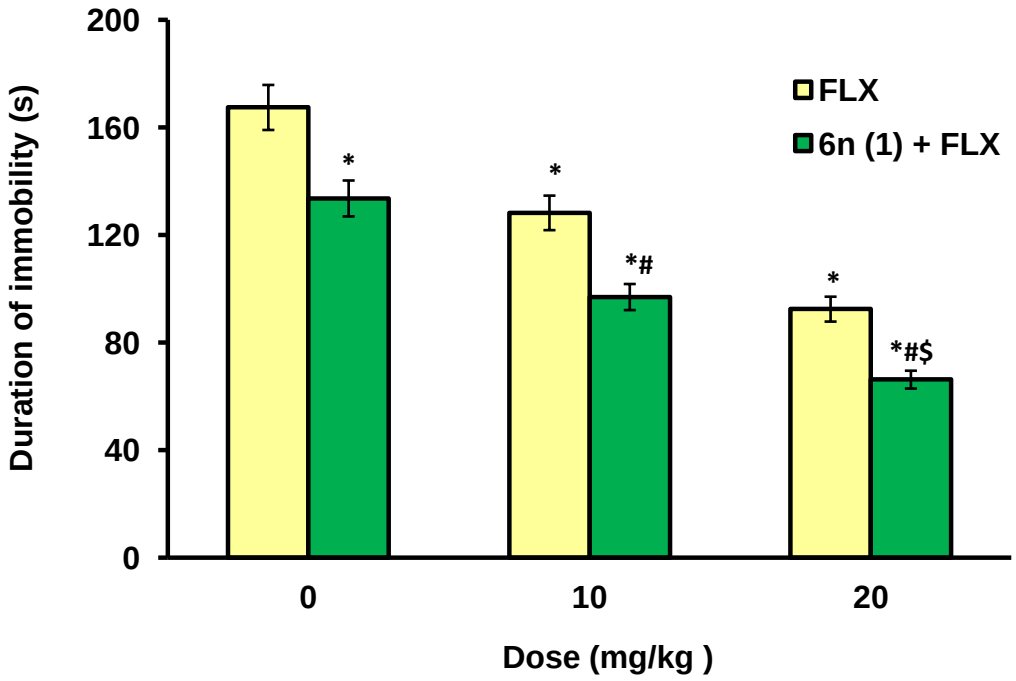


Fig. 70. The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n = 8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with FLX (10 and 20 mg/kg) treated group and $P < 0.05 compared with alone **"6n"** treated group. FLX= Fluoxetine.

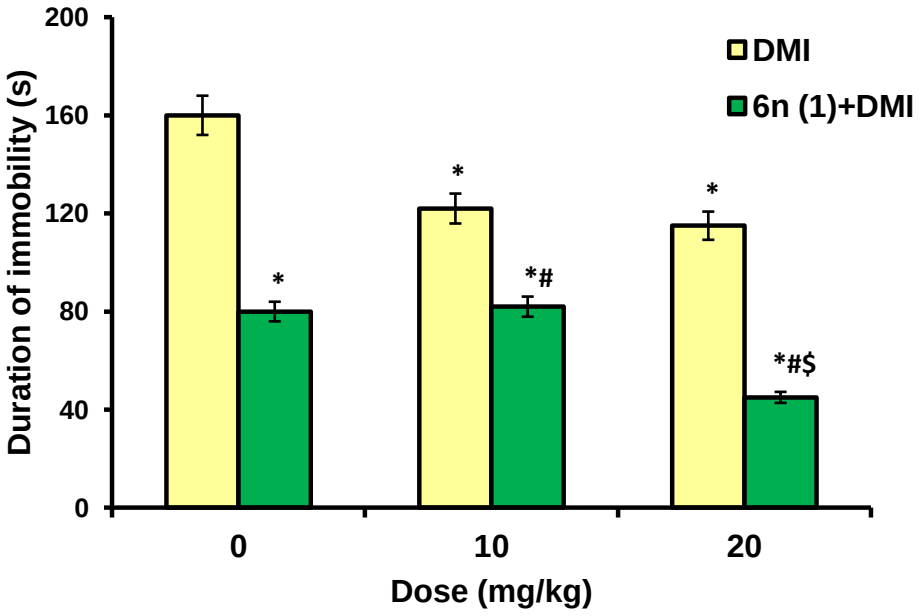


Fig. 71. The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n = 8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with DMI (10 and 20 mg/kg) treated group alone and $P < 0.05 compared with alone **"6n"** treated group. DMI=Desipramine.

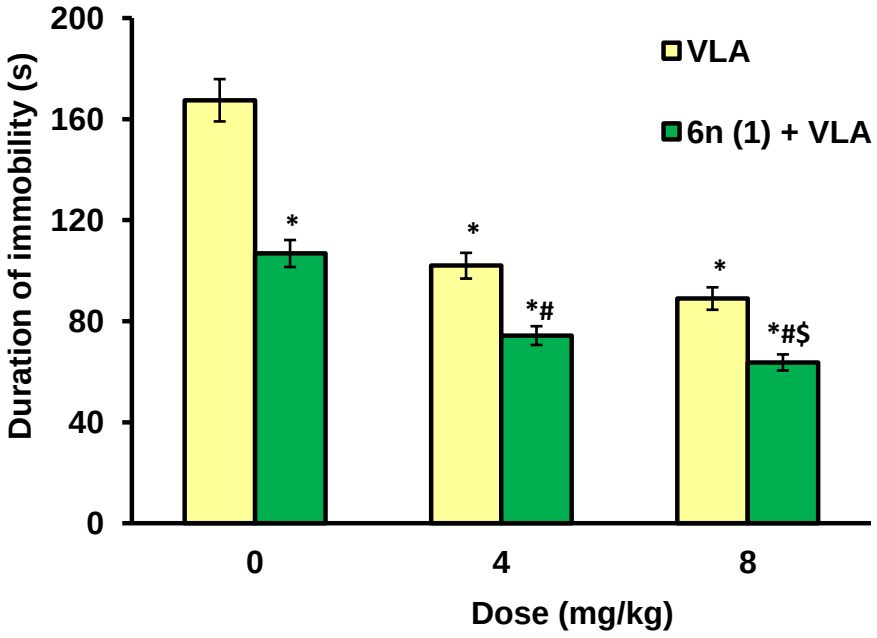


Fig.72. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n = 8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with VLA (4 and 8 mg/kg) treated group and $P < 0.05 compared with alone **"6n"** treated group. VLA= Venlafxine.

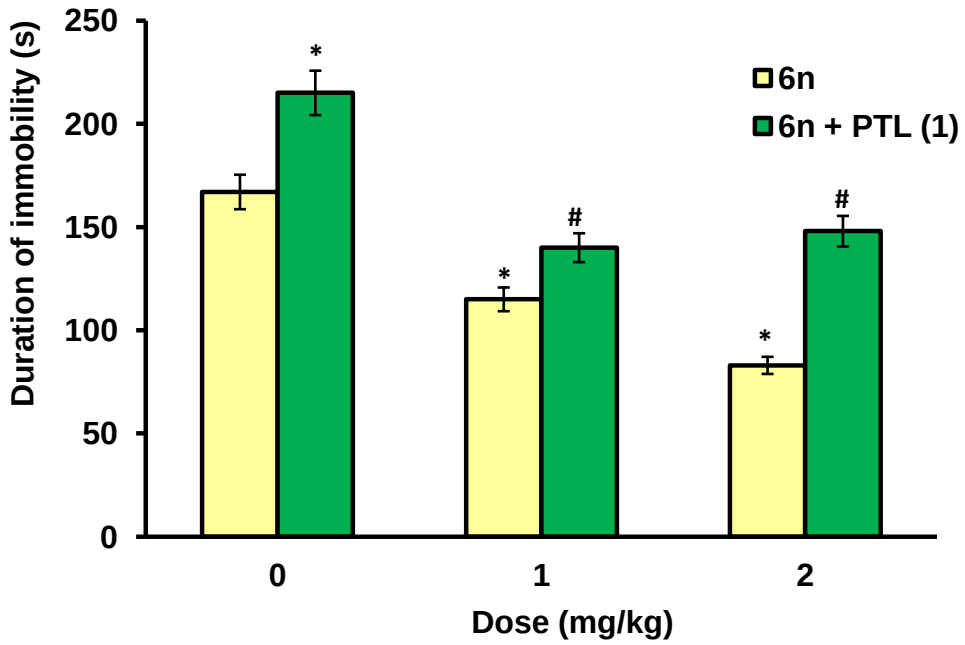


Fig. 73. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8/group. *P < 0.05 vs. vehicle treated group and # P < 0.05 compared with PTL (1 mg/kg) treated group. PTL=Parthenolide.

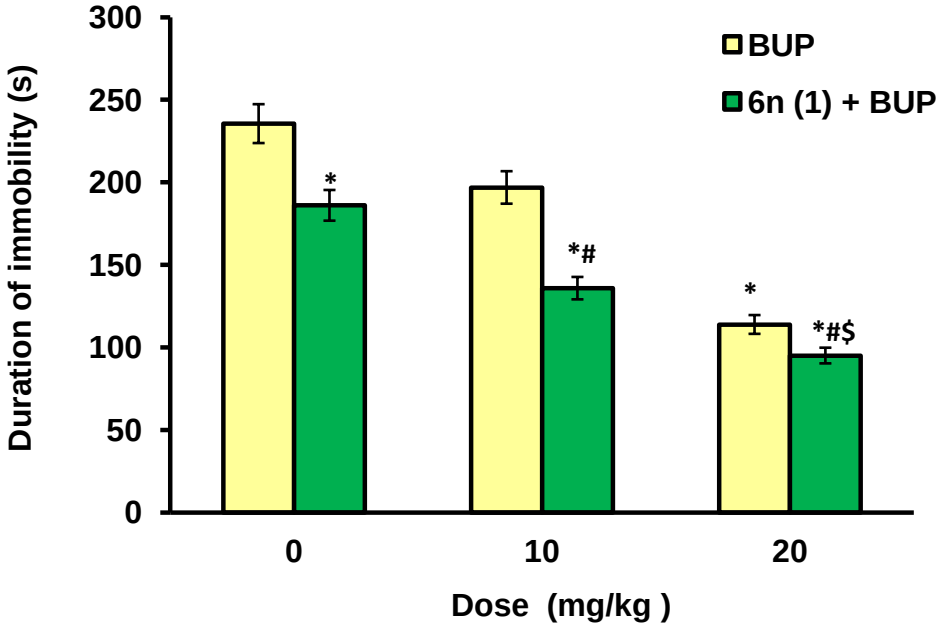


Fig. 74. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n =8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with BUP(10 and 20 mg/kg) treated group and $P < 0.05 compared with alone **"6n"** treated group. BUP=Bupropion.

5.8. Evaluation of "6n" in Animal Models of Anxiety

5.8.1. Effect of "6n" on behaviour of mice in EPM test

Acute treatment with **"6n"** (1 and 2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) markedly increased the percentage of both OAE [F (3, 28)=12.45, P<0.05] and TSOA [F (3, 28)=4.189, P<0.05] as compared to vehicle control group (Table 36).

Table 36: Effect of "6n" on behaviour of mice in EPM test

Treatment (mg/kg)	% TSOA	% OAE
Vehicle Control	2.00 ± 0.68	11.17 ± 1.97
DZM (2)	11.17 ± 1.47*	37.7 ± 4.73*
"6n" (1)	11.50 ± 3.30*	36.11 ± 7.95*
"6n" (2)	13.33 ± 3.35*	60.03 ± 6.22*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.8.2. Effect of "6n" on behaviour of mice in L/D test

Compound **"6n"** (1 and 2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment predominantly [F (3, 28)=10.91, P<0.05] increased the number of entries from one compartment to other as well as significantly [F (3, 28)=30.52, P<0.05] increased the total time spent in lit area (Table 37).

Table 37: Effect of "6n" on behaviour of mice in L/D test

Treatment (mg/kg)	Time spent in Lit area (s)	No. of transitions
Vehicle Control	49.67 ± 4.90	7.20 ± 0.62
DZM (2)	101.83 ± 5.08*	15.43 ± 1.33*
"6n" (1)	69.17 ± 3.93*	12.02 ± 0.97*
"6n" (2)	99.00 ± 4.08 *	14.39 ± 1.35*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.8.3. Effect of "6n" on behaviour of mice in HB test

Compound **"6n"** (1 and 2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment showed marked increase in the number of head dips [F (3, 28)=11.79, P<0.05], number of square crossed [F (3, 28)=14.96, P<0.05] and decreased the head dipping latency [F (3, 28)=3.996, P<0.05] (Table 38). DZM=Diazepam.

Table 38: Effect of "6n" on behaviour of mice in HB test

Treatment (mg/kg)	No. of head dips	No. of square crossed	Latency time (s)
Vehicle Control	8.50 ± 1.06	2.83 ± 0.87	9.67 ± 0.67
DZM (2)	26.5 ± 4.03*	18.50 ± 2.01*	2.33 ± 2.01*
"6n" (1)	25.83 ± 2.77*	12.82 ± 1.49*	6.83 ± 1.22*
"6n" (2)	12.50 ± 1.91*	15.50 ± 2.31*	3.33 ± 2.31*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.8.4. Effect of "6n" on behaviour of mice in OFT

Compound **"6n"** (2 mg/kg, i.p.) and DZM (2mg/kg, i.p.) treatment showed pronounced increase in the ambulation score [F (3, 28)=37.03, P<0.05] (Table 39). While **"6n**"(1 mg/kg, i.p) was not able to show any significant effect on ambulation. None of the tested doses of compounds and standard significantly [F (3, 28)=4.997, P>0.05] affect rearing numbers.

Table 39: Effect of "6n" on behaviour of mice in OFT

Treatment (mg/kg)	Ambulation scores	Rearing
Vehicle Control	145.67 ± 4.08	9.40 ± 0.64
DZM (2)	205.23 ± 5.09*	10.20 ± 0.45
"6n" (1)	172.25 ± 3.84	7.40 ± 0.56
"6n" (2)	190.76 ± 3.75*	8.00 ± 0.62

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.9. Co-morbid Evaluation in OBX Rats Using Behavioural Test Battery

5.9.1. Effect of OBX on body weight of rats

Body weight of sham and OBX rats were continuously observed till the behavioural tests were started. Decrease in body weight were observed in OBX rats for few days, post surgery. Statistical analysis revealed that weight gained in OBX rats was significanlty (P<0.01) less as compared to sham rats as shown in Table 40.

Table 40: Effect of OBX on change in body weight of rats

	Dose (mg/kg)	Initial weight	Final weight
Sham control	0	250.00 ± 3.00	295.0 ± 5.00
Sham + **"6n"**	1	265.00 ± 5.50	294.0 ± 4.00
Sham + **"6n"**	2	253.00 ± 7.00	289.5 ± 4.50
Sham+ PAR	10	252.00 ± 5.50	288.5 ± 3.50
OBX control	**0**	**256.00 ± 5.50**	**266.00 ± 7.50***
OBX + **"6n"**	1	260.00 ± 5.20	284.00 ± 4.00
OBX + **"6n"**	2	254.00 ± 6.00	275.33 ± 4.50
OBX + PAR	10	259.00 ± 5.35	282.00 ± 8.50

Each value represents mean ± S.E.M. *P < 0.05 represent the mean change in body weight. **"6n"**/PAR vehicle (mg/kg) were administered p.o. once a day for 14 days. *P<0.05 vs sham control. n= 6/group. PAR= Paroxetine.

5.9.2. Open Field Test (OFT)

The effects of **"6n"** on the behaviour of OBX/sham rats were analyzed in different circumstances as shown in (Table 41). Chronic (14 days, p.o) treatment with **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) predominantly (P<0.05) reduced the number of ambulation [F (7, 40)=2.272, P<0.05], rearing [F (7, 40)=15.51, P<0.05] and fecal pellets [F (7, 40)=2.083, P<0.05] in OBX rats as compared to the vehicle treated OBX rats.

5.9.3. Sucrose Consumption Test

Sucrose (1%) consumption test was performed to measure anhedonia (loss of pleasure) in rodents. Sucrose consumption was tested in sham and OBX rats. Marked decrease in sucrose consumption (P<0.05) was observed in OBX rats as compared to sham rats. Chronic **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment significantly [F (7, 40)=5.294, P<0.05] increased the sucrose consumption in OBX treated rats as compared to vehicle treated OBX rats (Table 42).

Table 41: Effect of "6n" on open field behaviour in sham and OBX rats

Treatment (mg/kg)	No. of Ambulation	No. of Rearing	No. of Fecal Pellets
Sham Control	91.17 ± 6.88	10.00 ± 1.24	2.17 ± 0.47
Sham+**"6n"** (1)	103.00 ± 8.87	9.33 ± 1.05	2.33 ± 0.71
Sham+**"6n"** (2)	102.17 ± 7.34	8.33 ± 1.15	2.00 ± 0.86
Sham+PAR (10)	99.67 ± 9.37	8.67 ± 0.71	2.00 ± 0.58
OBX Control	225.00 ± 7.48*	30.67 ± 4.58*	4.33 ± 0.76*
OBX+**"6n"** (1)	140.00 ± 8.82#	15.00 ± 1.01#	3.80 ± 0.62#
OBX+**"6n"** (2)	138.00 ± 8.95#	11.4 ± 1.19#	3.67 ± 0.65#
OBX+PAR (10)	113.00 ± 7.34#	11.83 ± 0.59#	2.33 ± 0.47#

Each value represents ambulation, rearing score and No. of fecal pellet and error bars indicate S.E.M.,* P < 0.05 when compared to the sham operated rats, #P < 0.05 when compared to the vehicle-treated OBX rats (n = 6/group). PAR=Paroxetine.

5.9.4. Elevated Plus Maze (EPM)

EPM was employed for the anxiolytic test in the laboratory set-up. Percentage entries and time spent in open arms were measured in EPM test. OBX rats exhibited increased percentage of both OAE [P<0.05] and TSOA [P<0.05] of the maze in comparison with sham-operated rats (opposite to that observed in anxiety). Chronic **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment predominantly reversed the OBX behaviour by decreasing percentage of OAE [F (7, 40)=4.796, P<0.05] and TSOA of the maze [F (7, 40)=10.54, P<0.05] significantly as compared to vehicle treated OBX rats (Table 43).

Table 42: Effect of "6n" on sucrose consumption in sham and OBX rats

Treatment (mg/kg)	% Sucrose Consumption
Sham control	66.75 ± 5.49
Sham + **"6n"** (1)	60.25 ± 5.68
Sham + **"6n"** (2)	62.25 ± 6.03
Sham + PAR (10)	66.75 ± 3.47
OBX Control	30.50 ± 4.05*
OBX+ **"6n"** (1)	51.25 ± 4.25#
OBX+ **"6n"** (2)	56.75 ± 4.82#
OBX+ PAR (10)	57.60 ± 6.25#

Results are expressed as mean ± S.E.M. *P < 0.05 vs sham control, #P<0.05 vs OBX control. n =6/ group. PAR= Paroxetine

Table 43: Effect of "6n" on % OAE and % TSOA in sham and OBX rats

Treatment (mg/kg)	% OAE	% TSOA
Sham control	44.25 ± 3.28	25.00 ± 3.45
Sham + **"6n"** (1)	49.00 ± 5.62	27.25 ± 3.78
Sham + **"6n"** (2)	49.25 ± 6.02	22.55 ± 4.65
Sham + PAR (10)	41.25 ± 3.78	29.58 ± 3.01
OBX Control	83.50 ± 9.52*	69.24 ± 7.64*
OBX+ **"6n"** (1)	61.24 ± 7.51#	49.00 ± 5.27#
OBX+ **"6n"** (2)	58.56 ± 5.72#	41.62 ± 5.72#
OBX+ PAR (10)	53.00 ± 4.82#	38.50 ± 3.02#

Each value represents mean percentage of OAE and TSOA. Results were expressed in mean ± S.E.M. *P<0.05 vs sham control , #P < 0.05 vs OBX control. n = 6/group. PAR= Paroxetine

5.9.5. Hyper-emotionality Test

Fig.75. displays mean scores for hyper-emotionality in sham and OBX rats. OBX rats showed significant (P<0.05) increase in hyper-emotional behaviour such as startle, struggle and fight response, as compared to sham rats. Hyper-emotional behaviour exhibited by OBX rats was markedly [F (7, 40)=27.63, P<0.05] reversed by chronic treatment with **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.).

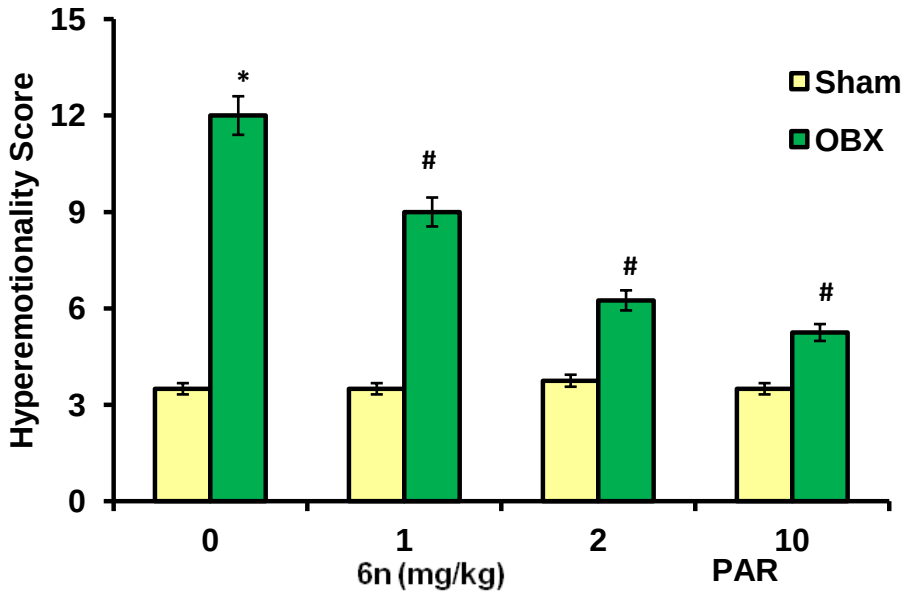


Fig 75. Effect of **"6n"** (1 and 2mg/kg) and PAR (10 mg/kg) and on hyper-emotionality scores of OBX and sham rats. Results are expressed as mean hyper-emotionality scores. Error bars represent S.E.M. *P <0.05 vs sham control, # P < 0.05 vs OBX control. n =6/group. PAR=Paroxetine.

5.9.6. Effect of "6n" on the levels of 5-HT, NE and DA in sham and OBX Rats

OBX significantly (P<0.05) reduced the 5-HT (ng/g wet brain tissue), NE (pg/g wet brain tissue) and DA (pg/g wet brain tissue) levels in rat brain as compared to the sham operated controls. Treatment with **"6n"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) predominantly increased the 5-HT levels [F (7, 40)=16.86, P<0.05] as compared to OBX control. **"6n"** (1 mg/kg, p.o.) was not able to produce any pronounced effect on 5-HT level as compared to OBX control rats (Table 44). Moreover the marked [F (7, 40)=98.61, P<0.05] increase on NE levels was observed after treatment with **"6n"** (2 mg/kg) and PAR (10 mg/kg) as compared to OBX control. **"6n"** (1 mg/kg) was not able to produced any significant effect on NE levels as compared to OBX control rats (Table 44). Furthermore treatment with **"6n"** (1 and 2 mg/kg) and PAR (10 mg/kg) were not able to produce any significant [F (7, 40)=12.56, P<0.05] change on DA levels as compared to OBX control rats (Table 44).

Table 44: Effect of "6n" on the levels of 5-HT, NE and DA in sham and OBX Rats

Treatment groups (mg/kg)	5-HT	NE	DA
Sham control	540.33 ± 21.67	425.56 ± 10.50	381.38 ± 11.55
Sham + **"6n"** (1)	535.00 ± 11.55	429.00± 20.04	380.00 ±9.25
Sham + **"6n"** (2)	540.50 ± 12.55	431.00 ± 15.55	375.00 ± 14.45
Sham + PAR (10)	545.45 ± 13.65	440.00 ± 11.00	379.65 ±17.19
OBX+ Vehicle	**393.36 ± 18.32***	**150.50 ± 5.50***	**307.93 ± 14.16***
OBX+ **"6n"** (1)	400.25 ± 9.45	141.50 ± 10.50	296.50 ± 10.55
OBX+ **"6n"** (2)	487.00 ± 14.00#	228.00 ± 12.35#	310.00 ± 12.75
OBX + PAR (10)	499.37 ± 17.45#	272.13 ± 15.27#	301.00± 16.74

Tabulated data represent the mean number of neuro-transmitter level [5-HT (ng/g), DA (pg/g) and NE (pg/g)]. Results are expressed as mean±S.E.M. **"6n"**/PAR/vehicle were administered once a day p.o. for 14 days. *P < 0.05 vs sham control, # P < 0.05 vs OBX control. n = 6/group.

5.9.7. Effect of "6n" on the level of BDNF in Sham and OBX Rats

OBX significantly (P<0.05) reduced the BDNF levels in rat brain as compared to the sham operated controls. Treatment with **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) markedly increased the brain BDNF levels [F (7, 40)=11.18, P<0.05] as compared to OBX control (Table 45).

Table 45: Effect of "6n" treatment on BDNF levels in sham and OBX rats

Groups	Dose (mg/kg)	BDNF(ng/mg protein)
Sham control	0	82.95 ± 2.37
Sham + **"6n"**	1	77.05 ± 5.50
Sham + **"6n"**	2	82.00 ± 6.86
Sham + PAR	10	85.77 ± 6.21
OBX Control	0	31.60 ± 2.68*
OBX + **"6n"**	1	66.35 ± 4.00$^{\#}$
OBX + **"6n"**	2	69.50 ± 5.00$^{\#}$
OBX + PAR	10	65.00 ± 7.25$^{\#}$

The values are expressed as mean ± S.E.M. The drug/vehicle treatments were carried out once a day for 14 days. *P<0.05 compared with sham control, $^{\#}$P<0.05 compared with vehicle treated OBX group; n = 6/ group. PAR=Paroxetine

5.10. Co-morbid Evaluation in Traumatic Brain Injury (TBI) Rats Using Behavioural Test Battery

5.10.1.Effect of TBI on body weight of rats

Body weight of sham and TBI rats were continuously observed till the behavioural tests were started. Initially decrease in body weight was observed in TBI rats post TBI. Statistical analysis revealed that TBI rats gained lesser weight than sham group (Table 46).

Table 46: Effect of TBI on body weight.

	Dose (mg/kg)	Initial weight	Final weight
Sham control	0	250.50 ± 3.50	301.00 ± 5.00
Sham + **"6n"**	1	270.50 ± 4.50	298.00 ± 8.00
Sham + **"6n"**	2	265.00 ± 4.00	299.50 ± 3.50
Sham+ PAR	10	265.00 ± 3.50	289.50 ± 9.50
TBI control	**0**	**268.00 ± 4.55**	**273.0 ± 4.50***
TBI + **"6n"**	1	255.00 ± 5.30	284.0 ± 4.05
TBI + **"6n"**	2	259.50 ± 3.50	292.33 ± 4.40
TBI + PAR	10	262.00 ± 4.55	292.67 ± 7.50

Each value represents the mean change in body weight. **"6n"**/ vehicle/ PAR (mg/kg) were administered p.o. once a day for 14 days. *P<0.05 vs sham control. n=6 /group. PAR=Paroxetine.

5.10.2. Open field test (OFT)

The OFT is the behavioural test used to evaluate the hyperactivity (characterized by ambulation, rearing and fecal pellets) in TBI rats as summarized in Table 47. TBI rats exhibited significant increase in ambulation [F (7, 40)=24.81, P<0.05], rearing [F (7, 40)=15.35, P<0.05] and number of fecal pellets [F (7, 40)=10.53, P<0.05], behaviour for 5 min after being exposed to the open field arena (Table 47). Increased frequencies of ambulation, rearing and fecal pellets were observed to be ameliorated by chronic **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment.

