Behavioral and Neuro-pharmacological Screening of Potential Serotonergic Modulators for Depression Co-morbid with Obesity

THESIS

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Under the Supervision of

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CERTIFICATE

This is to certify that the thesis entitled "**Behavioral and Neuro-pharmacological Screening of Potential Serotonergic Modulators for Depression Co-morbid with Obesity**" and submitted by **Kurhe Yeshwant Vijay**, **ID No. 2011PHXF0409P**, for award of Ph.D. degree of the Institute, embodies original work done by him under my supervision.

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Abstract

Depression is a serious public health issue, concerned with psychiatric disorders that affects the quality of life, leads to disability and increases tendency of suicide. Current antidepressants suffer from severe drawbacks because of limited effectiveness in less than 50% of depressed population. Another psychiatric disorder anxiety, which is a known trait in personality disorders, globally imposes heavy burden on socio-economic status of the affected individuals. The co-morbid association of psychiatric disorders depression and anxiety remains an area of talk since long time due to very narrow margin of symptoms, consistence appearance and positive association of both disorders.

Obesity is one of the key risk factor for depression. Obesity contributes to severe socio-economic burden on the health sector, globally. Both depression and obesity are very prevalent and associated with various health complications such as coronary heart diseases, hypertension and increased mortality rate. Association of depression in obese individuals is one of the most common co-morbid conditions growing across the world. More than 50% of obese individuals are twice likely to develop depression compared to non-obese individuals. The biological mechanisms linking depression and obesity, and effective pharmacotherapy for severe depressive-like behavior in obese population remains a mystery in the field of neuropsychopharmacology.

Several biological mechanisms linking depression and obesity are hypothalamic pituitary adrenal (HPA) axis hyperactivity, dysregulation of oxidant/antioxidant balance, leptin resistance, insulin resistance, reduced hippocampal brain derived neurotrophic factor (BDNF), altered hippocampal morphology and decreased serotonergic neurotransmission.

Serotonin (5-HT), a neurotransmitter regulates appetite, mood and sleep. 5-HT turnover is severely affected both in depression and obesity. Serotonergic neurotransmission have effective control over the biological mechanisms linking depression and obesity such as HPA axis hyperactivity, increased oxidative stress, leptin and insulin resistance, reduced hippocampal BDNF, and increased hippocampal neuronal degeneration. Hence, novel therapeutic targets that act by improving the serotonergic neurotransmission could be a sensible approach in the management of depression co-morbid with obesity.

One of the interesting identified potential targets for neuropsychiatric disorders including depression and anxiety is 5-HT_3 receptors. The antidepressant-like effect of 5-HT_3 receptor antagonists through modulation of brain serotonergic neurotransmission is well evident from several research investigations. In addition, 5-HT_3 receptor antagonists are suggested to have quick onset of action, specific and dose dependent therapeutic potential and wide safety margin.

Hence, the present investigation was designed to evaluate the potential effect of standard ondansetron (**OND**) and in-house newly synthesized 5-HT_3 receptor antagonists **QCM-4** and **4a** on depression co-morbid with obesity.

The research work was planned in accordance to four major objectives namely, (1) to address the biological mechanisms linking depression and obesity, (2) standardize suitable in-vivo rodent model(s) that mimic behavioral, biochemical and molecular alterations involved in depression co-morbid with obesity, (3) to evaluate the effect of standard and novel 5-HT_3 receptor antagonists on depression co-morbid with obesity, and (4) to study the plausible role of 5-HT_3 receptors in mediating antidepressant-like effect in depression co-morbid with obesity

Preliminary screening of novel 5-HT_3 receptor antagonists **QCM-4** and **4a** involved the dose response curve studies on basal locomotor activity score followed by the effect on high predictive validity behavioral assays of depression, including forced swim test (FST), tail suspension test (TST), and anxiety such as elevated plus maze (EPM), hole board test (HBT) and light/dark (L/D) test. Then, the potency of **QCM-4** and **4a** was assessed in chronic models of depression, mainly olfactory balbectomy (OBX) and chronic unpredictable mild stress (CUMS). Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and **4a** (2 and 4 mg/kg, p.o.) inhibited the OBX and CUMS induced behavioral alterations and showed antidepressant-like effect.

High fat diet (HFD) induced obesity model was standardized to study the depressive phenotypes by using biochemical parameters such as plasma glucose and lipid profile followed by behavioral assays such as sucrose preference test (SPT), FST, TST, EPM, HBT and L/D.

The effect of 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** on depression co-morbid with obesity in HFD fed mice was investigated. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.), **QCM-4** (1 and 2 mg/kg, p.o.) and **4a** (2 and 4 mg/kg, p.o.) inhibited the depressive phenotypes in HFD fed mice in SPT, FST, TST, EPM, HBT and L/D.

Then, effect of **OND**, **QCM-4** and **4a** on biochemical parameters such as plasma glucose, lipids, brain hippocampal oxidative stress, corticosterone (CORT), oral glucose tolerance test (OGTT), insulin and leptin resistance was studied. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.), **QCM-4** (1 and 2 mg/kg, p.o.) and **4a** (2 and 4 mg/kg, p.o.) reversed the abnormally elevated plasma glucose, lipids, oxidative stress marker malonaldehyde (MDA) and increased anti-oxidant reduced glutathione (GSH), inhibited HPA axis hyperactivity by reducing plasma CORT, inhibited leptin and insulin resistance involved in depression co-morbid with obesity in HFD fed mice.

In the molecular studies, chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.), **QCM-4** (1 and 2 mg/kg, p.o.) and **4a** (2 and 4 mg/kg, p.o.) improved the hippocampal neurotrophic factors such as cyclic adenosine monophosphate (cAMP) and BDNF, neurotransmitter 5-HT and hippocampal dentate gyrus (DG) morphology in histopathological study using hematoxylin and eosin (H & E) staining, and inhibited p53 mediated hippocampal DG damage in immunohistochemistry (IHC) assay, thus attenuated depression co-morbid with obesity in HFD fed mice.

Furthermore, HFD fed mice were subjected to CUMS procedure to evaluate whether CUMS worsens the depressive phenotypes in HFD fed mice or not. HFD fed mice subjected to CUMS procedure showed aggravated depressive behavior as evaluated in behavioral, biochemical and molecular assays. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.), **QCM-4** (1 and 2 mg/kg, p.o.) and **4a** (2 and 4 mg/kg, p.o.) reversed the depressive phenotypes associated with obesity by inhibiting the behavioral, biochemical and molecular alterations in HFD fed mice subjected to CUMS.

Importantly, serotonergic neurotransmission is affected in depression co-morbid with obesity. 5-HT_3 receptor antagonists act by modulating serotonergic neurotransmission and show antidepressant-like effect. Hence, in the present study, the role of serotonergic neurotransmission in regulation of various biological mechanisms such as HPA axis hyperactivity, oxidative stress, hyperlipidemia, leptin and insulin resistance, suppressed BDNF and significant neuronal damage involved in depression co-morbid with obesity is discussed.

Subsequently, the role of 5-HT_3 receptors, in mediating antidepressant response of **OND** (0.5 and 1 mg/kg, p.o.), **QCM-4** (1 and 2 mg/kg, p.o.) and **4a** (2 and 4 mg/kg, p.o.), was studied. Concomitant administration of a selective 5-HT_3 receptor agonist, mCPBG (m-chloro-phenyl-biguinide, 10 mg/kg, i.p.), inhibited the antidepressant-like effect of **OND**, **QCM-4** and **4a** in HFD fed mice. This suggests that 5-HT_3 receptor play a key role in the cellular processes that regulate depressive phenotypes, and antagonism of 5-HT_3 receptors is involved in mediating the antidepressant-like response of **OND**, **QCM-4** and **4a** in depression co-morbid with obesity.

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- Kurhe Yeshwant Vijay

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2-Me-5-HT	2-methyl-5-HT
4a	(4-phenylpiperazin-1-yl)(quinoxalin-2-yl) methanone
4-AP	4-aminophenazone
5-HT_1	Serotonin type 1
5-HT_2	Serotonin type 2
5-HT_3	Serotonin type 3
5-HT_4	Serotonin type 4
5-HT_7	Serotonin type 7
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
8-OH-DPAT	8-hydroxy-2-(din-propylamino)tetralin
Ach	Acetylcholine
ACTH	Adrenocorticotropic hormone
AD	Alzheimer’s disease
ADP	Adenosine-5-diphosphate
AgRP	Agouti related protien
ANOVA	Analysis of variance
APA	American Psychiatric Association
Arc	Arcuate nucleus
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
BMI	Body mass index
BSA	Bovine serum albumin
CA	Competitive antagonist
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CHE	Cholesterol esterase
CHOD-POD	Cholesterol oxidase-peroxidase
CMC	Carboxymethyl cellulose
CNS	Central nervous system
CORT	Corticosterone

COX	Cyclooxygenase
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CRF	Corticotropin releasing factor
CREB	CAMP response element Binding
CRH	Corticotropic releasing hormone
CUMS	Chronic unpredictable mild stress
$CuSO_4$	Copper sulfate
CVD	Cardiovascular disease
CVS	Cardiovascular system
DA	Dopamine
DAB	Diaminobenzidine
DAG	Diacylglycerol
DAP	Dihydroxyacetone phosphate
DG	Dentate gyrus
DIO	Diet induced obesity
DNA	Deoxyribonucleic acid
DRC	Dose response curve
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTNB	5,5′-dithiobis (2-nitrobenzoic acid)
DZM	Diazepam
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
EPM	Elevated plus maze
ESC	Escitalopram
FFA	Free fatty acid
Fig	Figure
FST	Forced swim test
G3P	Glycerol phosphate
GAA	Gacial acetic acid
GABA	Aminobutyric acid
GAD	Generalized Anxiety Disorder
GCs	Glucocorticoids
GLUT-4	Glucose transporter 4

G6PDH	Glucose-6-phosphate dehydrogenase
GOD-POD	Glucose oxidase-peroxidase
GPCR	G-protein coupled receptor
GPO-POD	Glycerol phosphate dehydrogenase-peroxidase
GR	Glucocorticoid receptors
GSH	Reduced glutathione
GSSG	Glutathione disulfide
H & E	Heamotoxylin-eosin
H_2O	Water
H_2O_2	Hydrogen peroxide
HBT	Hole board test
HDL	High density lipoprotein
HFD	High fat diet
HPA	Hypothalamic-pituitary-adrenal
i.p.	Intraperitoneal
IAEC	Institutional Animal Ethics Committee
IHC	Immunohistochemistry
IL	Interleukin
INS-1	Insulin producing beta-cell lines
IP3	Inositol triphosphate
K^+	Potassium
L/D	Light-dark
LepRb	Leptin receptors
LP	Lipophilic drugs
LPL	Lipoprotein lipase
MAOIs	Monoamine oxidase inhibitors
MC3R	Melanocortin 3 receptors
MC4R	Melanocortin 4 receptors
mCPBG	1-(m-Chlorophenyl)-biguanide
MDA	Malonaldehyde
MDD	Major Depressive Disorder
MetS	Metabolic syndrome
MUFA	Monounsaturated fatty acid
mRNA	Messenger ribonucleic acid

NA	Nor-adrenaline
Na^+	Sodium
NA2	2-(4-methyl piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile
Na_2CO_3	Sodium carbonate
nAChR	Nicotinic acetylcholine receptor
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NaSSA	Noradrenergic and specific serotonergic antidepressants
NCA	Non-competitive antagonist
NCE	New chemical entitie
NDRIs	Noradrenaline-Dopamine reuptake inhibitors
NE	Nor epinephrine
Non-REM	Non rapid eye movement
NPD	Normal pellet diet
NPY	Neuropeptide Y
NRIs	Noradrenaline reuptake inhibitors
NSB	Non-specific binding
NT	Neurotransmitter
O_2	Oxygen
OAE	Open arm entries
OAT	Open arm time
OBX	Olfactory bulbectomy
OCD	Obsessive-Compulsive Disorder
OFT	Open field test
OND	Ondansetron
p.o.	**Per-**oral
PBS	Phosphate buffer saline
PD	Parkinson’s disease
PGE2	Prostaglandin E2
PKA	Protein kinase A
pmol	Picomol
PNS	Peripheral nervous system
POMC	Propiomelanocortin
PSP	Progressive supranuclear palsy

PUFA	Polyunsaturated fatty acids
PVN	Paraventricular nucleus
QCM-4	3-methoxy-N-p-tolylquinoxalin-2-carboxamide
REM	Rapid eye movement
ROS	Reactive oxygen species
rpm	Revolutions per minute
SARIs	**Serotonin antagonist and reuptake inhibitors**
SARIs	Serotonin antagonists and reuptake inhibitors
SERT	Serotonin transporter
SFA	Saturated fatty acid
SDS	Sodium dodecyl sulfate
SLA	Spontaneous locomotor activity
SNRIs	Serotonin-Noradrenaline reuptake inhibitors
SPT	Sucrose preference test
SREs	Serotonin reuptake enhancers
SSRIs	Selective serotonin reuptake inhibitors
T2DM	Type 2 diabetes mellitus
TBA	Thiobarbituric acid
TCAs	Tricyclic antidepressants
T Gase	Transglutaminase
TM	Trans membrane
TNF- α	Tumor necrosis factor-alpha
TRD	Treatment Resistant Depression
TRIs	Triple reuptake inhibitors
TrkB	Tropomyosin-related kinase B
TST	Tail suspension test
VMN	Ventromedial nucleus
WHO	World Health Organization
α-MSH	Alpha-melanocyte stimulating harmone
°C	Degree Celsius
cm	Centimeter
g	Gram
h	Hour

Kg	Kilogram
M	Meter
mg/dl	Milligram per deciliters
mg/kg	Milligram per kilogram
min	Minute
ml	Milliliter
mmol	Millimol
µl	Microlitre
µg	Microgram
µg/ml	Microgram per milliliter
mg	Milligram
ng	Nanogram
pg	Picogram
pmol	Picomol
v/v	volume by volume

1. INTRODUCTION

1.1. Depression: A Neuropsychiatric problem and prevalence

Depression is a serious public health issue and most prevalent of all psychiatric disorders that affects the quality of life, leads to disability and increases tendency of suicide (Levinstein and Samuels, 2014). According to the World Health Organization (WHO), depression will be the foremost contributor to the global burden of disease severity and disability by 2030 (Manji et al., 2001, Nestler et al., 2002, Mathers et al., 2008). Depression affect around 120 million population, has lifelong prevalence of around 10-15%, and is leading cause of disability, suicidal tendency and poor quality of life (Lépine and Briley, 2011, Murray et al., 2013). In India, a recent meta-analysis study noticed the raised prevalence rate over past few decades of around 15.9% for depression. Data obtained from the primary health care settings in India, showed 21-84% of the cases of depression (Pattanayak and Sagar, 2014). As per a meta-analysis study, it is expected that more than 30 million adult population would experience a significant episodes of clinical depression at some stage in their lives (Gotlib and Joormann, 2010). Moreover, depression is known to be highly recurrent psychiatric disorder, as around 75% of depressed patients have more than one episode of depression, often relapsing within couple of years of recovery from the depressive episode (Boland et al., 2002).

Despite several available therapies, treatment resistant depression (TRD), has been reported in 15% to 30% of the depressed patients, that ultimately affects socio-economic status (Nemeroff, 2007, Fournier et al., 2010). Two major drawbacks of the current antidepressants include delayed clinical benefit and treatment resistance (Samuels et al., 2011). Selective serotonin reuptake inhibitors (SSRIs), the most commonly prescribed antidepressant agents, tend to show beneficial effect after 2 weeks of treatment and the maximal effect, is achieved after 6-9 weeks of treatment (Artigas et al., 1996, Gardier et al., 1996). Response and remittance rate for commonly used antidepressant class of drugs are very low due to diverse set of symptoms among the affected individuals and the complex nature of genetic mechanisms underlying depression (Trivedi et al., 2006). TRD often results from the failure to respond to two to three antidepressant treatment courses (Souery et al., 2006). Additionally, TRD leads to heavy socio-economic burden by increasing societal cost by $29-$48 billion thus making the total annual societal costs of Major Depressive Disorder (MDD) $106-$118 billion in America (Mrazek et al., 2014).

Hence, it remains a challenge for the researchers to investigate the novel therapeutic interventions for depression with better efficacy, fast action and novel targets that will achieve and maintain remission.

1.2. Anxiety its co-morbid association with depression

Anxiety disorder is another common psychiatric disorder usually associated with fear, nervousness, apprehension and panic but may also involve several systems individually or in combination such as the cardiovascular, respiratory, gastrointestinal, or nervous systems (Rakel, 1981). Anxiety disorder including panic disorders, and Obsessive-Compulsive Disorder (OCD) have lifetime prevalence of about 2%, social anxiety disorder (also known as social phobias) have lifetime prevalence of about 8-13% and affects a significant proportion of general community and Generalized Anxiety Disorder (GAD) affecting the major proportion of community with a prevalence rate of 3%-30% (Klein, 1980, Martin, 2003, Ruscio et al., 2008, Bandelow and Michaelis, 2015).

Anxiety is a known trait in personality disorders, imposes heavy burden on the socio-economic status of the affected individuals across the globe (Baxter et al., 2013). The co-morbid association of depression with anxiety, is well evidenced since several decades due to very narrow margin of clinical symptoms existing between them (Hirschfeld, 2001, Hranov, 2007). Around two-third of the depressed patients, exhibit prominent symptoms of anxiety, and more than 50% of affected population with OCD, exhibit symptoms of depression (Fainman, 2004, Kessler et al., 2005). Anxiety symptoms are commonly observed in 60% of the patients experiencing MDD and co-morbid anxiety is being reported in around 50% of the patients with depression (DS et al., 2009). One of the important studies suggested the frequent co-morbid association between anxiety disorder and depression, further advise the importance for assessment of anxiety symptoms in depressive disorders (Goldberg and Fawcett, 2012). Moreover, the severity of symptoms and impairment is greater in case of co-morbid mood and anxiety disorders (Brown et al., 1996). Clinical studies suggest that the presence of co-morbid anxiety in depressive patients increases suicidal thoughts, psychomotor retardation, sexual dissatisfaction, weight loss and diurnal variations compared to patients of depression alone (Altamura et al., 2004). Co-morbid association of anxiety and depression lead to poor clinical outcome in affected patients, compared to patient affected alone with depression or anxiety disorders (Emmanuel et al., 1997).

Most importantly, the co-morbid association of depression and anxiety disorders, results in poor response rates to treatment and less remission of symptoms during the treatment period. Most of the non-responding patients to the antidepressant therapy were found to be associated with anxiety disorders (Trivedi et al., 2006, Schaffer et al., 2007).

Hence, depression and anxiety are observed to be the most important co-morbid psychiatric disorders and to study their underlying neurobiological mechanisms remains a challenge which is an interesting area, for Neuropsychopharmacology researchers.

1.3. Clinical assessment of depression on the basis of Diagnostic and Statistical Manual of Mental Disorders (DSM)

The DSM guidelines designed by the American Psychiatric Association (APA) for mental disorders is considered as a standard criteria, for diagnosis of depressive and other psychiatric disorders. DSM criteria are used in almost all the clinical settings by clinicians from different background. DSM guidelines are also referred by mental health and other health professionals such as psychologist, biological, cognitive, psychiatrists, occupational and rehabilitation therapists. DSM-I was established in 1968 and now the recently updated DSM guidelines are DSM-V in 2013. DSM-IV (1994) remained the standard guidelines for long period of time and DSM-V was introduced with several modifications and updates with respect to new definitions and diagnostic criteria that were not known or not well defined in earlier manuals. Apart from DSM-IV/V, several common symptoms that are excluded from diagnostic criteria are reduced salivation, diurnal alterations and constipation. A table below represents the DSM-IV/V criteria for diagnosis of clinical depression (**Table 1.1**).

According to DSM-IV/V for a person to be diagnosed as depressed, five or more of the following symptoms must be present daily for two weeks and one of the symptoms must be depressed mood or loss of interest/pleasure as mentioned in **Table 1.1, 1.2 and 1.3** respectively.

Table 1.1: DSM-IV/V criteria for diagnosis of clinical depression

Symptoms	DSM-IV/V
Depressed mood	nearly every day, indicated by subjective reports such as feeling sad or empty and observations made by others including appearing tearful
Diminished interest/pleasure	In almost all or most of the activities of day, nearly every day
Weight change (5%)	change in the body weight by more than 5% in a month-either increase or decrease, accompanied by change in appetite
Altered sleep pattern	Insomnia or hypersomnia nearly every day
Psychomotor agitation or retardation	nearly every day and generally observed by others
Fatigue or loss of energy	nearly every day
Feeling of guilt/worthlessness	nearly every day
Altered concentration	Reduced ability to concentrate/think/indecisiveness, nearly every day
Suicidality	No fear of dying, recurrent suicidal thoughts with or without a specific plan for committing suicide

Table 1.2: DSM-IV Criteria A and B for depressive episode

A	B
• Depressed mood • Loss of interest and pleasure/enjoyments in almost all daily activities • Fatigue/reduced energy and physical activity	• Low self esteem and reduced confidence • Feeling of guilt and worthlessness • Pessimistic thoughts/always expect the worst as outcome • Altered sleeping pattern • Reduced appetite • Ideas of self harm

Table 1.3: DSM-V Criteria A, B and C for depressive episode

A	B	C
• Depressed mood/irritable • Loss of interest/pleasure • Weight change • Change in sleep • Change in activity • Fatigue/reduced energy and physical activity • Guilt/worthlessness • Problem of concentration • Suicidal thoughts	Symptoms that results in significant distress or impairment in; • Social • Occupational • Other functional areas	Symptoms that are not resulted from; • direct physiological effects of a substance such as a drug of abuse, a medication • general medical condition such as hypothyroidism

However, with concern to major depressive disorder and anxiety disorders several modifications were implemented in DSM-V. **Table 1.4** below represents the critical evaluation of modifications in DSM-IV and DSM-V manuals.

Table 1.4: Highlights of the changes from DSM-IV to DSM-V in metal health disorders depression and anxiety

Parameter	DSM-IV	DSM-V
(a) Depressive Disorders	i) Persistent depressive disorder/ Dysthymia included as separate from MDD ii) "Depressive Disorder" included in "Bipolar and Related Disorder" iii) Disruptive mood and dysregulated disorder mainly including potential of overdiagnosis and treatment for bipolar disorders in children was not well defined iv) Premenustral dysphoric disorder is mentioned in the appendix named as "Criteria Sets and Axes Provided for Further Study"	i) Included in the category of persistent depressive disorder that includes both MDD and dysthymia ii) Depressive Disorders is represented as separate/individual section iii) Disruptive mood and dysregulated disorder is included which refers to the children up to 12 years with persistent irritability and frequent episodes of extreme behavioral dyscontrol iv) Premenustral dysphoric disorder is mentioned is mentioned in section-II of DSM-V
(a1) Bereavement exclusion for depression	Exclusion criteria for major depressive episode that was applicable in depressive symptoms lasting for less than 2 months period post death of loved one (termed as bereavement exclusion)	This exclusion criteria was removed from DSM-V because; i) according to physician and grief counselor the bereavement last for 1 to 2 years rather than 2 months ii) Bereavement is a psychosocial stressor that can result in major depressive episode in vulnerable individual iii) Bereavement related depressive episodes are most likely to occur in individuals with past personal and family history of MDD
(a2) Specifiers for Depressive Disorders	Specifiers were not clearly defined for depressive disorders with respect to; i) mixed symptoms (bipolar and depressive disorders) ii) importance of anxious distress	i) Specifiers indicating mixed symptoms have been added to bipolar and depressive disorders. ii) Importance of anxiety as relevant to prognosis and treatment decision making (after several research conducted over last two decades). Anxious distress as specifier provides an opportunity for clinicians/physicians to rate the severity of anxious distress in affected individuals with bipolar or depressive disorders

Parameter	DSM-IV	DSM-V
(b) Anxiety Disorders	Includes obsessive compulsive disorder (OCD), posttraumatic stress disorder (PTSD) and acute stress disorder (ASD)	Does not includes OCD, PSTD and ASD
(b1) Agoraphobia, Specific Phobia, and Social Anxiety Disorder (Social Phobia)	i) Individuals over 18 years recognize that their anxiety is unreasonable or excessive ii) 6 months period for individuals under 18 years of age	i) Omission of that individuals over 18 years recognize that their anxiety is unreasonable or excessive based on ii) 6 months period for individuals under 18 years of age is now extended to all ages in order to minimize the overdiagnosis of transient fear
(b2) Panic Attack	Different types of panic attack; situationally bound/cued, situationally predisposed, and unexpected/uncued	Different types of panic attacks are replaced with unexpected and expected panic attacks
(b3) Panic Disorder and Agoraphobia	Included; i) panic disorders with agoraphobia ii) panic disorders without agoraphobia iii) agoraphobia without any history of panic disorder	Replaced by two separate diagnosis; i) panic disorders ii) agoraphobias
(b4) Specific Phobias	i) Requirement that individuals over 18 years must consider that their fear and anxiety are excessive or unreasonable ii) Duration requirement mainly last for 6 months or more with respect to specific phobia	i) Not required that individuals over 18 years must consider that their fear and anxiety are excessive or unreasonable as it is extended to all ages ii) No longer the duration of 6 months or more considered as it applies to all ages
(b5) Social Anxiety Disorder (Social Phobia)	i) Individuals over 18 years must consider that their fear and anxiety are excessive or unreasonable ii) Duration requirement of 6 months or more	i) It is extended to all ages ii) It applies to all ages.
(b6) Separation Anxiety Disorder	i) Included in the section - "Disorders Usually First Diagnosed in Infancy, Childhood, or Adolescence" ii) Age of onset before 18 years iii) Does not include duration criteria mainly lasting for 6 months of period for adults	i) It is now included/classified as anxiety disorder ii) Applies to all ages iii) Includes duration criteria of 6 months for adults to minimize overdiagnosis of transient fear

1.4. Association of depression and other neurodegenerative disorders

The association of depression and other neurological diseases is quiet difficult to understand and identify clinically, at first instance/glance. Most of the time, depressive behavior in neurodegenerative disorders may occur as an early symptom, where depression could be the main manifestation. These depressive symptoms in neurological disorders are very important in medical practice as they have heavy impact on the quality of life, of affected patients, raising caregiver burden, disability, reduced functional ability and premature mortality rate (Baquero and Martín, 2015).

Depression and dementia, often remain important co-morbid condition, from the clinical perspective. It is suggested that late onset of depressive symptoms could be considered as one of the marker risk factors or an early manifestation that would later result in the development of dementia (Rickards, 2006). Reports mentioned the association of depressive symptoms in the patients with Alzheimer's disease (AD), as in the whole course of disease, around 80% of the affected patients with AD can develop the symptoms of depression to a greater or lesser degree (Lyketsos and Lee, 2003).

Another important co-morbid association with depression is the Parkinson's disease (PD). As reports suggest, the prevalence rate of depressive symptoms in PD patients is around 20% to 50% and frequently associated with impairment of cognitive functions, greater disability, and faster progression of motor symptoms (Schrag et al., 2007). This is recognized as a serious combination, as depression symptoms are considered as the marker of negative factors, hampering the quality of life and that may precede motor symptoms for years in PD affected patients (Aarsland et al., 2009). Huntington's disease is other neurodegenerative disorder that results in incoordination, affected cognitive tasks, dystonia, accompanied by behavioral deficits. Depressive symptoms are diagnosed in around 40% of the cases affected with Huntington's disease (Paulsen et al., 2001). Apart from this, behavioral and psychological symtomatological aspect, vascular dementia represents most of the symptoms of depression and apathy (Gupta et al., 2014). Progressive supranuclear palsy (PSP), is known to be a rare neurodegenerative disorder clinically characterized by symmetrical Parkinsonism, frontal lobe syndrome, postural instability and falls.

Clinically, it is found that PSP patients often suffer from behavioral alterations and more than 50% of the affected patients, experience depression, apathy and sleeping problems, and important symptom of mood disorder apathy is observed in around 90% case of PSP (Gerstenecker et al., 2013). This information suggests, the serious impact of depression and associated symptoms in several neurodegenerative disorders leading to heavy impact on quality of life of the affected individuals.

1.5. Association of depression with inflammation and metabolic disorders

In the last decade, researchers focused on the inflammatory and metabolic dysregulation, in depression. Several evidence based studies suggested the association of inflammatory and metabolic dysregulation in chronic forms of depression (Duivis et al., 2011, Pan et al., 2012). Metabolic syndrome (MetS) consists of the cluster of events including central obesity, hypertension, hyperglycemia, hypertriglyceridemia and reduced high density lipoprotein (HDL) cholesterol. Evidence based studies, revealed depression as a risk factor for type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Musselman et al., 1998, Glassman, 2008, Mezuk et al., 2008). As depression and MetS, contribute significantly to the public health challenges, the co-morbid association of depression and MetS, has attracted the attention of scientists and researchers in the recent past (Pan et al., 2012).

Inflammation, and in particular, the inflammatory cytokines, are known to be involved in depression through various pathways such as monoamine metabolism, neuroendocrine function, synaptic plasticity and neurocircuits relevant to mood regulation (Kurhe et al., 2014). The pro-inflammatory cytokines, mainly interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), are higher in obese and depressive subjects (McMurray et al., 2007, Dowlati et al., 2010). ‘Sickness behavior,’ a characteristic feature is induced by pro-inflammatory cytokines IL-6 and prostaglandin E2 (PGE2) which leads to cyclooxygenase (COX) 2 expression in depressed patients (Yirmiya et al., 1997). On the other hand, the role of COX-2 in obesity and COX-2 mediated inflammation in fat is crucial, in the development of insulin resistance (Hsieh et al., 2009). Furthermore, patients with metabolic syndrome or insulin resistance syndrome, experience higher risk of developing depression (Almeida et al., 2009).

Hence, depression associated with metabolic disorders, particularly obesity and diabetes, remain an important issue to study for the welfare of public health and to reduce global burden of diseases with respect to biological mechanisms linking such co-morbid disorders, and the novel therapeutic approach to combat such co-morbidities.

1.6. Obesity

Obesity is a multifoctorial condition and combination of several pathological events. Obesity is generally known as deposition of excess fat in the body. The rising prevalence of obesity in children, adolescents and adults since last three decades, strikes as major health concern that imparts heavy burden on the socioeconomic status of the public health in 21st century across the globe (Güngör, 2014). According to WHO, obesity remains one of the most common and most neglected public health problems in both developed and developing nations across the world (Pednekar et al., 2008, Kalra and Unnikrishnan, 2012). As per the new data from 188 countries, approximately 2.1 billion people (rating 30% of the global population), are either obese or overweight. Countries with leading obese population include United States (13%), and India and China together represent 15%. The prevalence of overweight or obesity in children and adolescents has been raised by nearly 50% since last three decades (1980-2013) (Hua et al., 2014).

Assessing the total body fat properly, is a very important aspect, as the perfect method or the sophisticated technology, is not readily available. WHO recommends the measurement of total body fat in overweight and obesity by body mass index (BMI), which is calculated by dividing the body weight in kilo gram (kg) by square of the height in meter (m) (Organization, 2009, Flegal et al., 2013), as described in **Table 1.5** below.

Body mass index (BMI) = Weight (kg)/ Height (m^2)

Table 1.5: Classification of obesity on the basis of BMI

BMI (kg/m^2)	**20-25**	**25-30**	**30-35**	**35-40**	**>40**
Class	Normal	Overweight	Obese (I)	Obese (II)	Obese (III)
Risk	Very low	Low	Moderate	High	Very high
Fat cell no.	Normal	Normal	Normal or Increased	Increased	Increased

Obesity is one of the major health issues that have great impact on quality of life, mainly due to several genetic and behavioral factors. Obesity leads to several metabolic disorders including dyslipidemia, type 2 diabetes mellitus (T2DM), hypertension and psychological disorders, thus increasing the morbidity and mortality rate (Luppino et al., 2010, Griffiths et al., 2014, Gao et al., 2015) as mentioned in **Fig 1.1**.

Owing to these co-morbidities, obesity is no longer considered as just a major health issue but is also considered as economic predicament, as it imposes heavy economic burden on society for maintenance of obese patients with respect to care and treatment (Varela and Horvath, 2012). In the next 20 years, the healthcare costs for care and treatment of obesity is expected to rise by 16% of the total costs in western countries (Wang et al., 2008, Wang et al., 2011).

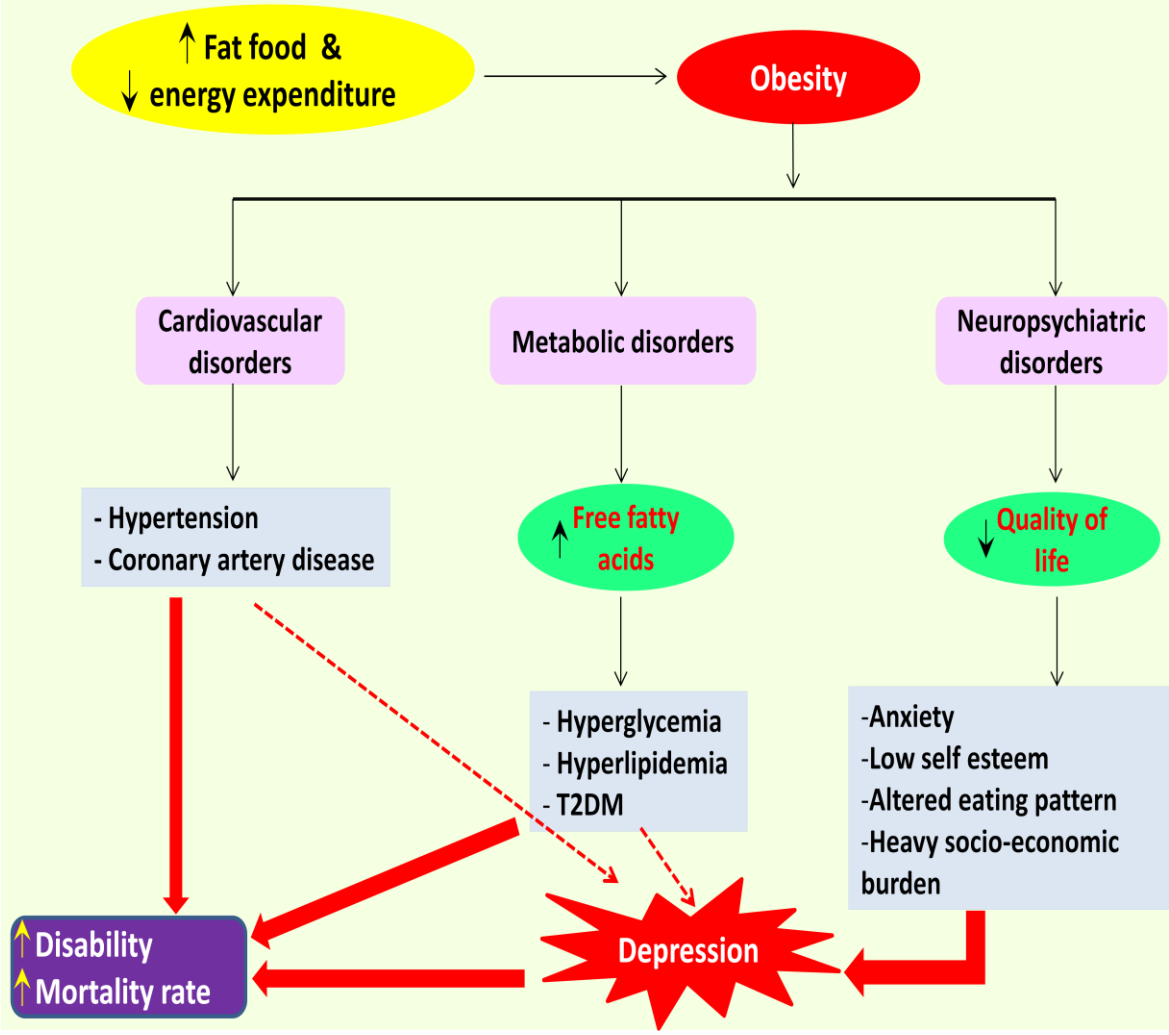


Fig 1.1: Various complications associated with obesity

1.7. Model for Central Nervous System (CNS) control of energy and glucose homeostasis

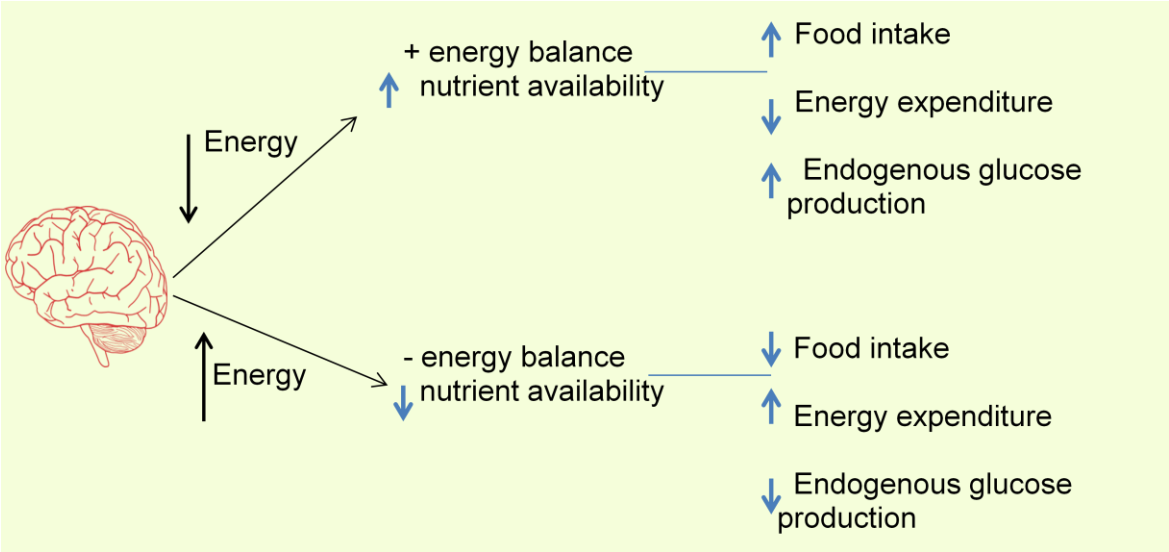


Fig 1.2: CNS control of energy and glucose homeostasis

The schematic representation mentioned above, in **Fig 1.2** briefly explains the control of energy and glucose homeostasis by CNS. Brain is involved in transformation of input signals from neurons, hormones and nutrient and thus maintain the balance between energy and glucose homeostasis. Long term energy signals are conveyed by hormones such as leptin and insulin whereas, short term energy signals are carried by nutrients such as glucose and free fatty acids.

Brain receives input signals and responds to them, by making adjustments in the output system involved in regulation of food intake, energy expenditure, hepatic insulin sensitivity and glucose uptake. Generally, brain plays a pivotal role in restoring the homeostsis as in case of decreased energy availability, brain makes changes in the output systems by promoting the positive energy balance and raising nutrient availability. Likewise, in case of excess nutrient or energy availability, brain sends the output signals in such a manner that it promotes the negative energy balance and reduces the endogeneous glucose production so that homesostasis is maintained between CNS control of energy and glucose.

A significant research outcome, confirmed that the hormone secreted in proportion to the body fat mass named as ‘leptin,’ is involved in the negative feed back loop, to promote energy homeastasis by acting in the CNS (Morton and Schwartz, 2011).

1.8. Regulation of food intake and energy expenditure

In 1953, Scientist Kennedy hypothesized that the body fat mass or adiposity is controlled by an endogeneous factor secreted in proportion to the adipose tissue mass by acting in the specific areas of brain to maintain the energy balance (Kennedy, 1953). Further research by Scientists Zahng and his colleagues in 1994, confirmed that the adipose tissue expresssed *ob* gene encodes a peptide hormone named as 'leptin' (Zhang et al., 1994).

Hypothalamic neurons mainly located in the arcuate nucleus (Arc), have been implicated in the regulation of energy homeostasis. There exist two types of neuronal control systems in hypothalamic Arc, group one consists of Neuropeptide Y (NPY)/ Agouti related protien (AgRP), whereas, group two includes propiomelanocortin (POMC). The neuronal balance is regulated by certain hormones, mainly leptin signalling (Varela and Horvath, 2012).