5.10.3. Elevated plus maze (EPM)

TBI and **"6n"** treated rats demonstrated variable responses in EPM task. TBI rats exhibited a significant (P<0.05) increase in percent OAE and TSOA as compared to sham rats. Chronic **"6n"** treatment (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) predominantly decreased the number of OAE [F (7, 40)=4.428, P<0.05] and TSOA [F (7, 40)=4.063, P<0.05] as compared to vehicle treated TBI rats in EPM test (Table 48).

Table 47: Effect of TBI and "6n" on the ambulation, rearing and fecal pellets of rats in OFT

Treatment (mg/kg)	No. of Ambulation	No. of Rearing	No. of Fecal pellets
Sham control	102.25 ± 0.85	10.00 ± 1.24	3.00 ± 0.40
Sham + **"6n"** (1)	119.75 ± 7.69	10.75 ± 1.93	2.50 ± 0.64
Sham + **"6n"** (2)	105.25 ± 4.75	8.50 ± 1.55	2.25 ± 0.47
Sham + PAR (10)	99.67 ± 9.37	8.67 ± 0.71	2.00 ± 0.58
TBI Control	231.00 ± 7.92*	31.00 ± 3.50*	8.75 ± 0.85*
TBI+ **"6n"** (1)	174.75 ± 17.76#	17.50 ± 1.55#	4.75 ± 1.37#
TBI+ **"6n"** (2)	163.50 ± 13.67#	17.00 ± 2.79#	4.25 ± 0.25#
TBI+ PAR (10)	101.75 ± 2.65#	8.50 ± 1.04#	2.50 ± 0.28#

Tabulated data represent the mean number of ambulation, rearing and fecal pellets. Results are expressed as means ± S.E.M. **"6n"**/ vehicle/ PAR (mg/kg) were administered once a day p.o. for 14 days. *P < 0.05 vs sham control, # P < 0.05 vs TBI control. n = 6 /group. PAR=Paroxetine

Table 48: Effect of TBI and "6n" on the % OAE and % TSOA of Rats in EPM

Treatment (mg/kg)	% OAE	% TSOA
Sham control	24.00 ± 3.80	36.50 ± 4.72
Sham + **"6n"** (1)	22.50 ± 2.74	34.00 ± 3.08
Sham + **"6n"** (2)	31.52 ± 4.31	40.75 ± 3.29
Sham + PAR (10)	29.27 ± 2.52	38.75 ± 4.45
TBI Control	48.50 ± 8.82*	61.08 ± 7.23*
TBI+ **"6n"** (1)	24.75 ± 2.60#	33.16 ± 5.60#
TBI+ **"6n"** (2)	19.00 ± 2.01#	31.00 ± 1.84#
TBI+ PAR (10)	29.23 ± 3.44#	38.23 ± 5.01#

Tabulated results are expressed as mean percentage OAE and TSOA. Error bars represent mean S.E.M. *P <0.05 vs sham control, #P < 0.05 vs TBI control. n =6 /group. PAR=Paroxetine

5.10.4. Marble burying behaviour

The effect of TBI and **"6n"** treatment on marble burying behaviour, as one of many anxiety-related sequelae is shown in Fig.76. TBI significantly (P<0.05) increased the marble burying behaviour as compared to sham rats. The chronic **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment markedly [F (7, 40)=5.794, P<0.05] suppressed the aversive marble burying behaviour as compared to vehicle treated TBI rats. Marble burying behaviour exhibited by the sham group was significantly reduced by the PAR (10 mg/kg, p.o.) treatment.

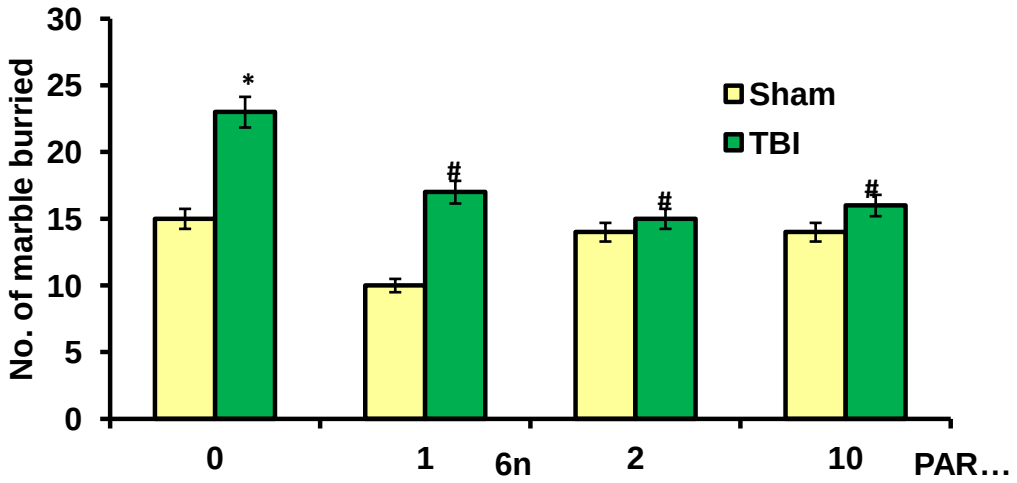


Fig.76. Effect of **"6n"** (1 and 2 mg/kg) and PAR (10 mg/kg) on the number of marbles buried by TBI and sham rats in marble burying test. Results are expressed as mean number of marbles buried. Error bars represent S.E.M. * P<0.05 vs sham control, # P < 0.05 vs TBI control. n =6 /group. PAR=Paroxetine

5.10.5. Sucrose consumption test

Sucrose (1%) consumption test was performed to measure anhedonia (loss of pleasure) in rodents. Sucrose consumption was tested in sham and OBX rats. Significant decrease in sucrose consumption (P<0.05) was observed in OBX rats as compared to sham rats. Chronic **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment showed pronounced [F (7, 40)=4.389, P<0.05] increase in the sucrose consumption in OBX treated rats as compared to vehicle treated OBX rats (Table 49).

Table 49: Effect of TBI and "6n" on the % sucrose consumption

% Sucrose Consumption	
Sham control	65.75 ± 5.49
Sham + **"6n"** (1)	61.25 ± 6.44
Sham + **"6n"** (2)	59.00 ± 6.01
Sham + PAR (10)	66.75 ± 6.47
TBI Control	33.00 ± 2.97*
TBI+ **"6n"** (1)	61.50 ± 6.51#
TBI+ **"6n"** (2)	66.25 ± 3.92#
TBI+ PAR (10)	67.75 ± 4.60#

Tabulated results are expressed as mean percent sucrose consumption. Error bars represent mean S.E.M. *P < 0.05 vs sham control, # P < 0.05 vs TBI control. n =6 /group. PAR=Paroxetine

5.10.6. Effect of "6n" on the levels of 5-HT, NE and DA in sham and TBI Rats

TBI significantly (P<0.05) reduced the 5-HT (ng/g wet brain tissue), NE (pg/g wet brain tissue) and DA (pg/g wet brain tissue) levels in rat brain as compared to sham operated controls. **"6n"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) significantly [F (7, 40)=55.71, P<0.05] increased serotonin levels in TBI rats as compared to TBI control rats. **"6n"** (1 mg/kg, p.o.) was not able to produce any significant effect on 5-HT levels as compared to TBI control rats (Table 50). The significant [F (7, 40)=15.83, P<0.05] increase in NE levels were observed after treatment with **"6n"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) as compared to TBI control. **"6n"** (1 mg/kg, p.o.) was not able to produced any marked effect on NE levels as compared to TBI control rats (Table 50). Moreover treatment with **"6n"** (1and 2 mg/kg) and PAR (10 mg/kg) predominantly [F (7, 40)=104.5, P>0.05] were not able to affect the DA levels as compared to TBI control rats (Table 50).

Table 50: Effect of "6n" on the levels of 5-HT, NE and DA in sham and TBI Rats

Treatment (mg/kg)	5-HT	NE	DA
Sham control	470.22 ± 12.63	476.56 ± 10.52	580.25 ± 20.55
Sham + **"6n"** (1)	472.50 ± 11.50	471.00± 10.45	596.45 ±12.25
Sham + **"6n"** (2)	481.00 ± 9.25	485.23 ± 15.00	591.25 ± 10.45
Sham + PAR (10)	484.23 ± 10.32	481.14 ± 15.78	578.90 ±12.98
TBI + Vehicle	**270.00 ± 12.26***	361.33 ± 12.48*	**497.33 ± 15.33***
TBI + **"6n"** (1)	301.00 ± 10.45	360.00 ± 10.45	491.00 ± 14.52
TBI + **"6n"** (2)	355.00 ± 14.50#	415.00 ± 12.35#	497.50± 17.75
TBI + PAR (10)	389.22 ± 11.22#	442.23 ± 16.33#	483.25 ± 10.25

Tabulated data represent the mean number of neuro-transmitter level [5-HT (ng/g), DA (pg/g) and NE (pg/g)]. Results are expressed as means ± S.E.M. *P < 0.05 vs sham control, # P < 0.05 vs TBI control. n = 6/group. PAR=Paroxetine

5.10.7. Effect of "6n" on the level of BDNF in sham and TBI Rats

TBI significantly (P<0.05) reduced the BDNF levels in rat brain as compared to the sham operated controls. Treatment with **"6n"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) markedly increased the brain BDNF levels [F (7, 40)=11.18, P<0.05] as compared to TBI control (Table 51).

Table 51: Effect of "6n" treatment on BDNF levels of sham and TBI rats

Groups	Dose (mg/kg)	BDNF (ng/mg protein)
Sham control	0	74.65 ± 5.23
Sham + **"6n"**	1	88.00 ± 4.50
Sham + **"6n"**	2	92.05 ± 5.20*
Sham + PAR	10	85.00 ± 4.50
TBI control	0	20.72 ± 4.87*
TBI + **"6n"**	1	43.00 ± 2.35#
TBI + **"6n"**	2	41.50 ± 3.00#
TBI + PAR	10	48.25±3.15#

The values are expressed as mean ± S.E.M. The drug/vehicle treatments were carried out once a day for 14 days. *P < 0.05 compared with sham control, #P < 0.05 compared with vehicle treated OBX group; n = 6/ group. PAR=Paroxetine

5.11. Co-morbid Evaluation of Mice Under CUMS Using Behavioural Test Battery

5.11.1. Behavioural Observations

A. Effect of "6n" on SLA of mice

The locomotor activity was evaluated after 28 day of CUMS procedure using actophotometer. There was no significant [F (4, 35)=0.52, P>0.05] difference observed in the locomotor scores in mice of different groups (Fig.77)**.**

B. Effect of "6n" on immobility time in FST

Mice subjected to CUMS showed significant increase in duration of immobility as compared to control mice. Chronic treatment with **"6n"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) predominantly [F (4, 35)=21.75, P<0.05] decreased the immobility duration of stressed mice as compared to stress control group (Fig. 78).

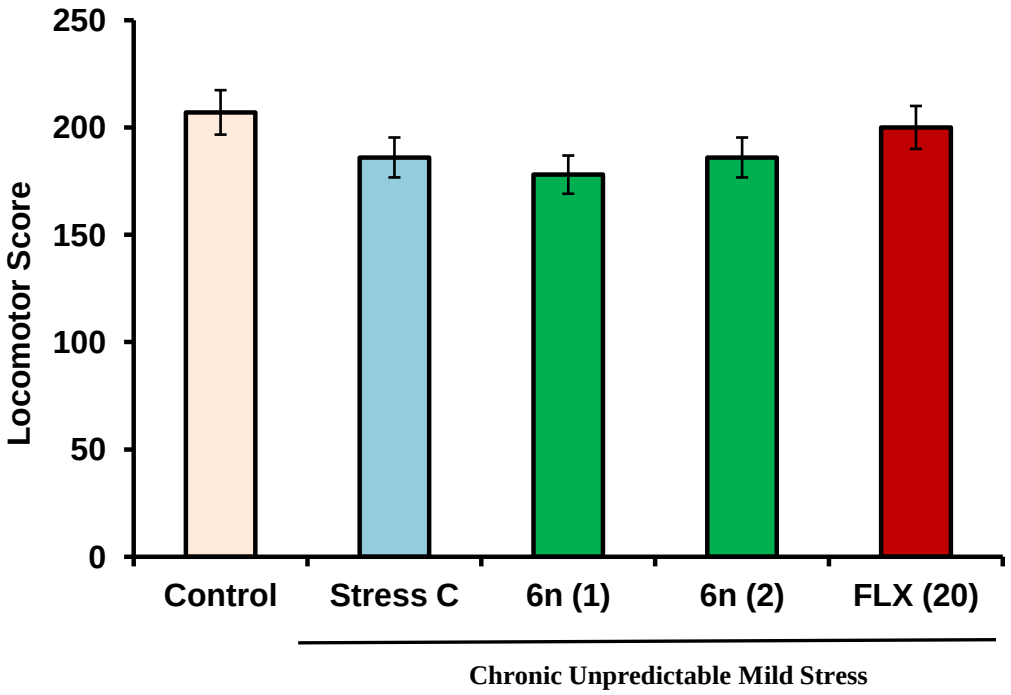


Fig.77. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on locomotor scores in stressed mice. Each column represents mean locomotor scores recorded in 8 min observation period. The error bars indicate S.E.M.; n = 8/group. FLX=Fluoxetine.

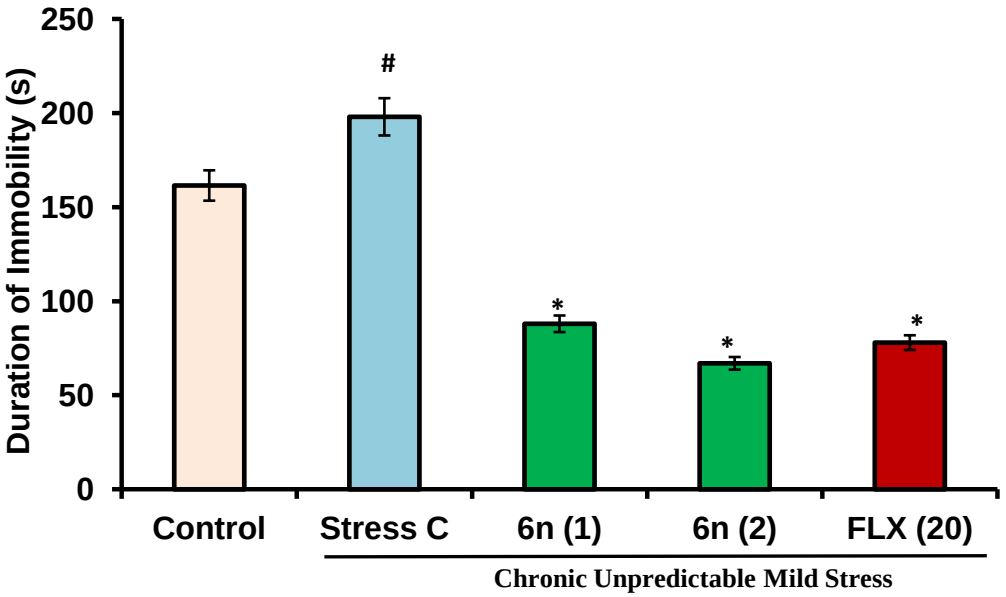


Fig.78. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on duration of immobility in stressed mice. Each column represents mean duration of immobility (s). The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group; n = 8/group. FLX=Fluoxetine.

C. Effect of "6n" on the percentage of sucrose consumption

The effect of **"6n"** treatment on sucrose consumption is shown in Fig. 79. The stressed mice showed a significant reduction in the percentage of sucrose consumption when compared to control mice. The chronic administration of **"6n"** (1 and 2 mg/kg, p.o.) notably [F (4, 35)=5.32, P<0.05] restored the percentage of sucrose consumption in stressed mice as compared to stress vehicle treated group. The positive control, FLX (20 mg/kg, p.o.) also markedly **(**P<0.05) reversed the reduction in the percentage of sucrose consumption in stressed mice as compared to stress control group.

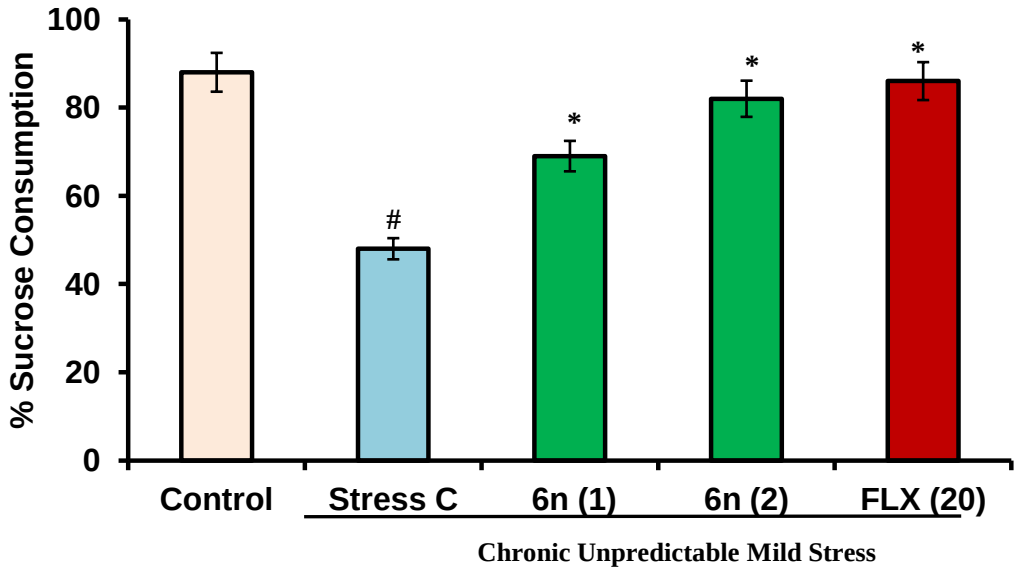


Fig.79. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on sucrose preference (%) in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group; n = 8/group. FLX=Fluoxetine.

D. Effect of "6n" on immobility time in TST

Mice subjected to CUMS showed significant increase in duration of immobility as compared to control mice. Chronic treatment with **"6n"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) predominantly [F (4, 35)=5.354, P<0.05] decreased the immobility duration of stressed mice as compared to stress control group (Fig. 80) .

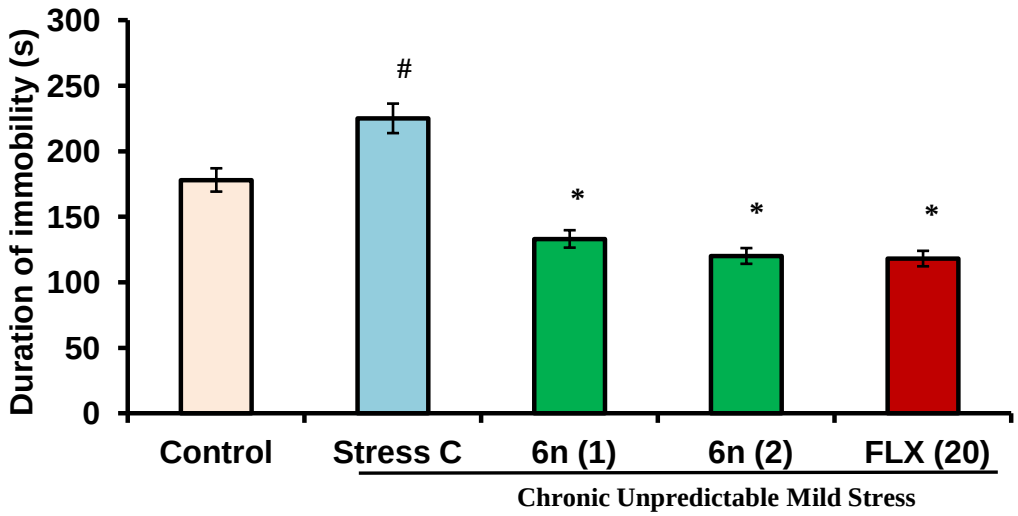


Fig.80. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on duration of immobility in stressed mice. Each column represents mean duration of immobility (s). The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group; n = 8/group. FLX=Fluoxetine.

E. Effect of "6n" on percentage of both TSOA and OAE in EPM test

Mice subjected to CUMS showed significant decrease in % TSOA [F (4, 35)=5.356, P<0.05] and % OAE [F (4, 35)=10.54, P<0.05] as compared to control mice. Chronic treatment with **"6n"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) substantial increase in % TSOA and % OAE of stressed mice as compared to stress control group (Table 52).

Table 52: Effect of "6n" on % TSOA and % OAE in EPM test

Treatment	Dose (mg/kg)	% TSOA	% OAE
Normal Control	0	31.24 ± 3.21	39.17 ± 1.97
Stress C	1	9.93± 0.95#	5.27 ± 2.34#
FLX	20	24.72 ± 1.47*	37.7 ± 4.73*
"6n"	1	21.6 ± 3.30*	36.11 ± 2.95*
"6n"	2	25.53± 3.35*	60.03 ± 6.22*

Tabulated results are expressed as mean percent TSOA and OAE. Error bars represent mean S.E.M. #P <0.05 vs normal control, *P < 0.05 vs stress control group. n =8 /group. FLX=Fluoxetine.

5.11.2. Biochemical Parameters

A. Effect of "6n" treatment on brain lipid peroxidation levels

The TBARS level was significantly increased in brain of stressed mice as compared to the control mice (Fig 81). Repeated treatment with **"6n"** (1 and 2 mg/kg, p.o.) produced a marked [F (4, 35)=36.49, P<0.05] reduction in TBARS levels in the brain of stressed mice as compared to stress control group. Moreover, FLX (20 mg/kg, p.o.) treatment also significantly reduced TBARS levels in the brain of stressed mice.

B. Effect of "6n" treatment on brain GSH levels

The brain GSH levels were found to be significantly depleted in brain of stressed mice compared to control mice (Fig 82). Chronic treatment with **"6n"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) showed a noticeable [F (4, 35)=13.05, P<0.05] increase in brain GSH level as compared to stress control group.

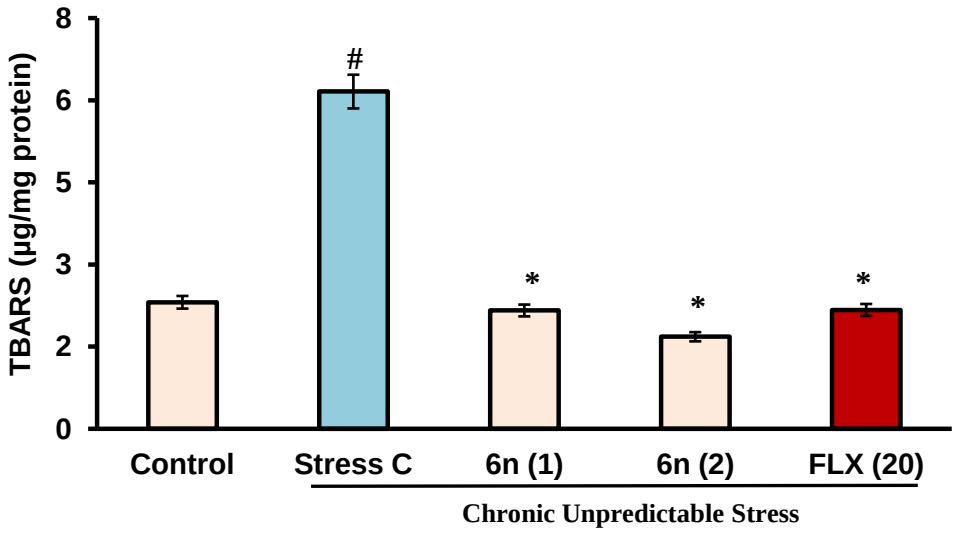


Fig.81. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain lipid peroxidation levels in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group; n = 8/group. FLX=Fluoxetine.

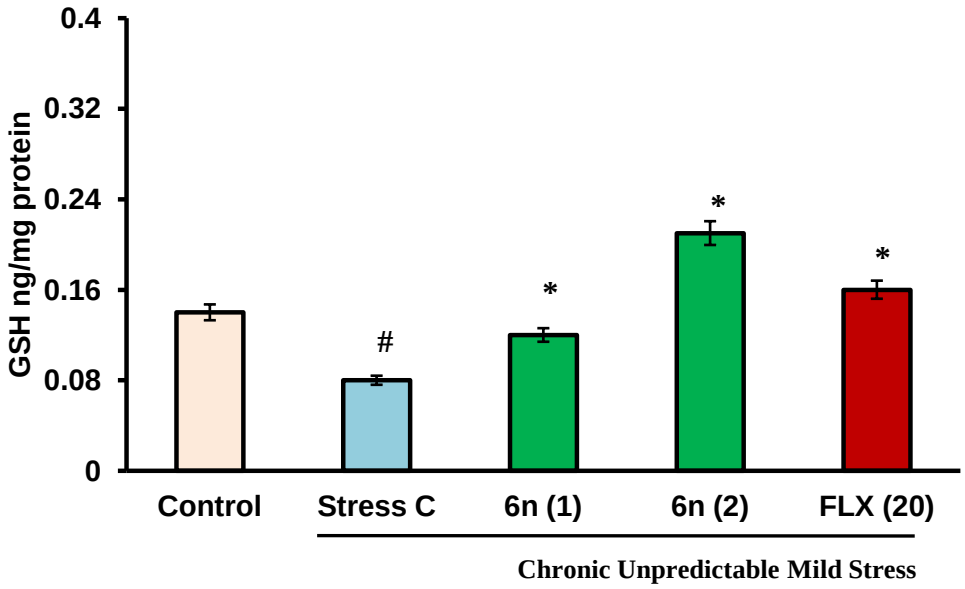


Fig.82. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain GSH levels in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group; n = 8/group. FLX=Fluoxetine.

C. Effect of "6n" treatment on brain CAT levels

As depicted in Fig. 83., CUMS subjected mice showed a significant (P < 0.05) reduction in brain CAT levels of as compared to control mice. Chronic treatment with **"6n"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) significantly [F (4, 25)=16.76, P<0.05] restored CAT levels in the brain as compared to stress control group.

D. Effect of "6n" treatment on brain SOD levels

The effect of **"6n"** on the brain SOD activity is shown in Fig.84. The enzymatic activity of SOD was observed to be substantially lower in brain of stressed mice as compared to control mice. Repeated treatment with **"6n"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) showed marked [F (4, 35)=23.06, P<0.05] improvement in the SOD activity in stressed mice brain as compared to stress control group.

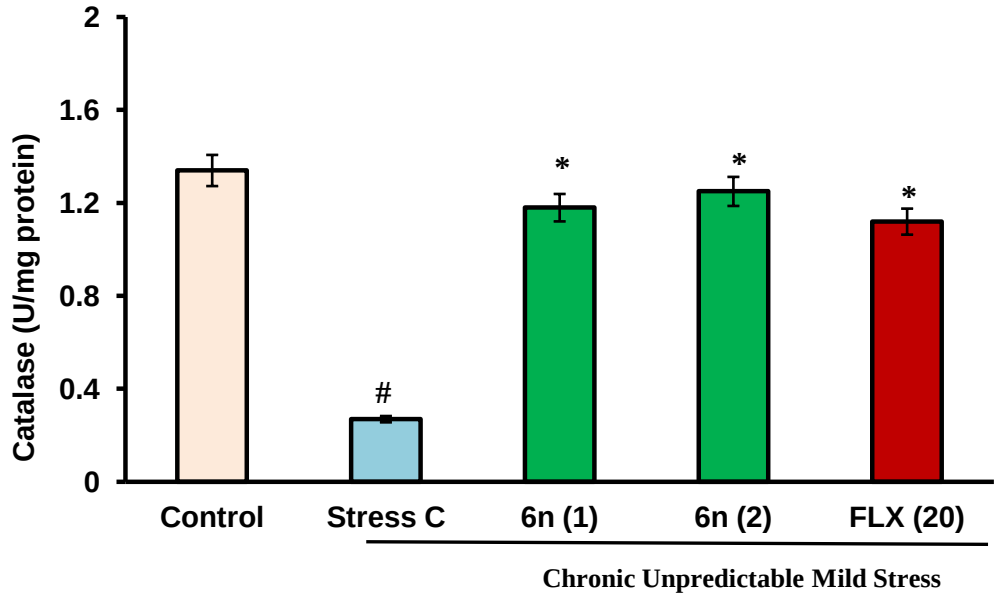


Fig.83. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain CAT levels in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group n = 8/group FLX=Fluoxetine.