When there is an increase in leptin level, POMC is cleaved into alpha-melanocyte stimulating harmone (α-MSH), that activates melanocortin 4 receptors (MC4R), expressed in the paraventricular nucleus (PVN) of the hypothalamus, resulting in decreased food intake and increased in energy expenditure. With reduced leptin signalling, NPY/AgRP is activated, which in turn releases NPY and AgRP, that antagonises the effect of α-MSH, resulting in increased food intake and reduced the energy expenditure. This is how food intake and energy expenditure balance is maintained, briefly explained in **Fig 1.3**. Whenever there is disturbance of this balance, it results in obesity (Belgardt et al., 2009, Varela and Horvath, 2012).

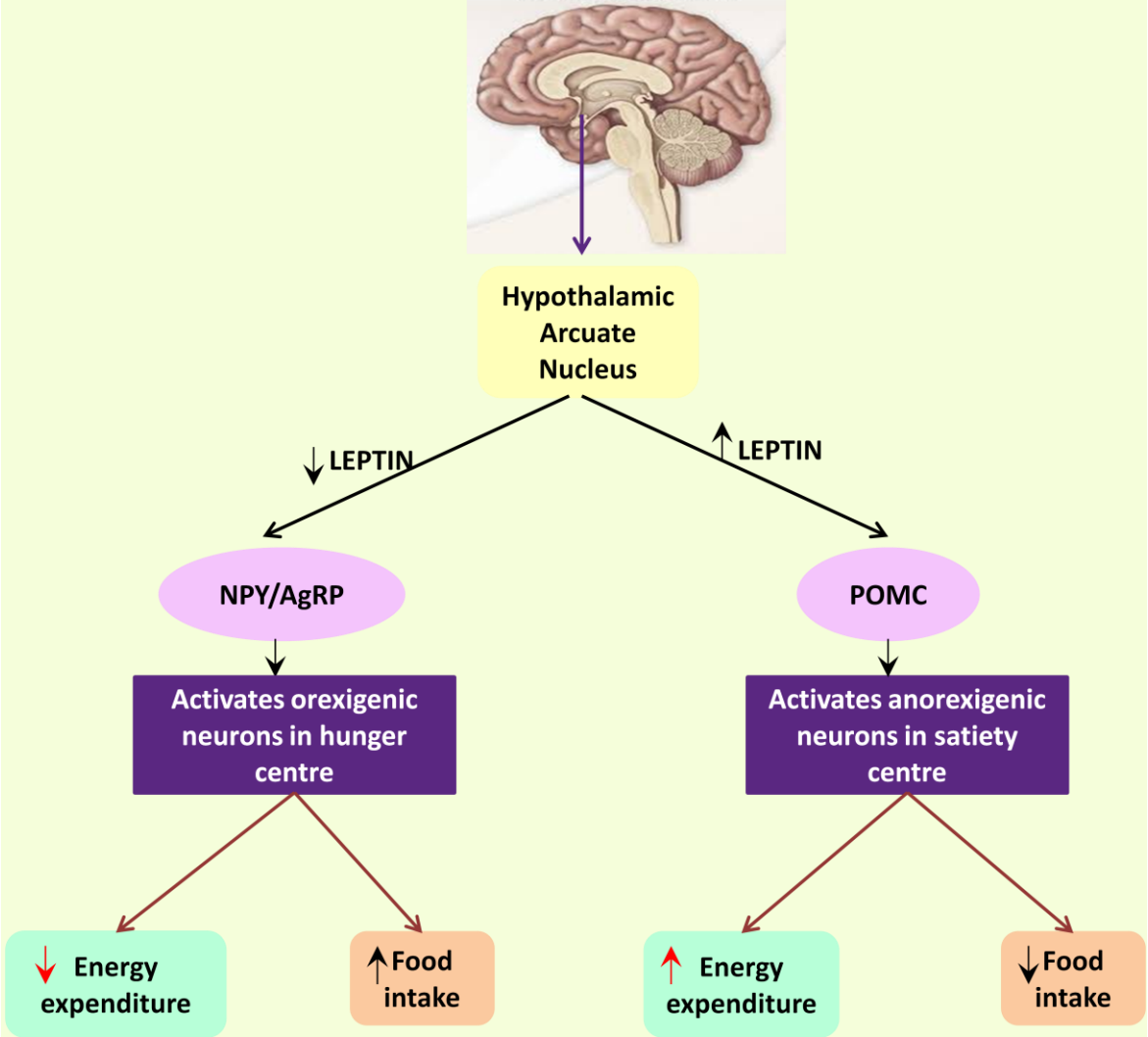


Fig 1.3: Regulation of food intake and energy expenditure by leptin

1.9. Neurodegenerative disorders co-morbid with obesity

Obesity has been known to accelerate the aging process (Tzanetakou et al., 2012). Several physiological changes are reflected in obese population, especially insulin resistance that disturbs the glucose homeostasis, dyslipidemia termed as 'metabolic syndrome' (Grattagliano et al., 2008). Obesity and metabolic syndrome together lead to oxidative damage and raise pro-inflammatory cytokines (Sutherland et al., 2004). Obesity has been linked with various progressive age related neurodegenerative disorders such as AD, PD, and multiple sclerosis (Ashrafian et al., 2013). Obesity affects the cognitive functions due to impaired insulin metabolism and signaling pathways along with defects in glucose transport and homeostasis in the brain (Naderali et al., 2009).

2.0. Depression co-morbid with obesity

The association of obesity and various neurological disorders such as sleep apnea, anxiety and depression are well reported (Ravanan et al., 2010, Awada et al., 2013, Nemiary et al., 2012). Depression and obesity have been known for long time as major public health issues in youths. Data from National Health and Nutrition Examination Survey, estimated that, 17% of youths aged between 2-19 years were overweight, which is remarkably higher compared to just 5%, a few decades ago (Ogden et al., 2006).

Depression and obesity are very prevalent disorders and are associated with various health complications such as coronary heart diseases, hypertension and raised mortality rate (Luppino et al., 2010). Interestingly, obesity has been known to be a major risk factor for depression and anxiety (Abildgaard et al., 2011, Mizunoya et al., 2013). Association of depression in obese individuals is one of the most common co-morbid conditions growing globally, especially in children and women (Ma and Xiao, 2010). Around 50% of the obese individuals, are twice as likely to develop depression as non-obese individuals (Fabricatore and Wadden, 2004, Gadalia, 2009, Luppino et al., 2010). Association of various types of anxiety disorders in obese individuals have been described in several meta-analysis studies (Gariepy et al., 2010, Lykouras and Michopoulos, 2011). At present, very little information is available on the pathological factors involved in depression co-morbid with obesity. The high prevalence rate of such co-morbid disorders demand extensive research in the area of Neuropsychopharmacology to identify the underlying mechanisms (Vogelzangs et al., 2009, Abildgaard et al., 2011).

2.1. Similarities in clinical features for association of depression and obesity

Depression and obesity have become important health concerns, as both lead to heavy socio-economic burden on the society and public health sector. Depression and obesity, may possibly lead to other neuropsychiatric disorders and increase the mortality rate across the globe. Hence, it is very essential to understand the nature of the disease with respect to behavioral, biochemical and molecular alterations that are observed in clinical patients suffering from such co-morbid disorders.

Even though obesity and depression are known as different health problems of physical and emotional nature, respectively (Faith et al., 2002), they share some common symptoms such as sleep problem, sedentary behavior, dysregulated food intake and negative self image (Reeves et al., 2008), as outlined in **Fig 1.4** below.

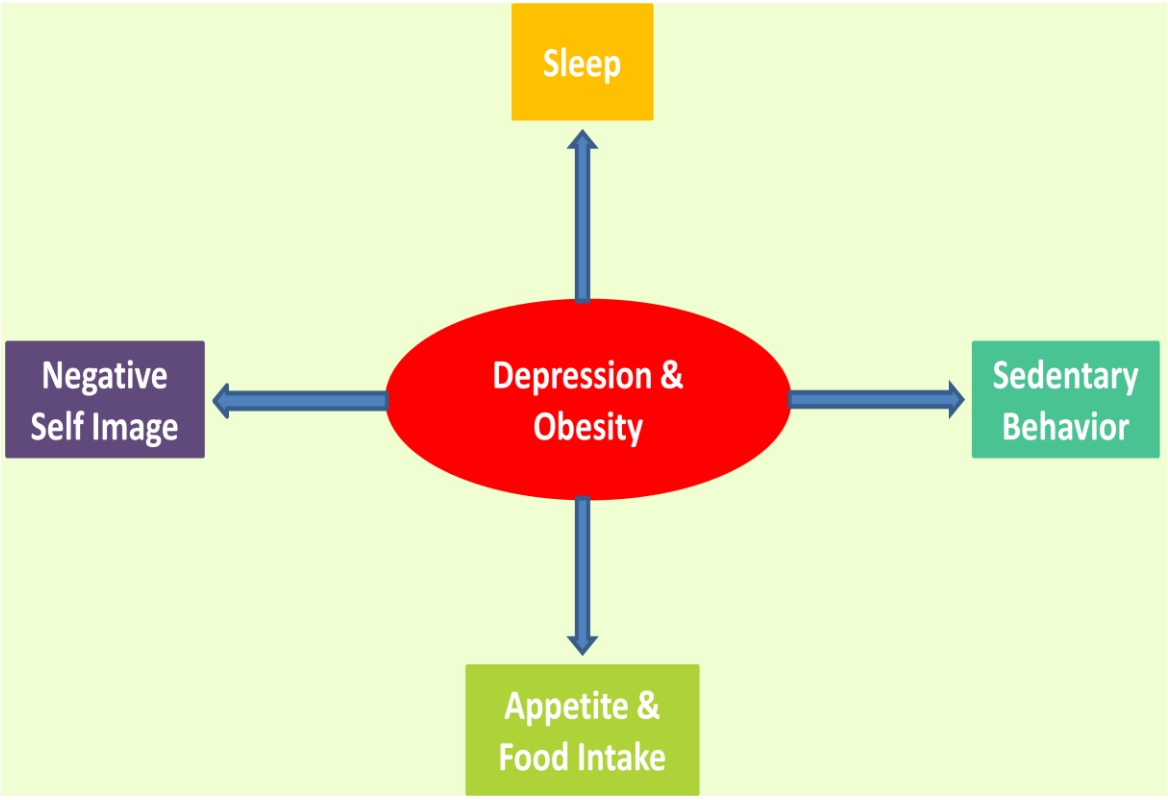


Fig 1.4: Similarities of clinical features between depression and obesity

2.1.1. Sleep

Sleep disturbances are very prominent features of adolescent depression, mainly reflected by prolonged sleep latency (Emslie et al., 2001). In depressed adolescents, insomnia is associated with suicidal thoughts (Barbe et al., 2005). Obesity also, has connections with sleep problems.

Overweight children/adults, are observed at higher risk for sleep apnea, along with obesity hypoventilation syndrome, that result in the daytime somnolence and reduced night time sleep, thus experiencing less total sleep time (Barlow and Dietz, 1998). The sleep alterations in depression and obesity could be linked to two underlying dysfunctions;

(i) the neurotransmitter system that is involved in regulation of both mood and sleep, e.g. serotonin (5-hydroxytryptamine; 5-HT)
(ii) the other system that is altered due to sleep deprivation could be crucial for both depression and obesity, mainly the hypothalamic-pituitary-adrenal (HPA) axis (Capaldi et al., 2005).

2.1.2. Sedentary behavior

Reduced physical activity, is a cause of obesity. Mood disorder like depression may be a result of inactivity and hence, could promote obesity. One of the core symptoms/features of depression, is reduced interest or lack of motivation for activity. Predominant sedentary behavior may result in worsening depression and obesity, and could also directly link these two conditions. Increased sedentary behavior in depression may be secondary to depressed mood, fatigue and lack of motivation.

The combination of reduced physical activity and/or uncontrolled appetite can result in unhealthy weight gain. Playing favorite video games by children or social isolation and decreased physical activity could possibly lead to weight gain, thus promoting the sedentary behavior. Increased sedentary behavior may also sustain or worsen obesity, unless there is a significant reduction in food intake. Hence, prohibition of sedentary activities could improve the obesity by increasing energy expenditure and, improve mood by increasing social interaction/support (Anton et al., 2006, Reeves et al., 2008).

2.1.3. Appetite and food intake

Depression is associated with changes in appetite, which represents desire to eat. Both depressed mood and increased appetite are associated in adulthood depression, explaining that increase appetite is the central feature of depression (Pettit et al., 2006). Food intake is important with concern to obesity, as it is the result of imbalance between energy intake and expenditure. The physiological factors involved in regulation of food intake and satiety are significantly altered in obese subjects.

Leptin, a peptide hormone acts on the receptors in hypothalamus, a centre for satiety/appetite regulation (Reinehr et al., 2005). Higher leptin levels are associated with reduced appetite. Central and peripheral leptin resistance is commonly observed in obese youths, which upon weight reduction is normalized (Reinehr et al., 2005). Ghrelin is another peptide hormone which, unlike leptin stimulates the appetite and elevated in sleep deprivation, possibly explaining the associations of sleep and obesity (Spiegel et al., 2004).

2.1.4. Negative self image

Feelings of guilt and shame are the prominent characteristics of depressive episodes. In clinical report, depressed population between age group 11-20 years, reflected feeling of guilt, failure and self blame in both male and females, but more prominently in females than males (Bennett et al., 2005). Obesity has also been strongly associated with negative self image (Wardle and Cooke, 2005). Demographic characteristics like gender, weight cycling, and culture are the crucial moderators in the relationship between obesity-self-esteem, and depression-obesity (Petroni et al., 2007).

2.2. Relationship between obesity and common mental health disorder; depression

The relationship between obesity and common mental health disorders, such as depression and anxiety remains complex. The data from most of the meta-analysis/longitudinal studies, represented the bi-directional association between depression and obesity, stating that obese population are at risk (by 55%) for developing depression overtime, whereas, depressed patients are at risk (by 58%) for becoming obese (Luppino et al., 2010). Another, systemic longitudinal study suggested positive association of obesity and anxiety disorders (Gariepy et al., 2010).

The present study focuses on; (a) risk of developing depressive behavior in obese populations, (b) the underlying mechanisms involved, and, (c) the possibility of identification of novel therapeutic approach for the management of such co-morbid disorders. There are several mechanisms that could potentially explain the casual association between obesity and depression. This complex relationship is overviewed and simplified in **Fig 1.5** that showed two variables, i.e.,

(i) the moderator variable that might influence the strength of a relationship between two conditions

(ii) the mediator variables assists/help to explain the relationship between two conditions (Gatineau and Dent, 2011).

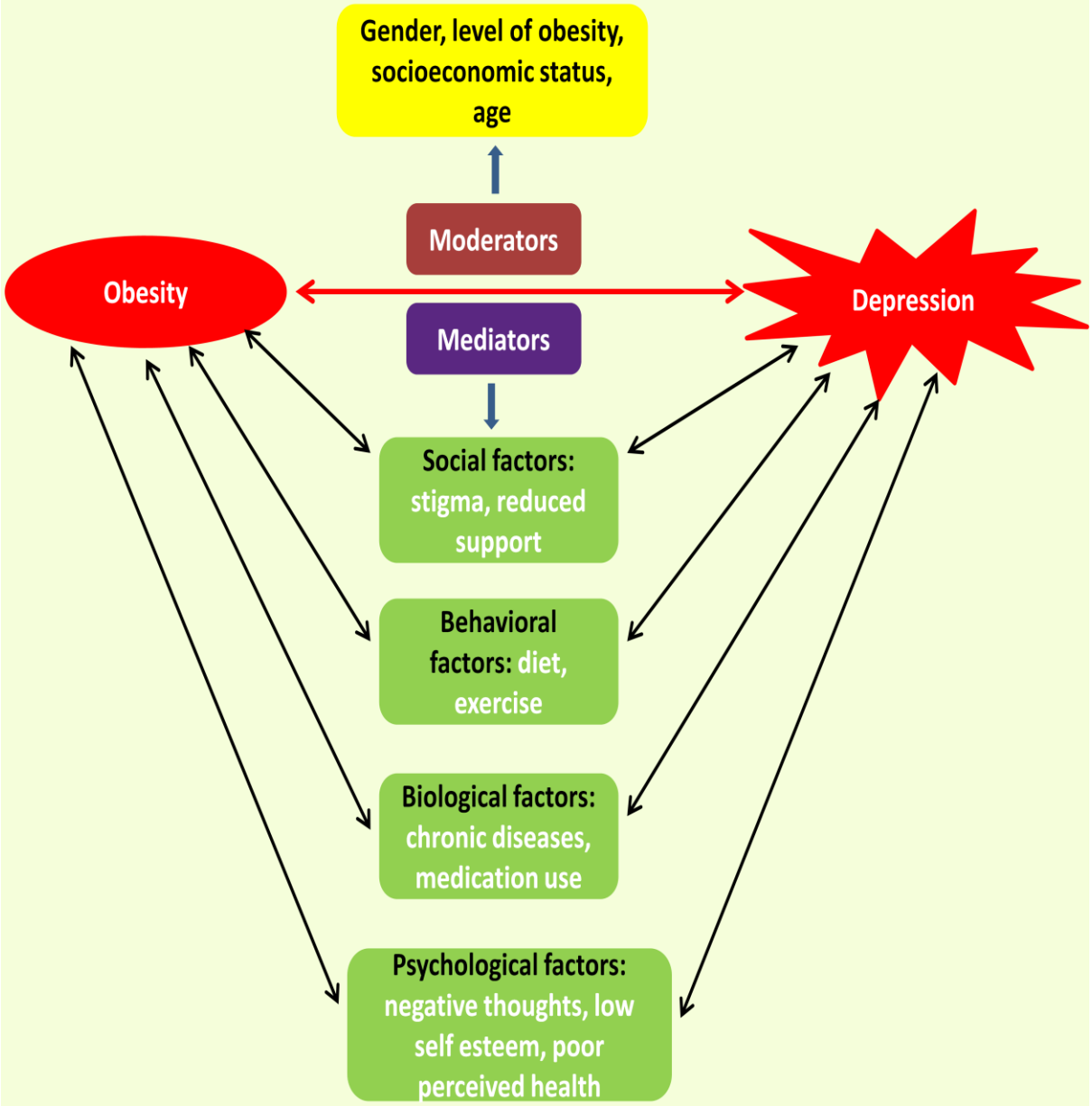


Fig 1.5: Representation of mediator/moderator relationship between obesity and depression *[Adopted from (Markowitz et al., 2008, Napolitano and Foster, 2008, Gatineau and Dent, 2011)].*

2.2.1. Mediators/mediating factors involved in obesity as cause of depression

Mediating factors relating to obesity and depression in adults and children include behavioral, biological, psychological and social factors. Various mediators involved in the pathways for developing obesity associated depression are mentioned in the following **Table 1.6** with behavior or symptoms. Mediators generally investigate the reasons and mechanisms of the agents such as age, socioeconomic status, ethnicity, and gender that affect the pathways involved in the development of obesity associated depression.

Table 1.6: Mediators/mediating factors involved in obesity as cause of depression

Mediators	Adult	Children
Behavioral	binge eating and dieting (Markowitz et al., 2008)	reduced physical activity (Mériaux et al., 2010), decreased interest in sports and athletics (Franklin et al., 2006), uncontrolled eating habits, unhealthy diet in high amount (Tanofsky-Kraff et al., 2004)
Biological	body pain (Atlantis and Baker, 2008), decreased physical activity (Markowitz et al., 2008), sleep disturbances (Aloia et al., 2005), higher rates of chronic disease (Markowitz et al., 2008), hormonal imbalance, side effects of medications (Luppino et al., 2010)	mainly disturbed hormonal pathways (Zametkin et al., 2004).
Psychological	low self-esteem (Vaidya, 2006), poorer perceived health (Markowitz et al., 2008), negative body image (Chen et al., 2009).	negative self image and reduced self esteem (Martyn-Nemeth et al., 2009), dissatisfaction of body, continues (Wardle and Cooke, 2005) and frequent perception of overweight (Ali et al., 2010).
Social	weight related issues, weight stigmas and weight bias (Markowitz et al., 2008), feeling of guilt, poor body image (Puhl and Heuer, 2009).	Weight stigma (Schwimmer et al., 2003), social withdrawal and isolation (Janssen et al., 2004), weight related teasing socially and among friends (Haines et al., 2006), social rejection (Eisenberg et al., 2003)

2.2.2. Moderators/moderating factors involved in obesity as cause of depression

Moderating factors as described below in **Table 1.7**, could affect the direction/strength of relationship between obesity and depression in adults and children include obesity level, gender, socioeconomic status and educational qualifications.

Table 1.7: Moderators/moderating factors involved in obesity as cause of depression

Moderators	Adult	Children
Obesity level	Severe obesity leads to higher risk for developing depression as accompanied with high depression score and lower self-esteem (Scott et al., 2008). Severe obesity leads to negative thoughts in life with respect to function, finance, social interaction, health and mobility (Vaidya, 2006).	Obesity level has less/no impact as a risk factor for relationship between childhood obesity and depression (Wardle and Cooke, 2005).
Sex/Gender	Women are more concerned to obesity as they experiences higher stigmas and dissatisfaction with their weight and shape than men (Atlantis and Baker, 2008)	Young girls are under more pressure with concern to weight than boys (Gray and Leyland, 2008).
Socioeconomic status and educational qualification level	Impact of socioeconomic status and education level on obesity remains unclear but it is fairly reported that people from low socioeconomic background experience negative impact of obesity on health related quality of life (Kinge and Morris, 2010). People of higher socioeconomic background are at increased risk of developing depression (Chen et al., 2009).	Socioeconomic status and educational qualification level does not appear to be a risk factor in the relationship between childhood obesity and depression. However, parental separation or unemployment in older adolescents have some impact on relationship between BMI and depression (Franklin et al., 2006).
Age	Age is an important moderator in depression and obesity. Young women are at high risk for obesity and depression (Van der Merwe, 2007). Older population due to ageing could also be at greater risk for obesity, depression and anxiety (Kivimäki et al., 2009).	Young children may have less impact of weight on friendship status. The risk increases as they move on from teenage to adolescent (Hill and Andrew, 2005).

2.3. Biological mechanisms linking/involved in depression co-morbid with obesity

Both obesity and depression have serious impact on the health related quality of life, self-esteem, and normal physiological functions. Affected population with both obesity and depression are always at higher risk with respect to health and well being as these co-morbid disorders could perpetuate one another at later stage, i.e., obesity may increase the risk for depression and depression may promote obesity. Hence, studying and understanding the underlying biological mechanisms that links obesity and depression is very important in order to design novel therapeutic strategies for individuals suffering from these co-morbid disorders. In order to understand the potential casual links between obesity and depression, in the earlier section the relationship between obesity and depression has been mentioned based on the clinical features, mediating factors and moderating factors.

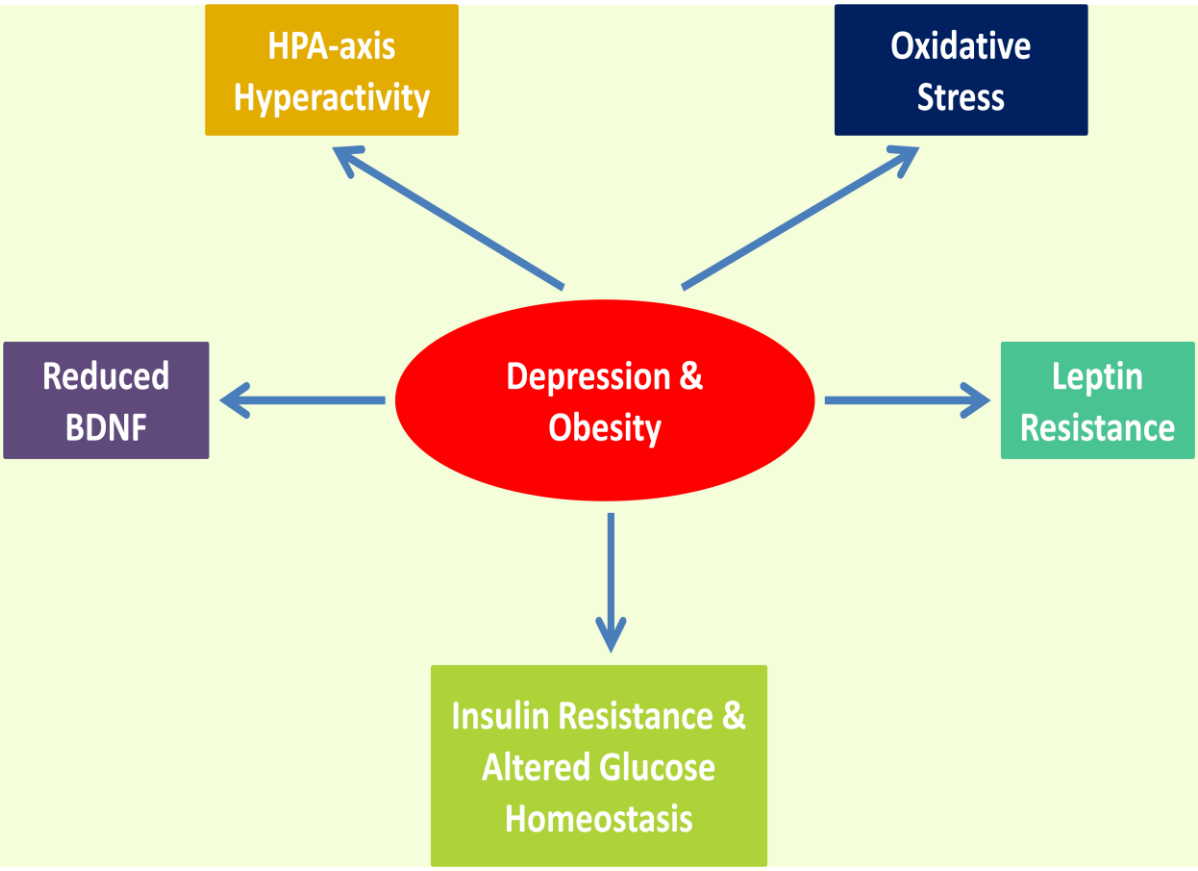


Fig 1.6: Biological mechanisms linking depression and obesity

Along with this information, to understand the casual mechanisms between obesity and depression one must study and understand the biological mechanisms linking these co-morbid conditions (Markowitz et al., 2008). Despite the advances in the Neuropsychopharmacological research, no clear biological mechanism has been drawn suggesting the pathological links between depression co-morbid with obesity (Sharma and Fulton, 2013). Some of the important biological mechanisms are outlined in **Fig 1.6**.

2.3.1. HPA axis hyperactivity

HPA axis is an important endocrinological axis of stress, that upon activation release corticosterone in rodents and cortisol in human (collectively referred as glucocorticoids) (Stetler and Miller, 2011). Various clinical studies have reported higher cortisol in depressive patients than the healthy volunteers (Stetler and Miller, 2011, Herbert, 2013).

HPA axis hyperactivity is characterized by elevated glucocorticoid due to altered circadian regulation of glucocorticoid secretion and the negative feedback mechanism mediated by the glucocorticoid receptors (GR) (Steiner et al., 2008). In depression, the negative feedback control is impaired in the hippocampus region of brain leading to excess glucocorticoids in the circulation due to dysregulation of HPA axis (Wulsin et al., 2010). Obesity is a pro-inflammatory stressful state associated with HPA axis hyperactivity thus, disturbing the negative feedback regulation of glucocorticoids (Bose et al., 2009).

Glucocorticoids inhibit the insulin receptors and have major impact on hyperplasia of beta-cells and insulin resistance due to deposition of visceral fat mass. A proportional relationship exists between severity of depression and insulin resistance, whereas, insulin resistance and thus, altered plasma glucose is well known in obesity (Qatanani and Lazar, 2007, Salim et al., 2010). A brief outline of HPA axis hyperactivity in depression co-morbid with obesity is demonstrated in **Fig 1.7**. High fat diet (HFD) feeding results in obesity, increased plasma total cholesterol, triglycerides and leads to several other metabolic complications including hormonal disturbances (Podrini et al., 2013). HPA axis dysregulation, leads to excess corticosteroids in the circulation that disturbs the lipid profile and blood glucose in obesity as well as depression (Mann and Thakore, 1999, Bray and Bellanger, 2006).

Hence, HPA axis hyperactivity remains one of the major common pathogenic factors shared by depression and obesity (Vreeburg et al., 2010, Waters and McCormick, 2011, Lucassen and Cizza, 2012).

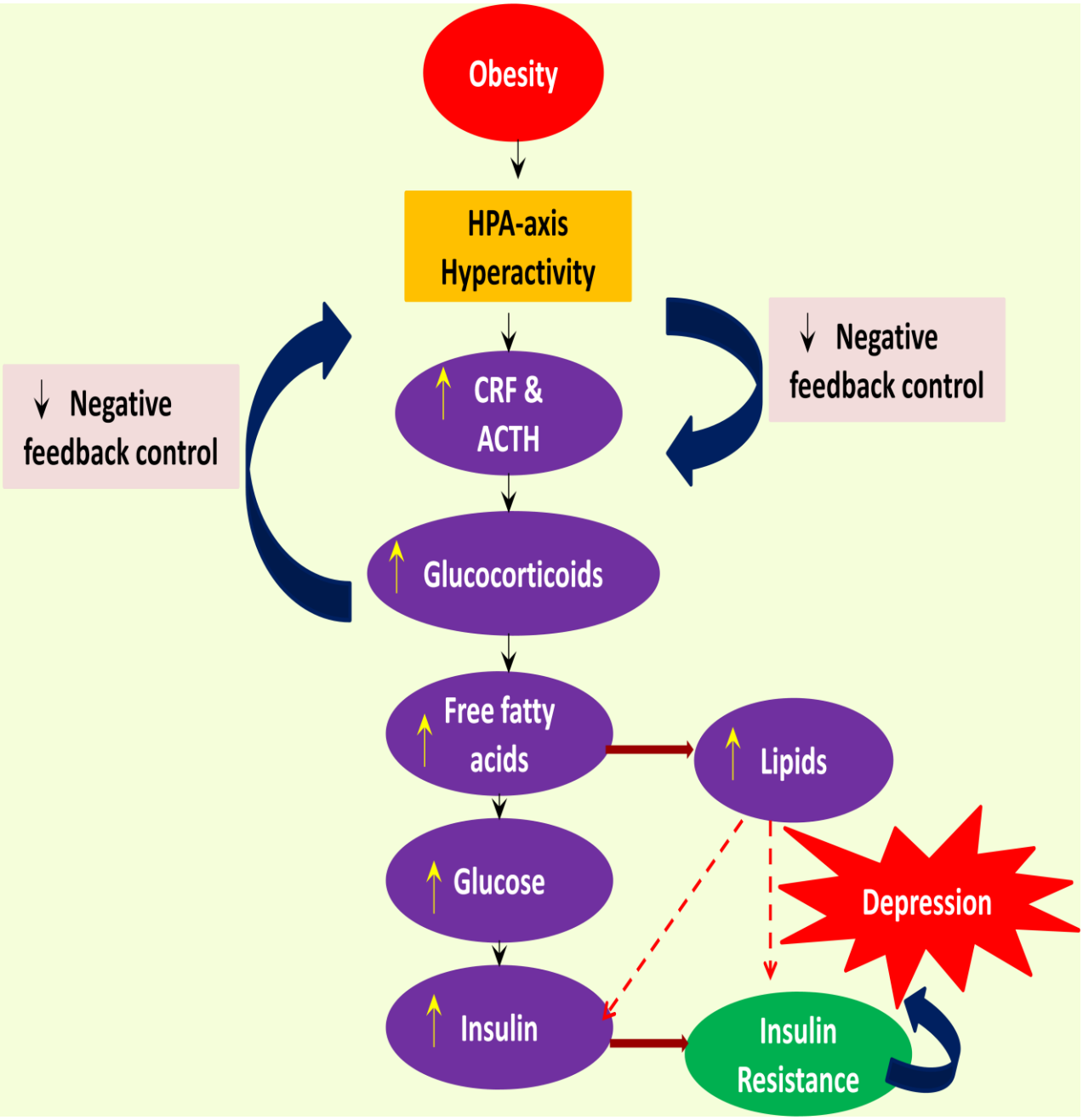


Fig 1.7: HPA axis hyperactivity in depression co-morbid with obesity

2.3.2. Oxidative stress

Oxidative stress induced damage, in the pathogenesis of depression and anxiety disorders, due to excessive glucocorticoids have been reported earlier (Liu et al., 1994, Schiepers et al., 2005, Chung et al., 2013). HPA axis dysregulation triggers oxidative stress, causing marked elevation of lipid peroxidation marker malonaldehyde (MDA), generated from free radicals, that damage the neuronal cells (Draper and Hadley, 1990). Oxidative stress is characterized by abnormally increased brain MDA and decreased anti-oxidant enzyme reduced glutathione (GSH) in depression. Obesity is a pro-inflammatory state that leads to increased oxidative stress marker MDA and diminished antioxidant enzyme GSH in the brain (Esposito et al., 2006).

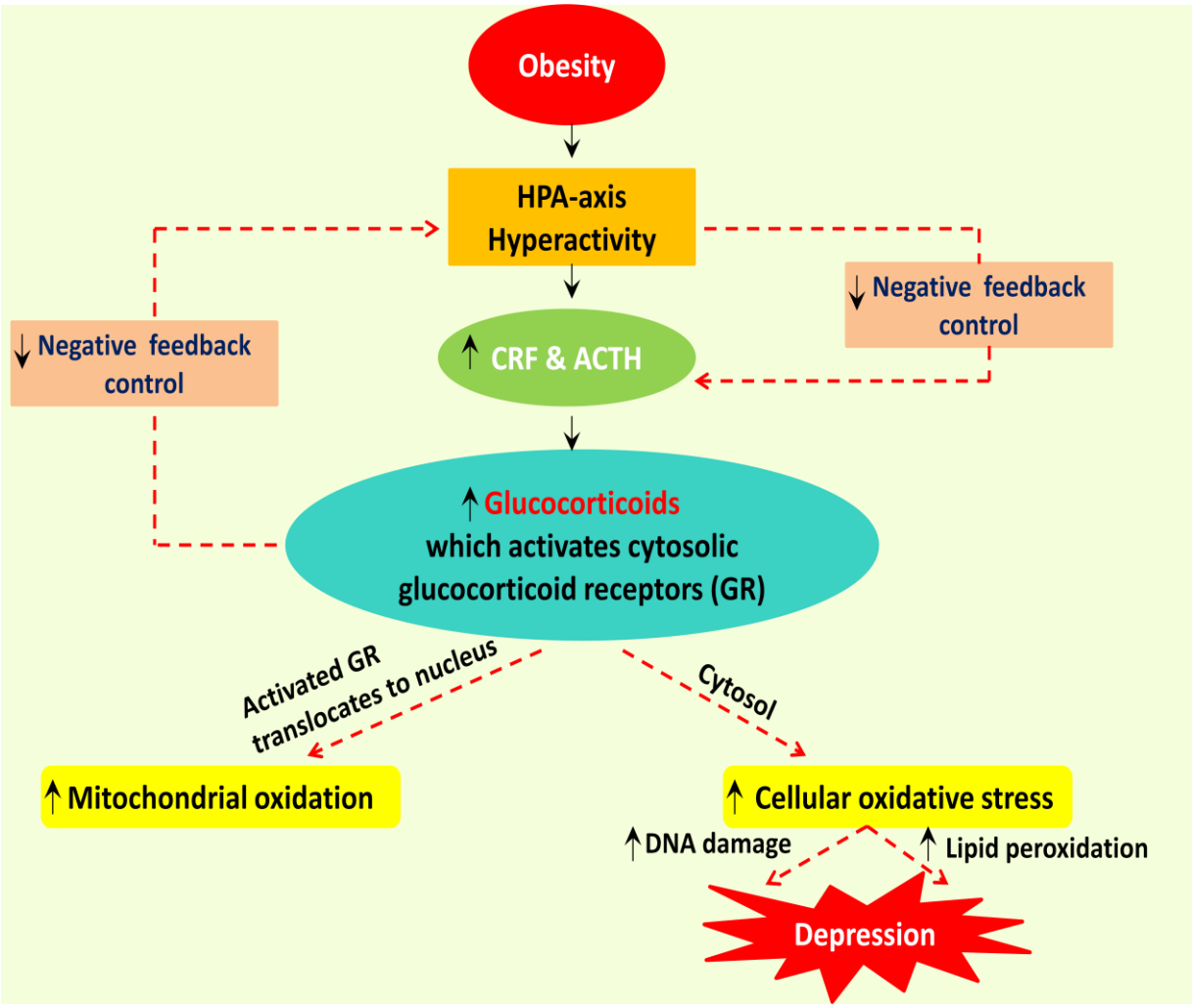


Fig 1.8: Oxidative stress in depression co-morbid with obesity [DNA- deoxyribonucleic acid]

As briefly depicted in the **Fig 1.8**, under stressful events, HPA axis becomes hyperactive and leads to excess release of glucocorticoids in the circulation, activate the GR in the cytosol, translocates in to the nucleus and modulate gene transcription which results in mitochondrial oxidation. On the other hand, outside the mitochondria in the cytosol, the superoxide is produced, which forms hydrogen peroxide upon dismutation. Hydrogen peroxide is neutralized by anti-oxidant enzymes such as catalase or reduced glutathione (GSH). Hydrogen peroxide can also interact with the superoxide radical to form hydroxyl radicals. This abnormally elevated production of hydrogen peroxide, superoxide and hydroxyl radicals leads to cellular oxidation that result in deoxyribonucleic acid (DNA) damage and increased membrane lipid peroxidation (Spiers et al., 2014).

2.3.3. Leptin hypothesis/resistance

Leptin is a peptide known as anti-obesity hormone secreted by adipose tissues in response to dietary fats. Chronic stress induced depression is a high predictive validity animal model of depression which showed diminished leptin levels (Willner, 2005, Lu et al., 2006, Guo and Lu, 2014). Rodents subjected to chronic stress showed significantly reduced sucrose consumption that was reversed by systemic administration of leptin (Lu et al., 2006). Clinical observation shows that dysregulation of leptin results in depression and anxiety disorders (Lawson et al., 2012).

Several clinical studies suggest a link between obesity and depression through the anti-obesity hormone leptin (Stunkard et al., 2003, Simon et al., 2006b, Kloiber et al., 2013). Leptin resistance in obesity are reported due to the defects in transport of leptin across blood brain barrier (BBB), altered function of leptin receptor and impairment in leptin signal transduction (Münzberg and Myers, 2005).

Moreover, leptin plays a role in regulation of 5-HT content and metabolism in the forebrain (Calapai et al., 1999, Hastings et al., 2002). Repetitive administration of leptin in ob/ob mice, reversed the hypercorticosteronemia accompanied with significant weight loss indicating the inverse relationship of leptin and glucocorticoids. This explains the role of leptin in regulation of HPA axis function independent of its effects on energy homeostasis (Stephens et al., 1995, Licinio et al., 1997). **Fig 1.9** describes the leptin resistance in obesity that could further lead to psychological disorder, depression.

Taken together, leptin hypothesis remains a crucial pathological link between the co-morbid obesity and depression that needs to be explored with a novel therapeutic approach.