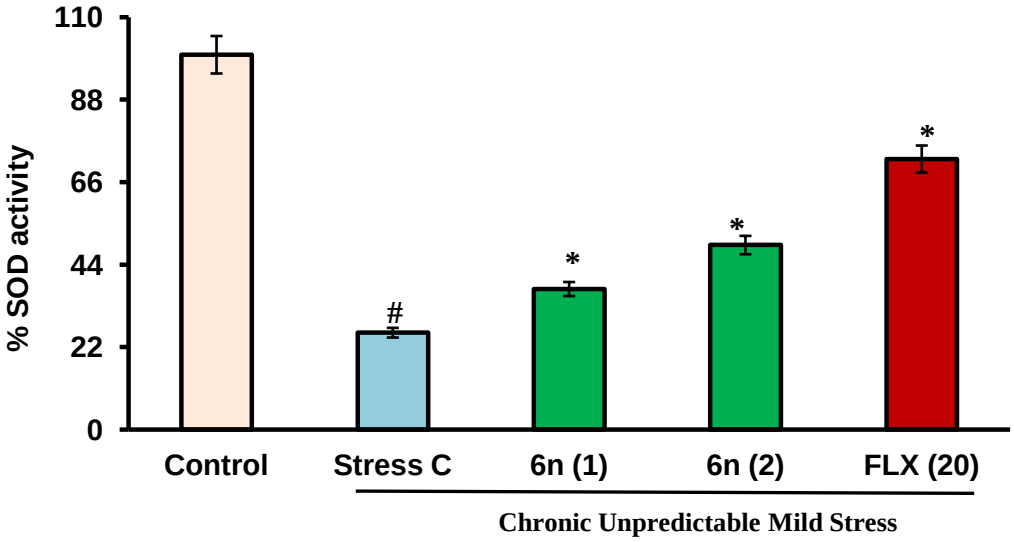


Fig.84. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain SOD activity in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group; n = 8/group. FLX=Fluoxetine.

E. Effect of "6n" treatment on brain nitrite level

Stressed mice showed a significant elevated brain nitrite levels as compared to control group mice (Fig. 85). The chronic administration of **"6n"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) showed marked [F (4, 35)=6.034, P<0.05] reduction in elevated brain nitrite level in stressed mice when compared with stressed control group.

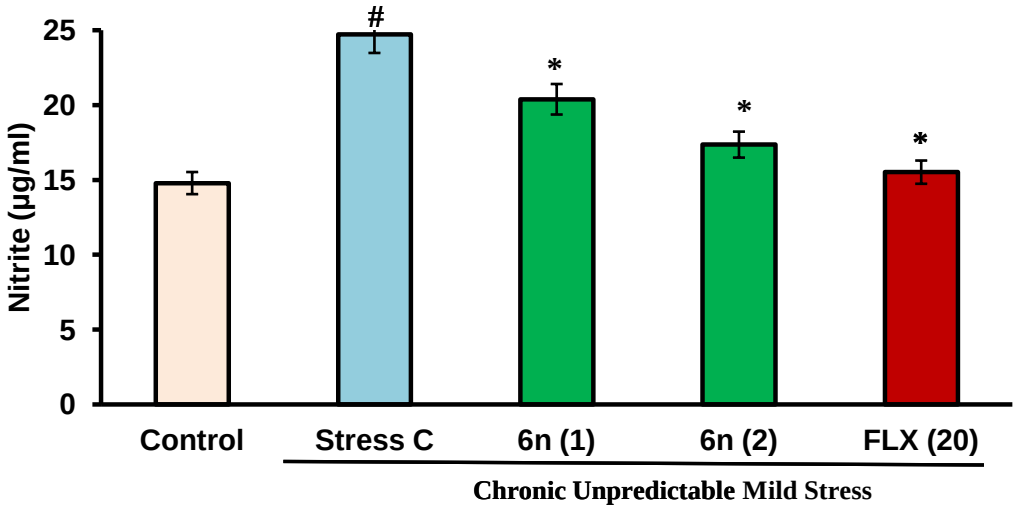


Fig.85. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on brain nitrite levels in stressed mice. The error bar indicates S.E.M., #P < 0.05 when compared with normal control; *P < 0.05 when compared with stress control group n = 8/group. FLX=Fluoxetine.

F. Effect of "6n" treatment on plasma corticosterone levels

CUMS mice showed pronounced [P<0.05] increase in plasma corticosterone levels as compared to normal control mice (Fig. 86). The chronic treatment with **"6n"** (1 and 2 mg/kg, p.o.) and FLX (20 mg/kg, p.o.) significanltly [F (4, 35)=254.9, P<0.05] reversed the increased corticosterone level as compared to stresse control group.

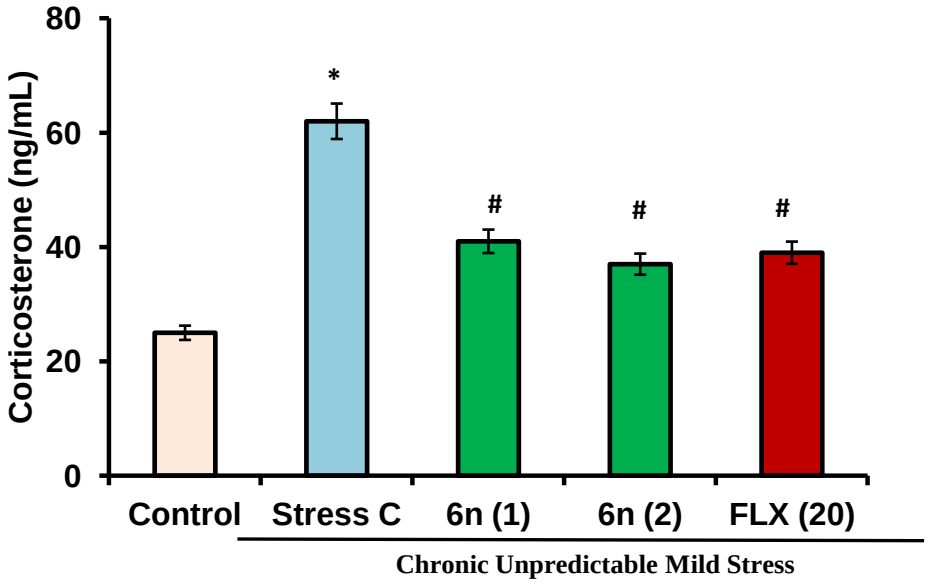


Fig.86. Effect of **"6n"** (1 and 2 mg/kg) and FLX (20 mg/kg) treatment on the plasma corticosterone levels in normal and CUMS mice. Each column represent mean corticosterone level. Error bars represent mean S.E.M. *P < 0.05 vs normal control, #P < 0.05 vs stress control group. n = 8 /group. FLX=Fluoxetine.

5.12. Evaluation of "6o" in Rodent Models of Depression

5.12.1. Effect of "6o" on SLA of mice

Compound **"6o"** (0.5, 1, 2 mg/kg, i.p.) did not show any change on [F (5, 42)=11.41, P>0.05] base line locomotions as compared to control. (Fig. 87).

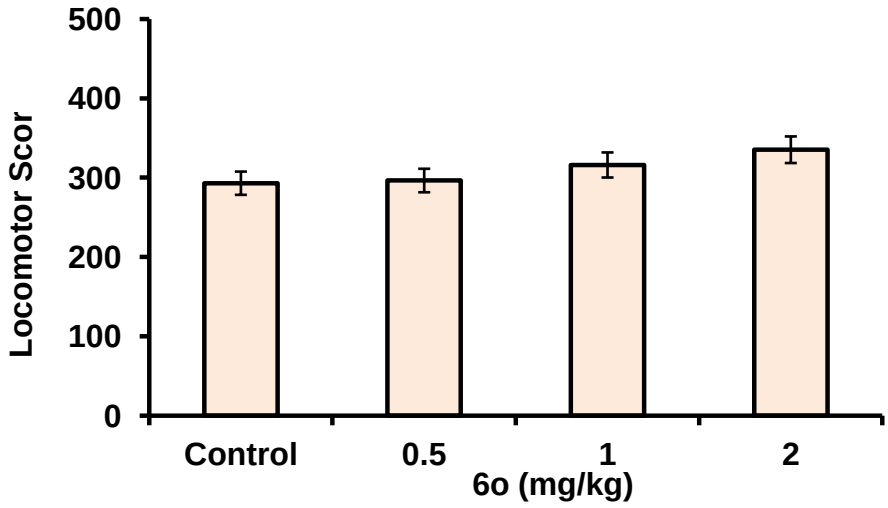


Fig. 87. The columns represent mean locomotor scores and error bars indicate S.E.M. n = 8 per group. *P < 0.05 compared with vehicle treated group**.**

5.12.2. Effect of "6o" on duration of immobility in mice using FST

In FST, the acute treatment with **"6o"** (1 and 2 mg/kg, i.p.) and ESC (10 mg/kg, i.p.) significantly [F (4, 35)=6.598, P<0.05] decreased the duration of immobility as compared to vehicle treatment (Fig. 88). While at lower dose **"6o"** (0.5 mg/kg, i.p.) was not able to produce marked change in duration of immobility.

5.12.3. Effect of "6o" on duration of immobility in mice using TST

In TST, the acute treatment with **"6o"** (1 and 2 mg/kg, i.p.) and BUP (20 mg/kg, i.p.) significantly [F (4, 35)=7.147, P<0.05] decreased the duration of immobility as compared to vehicle treatment (Fig. 89). While at lower dose **"6o"** (0.5 mg/kg, i.p.) was not able to produce any marked effect on duration of immobility

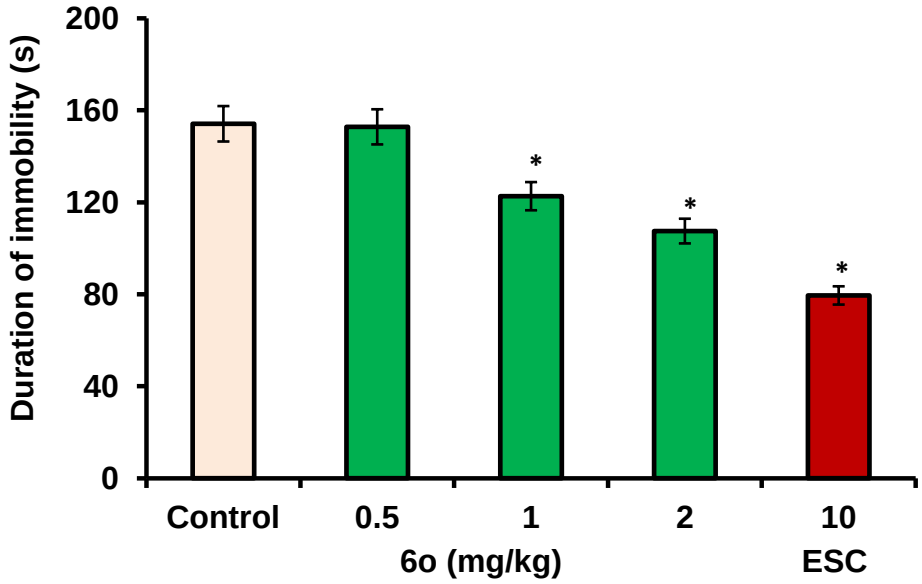


Fig. 88. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle treated group. ESC = Escitalopram.

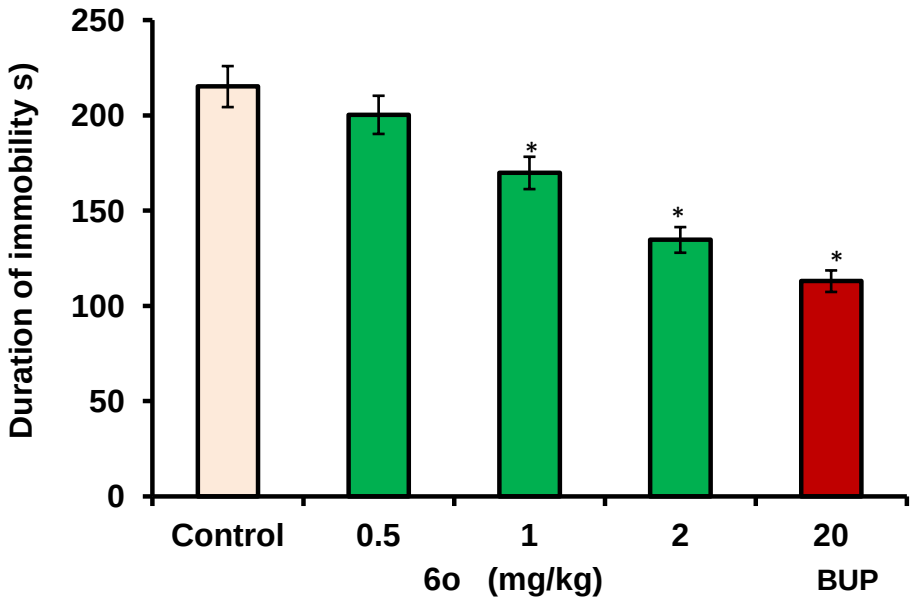


Fig. 89. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle treated group. BUP = Bupropion.

5.12.4. Effect of "6o" on RIH in mice

Reserpine (1 mg/kg i.p) elicited a pronounced decrease in core body temperature of rats. This effect was predominantly [F (3, 28)=51.29, P<0.05] reversed by **"6o",** (1 and 2 mg/kg) and ESC (10 mg/kg) treatments (Fig. 90).

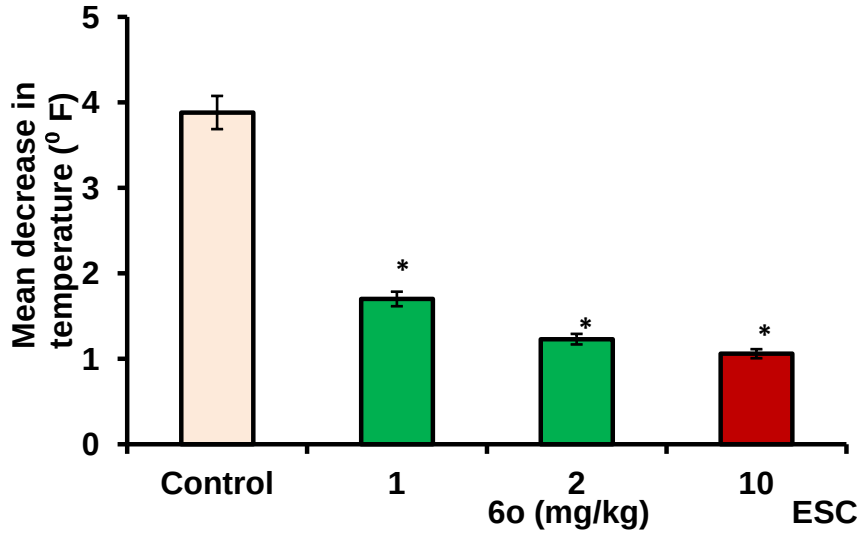


Fig. 90. The columns represent mean decrease in temperature (°F) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle treated group. ESC = Escitalopram.

5.12.5. Effect of "6o" on 5-HTP-HTR in mice

"6o" (2 mg/kg, i.p.) and FLX (20 mg/kg, i.p.) notably [F (3, 28)=26.53, P<0.05] potentiated the 5-HTP/PRG induced head twitches in mice (Fig. 91). While **"6o"** (1 mg/kg, i.p.) was not able to produce any pronounced effect on head twitch responses in mice.

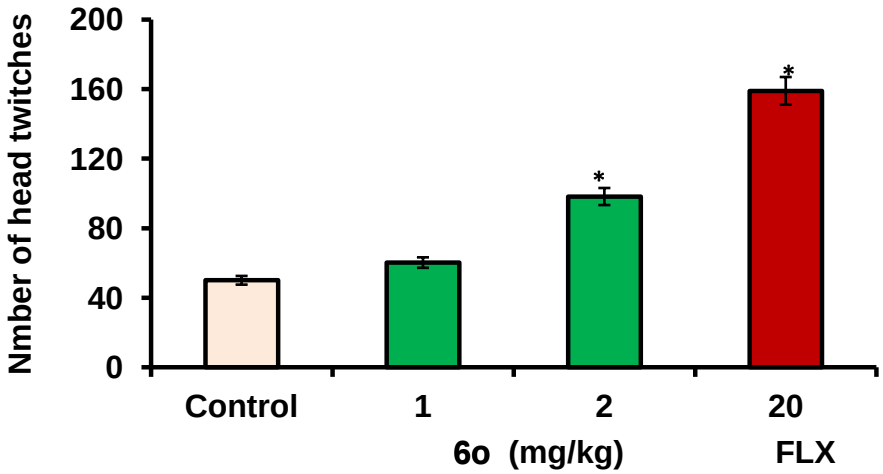


Fig. 91. The columns represent mean number of head twitches and error bars indicate S.E.M. n = 8/group. *P < 0.05 compared with vehicle-treated group. FLX = Fluoxetine

5.12.6. Interaction studies of "6o" with standard drugs

For a conclusive evaluation of anti-depressant potential of 5-HT_3 receptor antagonists, interaction studies with standard anti-depressants were carried out. **"6o"** pre-treatment (1mg /kg, i.p.) was found to enhance anti-depressant-like effects of FLX (10 and 20 mg/kg, i.p.) [F (1, 42)=19.11, P<0.05], VLA (4 and 8 mg/kg, i.p.) [F (1, 42)=18.79, P<0.05] and DMI (10 and 20 mg/kg, i.p.) [F (1, 42)=25.45, P<0.05] as shown in Fig. 92-94 respectively. Moreover, **"6o"** predominantly [F (1, 42)=30.25, P<0.05] reversed the depressant-like effect of PTL (1 mg/kg, i.p.) as shown (Fig. 95). Further, **"6o"** (1 mg/kg) noticeably [F (1, 42)=35.95, P<0.05] enhanced the anti-depressant activity of BUP (10 and 20 mg/kg) in mice TST (Fig. 96).

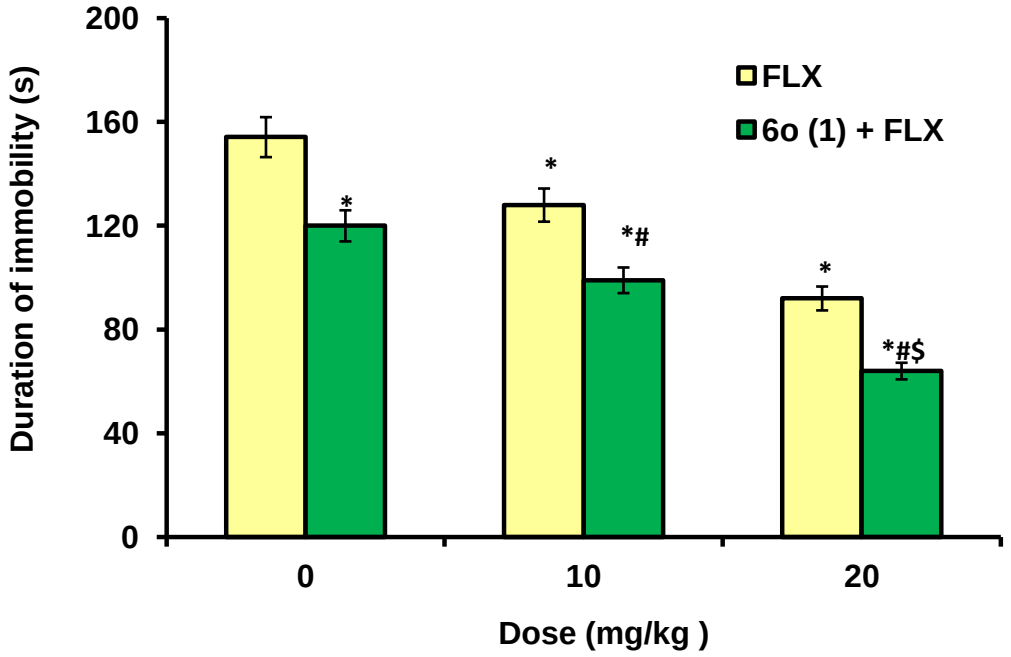


Fig. 92. The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n = 8 /group. *P < 0.05 compared with vehicle-treated group, $^{\#}$ P < 0.05 compared with FLX (10 and 20 mg/kg) treated group and $^{\$}$P < 0.05 compared with alone **"6o"** treated group. FLX=Fluoxetine

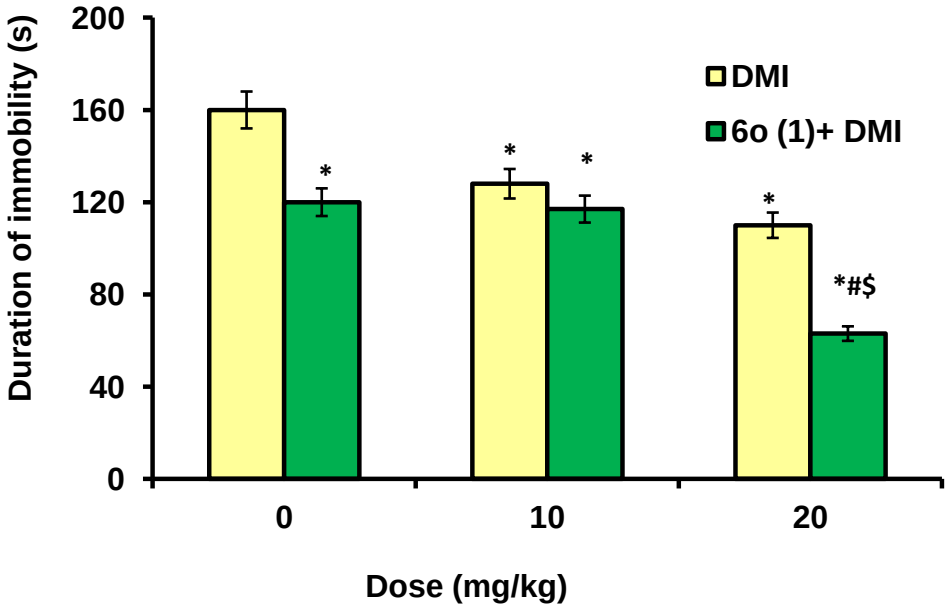


Fig. 93. The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n = 8 /group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with DMI (10 and 20 mg/kg) treated group alone and $P < 0.05 compared with alone **"6o"** treated group. DMI=Desipramine

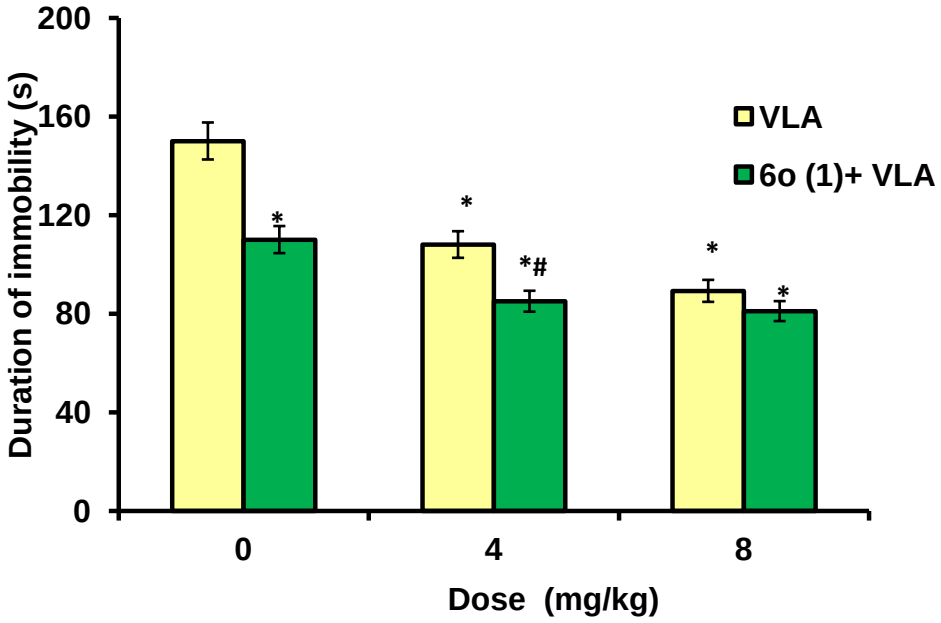


Fig. 94. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with VLA (4 and 8 mg/kg) treated group and $P < 0.05 compared with alone **"6o"** treated group. VLA=Venlafaxine

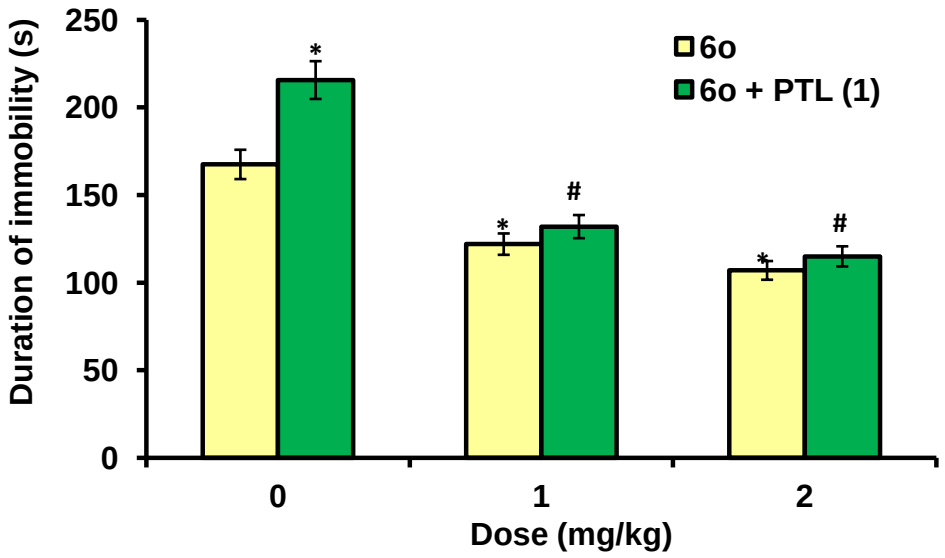


Fig. 95. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 vs. vehicle treated group and # P < 0.05 compared with PTL (1 mg/kg) treated group. PTL=Parthenolide

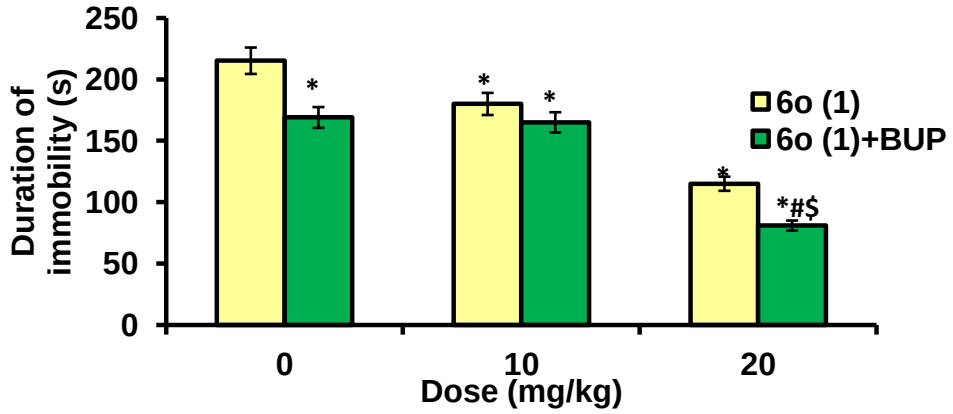


Fig. 96. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with BUP (10 and 20 mg/kg) treated group and $P < 0.05 compared with alone **"6o"** treated group. BUP=Bupropion

5.13. Evaluation of "6o" in Animal Models of Anxiety

5.13.1. Effect of "6o" on behaviour of mice in EPM test

Acute treatment with **"6o"** (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) significantly increased the percentage of both OAE [F (3, 28)=50.87, P<0.05] and TSOA [F (3, 28)=16.99, P<0.05] as compared to vehicle control group (Table 53). **"6o"** (1 mg/kg, i.p.) showed marked increase in percent TSOA and was not able to produce any significant change on percent OAE as compared to vehicle control group.