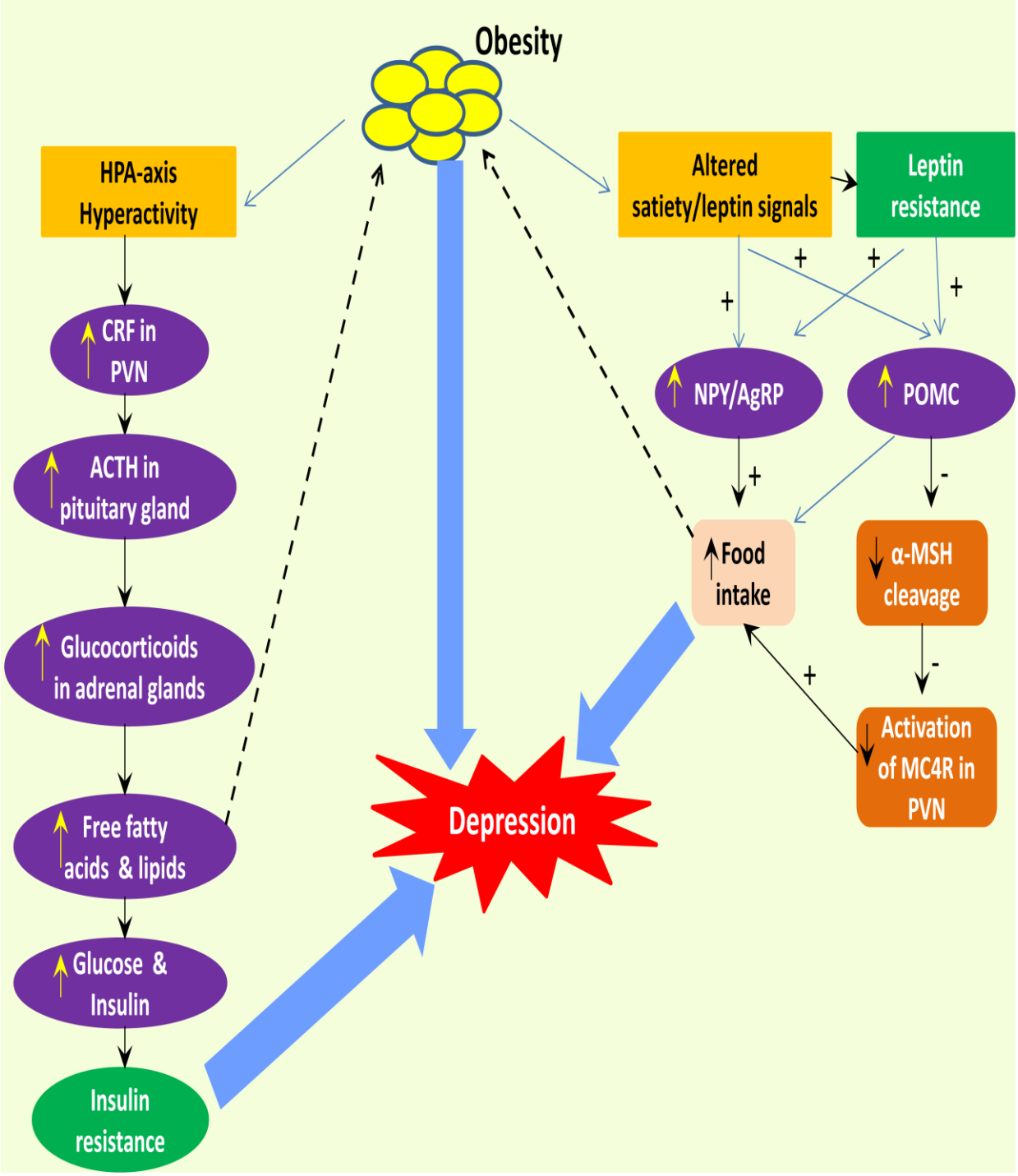


Fig 1.9: Leptin and Insulin resistance in depression co-morbid with obesity

2.3.4. Insulin resistance/altered glucose level

Insulin, a peptide hormone secreted by the beta-pancreatic cells, is responsible for glucose and lipid regulation (Saltiel and Kahn, 2001). In insulin resistance, increased insulin secretion from pancreatic cells or loss of insulin sensitivity, mainly results due to alterations in insulin receptors, insulin signaling and abnormalities in glucose transporter 4 (GLUT-4) (Quon, 2001, Jellinger, 2007). Insulin regulates several key processes of life such as energy homeostasis, reproduction, food intake and body weight (Vogt and Brüning, 2013). HPA axis hyperactivity results in abnormal cortisol release in the blood that causes insulin resistance or altered glucose level, which is observed in depressed and obese population, respectively (Brown et al., 2004, Almeida et al., 2009). Reduced insulin sensitivity or insulin resistance, is commonly associated with the pathogenesis of depression, anxiety and obesity (Adriaanse et al., 2006, Pan et al., 2008, Weber-Hamann et al., 2008, Salim et al., 2010, Hardy et al., 2012), as mentioned in **Fig 1.9**.

Insulin resistance in obesity, is due to the failure of excess insulin to normalize the raised plasma glucose (Qatanani and Lazar, 2007). Insulin resistance in obese population showed severe depressive behavior compared to non-obese individuals (Hannon et al., 2014). Clinical data supports the proportional relationship between insulin level and the severity of depression and antidepressant treatment regulates glucose homeostasis and reduces depression severity (Rosgan et al., 2002, Everson-Rose et al., 2004, McIntyre et al., 2006, Weber-Hamann et al., 2006, Pearson et al., 2010). Therefore, insulin resistance and glucose homeostasis are considered as one of the major pathological hallmarks linking biological mechanisms for depression co-morbid with obesity.

2.3.5. Brain derived neurotrophic factor (BDNF)

BDNF is an important regulatory protein that regulates neurogenesis, differentiation and preserves the viability of newly differentiated neurons through its high affinity receptor, tropomyosin-related kinase B (TrkB) (Tapia-Arancibia et al., 2004, Duman and Monteggia, 2006). BDNF and cyclic adenosine monophosphate (cAMP) are recognized as important biomarkers of depression as they play a central role in the synaptic plasticity (Duman and Monteggia, 2006) (Wuwongse et al., 2010). Reduced hippocampal volume and neuronal degeneration have been observed in postmortem brain samples of patients suffering from depression.

Depressive disorder involves neurodegeneration of hippocampus as characterized by reduced hippocampal volume due to reduced cAMP and BDNF in hippocampal dentate gyrus (DG) region (Martinowich and Lu, 2008, Wuwongse et al., 2010). Several clinical studies reported reduced serum and hippocampal BDNF expression, in depressive patients, which was reversed by chronic treatment with antidepressants (Bocchio-Chiavetto et al., 2010, Gass and Hellweg, 2010, Hashimoto, 2010).

Moreover, several animal studies with chronic stress model of depression have reported the diminished central BDNF expression (Smith et al., 1995, Ueyama et al., 1997) and BDNF administration has showed antidepressant-like effect in forced swim test (FST) and learned helplessness test (Siuciak et al., 1997).

Obesity is a stressful condition with elevated oxidative stress markers, inflammation, decreased neurogenesis factors that result in decreased neuronal volume in hippocampus region and neurodegeneration (Awada et al., 2013). A growing body of evidence suggested a pivotal role of BDNF in pathological processes leading to abnormal food intake and excessive weight gain (Rios, 2011). A pre-clinical study suggested that perturbing central BDNF signaling in mice leads to hyperphagia and development of obesity (Xu et al., 2003, Unger et al., 2007). In addition, BDNF level was altered by chronic feeding with HFD in rodents, thus showing the role of BDNF in energy homeostasis (Wang et al., 2010, Liu et al., 2014).

The above information suggests that BDNF has inhibitory effect on food intake and animal models showing BDNF disruption exhibit increased food intake and obesity (Gray et al., 2006), mechanism is briefly outlined in **Fig 1.10**. Therefore, BDNF can be considered as an important biomarker linking the biological mechanisms involved in depression co-morbid with obesity. It would be interesting to study and practice the therapeutic alternatives acting through modulation of BDNF pathways in depression co-morbid with obesity.

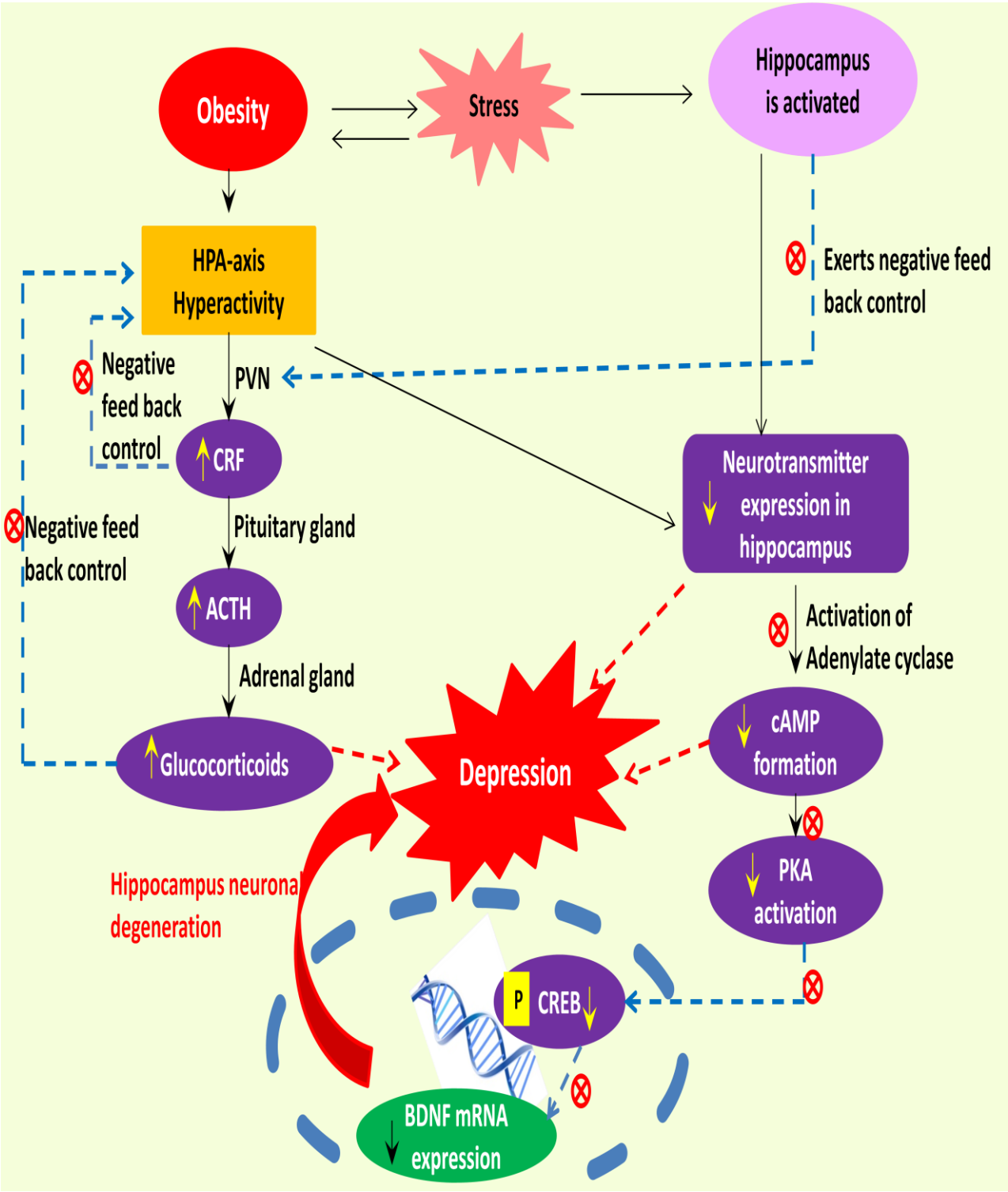


Fig 1.10: BDNF in depression co-morbid with obesity [cAMP- cyclic adenosine monophosphate; CREB-cAMP response element binding protein, PKA-protein kinase A, BDNF-brain derived neurotrophic factor, mRNA-messenger ribonucleic acid]

2.4. Current class of antidepressants with common side effects

Following **Table 1.8** briefs the currently used different class of antidepressants and their common side effects (Paykel, 1995, Ferguson, 2001, Montejo et al., 2001, Warner et al., 2006, Stone et al., 2009, Santarsieri and Schwartz, 2015).

Table 1.8: Current class of antidepressants and their common side effects

Class	Commonly used antidepressants	Common side effects
Tricyclic antidepressants (TCAs)	Imipramine, desipramine, amitriptyline	Dry mouth, blurred vision, cognitive & memory impairment, sexual dysfunction, anxiety, emotional blunting, tachycardia
Monoamine oxidase inhibitors (MAOIs)	Tranylcypromine, phenelzine, selegeline	Hypertensive crisis, withdrawal syndrome, weight gain, anxiety, fatigue, weakness, dizziness, headache
Selective serotonin reuptake inhibitors (SSRIs)	Fluoxetine, sertraline, paroxetine, citalopram, escitalopram	Sleep disturbance, sexual dysfunction, appetite changes, nausea, headache, dryness of mouth
Noradrenaline reuptake inhibitors (NRIs)	Reboxetine, amedalin, nisoxetine, talopram	Sleep problem, dry mouth, nausea, excessive sweating, anxiety, sexual dysfunction
Serotonin-Noradrenaline reuptake inhibitors (SNRIs)	Venlafaxine, duloxetine	Sexual dysfunction, loss of appetite, weight gain, sleep, fatigue headache, urinary retention, nausea, vomiting
Noradrenaline-Dopamine reuptake inhibitors (NDRIs)	Bupropion	Appetite changes, dry mouth, tachycardia, anxiety, dizziness, nervousness, sore throat, skin rashes
Noradrenergic and specific serotonergic antidepressants (NaSSA)	Mirtazapine	Weight gain, drowsiness, hypercholesterolemia
Serotonin antagonists and reuptake inhibitors (SARIs)	Etoperidone, trazodone	Nausea, sedation, priapism
Triple reuptake inhibitors (TRIs)	Mazindol, nefazodone, nefopam	Urinary retention, dry mouth, nervousness, tachycardia, sedation, allergic reactions, blurred vision, weakness
Tetracyclic antidepressants	Mianserin, amoxepine	Sedation, dry mouth, dizziness, weigh gain, vertigo

2.5. Limitations of the current antidepressant therapy

2.5.1. Modest efficacy

In any therapeutic approach, efficacy of the therapeutics/ligands, is always very crucial. Mood disorder research, has progressed through several meta-analysis studies, suggesting defining response and remission rate, being more clinically meaningful than the standard rating scale such as the Hamilton scale (Keller, 2004). The antidepressant class TCAs exhibited modest efficacy, as they showed 46% response rate against 31% with placebo (Storosum et al., 2001). Another meta-analysis study, reported 80% of the medication response, was duplicated in the placebo control groups (Kirsch et al., 2002).

The meta-analysis study have highlighted the clinical efficacy of SSRIs compared to placebo (Arroll et al., 2005). However, SSRIs did not offer any significant superior efficacy over TCAs, in several randomized trial studies (Williams et al., 2000, MacGillivray et al., 2003). Venlafaxine belonging to SNRIs class, showed greater efficacy in depressed patients, compared to fluoxetine belonging to SSRIs class, probably due to higher dose (150 mg) of venlafaxine (Montgomery and Andersen, 2006). Meta-analysis study have confirmed the better efficacy of escitalopram (ESC), over citalopram and other antidepressants, in case of depressive patients (Montgomery and Andersen, 2006).

2.5.2. Suicidality

Suicidality remains an important issue and controversy with the efficacy of current antidepressants. Reduced suicide rates with antidepressant treatment have increased the sale of antidepressants (Angst et al., 2005, Ludwig and Marcotte, 2005). In a major study with 65000 depressive cases, evaluation of suicidal phenomenon revealed that suicidal attempt reduced significantly with antidepressant treatment (Simon et al., 2006a). On the contrary, it was said that suicidal attempt could prompt the initiation of treatment, that results further, in declined suicidal attempts. Hence, the risks of attempted and completed suicide could be affected differently with antidepressant treatment. In a cohort study in Finland, including 15000 patients hospitalized for suicide attempt, in a follow up period of approximately 3.5 years, found that use of current antidepressants were effective in reducing the completed suicidal rates but were associated with markedly increased risk of attempted suicide (Tiihonen et al., 2006).

In United States, a comparative study highlighting the risk associated with different antidepressants, suggested that SSRIs showed lower suicide rates than other class of antidepressants whereas, a positive association between TCAs and suicidal rate was observed (Gibbons et al., 2005). Another study in the United Kingdom, suggested that suicidal risk was different for SSRIs compared to TCAs (Martinez et al., 2005). This could be due to prescription of different antidepressant to patients with different suicide risks and also created little doubt regarding higher safety of SSRIs in overdoses than TCAs (Cheeta et al., 2004). Hence, despite several treatments available, it is still not concluded that antidepressants reduced suicidal risks in depressive patients.

2.5.3. Tolerability

Tolerability remains an important concern of the currently used antidepressant therapy. TCAs are associated with a broad range of adverse effects as they have multiple pharmacological actions whereas SSRIs are associated with fewer adverse effects as they have a controlled range of actions. Various meta-analysis and survey studies have described the limitations of antidepressant in terms of tolerability. Williams and colleagues reported that TCAs are associated with more frequent dizziness, dry mouth, tremors, constipation, blurred vision, and urinary disturbances whereas SSRIs are associated with more frequent diarrhoea, headache, nausea, and insomnia (Williams et al., 2000). Common adverse events associated with antidepressants treatment for long-term, includes weight gain issue, sexual dysfunction, fatigue, cognitive impairment and sleep disturbances (Cassano and Fava, 2004).

2.5.4. Slow onset of response

One of the major limitations of antidepressants remains slow onset of efficacy that is achieved after 6 weeks of therapy. This delay in the clinical benefit results in risk of suicide in early antidepressant therapy showing non-adherence to therapy (Lader, 2007). However, as the delayed onset of response has become a challenge in antidepressant therapy, around 47 studies have suggested that 60% of the total improvement in patients occurs in first 2 weeks of therapy (Posternak and Zimmerman, 2005), while some showed clinical benefit in 1 week of therapy (Taylor et al., 2006). When the time for onset of response is considered, it is always important to distinguish between the time at which first benefit was achieved and the point at which maximal efficacy was attained (Lader, 2007).

Several antidepressants differ among themselves with respect to onset of response such as ESC shows faster onset of action than citalopram (Taylor et al., 2006), mirtazepine shows quicker action than venlafaxine (Benkert et al., 2006).

2.5.6. Withdrawal symptoms

Withdrawal symptoms with antidepressants are known for many years now. Discontinuation symptoms such as fatigue, dizziness, nausea, vomiting, tremor, anxiety, gait instability, headache and insomnia usually begin after 1-3 days of treatment withdrawal with SSRIs that last for around 10 days (Price et al., 1996, Black et al., 2000). Discontinuation symptoms with TCAs and MAOI generally are similar to those of SSRIs with some differences; mainly balance problem and parkinsonian signs observed with TCAs, and aggressiveness, psychotic symptoms, cognitive impairment with MAOI discontinuation (Warner et al., 2006).

In order to remember and rapid recognition of withdrawal symptoms with antidepressants, a collective term called 'FINISH' was coined, that represented flu-like symptoms, insomnia, nausea, imbalance, sensory disturbances, hyperarousal (Warner et al., 2006). In general, discontinuation symptoms may result into discomfort, absence from work, sometimes hospitalization that may give rise to chances of relapses. In addition, the suicidal tendency increases by nearly five-fold, post discontinuation of SSRIs and TCAs therapy (Yerevanian et al., 2004). This ultimately represents the protective effect of SSRIs and TCAs against the suicidal tendency of severe MDD patients.

2.5.7. Compliance

One of the major concerns with antidepressant therapy is non-compliance. Several meta-analysis and epidemiological studies represented the widely varied adherence 30% and 90%, 41% (Pampallona et al., 2002, Masand, 2003). Adverse effects could be only one of the factors affecting the compliance as SSRIs do not show better compliance over TCAs in clinical trials (Anderson and Tomenson, 1995). Personality and psychiatric characters are very crucial as extraversion is recognized as negative character of compliance and early SSRIs withdrawal is predicted by patient skepticism (Aikens et al., 2005).

2.5.8. Sleep disturbances and depression

Sleep disturbance is an important symptom of depression, observed in 50%-90% of the patients (Krupinski and Tiller, 2001, Almeida and Pfaff, 2005). Sleep disturbances, mainly represent restlessness/wakefulness during night, which leads to poor quality of life, low productivity with respect to work, day time drowsiness, increased risk of accident and suicidal behaviors (Ağargün et al., 1997, Léger et al., 2002, Mayers et al., 2003). Depressed patients often experience decreased latency to first rapid eye movement (REM) sleep, increased REM sleep duration, decreased slow wave activity experienced during non-REM sleep overall reducing non-REM sleep time causing poor quality of sleep (Tsuno et al., 2005). Hence, proper sleep cycle in the form of REM and non-REM sleep phases, is very important in the normal physiology and well being, of the individuals.

TCAs particularly improve the sleep continuity and efficiency, sometimes may lead to day time sedation, inhibit REM sleep, improve REM sleep latency, decrease the REM sleep proportion and accelerate slow wave sleep (Lader, 2007). Early treatment with SSRIs results in sleep disturbances that represents the major limitation of SSRI treatment. Results with SSRIs are different for different drugs such as fluoxetine and paroxetine disturbs the sleep at higher rate by reducing the sleep continuity and efficiency whereas sertraline and citalopram has minimal effect on sleep alterations, and ESC improves the sleep quality (Lader et al., 2005, Lader, 2007). MAOIs like phenelzine and tranylcypromine inhibits REM sleep, disturbs sleep continuity resulting in insomnia (Lader, 2007).

In brief, this section provide selective information on; depression, anxiety, co-morbid relationship of depression and anxiety, obesity, relationship of obesity and neuropsychiatric disorders, co-morbid association of obesity and depression, similarities and biological pathways involved in depression and obesity, current antidepressant therapy and their side effects and limitations.

Taken together, considering the growing prevalence rate of depression associated with obesity, it is immense need of the time to undertake studies specifically dealing with biological mechanisms involved in depression associated with obesity and to develop novel therapeutic strategy to combat such co-morbid disorders.

2. REVIEW OF LITERATURE

2.1. Shift in the pharmacological perspectives for depression

Various classes of antidepressant drugs have been utilized in the management of depressive behavior in the affected population. Almost nine classes (as seen in 2.4 section of introduction) of drugs are known for the treatment of depression. Most of the antidepressants (six out of nine) involve serotonergic transmission as mechanism in the treatment of depression as mentioned in **Table 2.1** (Rajkumar and Mahesh, 2008).

Table 2.1: Various antidepressant class acting through seronergic system

Class	Function/mechanism
Selective serotonin reuptake inhibitors (SSRIs)	Inhibits serotonin transporters
Serotonin-norepinephrine reuptake inhibitors (SNRIs)	Inhibits serotonin transporters
Serotonin antagonist and reuptake inhibitors (SARI)	Inhibits serotonin transporters and 5-HT_2 receptor antagonism that reduces treatment associated side effects
Noradrenergic and specific serotonergic (NaSSA) antidepressant	Apart from blocking adrenergic α_2 receptors, also antagonizes 5-HT_2 and 5-HT_3 receptors
Monoamine oxidase inhibitors (MAOIs)	Inhibits 5-HT degradation in synaptic cleft
Serotonin reuptake enhancers (SREs)	Reverse mechanisms of reuptake inhibitors, additionally raises the susceptibility of 5-HT to MAOIs

These altogether suggest an important role of serotonergic system in treatment of depression. This also hints the role of different 5-HT receptors in the management of mental health disorders such as depression and anxiety.

2.2. 5-HT receptor types

5-HT, is an important neurotransmitter that produces its pharmacological effect by acting on the various membrane bound 5-HT receptors present in CNS, peripheral nervous system (PNS), along with some non-neuronal sites mainly gut, cardiovascular system (CVS), and blood.

5-HT neurotransmitter has been found to be involved in pathophysiology of several diseases including depression, anxiety, Schizophrenia, migraine, eating disorders, vomiting, pulmonary hypertension and irritable bowel syndrome (Hoyer et al., 2002). 5-HT receptor family is vast family comprising of 5-HT_1 to 5-HT_7 receptors having several subtypes for each 5-HT_1, 5-HT_2 and 5-HT_3 receptors. All receptors belonging to 5-HT system except 5-HT_3 which is ion channel ligand gated receptor belong to G-protein coupled receptor (GPCR) family. Various 5-HT receptor subtypes with their location, drugs acting on them, blockers, second messenger mechanisms and their antidepressant-outcome effect are mentioned in the **Table 2.2** given below.

2.3. 5-HT receptors as novel target for depression

5-HT receptor family is vast and they are located in discrete brain regions. Earlier studies had reported the role of serotonergic system in depression and antidepressant drug action (Schechter et al., 2005, Rajkumar and Mahesh, 2008).

2.3.1. 5-HT_1 receptor

2.3.1.1. 5-HT_{1A} receptor

5-H_{1A} is an autoreceptor present throughout CNS. The postsynaptic receptors are majorly present in the hippocampus, frontal cortex and amygdala in rodents. Activation of somatodendritic autoreceptor 5-HT_{1A} inhibits the neuronal firing, 5-HT synthesis and release in the raphe nuclei (Barnes and Sharp, 1999). 5-HT_{1A} postsynaptic receptor activation has been reported for the antidepressant effect of 5-HT_{1A} receptor agonist such as buspirone through secondary messenger system (Newman et al., 1992). 5-HT_{1A} receptor selective agonist, 8-hydroxy-2-(din-propylamino)tetralin (8-OH-DPAT), leads to hypothermia and by acting on postsynaptic receptors, 8-OH-DOPAT exhibit its antidepressant effect (Goodwin et al., 1985, Rajkumar and Mahesh, 2008). Several animal studies have reported antidepressant effect of 5-HT_{1A} receptor agonist (Singh and Lucki, 1993, O'Neill and Conway, 2001).

Interaction studies have confirmed the potentiation of antidepressant effect of serotonergic agents, by blocking the $5HT_{1A}$ autoreceptor, suggesting that the antidepressant effect of SSRIs, could be mediated through activation of $5\text{-}HT_{1A}$ postsynaptic receptors (Romero et al., 1996, Tatarczyñska et al., 2002).

Table 2.2: 5-HT receptor sub-types with location, agonist, antagonist, signaling pathway and modulation of antidepressant-outcome

Type	Location	Agonist	Antagonist	Signaling pathways	Antidepressant-outcome
$5\text{-}HT_{1A}$	Hippocampus, cortex, raphe nuclei	Buspirone, 8-OH-DOPAT	Way 100635	↓ cAMP	selective agonism (full/partial)
$5\text{-}HT_{1B}$	Striatum, nucleus accumbens, ventral tigmental area (VTA)	Anpirtoline, CP 94253	SB 224289, GR 127935	↓ cAMP	Antagonism
$5\text{-}HT_{2A}$	Hippocampus, frontal cortex, basal ganglia	DOI	Ketanserin, M 100907	IP3 & DAG	Antagonism
$5\text{-}HT_{2C}$	Frontal cortex, olfactory structures, amygdala, hippocampus	m-CPP, R0 600175	Mesulergine, SB 200907	IP3 & DAG	Agonism
$5\text{-}HT_{3}$	Hippocampus, frontal cortex, amygdala	mCPBG, 2-CH3-5-HT	Ondansetron Tropisetron	Ion channel	antagonism
$5\text{-}HT_{4}$	Nigrostriatal & mesolimbic regions, nucleus accumbens, cortex	BIMU-8, RS 67333, Cisapride	GR 113808, SB 204070	↑ cAMP	antagonism
$5\text{-}HT_{6}$	Striatum, cortex, hippocampus, hypothalamus	EMDT, WAY181187, WAY 208466	SB 399885	↑ cAMP	Agonism
$5\text{-}HT_{7}$	Thalamus, limbic & cortical regions	8-OH-DPAT	Amisulpiride, SB 269970	↑ cAMP	Antagonism

2.3.1.2. 5-HT_{1B} receptor

Another subtype of 5-HT_1 receptor is 5-HT_{1B} receptor, known to be presynaptically localized in nucleus accumbens, caudate putamen and dorsal raphe nucleus (Sari et al., 1999). Activation of 5HT_{1B} receptor through agonist such as RU 24969, reduced the hippocampal 5-HT release and antagonising presynaptic 5-HT_{1B} receptors improved the SSRIs mediated 5-HT release in rodents (Davidson and Stamford, 1997). Overall, it is observed that 5-HT_{1B} agonists, result in depression-like behavior and 5-HT_{1B} antagonists, result in antidepressant-like effect (Rajkumar and Mahesh, 2008).

2.3.1.3. 5-HT_{1D} receptor

5-HT_{1D} receptor shows 63% structural homology with 5-HT_{1B} receptor with very low expression level compared to 5-HT_{1B} and hence, the functional role of 5-HT_{1D} receptors remains ill-understood (Hoyer et al., 2002). 5-HT_{1D} receptors are co-localized with 5-HT_{1B} in dorsal raphe, cerebellum and olfactory tubercle (Bonaventure et al., 1997). Moreover, dorsal raphe 5-HT_{1D} receptors are localized presynaptically (Roberts and Price, 2001). In a pre-clinical study with non-selective 5$HT_{1B/1D}$ receptor antagonist, showed facilitation of extracellular 5-HT suggesting that the antagonism of 5-HT_{1D}, may improve the onset of antidepressant action (Rajkumar and Mahesh, 2008).

2.3.2. 5-HT_2 receptor

2.3.2.1. 5-HT_{2A} receptor

5-HT_{2A} receptors are present in cortex, basal ganglia, hippocampus, amygdala, striatum and olfactory structures, mostly co-localized with 5-HT_{1A} receptors (Xu and Pandey, 2000). Most of classes of antidepressants down regulate 5-HT_{2A} receptors especially in hippocampus and frontal cortex as shown by desipramine in olfactory bulbectomized (OBX) animals (Goodwin et al., 1984, Mudunkotuwa and Horton, 1996). 5-HT_{2A} antagonism, result in potentiation of antidepressant effect of SSRIs and various 5-HT_{2A} antagonists have shown antidepressant effect in several pre-clinical studies (Patel et al., 2004, Marek et al., 2005).

2.3.2.2. 5-HT_{2C} receptor

5-HT_{2C} receptors are expressed in hippocampus, amygdala, olfactory components, substantia nigra, cingulated and piriform cortex. Several antidepressants such as mainserin, mirtazapine, trazadone, fluoxetine show good affinity for 5-HT_{2C} receptors (Hannon and Hoyer, 2008). Evidence based studies confirmed the antidepressant activity of 5-HT_{2C} receptor agonist in behavioral assays like forced swim test (FST), social stress model, OBX model of depression (Cryan and Lucki, 2000, Rosenzweig-Lipson et al., 2007). Moreover, behavioral effects of fluoxetine in FST were inhibited by selective 5-HT_{2C} receptor antagonist SB206533 (Cryan and Lucki, 2000). The antidepressant effect of 5-HT_{2C} receptor antagonist mianserin was believed due to alpha 2 adrenergic receptor antagonism (Cryan and Lucki, 2000).

2.3.3. 5-HT_3 receptor

5-HT_3 receptor is only the ligand gated ion channel type in the 5-HT receptor family. Central 5-HT_3 receptors are widely distributed in dorsal vagal complex, hippocampus, cortex and amygdala (Hannon and Hoyer, 2008). Evidence based studies have identified 5-HT_3 receptor as a potential target for antidepressant effect in laboratory animals. Selective 5HT_3 receptor antagonists such as ondansetron (OND) and tropisetron produced antidepressant-like effect in several animal models of depression, and in interaction studies, OND potentiated the antidepressant effect of SSRIs (Redrobe and Bourin, 1997, Mahesh et al., 2007, Rajkumar and Mahesh, 2010). Additionally, the antidepressant effect of 5-HT_3 receptor antagonists were blocked by 5-HT_3 receptor agonist mCPBG in rodents (Nakagawa et al., 1998). The antidepressant effect of SSRIs was observed through functional antagonism of 5-HT_3 receptor (Eisensamer et al., 2003).

2.3.4. 5-HT_4 receptor

5-HT_4 receptors are mainly located in nigrostriatal and mesolimbic regions, septum, hippocampus, and amygdala regions of the brain (Hannon and Hoyer, 2008). 5-HT_4 receptor agonist, RS 67333 produced antidepressant effect in acute and chronic rodent models of depression. 5-HT_4 receptor agonist leads to desensitization of presynaptic autoreceptor 5-HT_{1A} that leads to phosphorylation of CREB and thus hippocampal neurogenesis (Lucas et al., 2007). However, 5-HT_4 receptor agonist RS 67333 when combined with fluoxetine, citalopram potentiated the antidepressant effect.

5-HT_4 receptor antagonist SB 204070A, did not showed any effect in FST and when combined with fluoxetine did not altered the antidepressant effect of fluoxetine in FST (Cryan and Lucki, 2000, Lucas et al., 2010).

2.3.5. 5-HT_6 receptor

The principal area of brain where 5-HT_6 receptors are located includes striatum, cortex, amygdala, hippocampus, hypothalamus, thalamus and cerebellum (Hannon and Hoyer, 2008). 5-HT_6 receptor agonist EMDT showed antidepressant activity in tail suspension test (TST) rodent model of depression whereas, antagonist SB 271046 attenuated the antidepressant effect of EMDT and fluoxetine (Svenningsson et al., 2007). Moreover, 5-HT_6 receptor agonist, WAY-1811187 and WAY-208466 showed antidepressant effect in FST and anti-anxiety effect in marble burying test (Carr et al., 2011). However, 5-HT_6 receptor antagonists showed antidepressant-like effect through non-serotonergic mechanisms and facilitating the release of other neurotransmitters, mainly nor-adrenaline (NA) and dopamine (DA) (Wesolowska, 2007).

2.3.6. 5-HT_7 receptor

5-HT_7 receptors are densely located in thalamus, limbic and cortical regions of brain (Hannon and Hoyer, 2008). 5-HT_7 receptors are involved in depression as confirmed from a study in which chronic fluoxetine injection reduced binding sites on 5-HT_7 receptors and acute restrain stress raised the hippocampal 5-HT_7 receptor mRNA expression in rodents (Sleight et al., 1995, Yau et al., 2001). Pre-clinical studies reported the antidepressant effect of 5-HT_7 receptor knockout animals or administration of 5-HT_7 receptor blockers in high predictive validity model of depression (Guscott et al., 2005, Wesołowska et al., 2006). One of the studies suggested that 5-HT_7 receptor antagonist administration potentiated the antidepressant effect of citalopram in TST and REM sleep in rodents (Bonaventure et al., 2007). Moreover, genetic deletion of 5-HT_7 receptors in mice, showed antidepressant-like effect in FST, TST and REM sleep further confirming the involvement of antagonism of 5-HT_7 receptors in antidepressant activity (Hedlund et al., 2005).

2.4. 5-HT$_3$ receptors

2.4.1. Structure of 5-HT$_3$ receptor

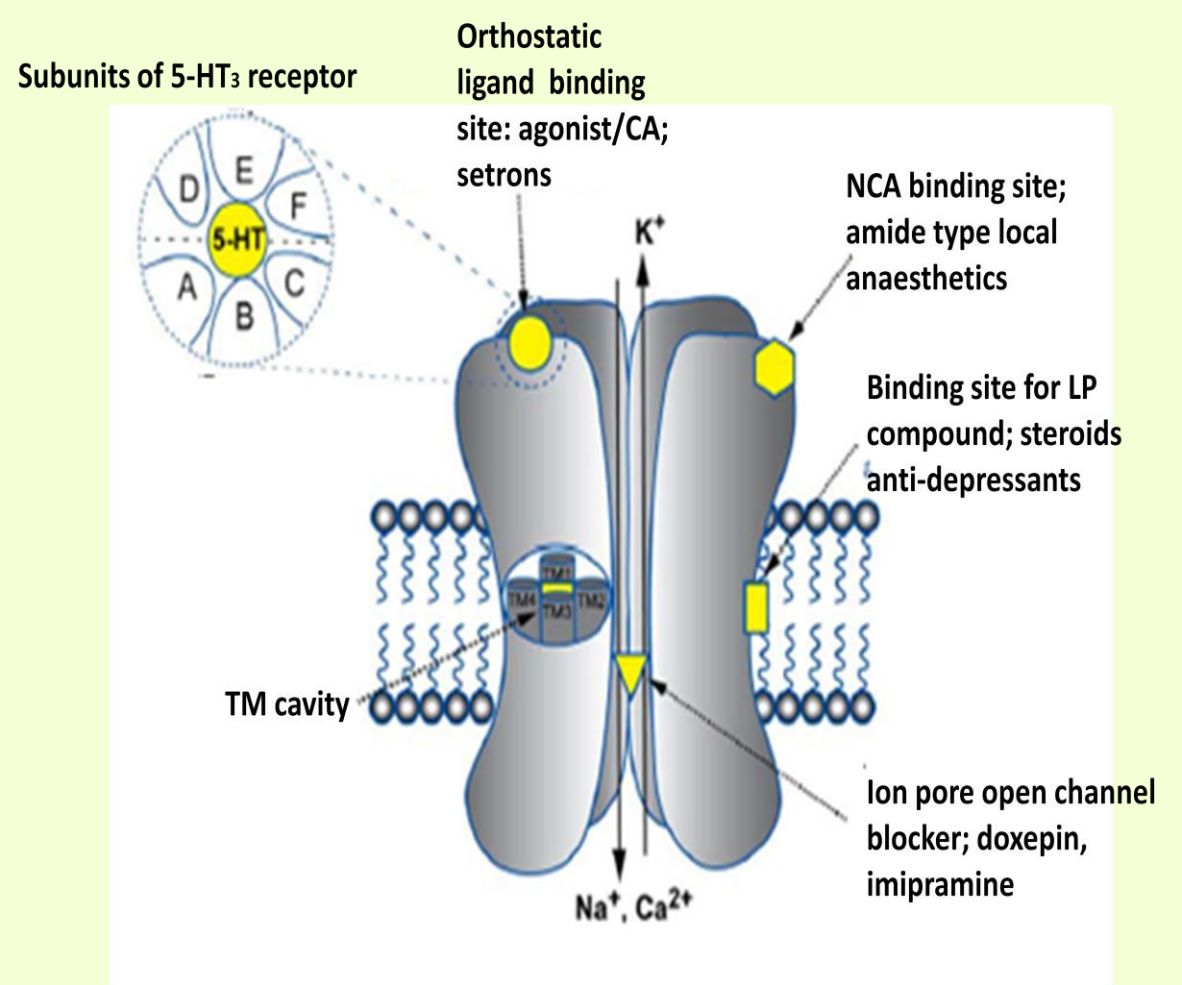


Fig 2.1: Structure of 5-HT$_3$ receptors; CA-competitive antagonist; NCA- noncompetitive antagonist; LP-lipophilic drugs; TM-trans membrane *[Adopted from (Walstab et al., 2010)]*

5-HT$_3$ receptor is a ligand gated ion channel coupled receptor (**Fig 2.1**) belonging to the cys-loop receptor superfamily as like gamma aminobutyric acid (GABA), glycine and nicotinic acetylcholine receptor (nAChR). 5-HT$_3$ receptors are composed of five symmetrically arranged subunits surrounding the ionic pore which is crossed by cations (Maricq et al., 1991). In rodents, two main subunits cloned successfully are 5-HT$_{3A}$ and 5-HT$_{3B}$ that are arranged in homomeric 5-HT$_{3A}$ receptors and heteromeric 5-HT$_{3A}$/5-HT$_{3B}$ receptors form (Davies et al., 1999, Dubin et al., 1999). Genes for other subunits such as 5-HT$_{3C}$, 5-HT$_{3D}$ and 5-HT$_{3E}$ are described in multiple mammalian species but not in rodents, usually forms heteromeric receptors with 5-HT$_{3A}$ subunit (Holbrook et al., 2009).

2.4.2. Distribution of 5-HT_3 receptors in brain

5-HT_3 receptors are localized in hippocampus, cortex, substantia nigra, nucleus accumbens, ventral tigmental area, area postrema and nucleus tractus solitarius (Tecott et al., 1993). 5-HT_3 receptors are more abundantly found in hindbrain regions than forebrain. Interestingly, significant proportion of 5-HT_3 receptors are found in the brain areas involved in the pathophysiology of depression mainly hippocampus, amygdala and cortex with different densities in various species (Bétry et al., 2011). Apart from these, significant amount of 5-HT_3 receptors are located on pre-synaptic nerve terminals (Waeber et al., 1988, Miquel et al., 2002) especially cortex and amygdala regions, whereas in hippocampus, postsynaptic receptors are majorly localized (Rondé and Nichols, 1998, Miquel et al., 2002). All the 5-HT_3 receptors in CNS and PNS contain an A subunit, whereas the distribution of B subunit remains unclear. Results of immunohistochemistry (IHC) studies have confirmed presence of 5-HT_{3B} receptor subunit in PNS only. However, in situ-hybridization studies, have shown the presence of 5-HT_{3B} receptor subunit mRNA expression in human brain and rat hippocampus thus, leading to their low or discrete presence in CNS (Davies et al., 1999, Thompson and Lummis, 2007).