Table 53: Effect of "6o" on behaviour of mice in EPM test

Treatment (mg/kg)	% TSOA	% OAE
Vehicle Control	2.67 ± 0.23	11.15 ± 1.13
Diazepam (2)	10.58 ± 1.04*	37.70 ± 2.56*
"6o" (1)	5.17 ± 0.67*	15.48 ± 1.64
"6o" (2)	10.67 ± 1.48*	33.89 ± 3.23*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n=8/group. DZM=Diazepam.

5.13.2. Effect of "6o" on behaviour of mice in L/D test

"6o" (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment significantly (P < 0.05) increased the total time spent in lit area [F (3, 28)=28.37, P<0.05] and number of transitions [F (3, 28)=10.39, P<0.05] from one compartment to other as compared to vehicle treated group. Lower dose of **"6o"** (1 mg/kg, i.p.) was not able to produce any substantial effect on total time spent in lit area and on number of entries as compared to vehicle treated group (Table 54).

Table 54: Effect of "6o" on behaviour of mice in L/D test

Treatment (mg/kg)	Time spent in Lit area (s)	No. of transitions
Vehicle Control	44.83 ± 4.14	8.00 ± 0.62
DZM (2)	101.83 ± 5.08*	15.73 ± 1.33*
"6o" (1)	56.33 ± 5.17	11.00 ± 0.97
"6o" (2)	68.17 ± 3.95*	14.83 ± 1.35*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.13.3. Effect of "6o" on behaviour of mice in HB test

The results of the HB test are shown in Table 55. Compound **"6o"** (1 and 2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment predominantly increased the number of head dips [F (3, 28)=5.179, P<0.05], number of square crossed [F (3, 28)=13.34, P<0.05] and decreased the head dipping latency [F (3, 28)=22.02, P<0.05] as compared to vehicle control group.

Table 55: Effect of "6o" on behaviour of mice in HB test

Treatment (mg/kg)	No. of head dips	No. of Square crossed	Latency Time (s)
Vehicle Control	7.25 ± 1.06	2.63 ± 0.87	9.47 ± 0.67
DZM (2)	25.38 ± 2.43*	19.50 ± 2.14 *	2.33 ± 0.42*
"6o" (1)	19.83 ± 3.93*	9.00 ± 1.71*	3.67* ± 0.80*
"6o" (2)	21.83 ± 5.06*	9.17 ± 2.52*	3.80 ± 0.75*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n= 8/group. DZM=Diazepam

5.13.4. Effect of "6o" on behaviour of mice in OFT

"6o" (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment showed pronounced [F (3, 28)=252.0, P<0.05] increase in the number of squares crossed as compared to vehicle treatment group (Table 56). While **"6o"** (1 mg/kg, i.p.) was not able to affect ambulation scores. However **"6o"** (1 and 2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment was not able to affect rearing score significantly as compared to vehicle treatment group.

Table 56: Effect of "6o" on behaviour of mice in OFT

Treatment (mg/kg)	Ambulation scores	Rearing
Vehicle Control	145.67 ± 4.08	4.40 ± 0.64
DZM (2)	267.23 ± 5.09*	4.20 ± 0.45
"6o" (1)	245.50 ± 3.54	3.76 ± 0.56
"6o" (2)	292.50 ± 3.25*	3.92 ± 0.62

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.14. Co-morbid Evaluation in OBX Rats Using Behavioural Test Battery

5.14.1. Effect of OBX on body weight of rats

Table 57. displays the effect of OBX on body weight. Body weight of sham and OBX rats was continuously observed till the behavioural tests were started. Decrease in body weight were observed in OBX rats for few days, post surgery. Statistical analysis revealed that weight gained in OBX rats was significanlty (P<0.05) less as compared to sham rats.

Table 57: Effect of OBX on change in body weight of rats.

	Dose (mg/kg)	Initial weight	Final weight
Sham control	0	260.00 ± 5.25	294.0 ± 4.00
Sham + **"6o"**	1	261.50 ± 2.25	292.0 ± 3.50
Sham + **"6o"**	2	265.00 ± 2.50	283.5 ± 4.50
Sham+ PAR	10	255.00 ± 3.50	280.5 ± 2.50
OBX control	**0**	**265.00 ± 11.55**	**269.0 ± 4.00***
OBX + **"6o"**	1	269.00 ± 5.30	291.0 ± 3.50
OBX + **"6o"**	2	267.00 ± 4.28	295.33 ± 4.25
OBX + PAR	10	265.00 ± 8.43	294.67 ± 4.00

Each value represents the mean change in body weight. **"6o"**/ PAR/ vehicle (mg/kg) were administered p.o. once a day for 14 days. *P < 0.05 vs sham control. n= 6 /group. PAR=Paroxetine

5.14.2. Open field test (OFT)

OFT was the first behavioural study to be performed in OBX rats, post 14 days of treatment (Table 58). The effects of **"6o"** on the behaviour of OBX/sham rats were analyzed in different circumstances. Chronic (14 days, p.o) treatment with **"6o"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) predominantly reduced the number of ambulation [$F(7, 40)=19.22$, $P<0.05$], rearing [$F(7, 40)=44.62$, $P<0.05$ and number of fecal pellets [$F(7, 40)=29.90$, $P<0.05$] as compared to the vehicle treated OBX rats (Table 58).

5.14.3. Sucrose consumption test

Sucrose (1%) consumption test was performed to measure anhedonia (loss of pleasure) in rodents. Sucrose consumption was tested in sham and OBX rats. Marked decrease in sucrose consumption ($P<0.05$) was observed in OBX rats as compared to sham rats. Chronic treatment with **"6o"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o. showed pronounced [$F(7, 40)=8.39$, $P<0.05$] increase in the sucrose consumption in OBX treated rats as compared to vehicle treated OBX rats (Table 59). Chronic treatment with **"6o"** (1 mg/kg, p.o.) treatment did not exhibit any increase in the sucrose consumption in OBX treated rats as compared to vehicle treated OBX rats.

Table 58: Effect of "6o" on open field behaviour in sham and oBX rats

Treatment (mg/kg)	No. of Ambulation	No. of Rearing	No. of Fecal Pellets
Sham Control	91.17 ± 6.88	10.00 ± 1.24	2.17 ± 0.47
Sham+**"6o"** (1)	103.00 ± 8.87	9.33 ± 1.05	2.33 ± 0.71
Sham+**"6o"** (2)	102.17 ± 7.34	8.33 ± 1.15	2.00 ± 0.86
Sham+PAR (10)	99.67 ± 9.37	8.67 ± 0.71	2.00 ± 0.58
OBX Control	206.00 ± 7.48*	27.33 ± 4.58*	7.00 ± 0.76*
OBX+**"6o"** (1)	133.00 ± 8.82#	13.50 ± 1.01#	6.00 ± 0.62#
OBX+**"6o"** (2)	113.50 ± 8.95#	22.83 ± 1.19#	5.00 ± 0.65#
OBX+PAR (10)	113.00 ± 7.34#	11.83 ± 0.59#	2.33 ± 0.47#

The values represent ambulation, rearing and fecal pellets and error bars indicate S.E.M.,* P < 0.05 when compared to the sham operated rats, #P < 0.05 when compared to the vehicle-treated OBX rats (n = 6 /group). PAR=Paroxetine

Table 59: Effect of "6o" on sucrose consumption in sham and OBX rats

Treatment (mg/kg)	% Sucrose Consumption
Sham Control	41.25 ± 4.49
Sham+**"6o"** (1)	42.38 ± 5.68
Sham+**"6o"** (2)	42.45 ± 4.51
Sham+ PAR (10)	46.52 ± 7.26
OBX Control	15.15 ± 2.47*
OBX+**"6o"** (1)	19.50 ± 3.28
OBX+**"6o"** (2)	29.50 ± 3.52#
OBX+ PAR (10)	36.45 ± 4.65#

Values represent mean ± S.E.M. *P < 0.05 vs sham control , $^{\#}$P < 0.05 vs OBX control. n = 6 /group. PARr= Paroxetine

5.14.4. Elevated plus maze (EPM)

EPM was employed for the anxiolytic test in the laboratory set-up. Percentage OAE and TSOA were measured in EPM test. OBX rats exhibited increased OAE [P<0.05] and TSOA [P<0.05] of the maze in comparison with sham-operated rats (opposite to that observed in anxiety). Chronic **"6o"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment showed marked decrease in both percentage OAE [F (7, 40)=20.41, P<0.05] and TSOA [F (7, 40)=31.78, P<0.05] in EPM as compared to vehicle treated OBX rats (Table 60).

Table 60: Effect of "6o" on % OAE and % TSOA in OBX rats

Treatment (mg/kg)	% OAE	% TSOA
Sham control	24.00 ± 3.80	35.50 ± 4.72
Sham + **"6o"** (1)	22.50 ± 4.12	33.00 ± 3.08
Sham + **"6o"** (2)	26.50 ± 5.17	33.75 ± 3.49
Sham + PAR (10)	29.75 ± 2.52	38.75 ± 4.49
OBX Control	48.50 ± 6.82*	63.08 ± 7.41*
OBX+ **"6o"** (1)	14.75 ± 2.60#	30.16 ± 7.60#
OBX+ **"6o"** (2)	6.00 ± 1.11#	3.00 ± 0.84#
OBX+ PAR (10)	24.25 ± 3.44#	22.45 ± 5.08#

The value represents mean percentage OAE and percentage TSOA. Results were expressed in mean ± S.E.M. *P < 0.05 vs sham control , #P < 0.05 vs OBX control. n = 6 /group. PAR=Paroxetine.

5.14.5. Hyper-emotionality test

Fig. 97. displays mean scores for hyper-emotionality in sham and OBX rats. OBX rats showed notable increase in hyper-emotional behaviour such as startle, struggle, and fight response, as compared to sham rats. Hyper-emotional behaviour exhibited by OBX rats was predominantly [F (7,40)=29.90, P<0.05] reversed by chronic treatment with **"6o"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.).

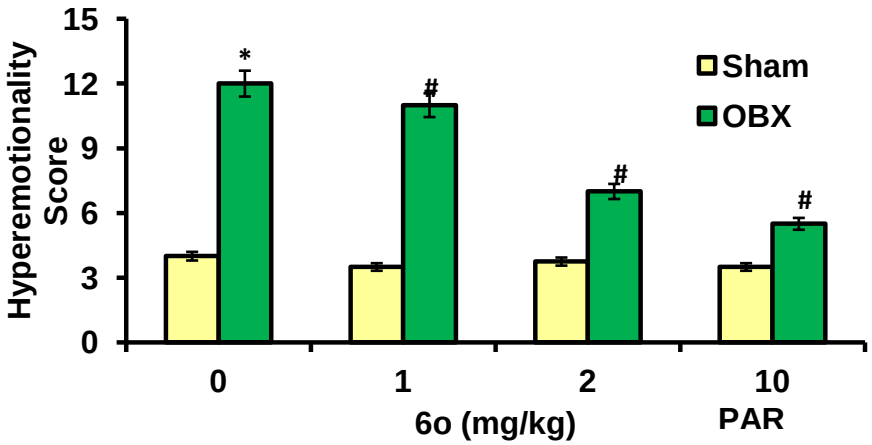


Fig. 97. Effect of **"6o"** (1 and 2mg/kg) and PAR (10 mg/kg) on hyper-emotionality scores of OBX and sham rats. Results are expressed as mean hyper-emotionality scores. Error bars represent mean S.E.M. *P <0.05 vs sham control, # P < 0.05 vs OBX control. n =6 /group. PAR=Paroxetine.

5.15. Evaluation of "6p" in Rodent Models of Depression

5.15.1. Effect of "6p" on SLA of mice

Compound **"6p"** (1, 2 and 4 mg/kg, i.p.) did not show any significant [F (5, 42)=8.685, P>0.05] effect on base line locomotions as compared to control. (Fig. 98).

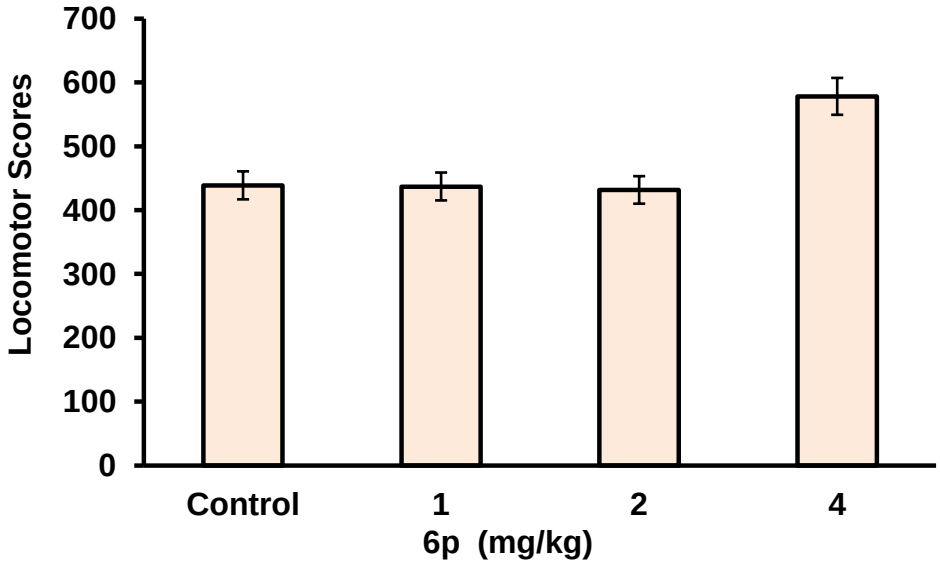


Fig. 98. The columns represent mean locomotor scores and error bars indicate S.E.M. n = 8 per group. *P < 0.05 compared with vehicle treated group.

5.15.2. Effect of "6p" on duration of immobility in mice using FST

In FST, the acute treatment with **"6p"** (1, 2 and 4 mg/kg, i.p.) and ESC (10 mg/kg, i.p.) showed marked [F (4, 35)=5.326, P<0.05] decrease in the duration of immobility as compared to vehicle treatment (Fig. 99).

5.15.3. Effect of "6p" on duration of immobility in mice using TST

In TST, acute treatment with **"6p"** (1, 2 and 4 mg/kg, i.p.) and BUP (20 mg/kg, i.p.) predominantly [F (4, 35)=10.86, P<0.05] decreased the duration of immobility as compared to vehicle treatment (Fig. 100).

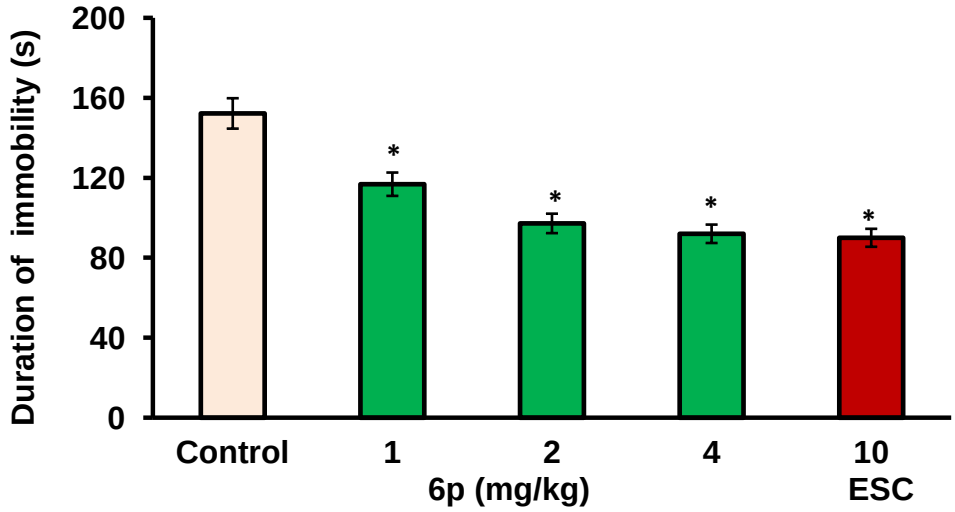


Fig. 99. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle treated group. ESC = Escitalopram.

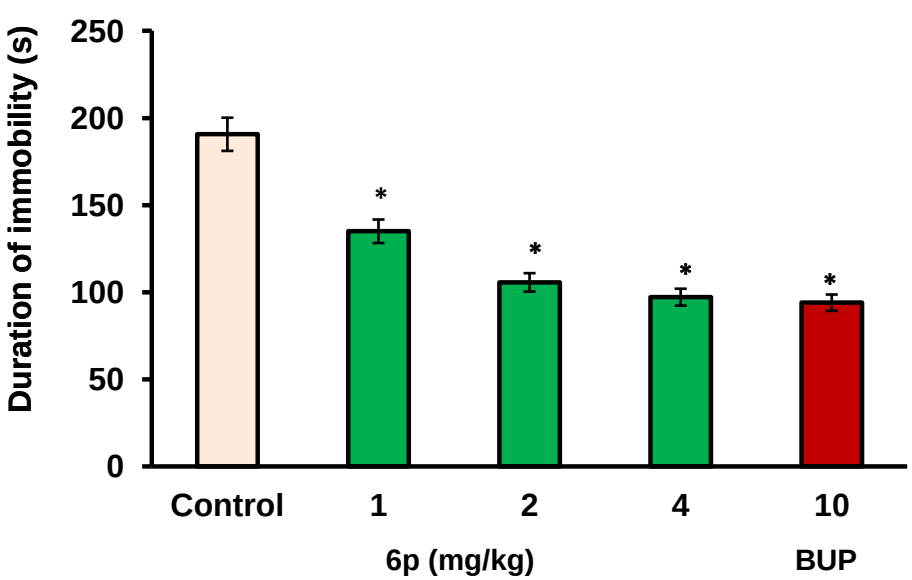


Fig. 100. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle treated group. BUP = Bupropion.

5.15.4. Effect of "6p" on RIH in mice

Reserpine (1 mg/kg i.p) elicited a pronounced decrease in core body temperature of rats. This effect was significantly [F (3, 28)=14.61, P<0.05] reversed by **"6p"** (2 mg/kg, i.p.) and ESC (10 mg/kg, i.p.) treatments (Fig. 101). **"6p"** (1 mg/kg, i.p.) was not able to produce any significant change in decreased temperature.

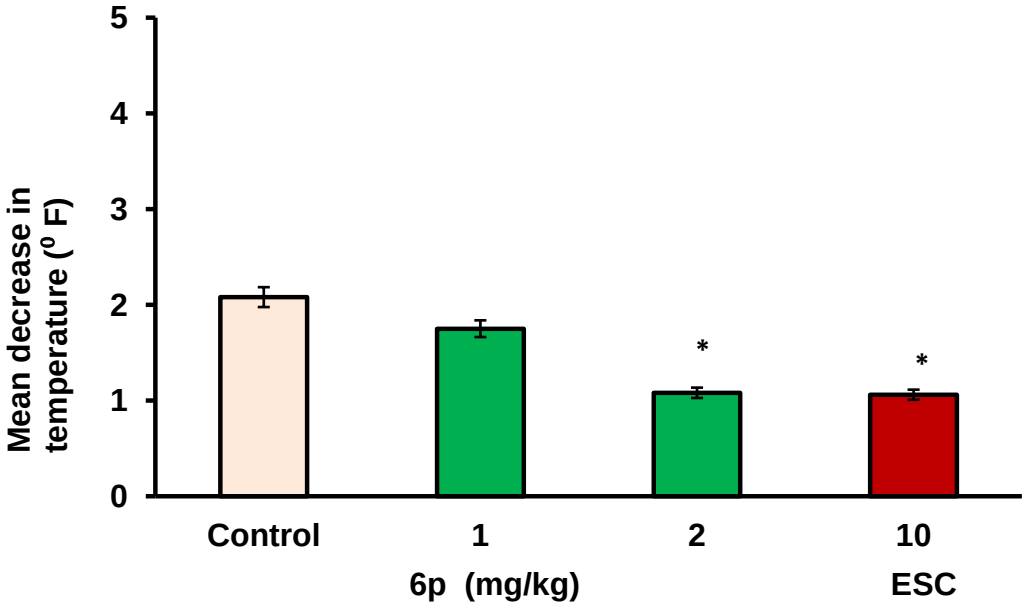


Fig. 101. The columns represent mean decrease in temperature °F and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle treated group. ESC= Escitalopram

5.15.5. Effect of "6p" on 5-HTP-HTR in mice

"6p" (2 mg/kg, i.p.) and FLX (20 mg/kg, i.p.) predominantly [F (3, 28)=25.78, P<0.05] potentiated the 5-HTP/PRG induced head twitches in mice (Fig. 102). **"6p"** (1 mg/kg, i.p.) was not able to produce any remarkable changes in effect.

5.15.6. Interaction Studies of "6p" with standard drugs

For a conclusive evaluation of anti-depressant potential of 5-HT_3 receptor antagonists, interaction studies with standard anti-depressants were carried out. **"6p"** pre-treatment (1mg /kg, i.p.) was found to enhance anti-depressant-like effects of FLX (10 and 20 mg/kg, i.p.) [F (1, 42)=48.28, P<0.05], DMI (10 and 20 mg/kg, i.p.) [F (1, 42)=46.47, P<0.05], VLA (4 and 8 mg/kg, i.p.) [F (1, 42)=24.69, P<0.05] as shown in Fig. 103-105, respectively. Moreover, **"6p"** markedly [F (1, 42)=74.68, P<0.05] reversed the depressant-like effect of PTL (1 mg/kg, i.p.) as shown (Fig. 106). Further **"6p"** (1 mg/kg) predominantly [F (1, 42)=43.37, P<0.05] enhanced the anti-depressant activity of BUP (10 and 20 mg/kg) in mice TST (Fig. 107).

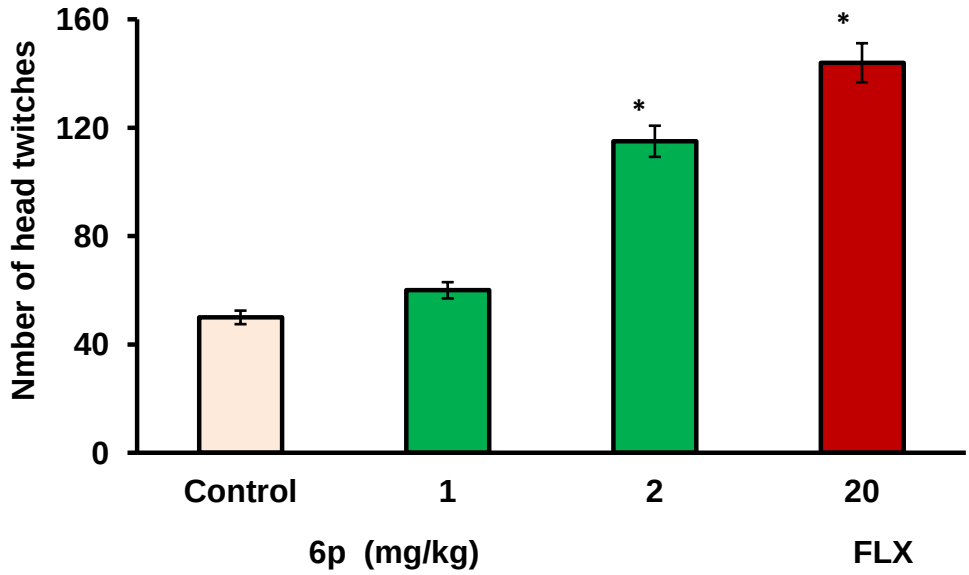


Fig. 102. The columns represent mean number of head twitches and error bars indicate S.E.M. n = 8 /group. *P < 0.05 compared with vehicle-treated group. FLX= Fluoxetine

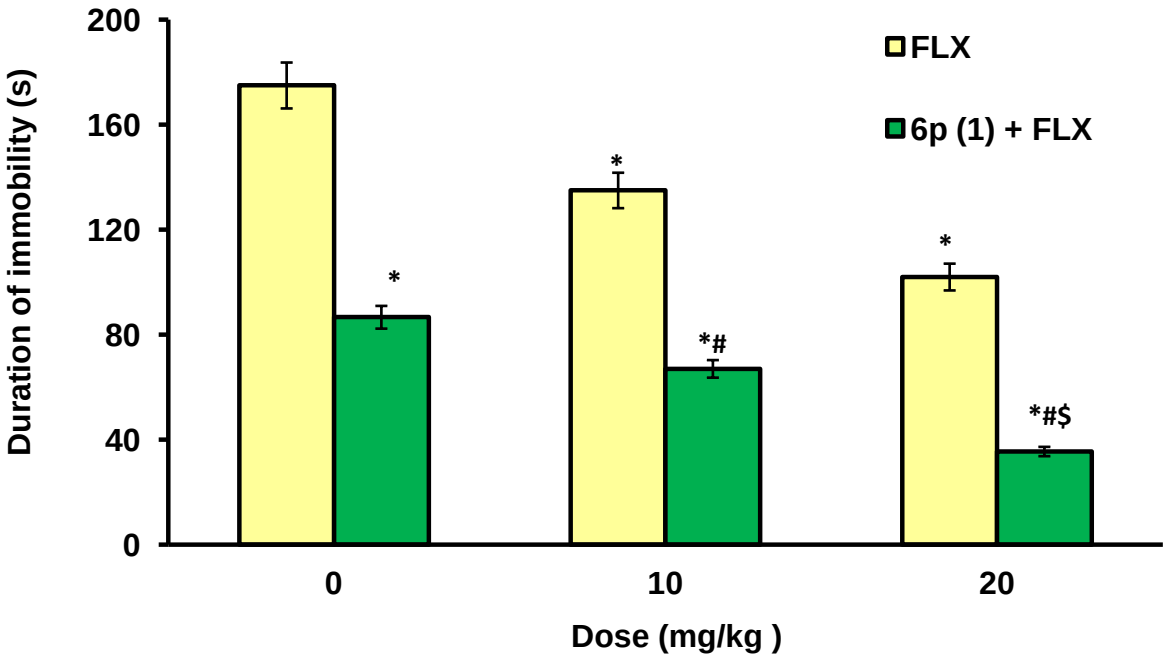


Fig. 103. The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n = 8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with FLX (10 and 20 mg/kg) treated group) and $P < 0.05 compared with alone **"6p"** treated group. FLX=Fluoxetine