2.4.3. Physiology of 5-HT_3 receptors

55-HT_3 receptors are ligand gated ion channel coupled receptors permeable to monovalent and divalent cations including Na^+, K^+ and Ca^{2+} which leads to membrane depolarization upon stimulation (Peters and Lambert, 1989, Yang, 1990). Activation of 5-HT_3 receptors on presynaptic terminals leads to release of several neurochemicals such as 5-HT, GABA or DA, whereas postsynaptic receptors are involved in fast synaptic transmission (Sugita et al., 1992, van Hooft and Vijverberg, 2000). Presynaptic 5-HT_3 receptors exhibits high permeability for Ca^{2+} whereas, postsynaptic 5-HT_3 receptors show low permeability for Ca^{2+} than Na^+ and K^+ (Yang, 1990, Nichols and Mollard, 1996). Moreover, homomeric 5-HT_3 receptors displays equal permeability for all the cations and shows slower activation and deactivation kinetics, whereas, heteromeric receptors shows low permeability to Ca^{2+} and additionally exhibits faster activation and deactivation kinetics (Stewart et al., 2003, Walstab et al., 2010).

2.4.4. Pharmacology of 5-HT_3 receptors

From the clinical perspectives, 5-HT_3 receptor agonists have gained less attention in research due to non-selectivity. Commonly used 5-HT_3 receptor agonist in preclinical research are mCPBG and 2-methyl-5-HT (2-Me-5-HT) (Campbell et al., 1995, Haddjeri and Blier, 1995). 5-HT_3 receptor antagonists are mainly used in chemotherapy induced emesis, in clinics.

Several other therapeutic actions of 5-HT_3 receptor antagonists have been suggested, such as in addiction, pain, psychiatric disorders, such as anxiety and depression. Various 5-HT_3 receptor antagonists namely OND, granisetron, tropisetron, palanosetron, itasetron, ricasetron, zatosetron, are being used clinically (Bétry et al., 2011).

Interestingly, responses of 5-HT_3 receptor agonist or antagonists are frequently associated with a bell shaped dose response curve (DRC), in pre-clinical as well as clinical research (Faerber et al., 2007). Importantly, it is also noted that they are effective at low doses and ineffective at higher doses as confirmed from pre-clinical models of depression such as FST, TST and learned helplessness (Martin et al., 1992, Ramamoorthy et al., 2008).

Inverse dose response relation curve was observed with 5-HT_3 receptor agonist possibly due to receptor desensitization. Cell culture studies confirmed the desensitization of receptor due to internalization of 5-HT_3 receptors upon treatment with 5-HT_3 receptor agonist mCPBG (Ilegems et al., 2004). This internalization was attenuated by 5-HT_3 receptor antagonists such as OND and granisetron (Freeman et al., 2006).

Later, due to inconsistent results with 5-HT_3 receptor agonists and antagonists, it was concluded that internalization of 5-HT_3 receptor alone is not enough to explain the bell shaped DRC under different conditions and brought forward two important concepts;
(i) hypothesis of steric hindrance at higher concentrations
(ii) different effect on homo/heteromeric receptors such that producing effect on some receptor at low concentration and on other at higher concentrations (Bétry et al., 2011).

2.5. Role of 5-HT_3 receptor antagonists in depression and anxiety-like disorders

Despite little clinical success with 5-HT_3 receptor antagonists, they remain an interesting target to study depression and anxiety, and demand further research studies. OND was the first 5-HT_3 receptor antagonist reported for anxiolytic effect in rodents in late eighties, followed by the investigation of anxiolytic profile of other 5-HT_3 receptor antagonists especially granisetron, tropisetron, and bemesteron in various animal species using different animal models for anxiety (Costall et al., 1987). Genetic deletion of 5-HT_3 receptors leads to anti-anxiety effects, thus making a point clear that 5-HT_3 receptor antagonists play an important role in anxiolytic effect (Barnes et al., 1992, Kelley et al., 2003, Bhatnagar et al., 2004). 5-HT_3 receptors were found to be an attractive target for anxiety treatment. Following chronic administration, 5-HT_3 receptor antagonists remained active, thus ruling out the chances of tolerance, rebound effects or dependence (Olivier et al., 2000).

The 5-HT_3 receptor remains one of the most interesting targets for potential antidepressants (Rajkumar and Mahesh, 2010, Kurhe and Mahesh, 2015). Since last decade, 5-HT_3 receptor antagonists have been used widely for depressive disorders in several pre-clinical studies (Martin et al., 1992, Mahesh et al., 2007). Initially, the inhibition of learned helplessness behavior in rodents by OND, tropisetron, zacopride gave a hint for antidepressant potential of chemically dissimilar 5-HT_3 receptor antagonists (Martin et al., 1992). In depression, the neuropeptide cholecystokinin, is released in the cortex and nucleus accumbens and treatment with 5-HT_3 receptor antagonist, has been found to inhibit this neuropeptide (Becker et al., 2008). SSRIs mediated antidepressant-like activity was studied to occur through K^+ channel-linked 5-HT_3 receptors (Redrobe and Bourin, 1997). Furthermore, the antidepressant effect of tropisetron, Imipramine, mianserin was inhibited by 5-HT_3 receptor agonist mCPBG administration (Nakagawa et al., 1998). An Interesting finding came up through a electrophysiological assay using human 5-HT_{3A} receptor and endogenous 5-HT_3 receptors showing the functional antagonism of 5-HT_3 receptors by currently used antidepressants TCAs, SSRIs, and NARIs confirming the antidepressant effect of chemically different agents (Eisensamer et al., 2003). Thus, researchers noticed that the inhibition of 5-HT_3 receptors could emerge as novel therapeutic approach for depression.

Moreover, administration of 5-HT_3 receptor antagonist, OND with sub-effective doses of SSRIs augmented the antidepressant effect in rodent models (Redrobe and Bourin, 1997, Ramamoorthy et al., 2008, Kos et al., 2006). Several studies in laboratory (in-house) with standard OND and novel 5-HT_3 receptor antagonists 2-(4-methyl piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA2) with comparable pA2 (indicating potency for 5-HT_3 receptors) and log p (indicating the ability to cross the BBB) values (Mahesh et al., 2007), exhibited antidepressant-like effect, in various acute and chronic models of depression in rodents, through postsynaptic 5-HT_3 receptors (Ramamoorthy et al., 2008).

2.6. Mechanism of 5-HT_3 receptor antagonists in antidepressant effect

Based on the behavioral and neuropharmacological research conducted in rodents, the putative mechanism for antidepressant effect of potential 5-HT_3 receptor antagonists, was investigated earlier (Rajkumar and Mahesh, 2010). Several behavioral tests battery with 5-HT_3 receptor antagonist treatment, found reduced immobility time in FST and TST, and improved swimming behavior in FST, potentiated the antidepressant effect of SSRIs and SNRIs, reversed reserpine induced hypothermia, potentiated 5-HTP induced head twitch responses, attenuated OBX induced hyperactivity, and chronic unpredictable mild stress (CUMS) induced behavioral and biochemical alterations (Bravo and Maswood, 2006, Mahesh et al., 2007, Ramamoorthy et al., 2008). Moreover, OND showed the "5-HT syndrome" in humans, which confirmed that 5-HT_3 receptor antagonists, improves 5-HT neurotransmission (Turkel et al., 2001). SSRIs were found as functional antagonists of 5-HT_3 receptors, thus blocking the inhibitory neurotransmission and facilitating the excitatory synaptic potential, further making a way for partial antidepressant effect through inhibition of presynaptic 5-HT_3 receptors (Fan, 1994c, Fan, 1994a, Fan, 1994b).

Effect of 5-HT_3 receptor antagonists was found to be dose dependent. At low concentrations, they blocked the post synaptic receptors, thereby inhibiting the fast excitatory potential in the limbic structures and increased the synaptic availability of 5-HT to other post synaptic 5-HT receptors mainly 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} further facilitating the 5-HT neurotransmission and produced antidepressant action (**Fig 2.2**) (Sugita et al., 1992, Sambunaris et al., 1997, Anttila and Leinonen, 2001). At higher concentrations, 5-HT_3 receptor antagonists, blocks the presynaptic and somatodendritic 5-HT_3 receptors, thereby inhibiting the synaptic cleft availability of 5-HT and finally resulting in depressive behavior (Ramamoorthy et al., 2008).

Apart from 5-HT, inhibition of 5-HT_3 hetero receptors present on presynaptic terminals alter the release of several other neurotransmitters, such as reduces NA (Matsumoto et al., 1995), DA (Dremencov et al., 2006), GABA (Dorostkar and Boehm, 2007) and increase Acetylcholine (Ach) (Chau et al., 2001) release, thus resulting in depression-like action.

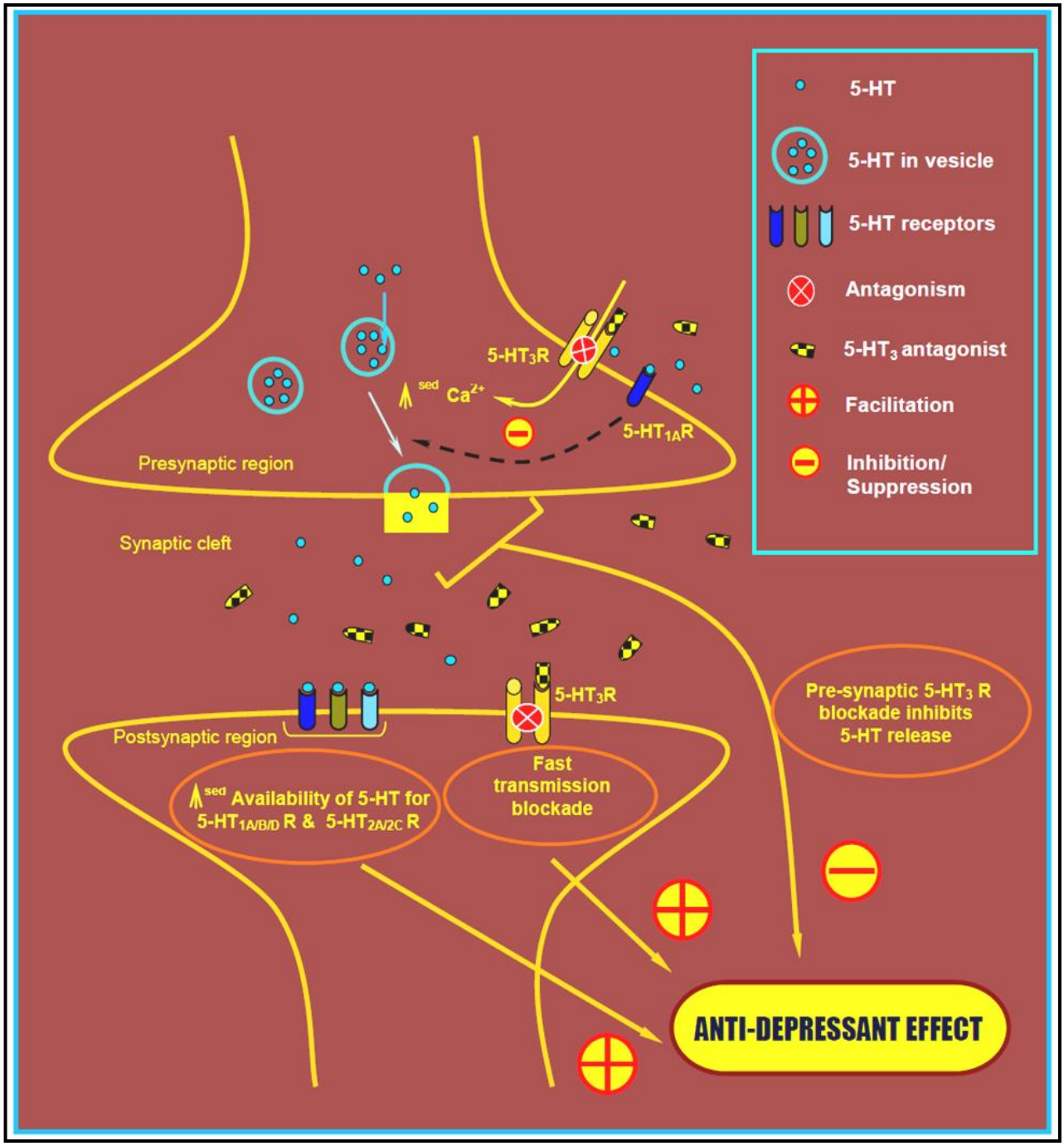


Fig 2.2: Schematic representation of antidepressant effect of 5-HT_3 receptor antagonists *[Adopted from (Rajkumar and Mahesh, 2010)]*

The above information suggest that as 5-HT_3 receptors are widely distributed in various sub cellular brain regions involved in depression, the antidepressant effect of 5-HT_3 receptor antagonists through modulatory effects on neurotransmitter system in particular 5-HT, cannot be neglected.

2.7. Role of 5-HT system in regulation of food intake/appetite/obesity

5-HT has inverse relationship with food intake and hence, serotonergic analogs are well known weight reducing agents (Garattini et al., 1986). It is specified that 5-HT induced appetite suppression is mediated through central serotonergic pathway (Samanin et al., 1980). Arc nucleus present in the hypothalamus plays an important role in regulation of energy homeostasis (Elmquist et al., 1999). Pharmacological and genetic research implicates that the energy balance effect of 5-HT is mediated through Gq-coupled 5-HT_{2C} receptor and the Gi-coupled 5-HT_{1B} receptor, that require melanocortin pathways to exert their effects on appetite. 5-HT and 5-HT_{2C} agonists, activate neurons expressing the endogenous anorectic melanocortin agonist POMC/α-MSH and 5-HT and 5-HT_{1B} agonists, inhibit the activity of neurons expressing the endogenous orexigenic melanocortin antagonist AgRP in the Arc nucleus of the hypothalamus (Broberger et al., 1998, Hahn et al., 1998). Within the brain, α-MSH and AgRP compete for action at MC3R and MC4R (Cone, 2005). MC4R are found to produce the most potent effects on the food intake, body weight, energy expenditure and insulin actions (Fan et al., 2000).

5-HT leads to the hyperpolarization and inhibition of AgRP and decreases an inhibitory drive onto POMC cells, through activation of 5-HT_{1B} receptors. Furthermore, 5-HT is found to activate POMC neurons, via activation of 5-HT_{2C} receptors. Thus, the reciprocal increase in α-MSH release and decreases in AgRP release at MC4R in target sites were observed (Heisler et al., 2006, Heisler and Lam, 2010). Extensive literature have specified that the probable role of 5-HT in reducing the body weight, could be through inducing hypophagia via the hypothalamic melanocortin system. These effects are probably mediated through a number of neuropeptides such as leptin, ghrelin and NP-Y. The other pathway suggests the ability of 5-HT, to stimulate insulin sensitivity and release, which in turn help in the metabolism of glucose.

In conclusion, the agents which increase the availability of 5-HT to act through 5-HT_{1B} and 5-HT_{2C} receptors, can play a role in the therapeutic management of obesity and its complications (Adeghate, 2007).

At present, it is believed that 5-HT and serotonergic receptor subtypes, mediate the ability to inhibit food intake. 5-HT, suppresses the food intake, that preserves the behavioral satiety sequence and ultimately indicates the natural physiological processes for meal termination and sustained post-meal satiety (Blundell, 1984). Moreover, it is reported that the serotonergic drugs fenfluramine and dexfenfluramine, inhibit the consumption of HFD in rodents (Duhault et al., 1975). Human studies reported the suppression of intake of highly palatable high fat foods, and a possible avoidance of fat, after the administration of dexfenfluramine and sumatriptan (Blundell and Halford, 1998). 5-HT, acts mainly in the medial hypothalamus. The PVN and ventromedial nuclei (VMN), are involved in controlling energy balance, while the suprachiasmatic nucleus regulates circadian patterns of eating (Halford et al., 2005). Several interesting studies evidenced an important role of 5-HT_{2C} receptors, in regulating energy balance and modulating glucose homeostasis (Heisler et al., 2003). Hypothalamic 5-HT_{2C} and 5-HT_{1B} receptors act by modulating melanocortin pathways and 5-HT release into the hypothalamus stimulate the sympathetic nerves innervating brown adipose tissue (Lam and Heisler, 2007).

2.8. 5-TH_3 receptor antagonists in the management of depression co-morbid with obesity

In the previous section, the biological mechanisms linking depression with obesity were discussed including mainly, HPA axis hyperactivity, oxidative stress, insulin resistance/altered plasma glucose, leptin resistance, reduced BDNF concentrations and hippocampal neurodegeneration. The mechanism of 5-HT_3 receptor antagonists through modulating the 5-HT neurotransmission in antidepressant effect is explained earlier.

Among various neurotransmitters, 5-HT plays a crucial role in regulation of sleep, appetite and mood, and hence, is an attractive target for both depression and obesity (Owens and Nemeroff, 1994, Lam et al., 2010, Sargent and Henderson, 2011).

SSRI fluvoxamine showed that serotonergic modulation improve the blood glucose by increasing the hepatic glucose uptake and glycogen storage in hyperglycemia and hyperinsulinemia (Moore et al., 2004, Moore et al., 2005). The association of 5-HT and insulin, has been a point of interest since last three decades (Lundquist et al., 1971). A close correlation between central 5-HT activity and peripheral insulin sensitivity has been observed in depressed and obese patients (Horáček et al., 1999, Almeida et al., 2009). 5-HT, regulates brain insulin activity, food intake and energy homeostasis and plays a very important role in the management therapy of depression (Yu et al., 2010). Moreover, one interesting finding, reported that 5-HT regulates the secretion of insulin by serotonylation of proteins (Paulmann et al., 2009). The association of 5-HT and peripheral insulin sensitivity, explains an important aspect in linking the biological mechanisms involved in depression co-morbid with obesity.

Similarly, leptin resistance is very common in depression and obesity. It has been reported that leptin increases the 5-HT content in brain (Hastings et al., 2002). Depression and obesity are associated with HPA axis hyperactivity resulting in excessive corticosteroids (CORT) in the plasma, further triggering several metabolic events. 5-HT exerts a modulatory effect on HPA axis hyperactivity and oxidative stress (Lanfumey et al., 2008). BDNF and 5-HT have been associated with neuronal regulatory functions such as synaptic plasticity of neuronal circuits and neurogenesis, and are majorly involved in the neuropsychiatric disorders including depression (Gatt et al., 2009). Obesity is a stressful condition leading to reduced BDNF and serotonergic neuronal survival, resulting in neurodegeneration. BDNF has been found to be co-expressed on the serotonergic neurons within the dorsal and median raphe neurons. Hence, 5-HT plays an important role in the hippocampal neurogenesis. Both, 5-HT and BDNF regulate the survival and differentiation of serotonergic neuronal survival in the brain (Mahar et al., 2014).

The 5-HT_3 receptor antagonist, tropisetron, modulates glucose induced obesity in mice, showing the involvement and role of the 5-HT_3 receptor antagonists in detection of sugar and short term regulation of food intake (Weber et al., 2009). Another study, suggested the role of 5-HT_3 receptor antagonists, in improving the obesity associated fatty liver diseases (Haub et al., 2011). OND, has been reported for regulation of hyperglycemia in rat raphe nucleus (Mitchell and Pratt, 1991).

In another study, prior administration of OND and LY-278584 inhibited hyperglycemia induced with quipazine (Carvalho et al., 2005). Tropisetron, 5-HT_3 receptor antagonist, has been reported to increase the insulin release in the insulin producing beta-cell lines (INS-1) (Heimes et al., 2009).

2.9. Animal models

Animal models are an essential part of research in neuropsychiatric disorders such as depression and anxiety as they help in understanding the etiopathogenesis of disease and in the development of novel therapeutic strategies to combat such psychiatric disorders (Abelaira et al., 2013). Despite several advantages of animal models in understanding psychiatric disorders, they do have some limitations, such as; in case of depression, animals are not able to experience feelings of guilt, sadness or suicidal thoughts, which are only observed in humans (Nestler and Hyman, 2010). Hence, animal models of depression, demand specific features, while designing (Belzung and Lemoine, 2011);

- Selection of proper species and strain, sex and age of animal
- The model should be sensitive and reproducible
- Animal model should offer an opportunity to understand and study the behavioral, biochemical, molecular, genetic and epigenetic modifications that may lead to depression
- Animal model should possess optimum construct, face and predictive validities
- The model should resemble the disease analogy as close as possible to that of human
- The symptoms or disease phenotypes exhibited by the animal model should be reversed by clinically effective pharmacotherapy

2.9.1. Validity criteria for animal models of neuropsychiatric disorders

Designing animal models to study neuropsychiatric disorders such as depression and anxiety, is very important and essential criteria. Importantly, the model should be as close as possible with respect to behavior, biochemistry, anatomy and physiology to the human disease. While designing an animal model, one should include the plausible risk factors of human disease or consider behavioral pathology that reflects human disease.