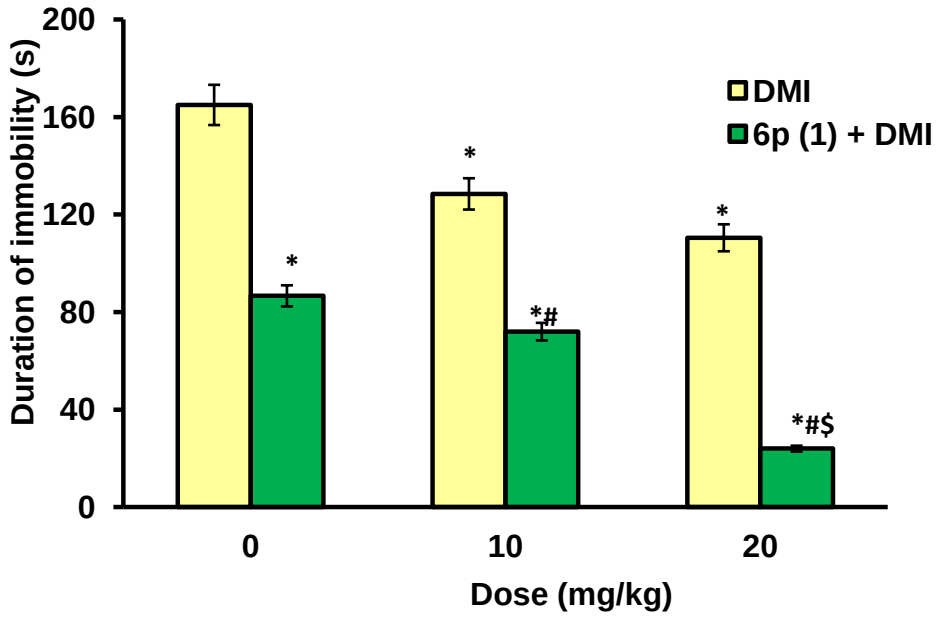


Fig. 104. The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n = 8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with DMI (10 and 20 mg/kg) treated group alone and $P < 0.05 compared with alone **"6p"** treated group. DMI=Desipramine

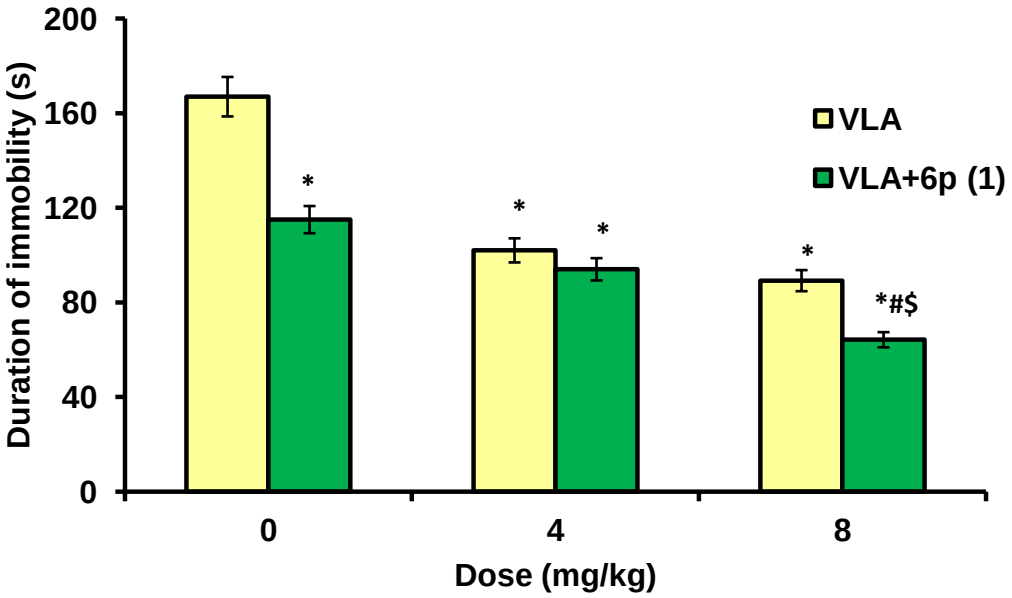


Fig. 105. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n = 8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with VLA (4 and 8 mg/kg) treated group and $P < 0.05 compared with alone **"6p"** treated group. VLA=Venlafaxine

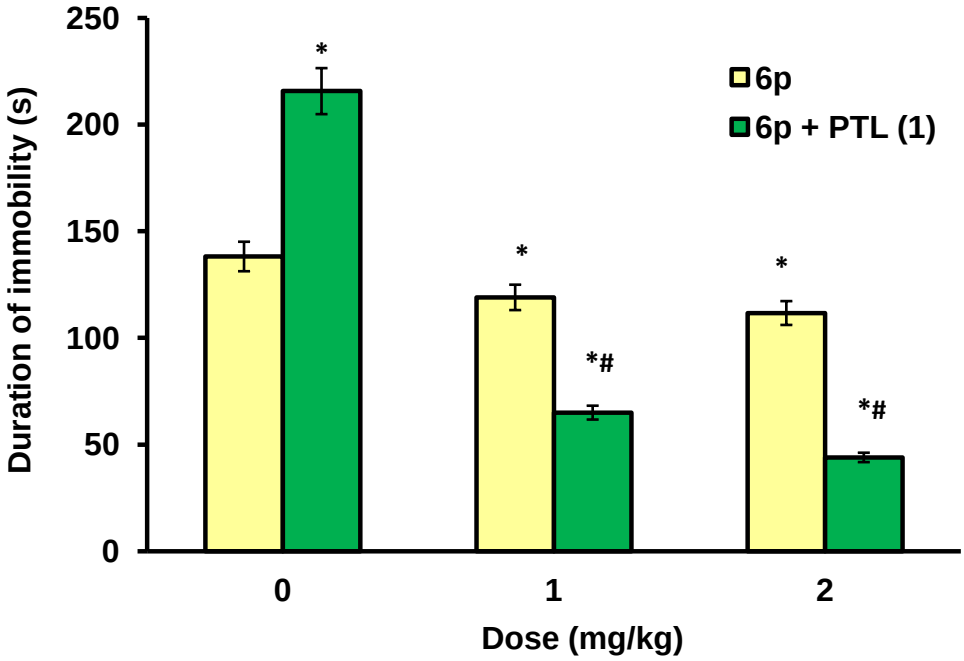


Fig. 106. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 8/group. *P < 0.05 vs. vehicle treated group and # P < 0.05 compared with PTL (1 mg/kg) treated group. PTL=Parthenolide

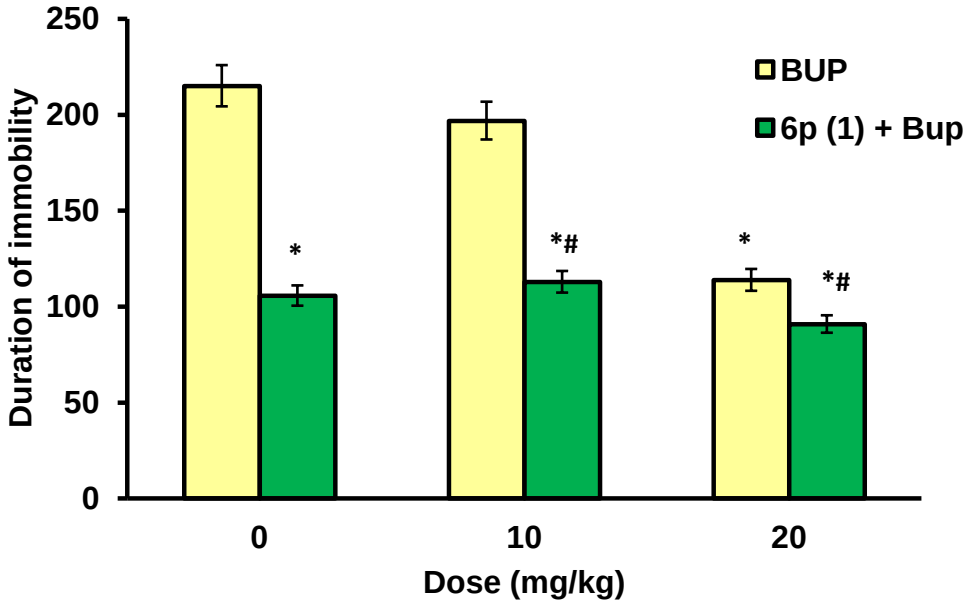


Fig. 107. The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n = 8/group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with BUP (10 and 20 mg/kg) treated group and $P < 0.05 compared with alone **"6p"** treated group. BUP=Bupropion.

5.16. Evaluation of "6p" in Animal Models of Anxiety

5.16.1. Effect of "6p" on behaviour of mice in EPM test

Acute treatment with **"6p"** (1 and 2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) showed pronounced increase in the percentage of both OAE [F (3, 28)=10.24, P<0.05] and TSOA [F (3, 28)=6.283, P<0.05] as compared to vehicle control group (Table 61).

Table 61: Effect of "6p" on behaviour of mice in EPM test

Treatment (mg/kg)	% TSOA	% OAE
Vehicle Control	2.00 ± 0.68	11.17 ± 1.97
Diazepam (2)	11.17 ± 1.47*	37.70 ± 4.73*
"6p" (1)	13.39 ± 3.39*	25.55 ± 3.50*
"6p" (2)	11.22 ± 1.50*	22.17 ± 2.85*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated; n = 8/group. DZM=Diazepam.

5.16.2. Effect of "6p" on behaviour of mice in L/D test

"6p" (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment significantly increased the number of entries [F (3, 28)=10.83, P<0.05] from one compartment to other as well as increased [F (3, 28)=23.79, P<0.05] the total time spent in lit area (Table 62). Lower dose of **"6p"** (1 mg/kg, i.p.) did not produce marked change in any of the parameters (Table 62).

Table 62: Effect of "6p" on behaviour of mice in L/D test

Treatment (mg/kg)	Time spent in Lit area (s)	No. of transitions
Vehicle Control	49.67 ± 4.90	7.20 ± 0.62
DZM (2)	101.83 ± 5.08*	15.43 ± 1.33*
"6p" (1)	65.17 ± 4.30	8.67 ± 1.23
"6p" (2)	76.67 ± 3.60*	13.17 ± 1.33*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.16.3. Effect of "6p" on behaviour of mice in HB test

The results of the HB test are shown in Table 63. Compound **"6p"** (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment, predominantly increase in the number of head dips [F (3, 28)=13.87, P<0.05] and number of square crossed [F (3, 28)=30.81, P<0.05], while decreased the head dipping latency [F (3, 28)=7.089, P<0.05]. Lower dose of **"6p"** (1mg/kg, i.p.) was not able to produce any significant effect on the number of head dips number of square crossed and latency time as compared to vehicle control.

Table 63: Effect of "6p" on behaviour of mice in HB test

Treatment (mg/kg)	No. of head dips	No. of square crossed	Latency time (s)
Vehicle Control	8.50 ± 1.06	2.83 ± 0.87	9.67 ± 0.67
DZM (2)	26.50 ± 4.03*	18.50 ± 2.01*	2.33 ± 2.01*
"6p" (1)	9.50 ± 1.05	3.01 ± 0.80	7.50 ± 0.92
"6p" (2)	17.50 ± 1.31*	12.50 ± 1.48*	4.50 ± 0.76*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; ; n = 8/group. DZM=Diazepam.

5.16.4. Effect of "6p" on behaviour of mice in OFT

"6p" (2 mg/kg, i.p.) and DZM (2 mg/kg, i.p.) treatment significantly increased the number of square crossed [F (3, 28)=16.28, P<0.05] and rearing scores ([F (3, 28)=25.47, P<0.05] as compared to vehicle treatment group (Table 64). While **"6p"** (1 mg/kg, i.p.) was not able to produce any pronounced effect on ambulation and rearing scores as compare to vehicle control group.

Table 64: Effect of "6p" on behaviour of mice in OFT

Treatment (mg/kg)	Ambulation scores	Rearing
Vehicle Control	149.67 ± 6.08	9.86 ± 0.64
DZM (2)	215.23 ± 8.23*	3.45 ± 0.49*
"6p" (1)	155.29 ± 8.26	7.95 ± 0.52
"6p" (2)	189.76 ± 7.73*	4.57 ± 0.68*

Values represent mean ± S.E.M. *P < 0.05 when compared with vehicle-treated group; n = 8/group. DZM=Diazepam.

5.17. Co-morbid Evaluation in OBX Rats Using Behavioural Test Battery

5.17.1. Effect of OBX on body weight of rats

Body weight of sham and OBX rats was continuously observed till the behavioural tests were started. Decreased body weight were observed in OBX rats for few days post surgery. Statistical analysis revealed that weight gained in OBX rats was significanlty (P<0.05) less as compared to sham rats (Table 65).

Table 65: Effect of OBX on change in body weight of rats.

	Dose (mg/kg)	Initial weight	Final weight
Sham control	0	255.50 ± 2.50	294.00 ± 8.00
Sham + **"6p"**	1	265.50 ± 2.50	295.00 ± 9.00
Sham + **"6p"**	2	251.00 ± 5.00	286.05 ± 4.50
Sham+ PAR	10	251.50 ± 3.50	280.5 ± 10.5
OBX control	**0**	**256.60 ± 11.55**	**261.0 ± 9.50***
OBX + **"6p"**	1	255.00 ± 5.30	282.0 ± 3.50
OBX + **"6p"**	2	254.00 ± 4.25	270.33 ± 4.50
OBX + PAR	10	258.00 ± 8.43	280.67 ± 7.76

Each value represents mean ± S.E.M. *P < 0.05 represent the mean change in body weight. **"6p"**/PAR vehicle (mg/kg) were administered p.o. once a day for 14 days. *P<0.05 vs sham control. n= 6 /group. PAR=Paroxetine.

5.17.2. Open field test (OFT)

OFT was the first behavioural study to be performed in OBX rats, post 14 days of treatment (Table 66). The effects of **"6p"** on the behaviour of OBX/sham rats were analyzed in different circumstances as shown in (Table 66). Chronic (14 days, p.o) treatment with **"6p"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) predominantly reduced the number of ambulation [F (7, 40)=16.23, P<0.05], rearing [F (7, 40)=49.71, P<0.05] number of fecal pellets in OBX rats [F (7, 40)=25.90, P<0.05] as compared to the vehicle treated OBX rats. **"6p"** (1 mg/kg) also showed marked reduction in the number of rearing and fecal pellets. While **"6p"** (1 mg/kg) did not able to produce any significant effect on ambulation score as compared to OBX control group.

5.17.3. Sucrose consumption test

Sucrose (1%) consumption test was performed to measure anhedonia (loss of pleasure) in rodents. Sucrose consumption was tested in sham and OBX rats. Significant decrease in sucrose consumption (P<0.05) was observed in OBX rats as compared to sham rats. Chronic **"6p"** (2 mg/kg, p.o.) and PAR (10 mg/kg) treatment showed pronounced increase in [F (7, 40)=4.515, P<0.05] sucrose consumption in OBX treated rats as compared to vehicle treated OBX rats (Table 67). Chronic treatment with **"6p"** (1 mg/kg, p.o.) was not able to produce any noticeable effect on sucrose consumption as compared to vehicle treated OBX rats

Table 66: Effect of "6p" on open field behaviour in sham and OBX rats

Treatment (mg/kg)	No. of Ambulation	No. of Rearing	No. of Fecal Pellets
Sham Control	91.17 ± 6.88	9.99 ± 1.24	2.47 ± 0.47
Sham+**"6p"** (1)	101.00 ± 8.87	9.33 ± 1.05	1.99 ± 0.71
Sham+**"6p"** (2)	105.17 ± 7.34	8.33 ± 1.15	2.05 ± 0.86
Sham+PAR (10)	96.48 ± 9.37	8.67 ± 0.71	2.00 ± 0.58
OBX Control	225.00 ± 8.28*	30.67 ± 4.58*	4.33 ± 0.76*
OBX+**"6p"** (1)	205.00 ± 6.42	15.00 ± 1.31$^{\#}$	3.80 ± 0.62$^{\#}$
OBX+**"6p"** (2)	136.00 ± 8.95$^{\#}$	11.40 ± 1.29$^{\#}$	3.67 ± 0.68$^{\#}$
OBX+PAR (10)	112.00 ± 7.34$^{\#}$	12.83 ± 0.59$^{\#}$	1.99 ± 0.49$^{\#}$

The columns represent rearing score and error bars indicate S.E.M.,* P < 0.05 when compared to the sham operated rats, $^{\#}$P < 0.05 when compared to the vehicle-treated OBX rats, n = 6 /group. PAR=Paroxetine.

Table 67: Effect of "6p" on sucrose consumption in sham and OBX rat

Treatment (mg/kg)	% Sucrose Consumption
Sham control	51.75 ± 4.25
Sham + **"6p"** (1)	52.25 ± 5.65
Sham + **"6p"** (2)	50.45 ± 6.03
Sham + PAR (10)	56.25 ± 6.24
OBX Control	27.50 ± 2.25*
OBX+ **"6p"** (1)	34.50 ± 3.65
OBX+ **"6p"** (2)	49.25 ± 5.25$^{\#}$
OBX+ PAR (10)	53.20 ± 5.82$^{\#}$

Results are expressed as mean ± S.E.M. *P<0.05 vs sham control , $^{\#}$P<0.05 vs OBX control. n = 6 /group. PAR= Paroxetine

5.17.4. Elevated plus maze (EPM)

EPM was employed for the anxiolytic test in the laboratory set-up. Percentage OAE and TSOA were measured in EPM test. OBX rats exhibited increased open arms entries [P<0.05] and time spent in open arm [P<0.05] of the maze in comparison with sham-operated rats (opposite to that observed in anxiety). Chronic **"6p"** (2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.) treatment predominantly decreased both percentage OAE [F (7, 40)=15.19, P<0.05] and TSOA [F (7, 40)=31.57, P<0.05] as compared to vehicle treated OBX rats (Table 68). Chronic **"6p"** (1 mg/kg, p.o.) treatment did not reverse OBX behaviour significantly, (P<0.05) in EPM as compared to vehicle treated OBX rats.

Table 68: Effect of "6g" on % OAE and % TSOA in OBX rats

Treatment (mg/kg)	% OAE	% TSOA
Sham control	32.25 ± 3.49	19.00 ± 2.49
Sham + **"6p"** (1)	38.01 ± 4.62	23.52 ± 3.21
Sham + **"6p"** (2)	36.42 ± 3.42	19.55 ± 3.62
Sham + PAR (10)	29.25 ± 2.58	18.08 ± 2.02
OBX Control	85.50 ± 9.52*	82.24 ± 8.84*
OBX+ **"6p"** (1)	72.53 ± 8.32	78.00 ± 6.05
OBX+ **"6p"** (2)	63.56 ± 5.74$^{\#}$	59.25 ± 5.25$^{\#}$
OBX+ PAR (10)	50.65 ± 5.40$^{\#}$	55.16 ± 5.24$^{\#}$

The column bar represent mean percentage of both OAE and TSOA. Results were expressed in mean ± S.E.M. *P<0.05 vs sham control , $^{\#}$P<0.05 vs OBX control. n = 6 /group.

5.17.5. Hyper-emotionality test

Fig. 108. displays mean scores for hyper-emotionality in sham and OBX rats. OBX rats showed significantly increase in hyper-emotional behaviour such as startle, struggle, and fight response, as compared to sham rats. Hyper-emotional behaviour exhibited by OBX rats was markedly [F (7, 40)=55.02, P<0.05] reversed by chronic treatment with **"6p"** (1 and 2 mg/kg, p.o.) and PAR (10 mg/kg, p.o.). While **"6p"** (1 mg/kg) was not able to produced any predominant effect on hyper-emotionality score as compared to OBX control rats.

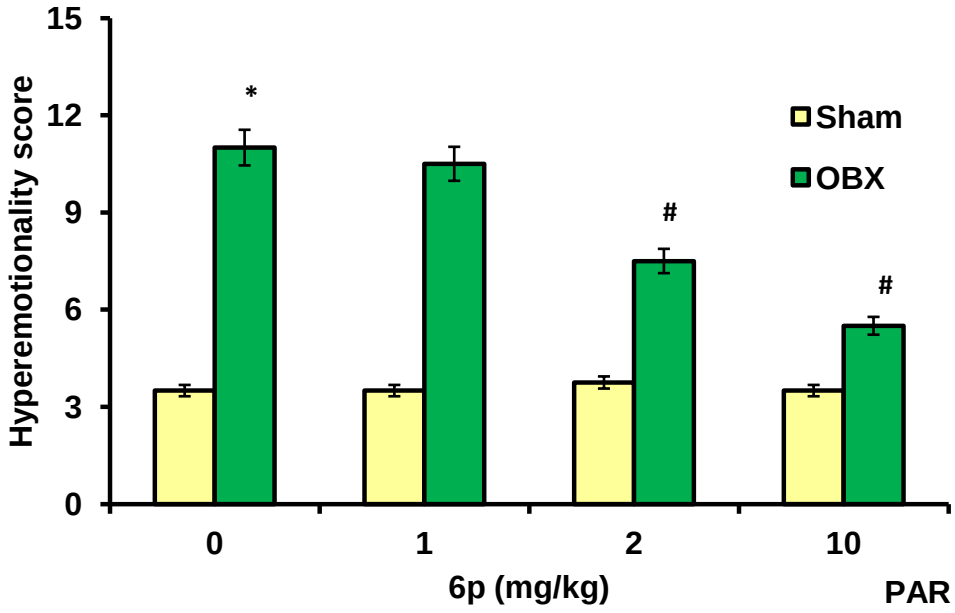


Fig. 108. Effect of **"6p"** (1 and 2mg/kg) and on hyperemotionality scores of OBX and sham rats. All drugs/vehicle were administered once a day p.o. for 14 days. Results are expressed as mean hyper-emotionality scores. Error bars represent mean S.E.M. *P <0.05 vs sham control, # P < 0.05 vs OBX control. n =6 /group. PAR=Paroxetine.

6. Discussion

The present neuro-psychopharmacological investigation, in various validated models of depression and co-morbid anxiety revealed the anti-depressant/anxiolytic-like effects of compounds **"6g", "6o", "6n"** and **"6p"** (novel quinoxaline derivatives; 5-HT_3 receptor antagonists). Acute and chronic treatment with NCE's exhibited anti-depressant-like effect in FST and TST at selected doses and also showed anxiolytic-like effect in EPM, L/D, HB and OFT models. The test substances reversed OBX and TBI induced behavioural deficits as indicated in the OFT, EPM, sucrose consumption, marble burying (TBI) and hyperemotionality (OBX) paradigms. The 5-HT and NE levels in the brain of OBX and TBI rats were increased/normalized by the drug treatments. In addition, NCE's pre-treatment potentiated 5-HTP/PRG induced head twitches in mice and reversed RIH in rats. Interaction of NCE's with various standard anti-depressants showed potentiation of effect with standards, SSRI, SNRI, NDRI and tricyclic anti-depressants. The test compounds also reversed the CUMS-induced depressive and anxiety-like symptoms (behavioural and bio-chemical oxidative stress parameters including corticosterone). The test compound **"6g"** also showed beneficial effects in LPS-induced depression and anxiety-like symptoms by changing both behavioural and oxidative stress parameters. The compound **"6g"** also increased the 5-HT levels in brain of mice as compared to LPS treated control group.

6.1. Anti-depressant-like Effects of 5-HT_3 Receptor Antagonists (6g, 6n, 6o, 6p) in SLA, FST and TST.

Assessment of anti-depressant potential is interfered by possible hyper-locomotive property of a test substance leading to false positive results (Porsolt et al., 1977). The psychomotor stimulation/sedation may increase/decrease the locomotor status (global motor activity) of mice in behavioural assays (FST and TST), when interpreting the depressant or anti-depressant-like effect of a new chemical entity (NCE). To rule out non-specific motor effects (false positive/negative) of NCE's **(6g, 6n, 6o, 6p)**, **spontaneous** locomotor activity was measured. The anti-depressant-like effects of in the FST and TST are may not be due to hyper-locomotive effects as indicated by the SLA. Single dose

treatment with NCE's at tested dose levels (1 and 2 mg/kg, i.p.) did not influence the SLA. The condition of immobility observed in TST is somewhat dissimilar from that seen in FST (Steru et al. 1985; O'Neill and Moore, 2003). The anti-depressant-like activity of a compound is determined by a decrease in the duration of immobility during FST and TST. Both of these models of depression are widely used to screen NCE's is for their anti-depressant potential (Bhatt et al., 2013a). These tests are sensitive and comparatively specific to all standard anti-depressants. The dose dependent effect was observed to be in consensus with earlier reports on 5-HT_3 antagonists tested in models of depression (Devadoss et al., 2010). Probable hypothesis for anti-depressant and anxiolytic-like effect of 5-HT_3 receptor antagonists (Martin et al. 1992, Rajkumar and Mahesh, 2010) in many other behavioural parameters are discussed later in this section.

Interaction studies with SSRIs, is essential for a conclusive evaluation of anti-depressant potential of 5-HT_3 receptor antagonists (Cryan et al. 2005). In one of the studies, ondansetron pre-treatment (0.01 µg/kg single dose) was found to enhance anti-depressant-like effects of FLX (Redrobe and Bourin, 1997). Interaction studies with ligands/conventional anti-depressants in FST and TST were carried out not only to predict the probable mechanism (possible receptor targets) of anti-depressant-like effects of NCE's, but also to pharmacologically validate the NCE's induced anti-depressant like behaviour in the above mentioned predictive tests. All the tested compounds at tested dose levels significantly enhanced the anti-depressant-like action of FLX showed involvement of serotonergic system for its action. All the NCE's also potentiated the effect of DMI by decreasing the duration of immobility, indicating the influence of nor-adrenergic system. In addition, VLA was observed to influence the serotonergic system and nor-adrenergic system (Redrobe et al. 1998). Therefore, it was inferred that the AD effects of NCE's in mice FST may not involve the NE neuro-transmitter system, but possibly mediated by modulation of 5-HT signaling as indicated by improved swimming pattern and augmentation of AD effects of FLX and VLA. Further to identify the involvement of serotonergic system, interaction study with PTL was performed. The test

compounds reversed the depressant-like effect of PTL by decreasing duration of immobility in FST. As PTL is a 5-HT release inhibitor, this effect further clearly describes the involvement of serotonergic neurotransmission as it inhibits the release of 5-HT from pre-synaptic neuron (Pandey et al., 2008).

To identify the role of dopaminergic system, following the DA hypothesis of depression (Randrup et al. 1975), an interaction study of NCE's was carried out with BUP. It has been reported to have anti-depressant effects (Yamada et al. 2004; Wilkes 2006). Due to species specific dependance, BUP was not able show anti-depressant-like effects in FST with Swiss albino mice (Bourin et al. 2005). Hence, interaction study in TST was performed to give idea on involvement of dopaminergic system. DA present in mesolimbic and mesocortical is involved in emotional behaviour (Simon and Le Moal 1984). ICS 205-930, a selective 5-HT_3 receptor antagonists antagonized the stress-induced activation of DA release, suggesting its importance in the prevention of stress-induced disorders. In the present study, NCE's pre-treatment at all doses enhance the effect of BUP by reduction of duration of immobility in TST indicating the role of dopaminergic system.

Reduction of biological amines (NE, 5-HT, DA) in the CNS was observed to induce catalepsy, ptosis and the most recorded parameter, hypothermia. The reduction in the body temperature induced by reserpine was proved to be antagonized by anti-depressants (Englert et al., 1973), MAO-inhibitors and central stimulants, which is a simple and reliable method to assess the anti-depressant activity of test substance (Bourin et al., 1983; Bourin 1990). In mechanistic models, reserpine used as a monoamine depleting agent, which acts by blocking the monoamine transport in synaptic vesicle. The depletion of brain biogenic amines affects the central nervous system characterized by hypothermia (Englert et al. 1973). The decrease in body temperature induced by reserpine was reported to be antagonized by anti-depressants (Bhatt et al., 2013a). All the NCE's at tested dose levels prevented the hypothermic effect of reserpine exhibiting anti-depressant effects in this sensitive model. As seen in earlier sections, one of the pharmacological mechanisms of anti-depressants is

the enhancement of synaptic concentrations of monoamines, 5-HT, in particular. 5-HTP is an immediate precursor of 5-HT, its administration was reported to amplify the 5-HT signaling inducing a characteristic feature known as HTR (head shaking movement) response in mice (Ortmann et al., 1981; Schreiber et al., 1995). Since 5-HTP is succeptible to enzymatic breakdown in blood, pre-treatment with MAO inhibitor like PRG is necessary (Nabeshima et al., 1991). In the present study, the combination of PRG (MAO-B inhibitor) and 5-HTP (5-HT precursor), was found to induced the typical HTR which was potentiated by FLX treatment. All the NCE's at tested dose levels increased the head twitch responses. These results strongly support that the anti-depressant effects of NCE's can be attributed to the increase in 5-HT concentrations in the synapse.

6.2. Anxiolytic-like Effects of 5-HT_3 Receptor Antagonists (6g, 6n, 6o, 6p) in EPM, L/D, HB and OF test.

All the above mentioned four NCE's (**6g, 6n, 6o, 6p**) were examined to assess depression associated with anxiety in animal models of anxiety, such as the EPM, L/D, HB and OFT (Bhatt et al., 2013b). The results of the present study verified the designed hypothesis that 5-HT_3 receptor antagonists plays an important role in pathogenesis of anxiety disorder via increasing the availability of 5-HT at other postsynaptic serotonergic receptors. Although, it is uncertain that any single animal model captures all of the components of the complex expression of anxiety, thus a battery of tests have been used to evaluate the potential anxiolytic effect of NCE's. The hypothetical mechanism of 5-HT_3 receptor antagonist has been shown in Fig. 117, later part of discussion.

EPM is considered as one of the well established model for unconditioned anxiety to detect anxiolytic/anxiogenic-like activity by investigating aspects of physiological and pharmacological behaviour. The EPM test is widely used to study the psychomotor function and emotional aspects in rodents. In this test increase in number of entries and time spent in open arms the most obvious index/ reliable indicator of decreased anxiety indicating the anxiolytic-like activity of a compound, while anxiogenic substances have the opposite effect

(Biala and Kurk, 2008; Yadav et al., 2008). All the tested novel compounds **(6g, 6n, 6o, 6p)** at selected doses produced anxiolytic-like effects in EPM test, as evidenced by increased percentages of both OAE and TSOA. In addition, diazepam used as reference anxiolytic also expressed the potential anxiolytic effects in EPM.

The L/D test is another universally accepted rodent model for evaluation of drugs produce anxiolytic-like effect by utilizing the animal's natural preference for dark spaces (Mi et al., 2005). Main basis of the L/D paradigm is natural aversion of mice towards intense light. Anxiolytic substances decrease the natural aversion to light and increase the time spent in light area and number of transition from one compartment to other based on the potential of compound (Bhatt et al., 2013b). All NCE's at selected doses significantly increased the time spent in lit compartment as well as number of transitions from one compartment to other. Some studies have reported that an anxiolytic drug(s) increased the transitions between the two compartments.

The anxiolytic-like effects of NCE's at selected doses were further confirmed using hole board test. Hole-board test has been popular as a model of anxiety and offers an easy method for determining the behavioural parameters of rodents to an unfamiliar environment (Takeda et al., 1998). The head dipping tendency of a mice in hole board is responsive to changes in emotional situation of the animal (Nolan et al., 1973). The results revealed that NCE's' treatment at selected doses significantly increased the number of head dips and a latency in head dipping reflected the anxiolytic activity of the test compound. This effect is in accordance with previous research studies in the area, which suggest that increase in the number of head dips and decreased latency of head dips reflect the anxiolytic like activity of a compound (Takeda et al., 1998; Bhatt et al., 2013b).

The OFT is also a popular model for the evaluation of anxiolytic/anxiogenic test substances. Normal aversion of a rodent to the brightly lit area produces anxiety and fear, which is characterized by alteration in the behavioural parameters of animal in open field. Previous reports suggest that anxiolytic

compound have a tendency to reduce the fearful behaviour of rodents in open field (Mechan et al., 2002).Treatment with novel compounds at selected doses increased the ambulation scores and deceased rearing in OFT indicating the anxiolytic effect of the test compounds. The overall results suggested the anxiolytic activity of NCE's at selected doses in animal models of anxiety. However, further studies are required in order to better evaluate the possible mechanisms, underlying the anxiolytic-like effects of all compounds (Bhatt et al, 2013b).

6.3. Evaluation of NCE's (6g, 6n, 6o, 6p) on Behaviourally Co-morbid Depression Associated with Anxiety in OBX

OBX is a well known chronic model of depression (Kelly et al., 1997). This study was conducted to investigate the possible co-morbid depression associated anxiety-like behaviour and the effect of NCE's in OBX rats through behavioural test battery of depression and anxiety. A set of depression and anxiety tests were performed in the OBX rats to simulate the symptoms of depression and anxiety. Depression and anxiety are common psychiatric illnesses (clinically mixed anxiety and depressive disorder) often associated with stressful events (Kessler, 1997; Shankman and Klein, 2003). The issue of prime importance in this report is the co-occurrence of anxiety and depression disorder. An impressive number of animal models to assess depression and anxiety, individually, are available today. However, the relationship between these models and the clinical syndromes of depression and anxiety are not always clear. A behavioural test is an important tool to simulate the symptoms of human co-morbid disorder. All four tested compounds **(6g, 6n, 6o, 6p)** were evaluated for anxiety associated depression post-OBX. All the compounds were tested individually in anxiety and depression tests in OBX rats. Anxiety and depression tests were selected and performed for co-morbidity in such a manner so that it can simulate the symptoms of co-morbid depression and anxiety as per DSM-IV/V.

6.3.1. Behaviour of OBX Rats in Depression Tests

OBX has been hypothesized as a model of co-morbid depression/anxiety and the effect of compounds on the behavioural tests in OBX and sham operated rats were studied. OBX rat has been authenticated as a model for screening of anti-depressant drugs over the past 20 years to uncover the neurobiological substrate of human MDD (Song and Leonard, 2005; Kelly et al, 1997). OBX induced lesion in rats exhibited depression and anxiety-like behavioural anomalies. These post-OBX behavioural anomalies were observed in six well documented rodent based assays on depression and anxiety. Slight reduction in the body weight was observed in the first week post OBX. However, a parallel increase in the body weight of sham and OBX rats were seen in subsequent weeks, post-OBX.

OFT is the most widely accepted indices of hyperactivity. The design of OFT area may be very important (Kelly et al.,1997). On placement in a unfamiliar 'open field' environment, OBX rats will exhibit behavioural hyper responsiveness that reflect the psychomotor retardation as one of the criteria of depression as according to DSM-IV and DSM-V. The loss of smell is also responsible for changed behavioural pattern. Open field test is the most widely employed test for emotional investigations in OBX rats as a validated model of major depressive disorder (Van Riezen and Leonard, 1990). In the present study, OBX rats exhibited increased ambulation, rearing and defecation as compared to sham-operated rats in open field arena in response to stressful environment. Hyperactivity exhibited by the OBX rats in OFT could be due to the neuronal damage to the pre-frontal cortex post OBX (Janscar and Leonard, 1983).

The "stressfulness" of the particular tasks may play a important role in the hyperactivity observed in OBX animals. Chronic treatment with NCE's significantly restores OBX induced changes in behavioural parameters, such as increased movement in unfamiliar environments (Janscar and Leonard, 1983; O'Connor and Leonard, 1988). Based on the results, NCE's were also evaluated for the co-morbid evaluation. Chronic treatment with NCE's

significantly reversed the hyperactivity exhibited by OBX rats in open field test. Effect of NCE's on OFT parameters has been shown in Fig. 109.

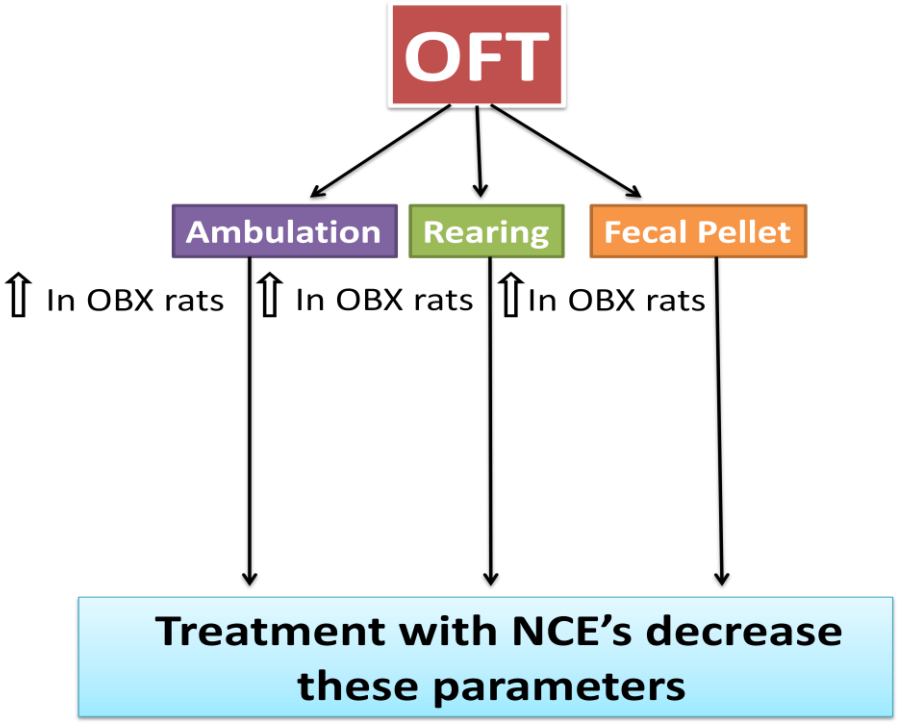


Fig. 109. Effect of NCE's on OFT parameters

It is well-known fact that anhedonia (in sucrose consumption test) is one of the core criteria for depression diagnosis (APA, 2000) that can be observed in OBX rats. The cellular mechanisms underlying anhedonia in OBX rats that induces such effects were not addressed in the present study, but these effects may be mediated by BDNF and such effects are observed to be reversed by chronic anti-depressant treatment (Muscat et al., 1992). In the current study, OBX rats consumed less sucrose solution (1%) than sham operated rats reflecting anhedonia (Rinwa et al., 2013). As olfaction is related to the emotion and memory, the deficits in the olfactory system leads to anhedonia. Chronic treatment with the compounds significantly reversed the Anhedonic behaviour in OBX rats as compared to vehicle treated OBX rats. OBX rats exhibited depression-like behaviour in OFT and sucrose consumption tests, respectively. Chronic treatment with NCE's significantly reversed the behavioural anomalies in aforementioned model(s) post OBX. Fig.110. has been shown the effect of OBX on neurons of limbic system.

In hyper-emotionality test, OBX rats exhibited significant increase in hyper-emotional responses to noxious stimuli reflecting psycho-motor retardation. Chronic treatment with NCE's at selected doses significantly decreased the hyper-emotional responses in OBX rats. Thus apart from having a strong theoretical rationale, the OBX was found to exhibit face and predictive validities. Though the exact mechanism of anti-depressant of 5-HT_3 antagonists in OBX rats is not clear, but it could be due to the release of monoaminergic neuro-transmitters by blocking the 5-HT_3 receptor (Devadoss et al., 2010).

6.3.2. Behaviour of OBX Rats in Anxiety Tests

Anxiety occurs commonly in patients with depressive disorder. OBX induced anxiety can be explained in two possible ways;

a) Bulbectomy causes behavioural disturbances, which are supposed to be caused by the destruction of GABAergic and serotonergic outputs (Janscar and Leonard, 1983) which projects from olfactory bulb to limbic system, mainly to hippocampus and amygdala (VanReizan and Leonard, 1990),

b) Behavioural disturbances can be observed in different situations including novel stressful environment, neophobic situation in elevated plus maze (Mcgrath and Norman,1999).

The EPM is the most widely used anxiety test (Hogg, 1996) across several laboratories. Predator-derived odours can be very effective stimuli for eliciting defensive behaviours in rodents (McGregor et al., 2004). It was hypothesized that the removal of olfactory bulbs can lessen or abolish anxiety in rats as well as decrease the defensive behaviour and may sometime make them aggressive and fearless (Marczynski and Urbancic, 1988).

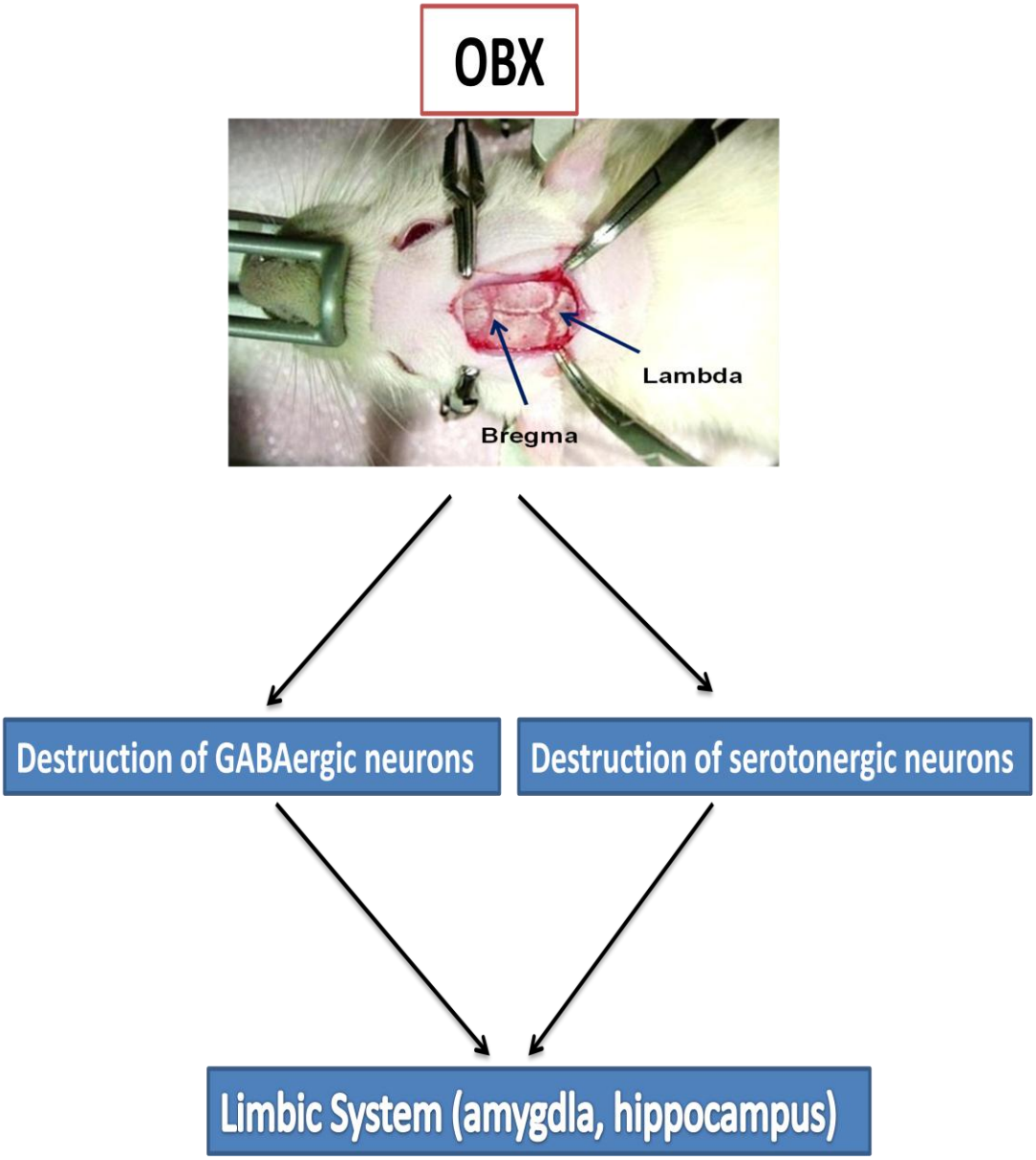


Fig. 110. Effect of OBX on GABAergic and serotonergic neurons

Increased time spent and entries in open arms were observed in OBX rat in EPM test. These results suggest the decreased defensive behaviour in OBX rat during exposure to neophobic situation in the EPM apparatus (Primeaux and Holmes, 1999; Song and Leonard, 2005; McGrath and Norman, 1999). In addition, current finding suggests that treatment with tested 5-HT_3 antagonists significantly reversed the OBX behaviour in EPM (Zhang et al., 2000). The location of olfactory bulb and olfactory tract in rats is shown in Fig. 111.

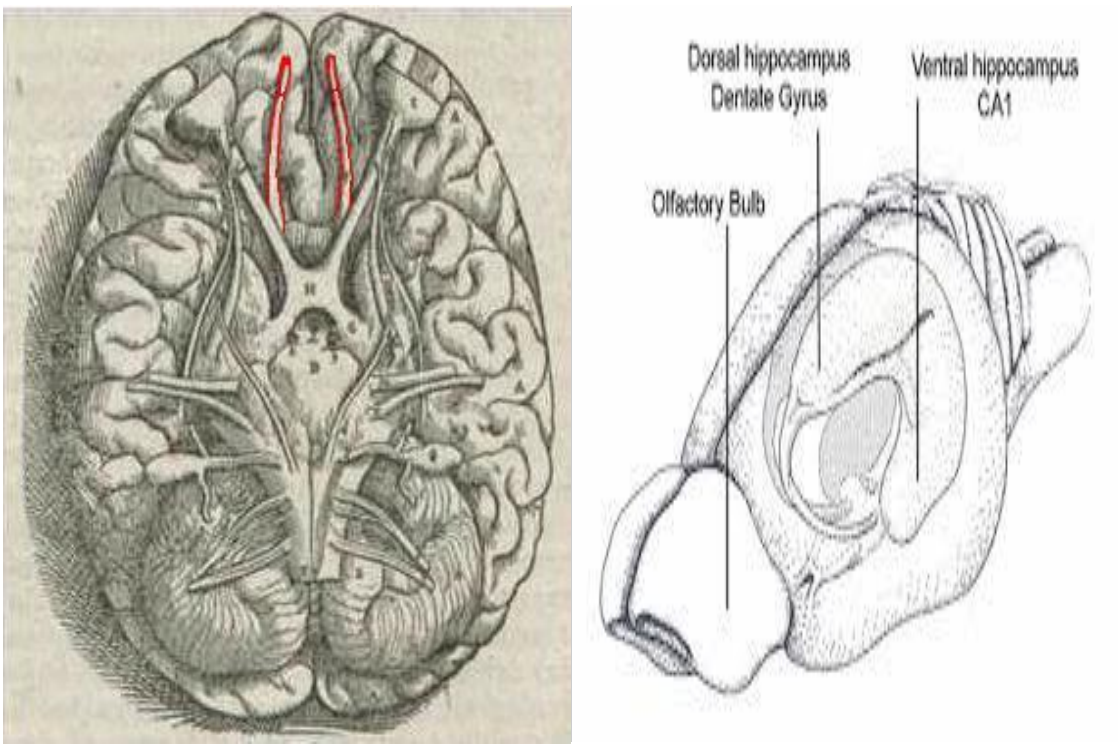


Fig. 111. Olfactory tract (outlined red) and Olfactory bulbs

Behavioural changes in OBX rats when subjected to stressful environment were evidenced with the changes in serotonin level. It is well known that a defect in serotonergic signaling pathways plays a important role in the pathophysisology of depressive and anxiety disorder (Janscar and Leonard 1984). The defect of serotonergic system in the amygdaloid cortex, frontal cortex and mid-brain (Redmond et al., 1997) involved in the regulation of both disorders (Deakin, 1996). Fig.112. has been shown schematic representation of co-morbid psychiatric disorder post olfactory bulbectomy.

The present results demonstrated that OBX result in decreased 5-HT, DA and NE levels in the rat brain. Moreover, the data suggested that chronic NCE's (**6g**, **6n**) treatment reversed OBX induced decrease in 5-HT and NE levels. The results were in accordance with our proposed hypothesis (Rajkumar and Mahesh, 2010) that binding of 5-HT_3 receptor antagonists to postsynaptic receptors influences the serotonergic transmission and also modifies serotonin mediated nor-epinephrine release. There was no significant effect dopaminergic levels was observed in OBX rats on the treatment with compound **"6g"** and **"6n"** as compared to OBX control rats. In addition the

chronic treatment with compounds (**6g, 6n**) shows a marked increase in BDNF levels, which indicates the possiblity of intracelluar signaling involvement in 5-HT_3 receptor mediated anti-depressant-like effect in OBX model.

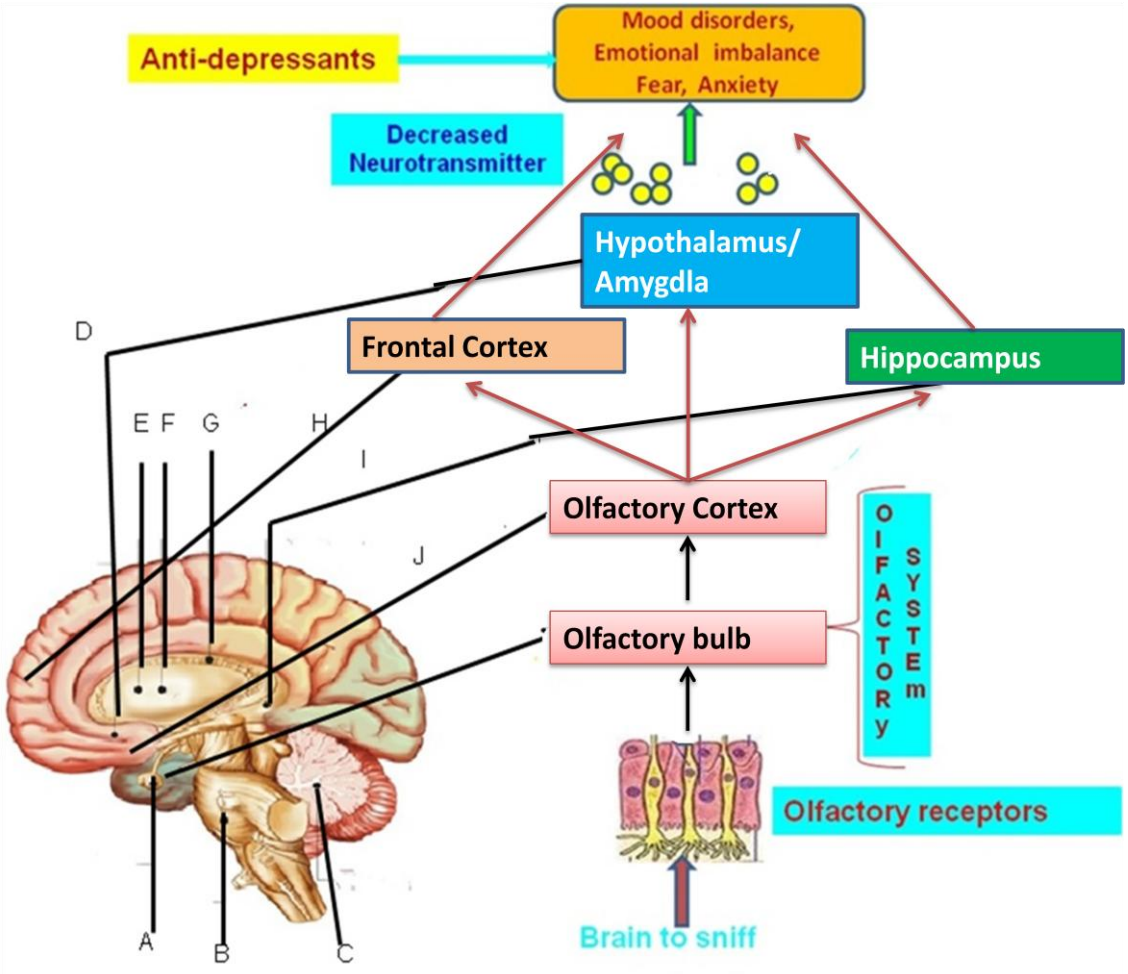


Fig.112. Proposed schematic representation of co-morbid psychiatric disorder post olfactory bulbectomy. Olfactory system is connected to neuroanatomic region. Removal of olfactory bulb leads to the dysregulation of neuronal circuit to anatomical region involved in modulation of depression and anxiety. Further dysregulation of neuronal circuit disturbed the level of monoamine neuro-transmitters. Abnormal neuronal circuit and decreased neuro-transmitter leads to the neurobehavioural disorders (Sugisaki et ., 1996). A-olfactory bulb; B-brain stem;C-cerebellum; D-amygdla; E-basal ganglia; F-thalamus; G-corpus callosum; H-Cortex, I-hippocampus; J-olfactory cortex

The various changes post-OBX would be observed as an idea for the rationale use of using OBX as a co-morbid model. The olfactory system in the rat is a part of the limbic region in which emotional and cognitive elements are governed by amygdala and hippocampus region (Kelly et al., 1997).

Olfactory bulbectomy disturbs the neuronal pathways that receiving projections from bulbs (Fig.112). Neuronal degeneration remodelling from olfactory bulb to brain areas such as cortex dorsal raphe and medial raphe are the regions involved in depression and anxiety. All the tested compounds significantly reversed the co-morbid depression and anxiety-like behaviour in OBX.

Argument emphasizing the loss of olfaction claimed that the removal of olfactory bulb produced a psychosocial stress since the sense of smell is the most important sensory modulator, by which rats obtain information from the environment, was lost following removal of olfactory bulbs. In addition to the behavioural changes, neuro-chemical alteration and imbalance in neuro-transmitter system occurs as consequence of bulbectomy. There is substantial evidence that abnormal neuro-transmitter system plays a role in the development of depression and anxiety.

6.4. Evaluation of NCE's (6g, 6n) in TBI Induced Co-morbid Depression and Anxiety in Rats

The majority of studies related to TBI in rodents were evaluated for deficits in learning and memory using fluid incursion method (Smith et al., 1991). However, TBI induces neurological impairment and patients with TBI may also indulge in abnormal goal-directed behaviours, which can further increase their emotional distress and cause social, occupational problems and therefore, the main aim of the present study was to determine general neuro-behavioural impairment and symptomatological correlation of depression and anxiety following TBI used in our laboratory setting. In the present study, a rodent's behavioural test battery was constructed to evaluate the anti-depressant and anxiolytic-like effects in the impact accelerated TBI model. Chronic administration of drugs is necessary in order to recover from the behaviour anomalies post TBI (Burt et al., 1995). In this study, weight drop TBI was standardized in the laboratory to assess the co-morbid depressive and anxiogenic-like symptoms in rats, although it is unclear which specific regions of brain are associated with behavioural fuctions. A number of research studies demonstrate that the synaptic plasticity in brain subparts such as hippocampus; amygdala and pre-frontal cortex are linked with mood disorders and anxiety

(Perera et al., 2007; Sairanen et al., 2007). Post induction of TBI, parameter assessment in behavioural tests shows the anxiogenic and depressive-like symptoms. Depression and anxiety assessment post-TBI were separately discussed in the study. The common characteristics of TBI induced neuronal degeneration is shown in Fig. 113. TBI accelerates degeneration of neurons mainly via increase in oxidative stress of brain, increase inflammatory mediators load and decrease in neuro-transmitter release in brain (Das et al., 2012; Skvronsky et al., 2006).

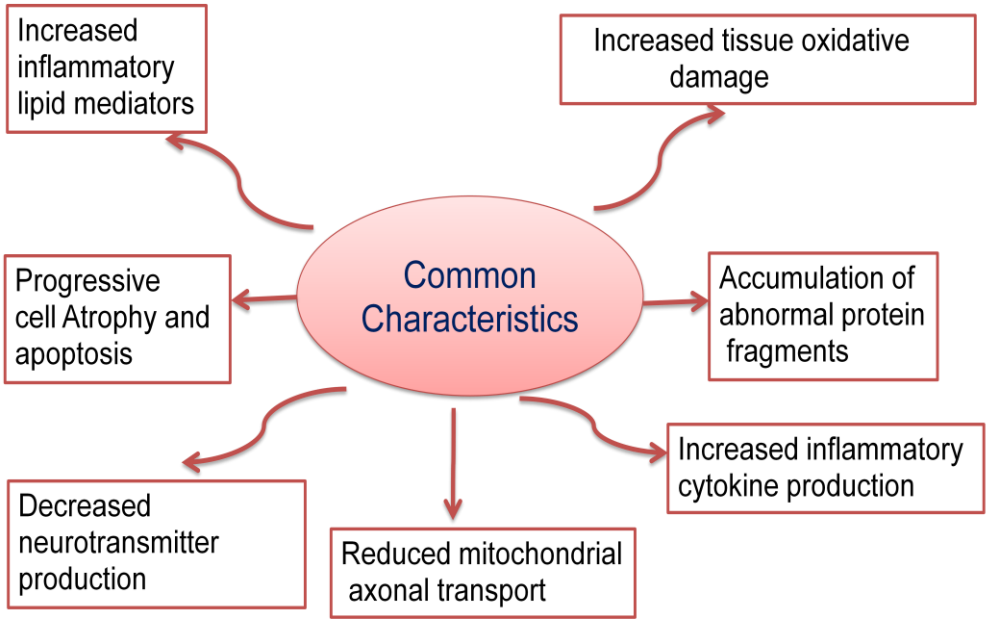


Fig. 113. Mechanism of neurodegeneration in TBI

6.4.1. Behavioural Deficits in TBI Rats Resembling the Symptoms of Depression

Depression is common, post-TBI and is observed to adversely influence the uptake of rehabilitation, psychosocial adjustment and return to work (Jorge and Robinson, 2002). Depression can develop years after injury and the early and late presentations can be different. Major depression (lasting six months or more) is more common when there is a history of psychiatric illness, substance abuse or poor social functioning – factors that also tend to prolong a major depressive episode. The symptoms of depression include psychomotor agitation, emotional imbalance and loss of interest but these are non-specific and can also reflect underlying medical problems (Kersel et al., 2001). Post TBI, rats were subjected to behavioural tests (comprising alternative depression/anxiety tests) simulating the core symptoms of human co-morbid

depression associated anxiety. Temporary suppression of feed intake and reduction in body weight was seen in the first week of injury. TBI rats gained lesser weight as compared to the sham rats.

TBI rats were first evaluated in OFT, a behavioural procedure most popularly used to find out exploratory hyperactivity (Ramamoorthy et al., 2008; Kulkarni, 1977). Elevated locomotor activity was observed during the first exposure to an unfamiliar arena, which is strongly related with other stress-induced behaviours. In stressful experimental environment, TBI rats exhibited hyperactivity, resembling the agitated symptom(s) of depressive patients, a psychopathological state that change a person's mind to commit suicide (Grahame et al., 2007). Post-TBI, suicide risk is higher than in the normal population due to feeling of hopelessness, worthlessness, guilt etc. The hyperactivity exhibited by TBI rats could be due to the changes in neuro-transmitter system and injury to the amygdala, hypothalamus, hippocampus, cerebellum, and temporal and prefrontal regions of the cerebral cortex, neuro-anatomical regions involved in aggression (Berkowitz, 1993). In the present study, increased frequencies of ambulation, rearing and fecal pellets (reflecting hyperactivity) were observed in TBI rats on exposure to aversive condition and the behavioural deficits as observed in open field arena was significantly reduced by the chronic treatment with compounds **"6g"** and **"6n"**. Treatment had no significant effect on the sham group. TBI induced hyperactivity in these tests confirmed one of the behavioural symptoms given by DSM-IV and DSM-V.

It is well-known fact that anhedonia (in sucrose consumption test) is one of the core criteria for depression diagnosis (APA, 2000) that can be observed in TBI rats. In the current study TBI rats consumed less sucrose solution (1%) than sham operated rats reflecting anhedonia (Jones et al., 2008). Chronic treatment with the compounds significantly reversed the anhedonic behaviour in TBI rats as compared to vehicle treated TBI rats. TBI rats exhibited depression-like behaviour in OFT and sucrose consumption tests, respectively. Chronic treatment with NCE's significantly reversed the behavioural anomalies in aforementioned model(s) post TBI.

6.4.2. Behaviour of TBI Rats in Anxiety

Data from various studies indicates that anxiety disorders are more prevalent after TBI surgery (Koponen et al., 2002). There is a higher degree of co-morbidity exists between mood and anxiety disorders among patients with TBI (Jorge et al., 2004).

EPM test exhibits the usual conflict between the force to find out a new condition and the inclination to avoid potentially hazardous area (Hogg, 1996). TBI rats exhibited increased entry and TSOA (phase aversion), opposite to that in normal anxiety test (Yamada et al., 2000; Wang, et al., 2007). These results from the EPM test suggested the decreased defensive behaviour in TBI rats during exposure to neophobic situation in the EPM apparatus, but not the anxiety-like behaviour (Yamada et al., 2000). In addition, the finding suggested that, the chronic treatment with tested compounds significantly reversed the TBI induced behavioural deficits in EPM test. The performance of the TBI animals on the EPM is quite controversial with increase in the time spent and the number of entries made into the open arms of EPM. This finding suggests that EPM alone may not be a reliable test for examining symptoms resembling anxiety behaviour in TBI animals. The current study suggests that EPM could be more valid model for defensive behaviour rather than anxiety in TBI rats.

Further, OCD in TBI rats was demonstrated in our laboratory as one of many anxiety-related sequel of brain injury, assessed (Michael and Brandon, 1988) using marble burying behaviour which reflects both compulsiveness and fear of novelty. In the present study, glass marbles provided the novel stimulus which TBI rats found aversive. Increased marble burying behaviour by TBI rats reflects fear of novelty and compulsiveness which indicates that the behaviour is more likely OCD (Broekkamp et al., 1986). TBI induced compulsive behaviour could be due to the damage of orbitofrontal cortex and frontal lobe. Disturbances in the neuro-transmitter system could be the possible reason for TBI induced OCD. The results are consistent with the previous finding that an SSRI showed positive response in marble burying behaviour (Ichimaru et al., 1995). In line with this observation, chronic administration of tested compounds

(**6g**, **6n**) significantly reversed the marble-burying behaviour in TBI rats suggesting the potential utility of this assay for the evaluation of anxiolytic agents.

Behavioural tests performed in the TBI rats significantly reflect the symptoms of co-morbid depression and anxiety. Behavioural anomalies post-TBI was correlated with change in neuro-transmitter level. Disruption of neuro-transmitter harmony was observed following TBI (Sziray et al., 2007). In the current study, TBI leads to a significant decrease in the concentration of 5-HT, NE and compared to sham control. The chronic treatment with NCE's increased the level of 5-HT and NE in treatment groups. The results were in accordance with earlier proposed hypothesis (Rajkumar and Mahesh, 2010) that binding of 5-HT_3 receptor antagonists to postsynaptic receptors influences the serotonergic transmission and also modifies serotonin mediated nor-epinephrine release. Moreover, there was no significant effect in dopamine levels were observed on treatment with compounds (**6g, 6n**). In addition the chronic treatment with compounds (**6g, 6n**) shows a marked increase in BDNF levels, which indicates the possibility of intracellular signaling involvement in 5-HT_3 receptor mediated anti-depressant-like effect in TBI model.

This study suggested that, the TBI can be a useful model for depression-co-morbid anxiety, rather than serving only as a model of major depression and this hypothesis was strengthened with the potential role of NCE's reversing the symptoms of co-morbid depression with anxiety. As mentioned in DSM-IV (1994), the depressive disorders have been extended with a new diagnosis: co-morbid depression with anxiety (Levine et al., 2001).

However, the exact mechanism behind the behavioural abnormalities post TBI is not clear. It could be due to the imbalance in the neuro-transmitter system and the neuro-transmitter turnover. Unfortunately, not enough studies have been performed to clarify the exact phenomenon. In TBI rats depression is related with distraction of neural circuits in prefrontal cortex, amygdala, hippocampus, basal ganglia, and thalamus area (Jorge and Robinson, 2002).

TBI leads to activation of glial cells followed by release of various inflammatory mediators and this leads to neuronal injury and death (Das et al., 2012). The probable mechanism and the relation between brain and systemic immunity after TBI has been shown in Fig. 114.

6.5. Anti-depressant and Anxiolytic-like Effects of 5-HT_3 Receptor Antagonists (NCEs: 6g, 6n) in CUMS Model

Chronic treatment with NCEs at tested dose levels reversed the CUMS-induced depressive-like behaviour in mice. Moreover, the tested compounds enhanced the beneficial effects against CUMS-induced alterations in oxidative stress-associated parameters in the mice brain.

The chronic administration of various uncontrollable stressors in a random manner is well-studied in rodent models for screening of anti-depressants (Katz et al., 1981; Willner et al., 1992). CUMS is considered as a most popular and valuable rodent model to study depression in rodents and to mimick several depressive-like symptoms in humans (Kumar et al., 2011).

The changes in spontaneous locomotor activity have been suggested to mimic anti-depressant/depressant-like effect of rodents in behavioural paradigms (Porsolt et al., 1977, Mahesh et al., 2012). However, there was no difference in spontaneous locomotor activity was observed in any of the experimental groups suggesting that changes in behaviour in vehicle treated and NCE's treated stressed mice does not have any influence on locomotor activity. The results were in accordance with the study performed in our lab (Jindal et al., 2013).

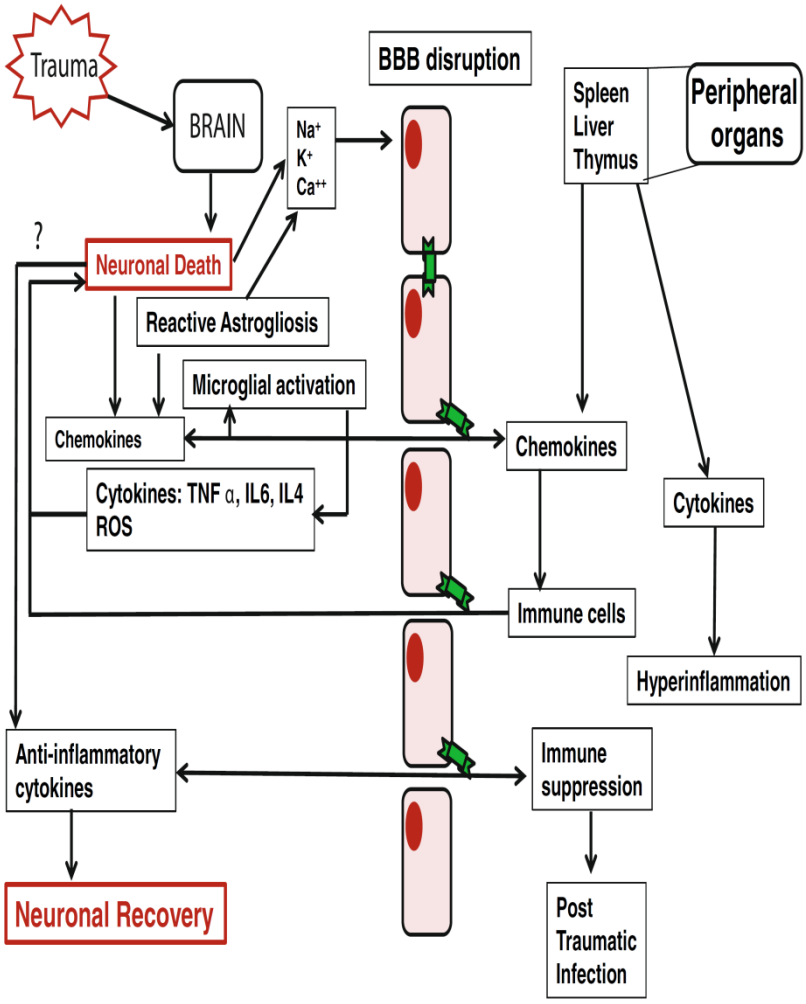


Fig. 114. Hypothetical mechanism and the relationship between brain and systemic immunity after TBI. (Das et al., 2012).

Sucrose preference test is considered as a important and justifiable behavioural test of CUMS in an animal paradigm. Anhedonia is a main characteristic of this test (Bekris et al., 2005; Kalueff et al., 2006; Strekalova et al,. 2006). Some previous studies suggested that CUMS is involved in death of neurons. According to previous reports, stressed mice showed a reduce preference and consumed less amount of sucrose solution as compared to unstressed mice. The results obtained are consistent with earlier findings that mice exposed to CUMS consumed less sucrose solution as compared to unstressed mice (Willner, 1997; Holsboer, 2000; Anisman, 2009). Chronic treatment with novel compounds significantly reversed this behavioural change, which represents the anti-depressant-like effect of 5-HT_3 receptor antagonists in CUMS model of depression. Considerable research have shown that anti-depressant treatment has an ability to reverse the chronic stress-induced reduction in sucrose consumption (McEwen and Olie, 2005; Kumar et al., 2011).

The FST, also known as the “behaviour despair” test is most frequently used to determine depression/anti-depressant-like behaviour in rodents after exposure to various stressors. The data of this investigation showed that mice subjected to chronic stress exhibited increase duration of immobility in FST. The data obtained were in agreement with previous reports that rodents exposed to chronic stress exhibited increase duration of immobility in FST (Zhou et al., 2007). Prolonged administration of the compounds **"6g"** and **"6n"** significantly decreased the duration of immobility in stressed mice, indicating the anti-depressant-like action. Moreover, both the compounds have shown their anti-depressant-like effect by decreasing the duration of immobility in mice FST (Bhatt et al., 2014; Mahesh et al., 2012) as discussed in earlier part of the thesis. Furthermore, numerous studies have shown that 5-HT_3 receptor antagonists decreased the duration of immobility in FST and could be used for anti-depressant-like effect (Devadoss et al., 2010).

TST is a well established screening paradigm, frequently used to determine depressant/anti-depressant-like behaviour in rodents after exposure to various

stressors (Steru et al., 1985; Kumar et al., 2011). The present data in TST is in agreement with previous findings that CUMS subjected mice showed an increased duration of immobility in TST as compared to normal control mice. It is well reported earlier that rodents exposed to chronic stress exhibit depressive-like behaviour, as evidenced by increased duration of immobility in behavioural despair test such as TST (Moretti et al., 2013). Chronic dosing with test compounds decreased the duration of immobility in stressed mice. Steru et al. (1985) reported that reduction in the immobility duration in TST is indicative of anti-depressant-like effect. In addition, chronic treatment with tested compounds significantly decreased duration of immobility in unstressed mice, which may be attributed to the anti-depressant potential of both drugs. At this juncture, it is worth mentioning that both the NCE's are effective in treating depression disorder (Bhatt et al., 2013a, Mahesh et al., 2012). In addition, fluoxetine which was used as reference drug also showed potential anti-depressant-like effect in unstressed and stressed mice in TST, which is consistent with previous reports (Zhang et al., 2002; Moretti et al., 2013).

Anxiety is also a disorder generally present with CUMS. Present study investigated anxiety or anxiolytic-like activity in one of the most validated rodent models namely, the EPM having predictive validity (Belzung and Griebel, 2001). The EPM test is considered as one of the well established model to detect anxiolytic/anxiety-like activity by investigating aspects of physiological and pharmacological behaviours (Hogg, 1996; Rex et al., 2004). Earlier studies have reported that most classical indices of anxiolytic-like behaviour in EPM test are the percentage increase in both open arm entries and open arm time (Andersen et al., 2000), while anxiogenic substances have the opposite effect. In the present study, stressed mice showed a significant decrease in percentage open arm entries. This result is in agreement with previous findings that stressed mice showed anxiety-like behaviour, as evidenced by decrease percentage open arm entries (Magarinos and McEwen, 1995). Chronic NCE's treatment increased the percentage open arm entries in stressed mice. Although, in unstressed mice significant increase in percentage of both open arm entries and time spent were observed, which may be responsible to the

anxiolytic potential of tested compounds. At this point, it is important to mention that the tested compounds are effective in treating depression disorder (Bhatt et al., 2013a, Mahesh et al., 2012). Fluoxetine used as a reference drug in the present study also showed potential anxiolytic-like effect in normal control and stressed mice.

It is reported that reactive oxygen species (ROS) also have a crucial role in the pathogenesis of neurological disorders including depression. Decreased antioxidant potential may not able to give protection against ROS causing damage to endogenous vital body molecules such as protein, fat, DNA etc. (Bilici et al., 2001; Khanzode et al., 2003; Eren et al., 2007). The increased oxidative stress leads to increase expression of NF-kβ which leads to increase levels of various pro-inflammatory cytokines and neuronal inflammation followed by neuronal death. In addition to neuronal inflammation oxidative stress also activated Caspase-3 mediated apoptotic neuronal death. The effect of increased oxidative load on neurons is shown in Fig. 115.

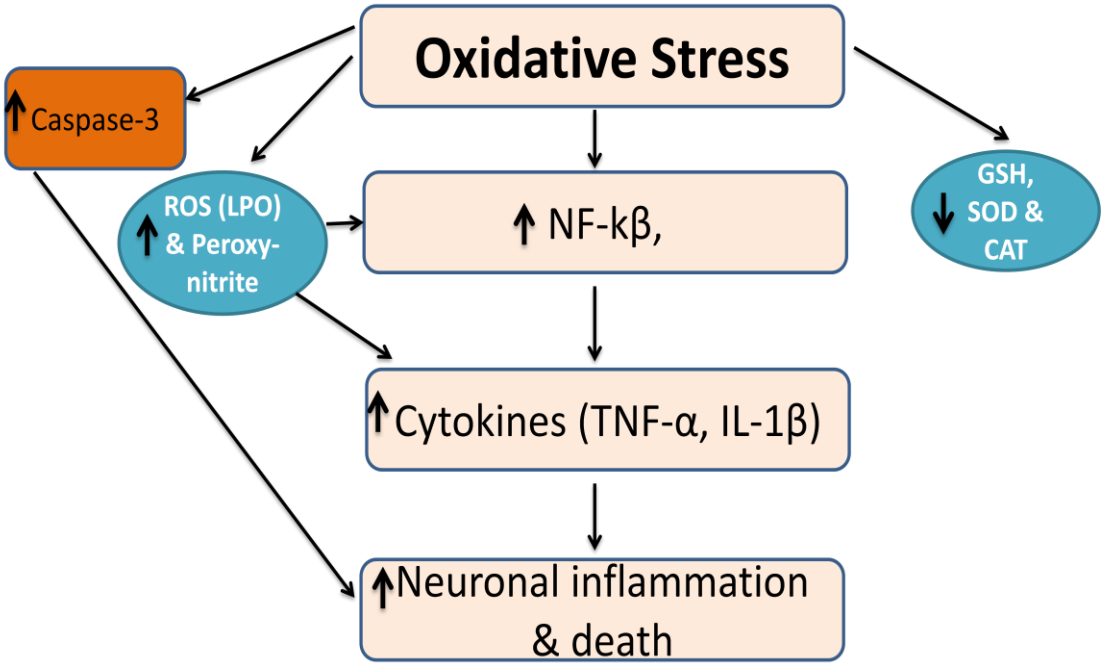


Fig. 115. Effect of increased oxidative stress on neurons

Coexistence of amplify oxidative stress with expression of depressive disorder as, evidenced by enhanced lipid peroxidation. In the present study, TBARS level that is proportional to lipid peroxidation and oxidative stress was

significantly increased in brain of stressed mice (Maes et al. 2000). Earlier studies have been shown that, malondialdehyde (MDA) a biotransformed output of lipid peroxidation was found in greater amount in biomatrix of depressive patients as compared to control subjects (Khanzode et al., 2003). Repeated treatment with NCE's (**6g**, **6n**) which inhibited CUMS-induced depressive-like behaviour also reinstated the CUMS-induced lipid peroxidation, suggesting a important link between both the events.

GSH, CAT and SOD enzyme levels/activity are important part of antioxidant defense mechanism. GSH levels are involved in the scavenging of free radicals. Some previous reports showed that lower levels of the antioxidant, GSH has important role in the pathophysiology of depression and lower activity may predispose towards an altered antioxidant defence (Kodydkova et al., 2009). The results showed, a predominant improvement in GSH activities in the brain of stressed mice, in response to chronic treatment with tested compounds (**6g**, **6n**). CAT enzyme catalyzes the reduction of hydrogen peroxide in to water and oxygen (Zhang et al., 2009). Previous studies report a decrease in CAT and SOD levels in the prefrontal cortex, the hippocampus and the striatum of stressed mice, indicating dysfunction in antioxidant defense mechanism in CUMS-induced depressive-like behaviour (Zhang et al., 2009). CAT mediates the signaling in abnormal cell growth, neuronal death, metabolism of sugars and platelet activation (Chelikani et al., 2004). Both the tested compound (**6g**, **6n**) significantly increased the CAT level in comparison to CUMS control mice.

SOD is another antioxidant enzyme that promotes dismutation of superoxide into oxygen and hydrogen peroxide. SOD is an important enzyme involved in depression, co-factored with copper and zinc. Mice deficient of SOD2 were exposed to great amount of oxidative stress and died soon just after birth (Li et al., 1995). The results showed, a significant increased in reduced CAT activities in the brain of stressed mice, in response to chronic treatment with tested compounds (**6g**, **6n**). The stressed mice also showed a nitrosative stress as evidenced by elevated brain nitrite level. NO is an important messenger entity that have a significant contribution in many pathophysiological processes such

as neurotransmission, immunomodulation, inflammation etc. A growing body of data have suggested that depressed patients show elevated nitrite levels (Suzuki et al., 2001). Compound **"6g"** at selected doses was not able to reduce elevated nitrite levels in brain of stressed mice. It is well known that there is increase in the level of oxidative stress markers is present during inflammation (Kumar et al., 2011). Compound **"6n"** at selected doses significantly reduced nitrosative stress by decreasing the elevated nitrite levels in brain of stressed mice.

6.6. Anti-depressant and Anxiolytic-like Effects of 5-HT_3 Receptor Antagonists (6g) in LPS Induced Depression and Anxiety Model.

LPS is a endotoxin obtained from bacteria, present in the cell wall of gram negative bacteria. Peripheral administration of LPS produce various inflammatory responses via release of inflammatory mediators like TNF-α, IL-1β, IL-1 α, IL-6 etc. This finally causes the sickness and depression- like symptoms (Yirmia et al., 1996, 2009).

The NCE (**6g**) might possibly causes the modification in serotonergic transmission as per the hypothesis described in earlier part of the thesis. Hence the compound **"6g"** was evaluated to change the various behavioural and biochemical parameters. The results of the present study state that chronic treatment with compound **"6g"** reversed the LPS-induced depressive-like behaviour in mice. Moreover, **"6g"** enhanced the beneficial effects against LPS-induced changes in oxidative stress-related parameters in the mice brain.

Peripheral administration of LPS causes decrease in locomotor activity and increase in immobility time (FST and TST). These results are in accordance with previous study (O'Connor et al., 2009). Chronic treatment with compound **"6g"** causes reduction in immobility time without affecting the base line locomotion.

Further, administration of LPS causes decrease in percentage of both OAE and TSOA in EPM, while treatment with **"6g"** increases both the parameters. In L/D

model, compound **"6g"** increases the time spent in lit area as well as no. of transitions from one compartment to other while it decreases the latency time. The results are in accordance with previous study (Sah et al., 2011).

Depression is accompanied by increased oxidative damage to fatty acid and hence lowered the level of omega-3 fatty acids (Maes et al., 2000). In the present study, TBARS level that is proportional to lipid peroxidation and oxidative stress was significantly increased in brain of LPS treated mice. Previous studies have been shown that, MDA a by-product of lipid peroxidation was found to be increased plasma of depressive subjects as compared to normal volunteers. (Khanzode et al., 2003). In the present study repeated treatment with **"6g"**, which inhibited LPS-induced depressive-like behaviour also restored the LPS-induced lipid peroxidation, suggesting a potential relationship between both events.

GSH, CAT and SOD enzymes are the important antioxidant enzymes. The present study shows a significant increase in GSH activities in the brain of LPS-control mice, in response to chronic **"6g"** treatment. There is increased activity of CAT observed in LPS-control mice as compared to treatment groups. This is in accordance with some study reports which demonstrated the activity of catalase enzyme in depressed individuals and reported increased catalase levels during short term depression as compared to healthy subjects (Galecki et al., 2009 a,b). Further some studies reported increased catalase activity as body responds against stressor (LPS) initially (Szuster-Ciesielska et al., 2008) .The present study found a significant decreased SOD activity in the brain of LPS control mice. Chronic treatment with **"6g"** at selected doses normalized the SOD activity in LPS-treated mice. The stressed mice also showed a nitrosative stress as evidenced by elevated brain nitrite level. NO is having a major role in inflammation. Previous research reported that LPS treated depressed patients showed elevated nitrite level (Nicholson et al., 2004). Chronic treatment with compound **"6g"** at selected doses was not able reduced the elevated nitrite levels in brain of stressed mice. The reason for this may be to reduce the nitrite levels a long term administration of compound **"6g"** will be

required. The proposed mechanism for compound **"6g"** is that it may influences the serotonergic transmission by inhibiting the increased level of inflammatory mediators responsible for degradation of tryptophan by highly activated indoleamine-2, 3-dioxigenase (IDO) enzyme (O'Connor et al., 2009). Degradation of tryptophan by IDO has been shown in Fig. 116.

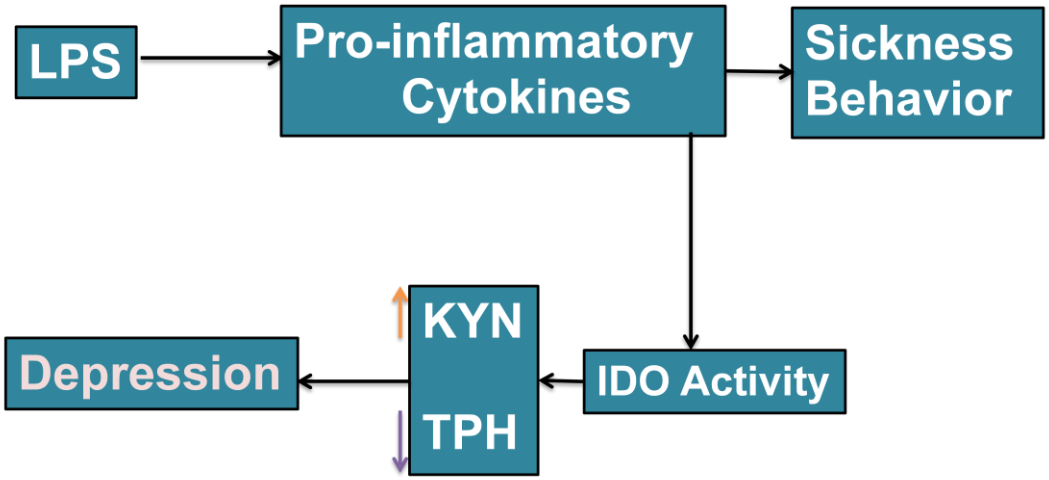


Fig. 116. Degradation of tryptophan in presence of IDO enzyme

6.7. Probable AD Mechanism of Action of 5-HT_3 Receptor Antagonists

Pre-synaptic 5-HT_3 receptor (auto-receptor) antagonism retards serotonin release (van Hooft et al., 2000); it purportedly enhances the availability of 5-HT in the synapse which can bind to 5-HT_{1A} pre-synaptic auto-receptor further inhibiting 5-HT release.

The situation may be viewed as (b) the antagonism at post-synaptic 5-HT_3 receptors. In serotonergic neurons post-synaptic 5-HT_3 antagonism can facilitate specific binding of 5-HT to other post-synaptic receptors viz. 5-HT_{1A}, 5-HT_{1B} (Bourin et al., 1998), 5-HT_{2A} and 5-HT_{2C} thereby aiding serotonergic transmission (Fig. 117). The increased availability of 5-HT to other post-synaptic receptors (such as 5-HT_{1A} receptor) can contribute to the AD effects. One of the proposed mechanisms of the novel AD, mirtazapine, that includes this indirect agonistic action at the post-synaptic 5-HT_{1A} receptors (Anttila and Leinonen, 2001).

In addition, 5-HT at post-synaptic receptors (5-$HT_{1A/B/1D}$, 5-$HT_{2A/2C}$) activates adenylyl cyclase and initiates the transformation of ATP to cAMP, that functions as a second messenger. cAMP further stimulates the phosphorylation enzyme protein kinase-A (PKA). Once PKA gets activated, phosphorylation of other intracellular protein molecules are initiated, thereby modifying the expression of CREB and BDNF in nucleus. This leads to anti-depressant-like effects by improving synaptic plasticity, neuronal survival and neurogenesis (Figure 117).

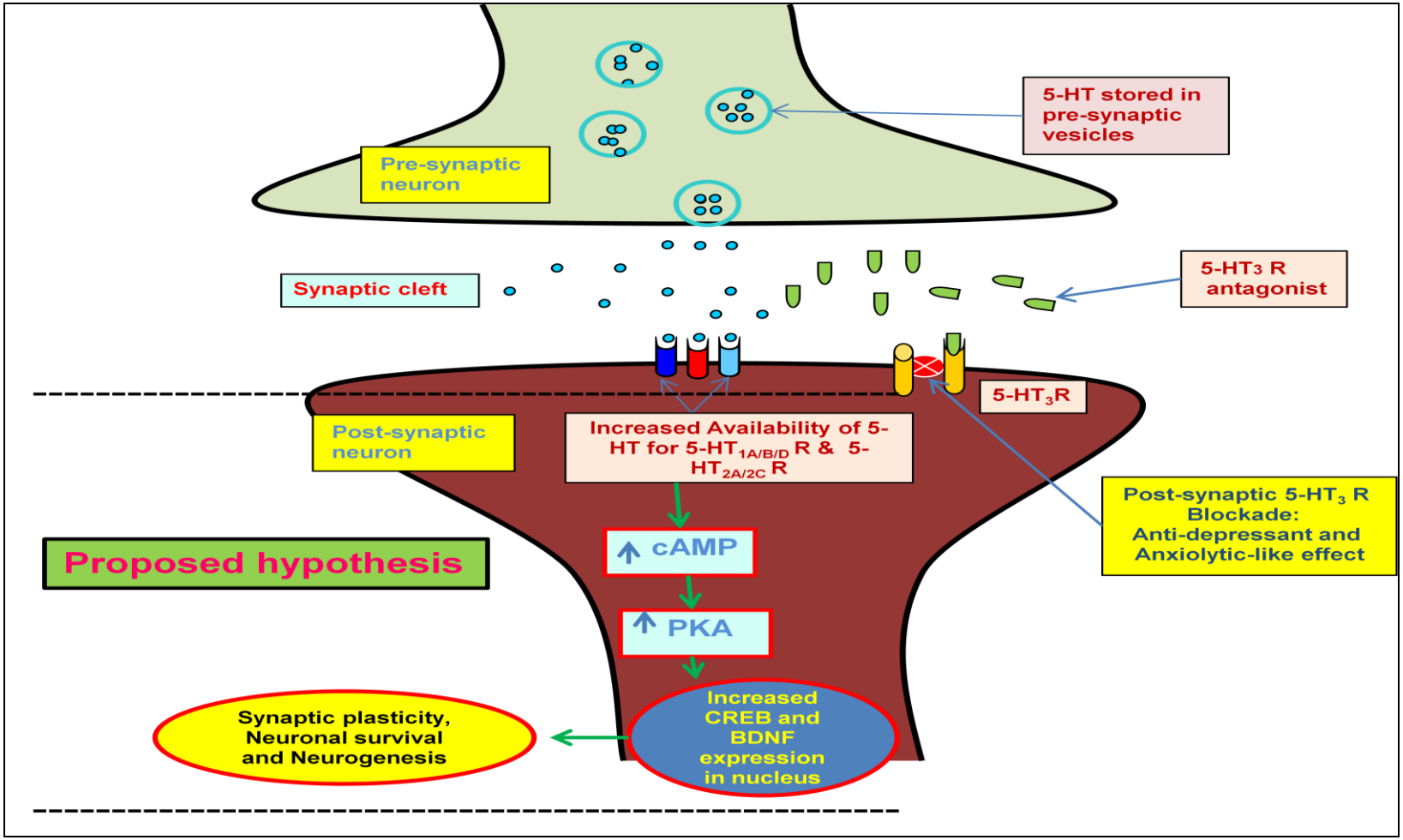


Fig.117. Modified schematic representation of the hypothesized mechanism behind the anti-depressant effect of selective $5HT_3$ receptor antagonists at the serotonergic synapse (Rajkumar and Mahesh, 2010)

The opposite effect is most likely to occur at higher doses, a condition in which the inhibition of 5-HT release due to both pre-synaptic 5-HT3 receptor blockade (ion channel type) and 5-HT1A receptor activation, eventually reduces the synaptic concentration of 5-HT. The positive influence of 5-HT3 receptor antagonism on the synaptic concentration of serotonin for a resultant AD-like effect can be explained convincingly by the above mechanism.

6.8. Clinical Evidence and Conclusions

Many clinical studies have investigated the efficacy of ondansetron in neuro-psychiatric conditions such as psychosis (Sirota et al., 2000), anxiety (Harmer et al., 2006), alcohol (Dawes et al., 2005a,b) and drug dependence (Johnson et al., 2007). The human trials with 5-HT3 receptor antagonists, have observed the influence of these drugs on the psychiatric manifestations (anxiety, depression, psychotic symptoms) co-morbid with other diseases/disorders such as cancer, hepatitis, bulimia, fibromyalgia, alcoholism, drug abuse, etc. Till date, no clinical trial with an ‘intention to treat’ design has been conducted to prove the AD efficacy. Treatment with 5-HT3 receptor antagonists were associated with an improvement in depression related symptoms in patients who suffered from other co-morbid conditions. Another reason that may be appended is that the antagonistic effects at pre-synaptic 5-HT3 receptor which is likely to cause depression-like effects (as explained in previous sections). The depressant effects at higher doses are questionable in humans since no clinical study has so far reported depression-like side-effects associated with 5-HT3 receptor antagonist treatment (Niesler et al., 2001; Yamada et al., 2006.

7. Summary and Conclusions

The 5-HT_3 receptor antagonists play a depression and anxiety mainly through modulation of serotonergic transmission. Targeting 5-HT_3 receptor will be of a notable interest for the development of newer anti-depressants. The beneficial effects of 5-HT_3 receptor antagonists (ondansetron, granisetron etc.) are well established in the treatment of cancer chemotherapy induced nausea and vomiting (with fewer or negligible side-effects profile) through several pre-clinical studies. According to the proposed hypothesis 5-HT_3 receptor antagonists are useful in depression and anxiety because they increase the availability of serotonin at post-synaptic 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A} and 5-HT_{2C} receptors. In fact, 5-HT_3 receptor antagonists have been shown to have anti-depressant- and anxiolytic-like effects in various studies.

The acute assays using mice such as spontaneous locomotor activity (for assessing locomotor status), FST, TST (AD dose response and interaction studies) and 5-HTP induced head twitch responses were used for preliminary anti-depressant studies. Besides these, the acute assays using mice such as EPM, L/D, HB and OFT were used for preliminary anxiolytic studies.

Interaction studies with existing ADs and research compounds such as FLX, VLA, DMI and PTL were carried out in FST and with bupropion in TST. The CUMS and LPS-induced depression model in mice were used as chronic models to assess the efficacy of 5-HT_3 receptor antagonists.

AD assays using rat as test system included in the present investigation were effects on chronic OBX and TBI model-induced behavioural anomalies in OFT, sucrose preference test, EPM exploration, hyper emotionality test and marble burying test and acute model reserpine induced hypothermia. Depressive disorder is linked with abnormalities of HPA axis, decrease neuro-transmitter (5HT, NE and DA) levels and altered oxidant/antioxidant system. There is also accumulative evidence BDNF mediated signaling is involved in the pathophysiology of major depression. Thus, in the present study we also explored the possible pathophysiological mechanism(s) and the effect of 5-HT_3

receptor antagonists. Moreover, 5-HT_3 receptor antagonists were also tested, to ascertain that whether there are direct effects of the hyperactive HPA axis, decrease neuro-transmitter (5HT, NE and DA) levels and alter oxidant/antioxidant system and the reduced levels of BDNF seen in depression responsible in neurodegeneration.

To our best knowledge, here we reported for the first time, beneficial effect of in housed synthesized compounds (**6g, 6n, 6o and 6p**) in the animal models of depression and anxiety. Moreover, pre-treatment with all NCE's, augmented the anti-depressant effects of FLX, VLA and DMI, indicating the potential efficacy of 5-HT_3 receptor antagonists. Further, all the tested compounds reversed the immobility effect induced by PTL in mice. All the compounds enhanced the effect of BUP in TST. In the study 5-HT_3 receptor antagonists (**6g, 6n, 6o and 6p**) were found to reverse in the behavioural anomalies induced by chronic experimental models (OBX). While 5-HT_3 receptor antagonists (**6g, 6n**) reversed the behavioural anomalies induced by chronic experimental models (TBI and CUMS). This observation has important clinical implications, suggesting that the common occurrence of depression disorders in humans following traumatic brain lesion may, at least in part, have a neurobiological basis. Additionally, it also signifies that the weight drop TBI and OBX in rats; CUMS and LPS injection in mice can be used as a model for depression and co-morbid anxiety disorder. Along with the TBI, OBX, CUMS and LPS injection models for depression and anxiety, this work reconstructs the various behavioural tests that possibly simulate the symptom(s) of depression in human. The LPS injection model has been first time standardized in our laboratory and can be used as a standard model to evaluate depression and co-morbid anxiety-like effect in mice. Beside these, the current work also stated the role of 5-HT_3 receptor antagonists in the intracellular transduction pathway for depression pathophysiology.

In the present work, the novel 5-HT_3 receptor antagonists were used for the reversal of depression behaviour following OBX, TBI, CUMS and LPS induced depression model. OBX, TBI, CUMS and LPS were observed to induce

behavioural deficits, possibly by affecting concentration of neuro-transmitter. The current study report the role of the tested compounds on HPA axis activity, oxidant/antioxidant system in CUMS (corticosterone estimation) and LPS induced depression.

The results from the current study give an idea that OBX rats are a model(s) for detecting anti-depressant activity. The major finding is that OBX rats show the same pattern of results that we would expect to see in patients with more severe depression symptoms. Treatment with compounds (**6g, 6n, 6o and 6p**) significantly reversed the OBX induced behavioural anomalies.

In the present study the weight drop TBI perfectly reflected the human form of TBI. Behavioural tests such as OFT, EPM, sucrose consumption performed and marble burying behaviour in TBI rats, successfully represents the behavioural symptom of and depression and co-morbid anxiety. The treatment with compound **"6g"** and **"6n"** also substantially decreased the TBI induced depression like behavioural anomalies in rats.

The CUMS subjected mice showed a significant alteration in the depression and anxiety like behaviour. The CUMS subjected mice showed symptom of anhedonia and increased duration of immobility in FST and TST that is more closely relevant to human depression symptom. Moreover CUMS subjected mice also decreased the percentage TSOA and OAE. Compounds **"6g"** and **"6n"** significantly reversed the behavioural deficits induced by CUMS.

The LPS injected mice showed a significant alteration in the depression and anxiety like behaviour. The single dose administration of LPS significantly increased the depression (FST, TST and sucrose consumption test) and anxiety (EPM & L/D model) symptoms. The chronic treatment with compound **"6g"** significantly decreased LPS injection induced depression- and anxiety-like behavioural anomalies in mice.

Besides this we also showed an alteration in the neuro-chemical and biochemical parameters that may be responsible for the development of the

Post OBX, TBI, CUMS and LPS injection induced behavioural alteration in rats and mice. These changes in neuro-chemical and biochemical parameters were reversed by the selected 5-HT_3 receptor anatgonists (compounds). In this study rats subjected to OBX and TBI showed a decrease in neurotrophic factor (BDNF) levels in rat brain which was reversed by treatment with compound (**6g, 6n**). In our studies rats subjected to OBX and TBI showed a decrease in neuro-transmitter (5-HT, NE) levels in the brain. The decrease in neuro-transmitter levels has been reversed by treatment with compounds (**6g, 6n**). Moreover in the present study results from CUMS (**6g, 6n**) and LPS (**6g**) induced co-morbid depression and anxiety models showed notable increase in oxidative stress markers. This effect was reversed by the tested compounds. CUMS subjected mice showed increase levels of plasma corticosterone. The increased plasma corticosterone levels were decreased by treatment with compounds (**6g, 6n**). Further decreased levels of serotonin neuro-transmitter was found in mice brain (in LPS induced depression only) post LPS treatment, which was reversed by treatment with compound **"6g"**.

Literature indicates that increase in oxidative stress marker mediated signaling plays an important role in the neurodegeneration associated with the development of psychiatric disorder like depression. In our study there was a correlation found in all these neuro-chemical and biochemical parameters. On the other hand, chronic treatment with tested compounds (in different models) significantly reversed the co-morbid behavioural deficits, biochemical and neuro-chemical alteration-induced by animal models.

Thus, molecules which selectively target intracellular signaling at receptor level by 5-HT_3 receptor antagonists are suitable as anti-depressants as well as useful in co-morbid anxiety disorder.

8.0. Salient Findings/Observations from the Work

8.1. Work Done

- Investigation of anti-depressant and anxiolytic potential of the novel 5-HT_3 receptor antagonists (**6g, 6n, 6o** and **6p**; quinoxaline derivatives)
- Standardization of animal model of lipopolysaccharide (LPS) administration in mice by using acute administration in our laboratory setup.
- Development of animal model(s) of depression like TBI, CUMS and OBX.
- Behavioural tests defined for the assessment of depressive symptoms.
- Experimentally, merging of various paradigms were conducted in a manner that emotional condition of a rodent becomes measurable via various non-exclusive tasks that could contribute to increased consistency, rapidity and completeness of behavoral measurement.
- Along with behavioural tests the possible involvement of the HPA axis activity, neuro-transmitter role, oxidant/antioxidant system activity and intracellular neurotrophic signaling transduction cascade were also assed and explored in the depression pathophysiology.
- The impact of each hypothesis (HPA axis, monoamine, oxidative stress and intracellular signaling transduction cascade hypothesis) on the regulation of the other hypothesis were assessed.

8.2. Observations

- 5-HT_3 receptor antagonists significantly reversed the behaviour deficits (depression and anxiety) induced by in TBI, OBX CUMS and LPS-injection model.
- 5-HT_3 receptor antagonists also normalized oxidant/antioxidant system, HPA axis activity and intracellular neurotrophic signalling mediators.

8.3. Findings

NCE's (**6g, 6n, 6o** and **6p)** showed anti-depressant and anxiolytic-like effects in various rodent models of depression and anxiety. All the compounds showed beneficial effects in co-morbid surgical models such as OBX (**6g, 6n, 6o** and **6p)** and TBI (**6g, 6n**). Selected compounds (**6g, 6n**) not only altered the defective behavioural pattern but also normalized the biochemical parameters

neurotrophic signaling (BDNF) and neurotransmitter levels (5-HT, NE) which proved their efficacy as potential anti-depressant and anxiolytic like compounds. Moreover, in the present study, tested compounds reversed the CUMS (**6g, 6n**) and LPS (**6g**) induced co-morbid depression and anxiety and also changed biochemical parameters related to the oxidative stress. The compounds (**6g, 6n**) normalized the increased plasma corticosterone levels in CUMS model. Further decreased levels of serotonin neurotransmitter was observed in mice brain (in LPS model), post LPS treatment, which was reversed by treatment with compound **"6g"**. The compound **"6g"** was also able to restore serotonergic levels in LPS-injection model which expressed its potential as a anti-depressant and anxiolytic compound.

8.4. Implications for Future Research

- Acute and Chronic toxicity study of the tested compound in rat and mouse can be conducted.
- Further studies are required to ascertain whether immunological, structural and physiological changes are involved in the pathology of affective disturbance following TBI, OBX, LPS and CUMS.
- Histological and immune-histochemistry of various brain regions are required to give more validity to these models.
- Further studies (such as combination treatment, chronic behavioural assessment and importance of other neuro-transmitter in co-morbidity, etc) are required to give more acceptance and validity to the animal models and to find out whether simultaneous treatment of depression and anxiety can able to possibly decrease somatic symptom burden, medical testing and polypharmacy.
- Focused studies on other psychiatric co-morbidity like depression associated insomnia and Parkinsonism, would be taken up.

8.5. Inclusion and Exclusion Criteria for Mice and Rats

A. Inclusion Criteria

- Age and weight matched rats (18-20 weeks old/ 250-275g) and mice (10-12 weeks old/ 22-30g) were included for the neuro-psychopharmacology screening.
- For the preliminary AD and anxiolytic screening rodents of either sex were used.
- For surgical models rats were preferred as it was easy to differentiate different parts of brain
- For surgical models rats were preferred as in rats survival rates were found to be better than mice
- In the present study, male rats were employed for chronic surgical models (OBX and TBI), whereas, male mice in non-surgical models (CUMS and LPS injection).

B. Exclusion Criteria

- Mice with abnormal exploratory behaviour in actophotometer test were excluded from the study.
- Female rats and mice were excluded from chronic study, because of low recovery rate post-surgery and hormonal alterations could influence the study parameters.
- Post-surgery all rats were examined for recovery and injured rats with abnormal behaviour patterns were excluded.
- Infected mice during the study protocol were excluded.

8.6. Inclusion and Exclusion Criteria for In-house Synthesized Compounds

A. Inclusion Criteria for In-house Synthesized Compounds

- All the in-house synthesized compounds satisfy the pharmacophore for 5-HT_3 receptor antagonists which consists of a heteroaromatic ring, carbonyl group and a basic nitrogen.

- The compounds (**6g, 6n, 6o** and **6p**) were selected on the basis of pA_2 value (>6.9, ondansetron)
- The compounds (**6g, 6n, 6o** and **6p**) were selected on the basis of lipophylicity (cLogP >2)
- The compounds (**6g, 6n, 6o** and **6p**) showed promising results in preliminary behavioural tests such as FST and TST

B. Exclusion Criteria for In-house Synthesized Compounds

- The compounds were excluded on the basis of pA_2 value (<6.9, ondansetron)
- The compounds were excluded on the basis of lipophylicity (cLogP <2)
- The compounds did not show promising results in preliminary behavioural tests such as FST and TST.

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Publications from thesis

1. Bhatt S, Mahesh R, Jindal A, Devadoss T. Protective effects of a novel 5-HT_3 receptor antagonist, *N*-n-butyl-3-methoxy quinoxaline-2-carboxamide (6o) against chronic unpredictable mild stress–induced behavioural changes and biochemical alterations. ***Pharmacology Biochemistry and Behaviour***, 2014;122, 234-239.

2. Bhatt S, Mahesh R, Jindal A, Devadoss T. Neuropharmacological Effect of Novel 5-HT_3 Receptor Antagonist, *N*-n-propyl-3-ethoxyquinoxaline-2-carboxamide (6n) on Chronic Unpredictable Mild Stress-Induced Molecular and Cellular Response: Behavioural and Biochemical Evidences. ***Pharmacological Reports***, 2014; 66, 804-810.

3. Bhatt S, Mahesh R, Jindal A, Devadoss T, Dhar AK. Neuropharmacological evaluation of a novel 5-HT3 receptor antagonist (6g) on chronic unpredictable mild stress-induced changes in behavioural and brain oxidative stress parameters in mice. ***Indian Journal of Pharmacology***, 2014; 46, 191-196.

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<u>Other Publications</u>

1. Jindal A, Mahesh R, Bhatt S. Etazolate rescues behavioural deficits in chronic unpredictable stress model: Modulation of hypothalamic-pituitary-adrenal axis activity and brain-derived neurotrophic factor level. ***Neurochemistry International***, 2013; 63, 465-475.

2. Mahesh R, Dhar AK, Jindal A, Bhatt S. Design, synthesis and evaluation of anti depressant activity of novel 2-methoxy 1, 8 naphthyridine 3-carboxamides as 5-HT3 receptor antagonists. ***Chemical Biology & Drug Design****,* 2014; 83, 583-91.

3. Jindal A, Mahesh R, Bhatt S. Etazolate, a Phosphodiesterase 4 Inhibitor Reverses Chronic Unpredictable Mild Stress-Induced Depression-Like Behaviour and Brain Oxidative Damage. ***Pharmacology Biochemistry Behaviour***, 2013; 105, 63–70.

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behaviour-based rodent models of depression. ***Indian Journal of Pharmacology***, 2012; 44, 560-565.

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PAPER PRESENTATIONS

1. Mahesh R, Bhatt S, Devadoss T, **Jindal A**. Neuropharmacological effect of (4-benzylpiperazin-1-yl) (3-methoxyquinoxalin-2-yl) methanone (qcm-15), a potential 5-ht3 receptor antagonist on chronic unpredictable mild stress-induced changes in behaviour and brain oxidative stress in mice. (**International Conference on Recent Advances in Molecular Mechanisms of Neurological Disorders, 21-23rd February 2013, AIIMS New Delhi)**

2. Mahesh R, Jindal A, Bhatt S. Etazolate rescues behavioural deficits in chronic unpredictable stress models of depression by the modulation of HPA axis, BDNF signaling and antioxidant defence system: possible mechanism(s) of neuroprotection. (**International Conference on Recent Advances in Molecular Mechanisms of Neurological Disorders, 21-23rd February 2013, AIIMS New Delhi).**

3. Mahesh R, Dhar AK, Jindal A, Bhatt S. 2-(4-substitutedpiperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids: novel 5-HT3 receptor antagonists a potential anxiolytics. (**International Conference on Recent Advances in Molecular Mechanisms of Neurological Disorders, 21-23rd February 2013, AIIMS New Delhi)**

4. Mahesh R, Bhatt S, Panjawani R, Devadoss T, Jindal A. Neuro-pharmacological evaluation of novel serotonin type-3 (5-HT3) receptor antagonists qcm-15, qcm-16, qct-16 & qct-22 (quinoxaline derivatives) for their anxiolytic potential. **(Association of Pharmaceutical Teachers in India Conference, 17-18th March 2012, Manipal)**

5. Gupta D, Mahesh R, Bhatt S, Devadoss T. Anti-depressant like activity of buspirone and role of HPA axis dysregulation in depression. National conference on pharmacy and pharmaceutical sciences-from bench to market. **Pharmanext (AIPER),** 28-29th April 2012, Madhya Pradesh.

6. Mahesh R, Kumar B, Jindal A, Pandey D, Bhatt S, Devadoss T. Anti-depressant like activity of (4-phenylpiperazin-1-yl) (quinoxalin-3-yl) methanone (4a), a novel 5 HT3 receptor antagonist: An investigation in behaviour-based rodent models. (**Indian Pharmacological Society Conference 19-21th December 2011, Manipal)** (Oral Presentation).

7. Mahesh R, Jindal A, Kumar B, Bhatt S, Pandey D. Rolipram, a phosphodiesterase 4 (PDE4) enzyme inhibitor as a potential anti-depressant: An investigation using behavioural tests battery of depression. (**Indian Pharmacological Society Conference 19-21th December 2011, Manipal)**

8. Mahesh R, Dhar A, Singh S, Jindal A, Sharma A, Bhatt S. Evaluation of antianxiety like activity of 2-(-4-phenylpiperazine-1-yl) 1.8-Naphthridine-3-carboxylic acid(7a) A serotonin type-3(5-HT3) receptor antagonist: An investigation using behavioural test battery of anxiety. (**Indian Pharmacological Society Conference 19-21th December 2011, Manipal).**

9. Mahesh R, Srinivasrao B, Devadoss T, Jindal A, Bhatt S, Kumar B. Studies on the analgesic and anti-inflammatory activity of n-[2-(1h-indol-3-yl)ethyl]quinoxalin-2-caboxalicacid (qcf-20), a novel serotonin type-3 (5-ht3) receptor antagonist. (**Indian Pharmacological Society Conference 19-21th December 2011, Manipal).**

10. Mahesh R, Jindal A, Kurdekar V, Jhadav H, Bhatt S, Gautam BK, Pandey DK, Evaluation of Anti-oxidant, Analgesic and Anti-inflammatory activity of Methanolic Bark extract of Atalantia monophylla. **(Indian Pharmacological Society Conference, 14-16th December 2010, Hyderabad)**

11. Mahesh R, Gumpally C, Bhatt S, Thangaraj Devadoss, Dilip Pandey, Gautam B, Jindal A. Evaluation of novel sertonergic type-3 receptor antagonist quinoxalin-2-carboxylic acid derivative (qct-22) for their anti-depressant potential. **(Indian Pharmacological Society Conference, 14-16th December 2010, Hyderabad)**

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13. Mahesh R, Modak N, Bhatt S, Devadoss T, Pandey D, Gautam B, Jindal A. Novel Quinoxaline Derivative: A Potential Anti Depressant For Treatment Resistant Depression. **(Indian Pharmacological Society Conference, 14-16th December 2010, Hyderabad)**

14. Mahesh R, Bhatt S, Devadoss T, Gautam B, **Jindal A**, Pandey D. QCM-13(n-cyclohexyl-3-methoxyquinoxalin-2carboxamide) a novel anti-depressant; pharmacological evaluation of the potential serotonin type 3 (5-HT3) receptor antagonist. **(Indian Pharmacological Society Conference, 14-16th December 2010, Hyderabad).**

15. Mahesh R, Bhatt S, Devadoss T, Gautam B, Jindal A, Pandey D. Evaluation of Novel 5-HT_3 antagonist as a potential Anti-depressant in co-morbid conditions. **Pharmanext Conference, 18-19th September 2010, Jaipur.**.

16. Mahesh R, Gautam B, Pandey D, Bhatt S, Ramamoorthy R. Hyposerotonergic consequences of parthenolide in a battery of rodents behavioural Assays of depression. **Pharmanext Conference, 18-19th September 2010, Jaipur.**

17. Mahesh R, Bhatt S, Devadoss T, Joshi A, Pandey DK, Yadav S. Evaluation of novel serotonin type 3 (5-HT3) receptor antagonist for their anti-depressant like Activity. **APTICON, 3-4th October 2009, Jodhpur, Rajasthan.**

SYMPOSIUM / WORKSHOPS PARTICIPATED:

1. Pre-Conference workshop on Scientific Writing-Biomedical Communication & Intellectual Property Rights, at SMS Medical College Jaipur, Rajasthan December 20, 2006.

2. UGC Training Course-“Hands-On-Exposure to Neuropharmacological & Molecular Biological Tools” under UGC Networking Resource Centre to be held at University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh from September 19- 24, 2011.

Biographies

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Prof. R. Mahesh is currently Professor, Pharmacy Department & Dean, Faculty Affairs Division, BITS, Pilani. He received his Ph.D. degree in 1997 from BITS, Pilani. He has vast experience in the field of Molecular Modeling and Drug Design, Neuropharmacology and Clinical Pharmacy which comprises of his major area of research. He is also currently involved in developing research in the area of biomedical instrumentation. He has more than 20 years of teaching, research and administrative experience at BITS-Pilani. He has guided 4 Ph.D students and 8 students are presently doing. He has guided more than 100 M. Pharma students for various projects. Several students have worked under various projects in their undergraduate or post graduate programs. Several of his projects have won awards in Academic Exhibitions held at BITS, Pilani. He is life time member of Association of Pharmaceutical Teachers of India and Indian Pharmacological Society. He has published more than 45 papers in international/national Journal and more than 45 papers in conferences of national or international repute.

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Mr. Shvetank Bhatt completed his B. Pharm. from L.M. College of Science and Technology, Jodhpur in 2004 and M. Pharm. (Pharmacology) from Manipal College of Pharmaceutical Sciences, Manipal in 2006. He worked in Ranbaxy Research Laboratories Ltd., Gurgaon for two years in Metabolism and Pharmacokinetics (R&D-III) Department. He is working as a Lecturer in Department of Pharmacy Since October, 2008. His area of interest is Neuropharmacology, Diabetes and Thrombotic disorders. His specialization is Pharmacology and in-vivo Pharmacokinetics.He is life time member of Association of Pharmaceutical Teachers of India (APTI) and Indian Pharmacological Society (IPS). He is also receipent of one minor reserach project granted by UGC. He has published more than 35 research papers and presented his research work in more than 22 conferences of national or international repute.