Animal models for neuropsychiatric disorders are generated through various means especially selective breeding, genetic engineering, brain lesions and environmental manipulations. Despite of wide approaches and challenges of validation, it is very important to know the ways of judging whether a particular disease model, is good enough to carry or conduct further research investments. Hence, for designing the animal models for neuropsychiatric disorders such as depression and anxiety, the validity criteria is very crucial which includes face, construct and predictive validities (Willner, 1984).

2.9.1.1. Face validity (symptom)

It refers to the similarities between the behavioral manifestations mimicked by an animal model and the specific clinical disease symptoms. Animal models are selected such that they represent, as close as possible, the human disease symptoms including behavioral, anatomical, biochemical, neuropathological, and neurobiological. An animal model of neuropsychiatric disorders such as depression and anxiety that parallels multiple symptoms of human depression and anxiety is considered valuable (Nestler and Hyman, 2010, Belzung and Lemoine, 2011).

2.9.1.2. Construct validity (pathology)

It refers to the theoretical concepts of an animal model for any neuropsychiatric disorder. Mainly, construct validity refers to the similarities between the clinical pathophysiological changes mimicked by the animal model of depression. However, it is quite difficult to take into consideration the various etiological factors such as psychological including adverse life experiences, stressful events of life, and biological factors including physical illness, genetic influences involved in neuropsychiatric disorders like depression and anxiety (Nestler and Hyman, 2010, Belzung and Lemoine, 2011).

2.9.1.3. Predictive validity (treatment)

It refers to the treatment/pharmacological criteria such that, whether an animal model correctly identifies treatment for neuropsychiatric disorders and whether the treatment potency in the animal model correlates with the clinical potency. In case of depression, whether the clinically effective antidepressant agents, are able to reverse the behavioral alterations in animal models of depression or not is indicated by predictive validity (Nestler and Hyman, 2010, Belzung and Lemoine, 2011).

2.9.1.4. Validating animal models of depression

Behavioral tests FST and TST are not models of depression, but rather considered as tests developed decades ago to screen drugs/novel compounds for antidepressant activity (Cryan et al., 2002, Nestler et al., 2002). A major limitation of these tests is that they are associated with short term stress applied to normal rodent (mice or rat) which are different from human depression which involves genetic vulnerability combined with stochastic and chronic environmental stress exposures to produce long lasting behavioral pathology. In addition, antidepressants are effective in these tests with acute treatment (single dose), which is in contrast to human depression treatment, which requires chronic treatment (weeks to months).

Another important test of depression related behavior, involves measurement of anhedonia, which has the advantage as it is based on symptoms of depression and hence, reflects higher face validity. Anhedonia represents a core symptom of depression, having neurobiological hypothesis and therefore gains importance for investigation in rodent models. Anhedonia, is measured in animals in terms of interest in pleasurable activities such as preference for sucrose solution over water. Animals displaying reduced sucrose preference, are considered as anhedonic behavior, indicating depression-like behavior (Krishnan et al., 2007, Nestler and Hyman, 2010).

The interpretation of anxiety-like phenomenon remains another major issue for extensively used current behavioral tests of depression. Several models based on stress such as elevated plus maze (EPM), hole board test (HBT), light/dark (L/D), open field test (OFT) exhibits anxiety-like behavior. In human frequently intermixed symptoms/features of depression and anxiety are observed and interestingly, chronic antidepressant administration treats anxiety disorders as well. Hence, it is important to understand which animal models of depression reflect anxiety-like symptoms by understanding the boundaries between several depression and anxiety syndromes in humans (Krishnan et al., 2007, Wallace et al., 2009). Below given **Table 2.3,** represents the acute and chronic models of depression with neurotransmitters (NT) involve and validity criteria.

Table 2.3: Animal models of depression and anxiety with validity criteria

Animal Model	Animal	Validity Criteria	NT	Reference
FST (Despair based test of depression)	Mice Rats	Predictive Face and construct (poor)	5-HT NE DA	(Petit-Demouliere et al., 2005, Castagné et al., 2011)
TST (Despair based test of depression)	Mice	Predictive Face and construct (poor)	DA 5-HT NE	(Cryan et al., 2005)
SPT (Anhedonia based test of depression)	Mice Rats	Predictive Face Construct (poor)	DA	(Powell et al., 2012)
HBT (exploratory and novelty based test of anxiety)	Mice	Predictive	GABA 5-HT	(Brown and Nemes, 2008)
EPM (exploration based test of anxiety)	Mice Rats	Face Predictive Construct	GABA 5-HT	(Walf and Frye, 2007)
OFT (novelty and exploratory based model)	Mice Rats	Predictive Construct	GABA 5-HT	(Royce, 1977) (Tachibana, 1980)
LDT (exploratory based test of anxiety)	Mice	Predictive	GABA 5-HT	(Rodgers and Shepherd, 1993)
CUMS (mild unpredictable stressor based chronic model for depression and anxiety)	Mice Rats	Face Predictive Construct	HPA-axis 5-HT NE DA	(Willner, 1997, Powell et al., 2012)
OBX (Lesion-based chronic model for depression and anxiety)	Rats	Face Predictive Construct	5-HT NE DA, GABA Ach	(Harkin et al., 2003)

2.9.2. Selection criteria of animal model based on metabolic disturbances connecting obesity and depression; Mood, food and adiposity

Emotional reactions (positive reactions such as rewarding, hedonic effects) play a significant role in promoting overeating, that further leads to development of obesity (Fulton et al., 2006). Various stressors, like external or psychological, may have different effects on feeding pattern, as observed in some individuals with increased food intake while others eat less (Dallman et al., 2006). In the similar way, conditions of chronic stress can cause reduced appetite and weight loss in some individuals while may exhibit opposite effect in some other cases by stimulating the intake of palatable and rewarding foods to which ultimately may inhibit stress response (Adam and Epel, 2007). It has been documented that, chronic stress leads to consumption of palatable food in several human and animal studies (Stone and Brownell, 1994, Cottone et al., 2009). On the other hand, it is well reported that consumption of palatable food, leads to signs and symptoms of stress and anxiety, when exposed to chronic stress conditions (Finger et al., 2011, Finger et al., 2012).

The choice of food and energy metabolism may be affected by the mood state. Generally, individuals with depressed mood prefer consumption of palatable or comfort food to get rid of the negative thoughts and feelings (Mathé et al., 2007). Short term consumption of palatable food, may lead to relief from negative thought, feeling and emotions. On the contrary, chronic consumption of palatable high calorie food can subsequently result in increased adiposity and may provoke vulnerability to mood disorder such as depression (Kloiber et al., 2007, Sharma and Fulton, 2013). It was reported that mice consuming high fat diet (HFD) for 12 weeks, exhibited depressive phenotypes in behavioral models of depression showing higher immobility time in FST and anxiety-like behavior representing decreased exploratory behavior in EPM and OFT (Sharma and Fulton, 2013)

2.9.3. HFD induced obesity; as a model to study depression co-morbid with obesity

Diet induced obesity (DIO) such as HFD is most commonly practiced in the research laboratories as it reflects and mimics most of the signs observed in obese humans. Diet rich in saturated fat and low in monounsaturated and polyunsaturated fats is involved in the pathogenesis of both mood and metabolic disorders during obesity. Consumption of food with high saturated and/or trans fat as observed in Western diet, is mainly linked with higher incidence of depression.

On the other hand, diet rich in unsaturated fatty acids as observed in Mediterranean diet generally reduces depression cases (Sanchez-Villegas and Martínez-González, 2013, Hryhorczuk et al., 2013). It is reported that inadequate amount of polyunsaturated fatty acids (PUFA) in diet are associated with increased incidence of depression (Peet et al., 1998) and consumption of omega 3 PUFA through fish diet may reduce the depression incidences in humans (Park et al., 2012) and rodents (Moranis et al., 2012), or have no significant effect on mood (Ruusunen et al., 2011). However, enough evidence based studies have documented that, saturated fats rich diet leads to adiposity and fat accumulation in the abdomen. Population consuming diet rich in saturated fats compared to Mediterranean diet represents weight gain, higher visceral adiposity, high waist circumference and increased mortality rate due to cardiovascular diseases (Mozaffarian et al., 2011, Nazare et al., 2013).

Deposition of adipose tissues in the abdominal stores, is more important than overall body fat, in order to promote various risks of complications associated with obesity such as insulin resistance and MetS (Tchernof and Després, 2013). Increased dysfunctional adipose tissue in obesity, exacerbates MetS, as well as neurobiological impairments causing mood disorders. In addition, central obesity remains more important predictor than body weight or BMI for risk of mood disorders such as depression (van Reedt Dortland et al., 2013). Overall, it is pretty clear that high abdominal fat gain through higher consumption of saturated fat and metabolic disturbances stands, as a common causative element for metabolic as well as neuropsychiatric disorders, such as depression and anxiety.

2.9.4. HFD fed mice subjected to CUMS; as a model to study depression co-morbid with obesity

Research concerned with the mechanisms of antidepressant agents, must address two important issues.

(i) Antidepressants do not possess the ability to improve mood in normal individuals, making questions on the studies carried out in normal animals and hence, designing proper animal model of depression is absolutely necessary

(ii) Clinical efficacy with antidepressant agents demands chronic treatment for several weeks.

In order to study the antidepressant effect of drugs over a clinical time scale, animal models should resemble and mimic the depressive symptoms persistent for a period of several months.

Hence, it is very important to design an animal model that mimics human depressive behavior and symptoms, in order to validate the antidepressant activity of a novel or known drug. CUMS model, represent one such model that reflects most of the signs of clinical depressive state. CUMS model was modified by Willner from the earlier designed by Katz and colleagues, mainly with respect to two criteria; (a) the severity of the stressors used was reduced (b) hedonic measure was considered important in this model.

CUMS procedure suggests that; (i) the behavioral alterations induced by CUMS may last for several weeks of continuous induction (ii) the stressors used in the protocol do not include any severe stressful element as used earlier by Katz and colleagues such as 48 h food and/or water deprivation, intense foot shock, etc. (Willner, 1997, 2005).

Construct validity of CUMS model

Construct validity for any preclinical model in neuropsychopharmacology is based on theoretical concepts of the clinical disease condition. In chronic stress model, the theoretical rational mimic anhedonia, a characteristic core symptom of clinical depression reflected by loss of pleasure or response to experience pleasure. In preclinical setting, it can be simulated based on two rationales; (i) consumption of sucrose solution is considered as valid measure of sensitivity to reward phenomenon and (ii) CUMS results in generalized reduction towards reward (anhedonia) sensitivity (Willner and Jones, 1996, Willner, 2005, Nollet et al., 2013).

Face validity of CUMS model

Face validity refers to the clinical symptomlogy profile of disease that is reflected/mimic by the animal model. Apart from anhedonia, CUMS model, also represents other symptoms of clinical depression including reduced sexual, investigative and aggressive behaviors, sleep disturbances, (Paolo et al., 1994, Cheeta et al., 1997), etc, as mentioned in **Table 2.4.**

To summarize, the face validity of CUMS model involves, reduced response towards reward which is considered important and it parallels to the symptom of clinical MDD. However, some of the features/symptoms, were not studied in animals subjected to CUMS as these were unique human symptoms and only possible with verbal communication (Willner, 1991). Overall, it CUMS model of depression, represents good face validity.

Table 2.4: Symptom profile of CUMS model of depression in rodents for MDD according to DSM-IV and DSM-V (Willner, 1997, 2005, Association, 2013)

Features	Depression (MDD)	CUMS
Duration	Minimum 2 weeks	CUMS induced persists minimum for 3 weeks
Core symptoms	Depressed mood,	N/A
	Significantly loss of interest or pleasure	Reduced sexual and investigative behaviors, reduced response towards reward
Other symptoms	Marked weight loss	Around 5% weight loss
	Insomnia or hypersomnia	Sleep disturbances
	Retardation or psychomotor agitation	Reduced locomotor activity
	Loss of energy or fatigue	Reduced active waking in electroencephalogram (EEG)
	Feeling of guilt or worthlessness	N/A
	Indecisiveness or reduced ability to think	Not tested in CUMS
	Suicidal thoughts	N/A

Predictive validity of CUMS model

Predictive validity refers to the treatment criteria where the clinically effective drugs are tested in the designed animal model of particular disease and whether that drug reverses the disease symptoms in a specific period of time as observed with humans is analyzed.

In CUMS model, anhedonia is considered as core symptom of clinical depression and requires 3-4 weeks of treatment for reversal.

This resembles the clinical time course of antidepressant onset of action. Another important feature of antidepressant treatment in animals that resemble with clinic, suggest that antidepressants are effective in only CUMS subjected animals, to reverse the reward behavior and does not alter reward behavior in control (non-stress group) animals (Willner, 1997).

Taken together, it is pretty clear from the **Table 2.5** given below, that various antidepressants, are effective in reversing the reward alterations in CUMS exposed animals and that time course for the therapeutic effect, closely resembles clinical effect of antidepressant agents.

Table 2.5: Pharmacological treatment profile in CUMS model (Willner, 1997)

Features	Hits	Misses
True	TCAs- imipramine, desipramine, amitriptyline	Anti-anxiety agent- Chlordiazepoxide
	SSRIs- fluoxetine, fluvoxamine, citalopram	Neuroleptics- haloperidol, Chlorprothixene, resperidone
	NARI- Maprotiline	Opioid- morphine
	MAO-A inhibitors- Moclobemide	
	Atypical- Mianserin	
	5-HT_{1A} agonist- Buspirone	
Probable	Corticosterone synthesis inhibitors- ketoconazole	5-HT1 agonist-Ipsapirone
	Anti-manic agents- lithium, carbamazapine	Aplha 2 agonist- Ethoxyidazoxan
Possible	Anti-histaminergic agent- mepyramine	None
	Anti-cholinergic agents- atropine	
False	None	None

Hits- compounds that reversed CUMS induced behavioral alterations in animal

Misses- compounds that were not effective in CUMS model

True- correctly classified compounds

False- compounds that not correctly classified

2.9.4.1. Justification for use of HFD fed mice exposed to CUMS as model to study depression co-morbid with obesity

It is clear that HFD induced obesity is a widely accepted and valid model, which is highly practiced, in research laboratories. Similarly, the CUMS model is highly sensitive, valid, reliable model, to study depression in rodents and the antidepressant effect of novel or known drugs as it has high construct, face and predictive validities. HFD induced obesity model, reflects most of the biological mechanisms, involved in depression associated with obesity including HPA axis hyperactivity, oxidative stress, leptin resistance, insulin resistance or altered glucose level, reduced neurotrophic factor BDNF and altered hippocampal neurogenesis. CUMS model of depression is very popular model and it was used in the present study, to aggravate the depressive phenotypes in HFD fed mice.

Although obesity itself is a stressful condition and it induces depression, HFD fed mice, were subjected to CUMS, to provoke and worsen the depressive phenotypes, in mice. With HFD+CUMS model, the severity of biological mechanisms linking depression and obesity, such as HPA axis hyperactivity, oxidative stress, leptin resistance, insulin resistance or altered glucose, reduced BDNF, hippocampal neurogenesis and hippocampal 5-HT levels, would be interesting to study.

In an interesting study, CUMS procedure was applied in two sessions, each consisting of 7 weeks separated by 6 weeks, respectively, and treatment with fluoxetine or vehicle was started from 3rd week onwards of CUMS procedure. In this model, fluoxetine failed to reverse the depressive phenotypes in HFD fed mice (Isingrini et al., 2010).

Hence, it is very important to identify the efficacy of potential known or novel antidepressants using HFD+CUMS model as an effective pharmacotherapy for depression co-morbid with obesity.

2.10. Gap in existing research

Obesity is defined by BMI 30 or higher, known as serious health concern, as it is associated with several health consequences, mainly type 2 diabetes mellitus (T2DM), hyperglycemia, hypertension, hyperlipidemia, cardiovascular problems affecting the quality of life (Segula, 2014). Obesity has become a major challenge in developed nations, such as United States, where more than 33% adult and 17% youth population are obese, along with 69% adult and 24% youth population as overweight, that results in heavy burden/cost of obesity treatment in United States, reports around $147 billion (Finkelstein et al., 2009, Ogden et al., 2014).

Obesity also has very serious impact on mental health disorders such as depression and anxiety as around 55% of the obese individuals are twice susceptible for depression compared to non-obese population (Luppino et al., 2010).

Depression associated with obesity results in serious life threatening events, with respect to health, as both are associated with low self-esteem, social stigma, and severe health conditions. Depression in obese subjects are commonly associated with poor quality of life, poor social functioning, emotional aspects, and mental health overall increasing the global burden of diseases and socio-economic status of the affected individuals (Carey et al., 2014).

In a general practice study, the prevalence of depression in overweight and obese subjects was found to be 48.4% and 51.6%, respectively, that was significantly higher compared to depression in general (non-obese) individuals which was 16% and 34%, respectively (Nikolic, 2015). In a cross sectional study in United States, 43% of the depressed adults were obese (Pratt et al., 2014).

In another study, very important findings were evaluated especially in women. Middle aged-women are prone to develop depression, which is strongly associated with obesity due to low physical exercise, reduced energy expenditure and higher calorie intake. BMI under 25 increases the prevalence of depression by 6.5% and BMI above 35 increases the prevalence of severe depression by 25.9% in middle-aged population (Simon et al., 2008).

Overall, depression associated with obesity is one of the major health concerns that imparts serious burden of diseases on public health globally.

Despite advances in neuropsychopharmacology and rapidly growing prevalence rate of co-morbid depression and obesity, the biological mechanisms linking these co-morbid disorders, are still not completely understood. Hence, it is very essential to study and understand the pathological hallmarks involved in depression co-morbid with obesity, in order to design novel pharmacotherapeutic approaches to combat these co-morbid disorders.

Several biological mechanisms link depression co-morbid with obesity such as HPA axis hyperactivity, oxidative stress, leptin resistance, insulin resistance and reduced BDNF and 5-HT level and dentate gyrus (DG) neuronal damage. The pharmacotherapeutic approach should be designed in such a way that, the therapy reverses the biological mechanisms involved in depression co-morbid with obesity.

Moreover, no single pharmacotherapeutic treatment has been effective, to reverse depression co-morbid with obesity.

In the current scenario, following are known;

- Screening of HFD fed animals for depressive-like phenotypes in various labs
- Researchers have been targeting neurotransmitter systems especially DA and 5-HT, for such co-morbid disorder
- 5-HT is a neurotransmitter that regulates sleep, mood and appetite and its predominant reduction has been known in both depression and obesity

Currently used antidepressants are associated with severe side effects and limitations. Important limitations of current antidepressant therapy are listed below;

- The major drawback of the current antidepressants remains the weight gain issue
    - i) Antidepressants impacts the body's metabolism
    - ii) Depressed patients feel eating more enjoyable
- Nausea, insomnia, headaches
- Late clinical benefit
- Treatment resistance
- Low remission rates

Hence, taking into consideration the higher prevalence of depression co-morbid with obesity, several timely needed steps that could possibly overcome the gap in the existing research are;

- Study the biological mechanisms linking depression and obesity
- Practice pharmacotherapy that inhibits biological mechanisms involved in depression co-morbid with obesity
- Design of novel and better therapeutic alternatives for depression co-morbid with obesity that can overcome the limitations of current antidepressants

3.0. BROAD OBJECTIVES

Depression is expected to stand second in the global burden of diseases by 2020. Obesity is one of the serious psychiatric consequences as it is associated with several health complications increasing the mortality rate. One of the major risk factor for mental health disorders such as depression and anxiety is obesity. Depression and obesity affects 350 and 500 million world population, respectively. The exact mechanism for how obesity heightens the risk for depression is still not understood clearly. In the pre-clinical studies high fat diet (HFD) feeding has showed depressive behavior in rodents. However, researchers are still engaged in finding out biochemical pathways involved in depression co-morbid with obesity through various studies. As discussed in earlier sections, co-morbid association of depression and obesity is growing rapidly and needs some serious attention considering following important aspects.,

- The biological mechanisms/pathological factors involved in depression co-morbid with obesity are not fully explored
- Lack of pharmacotherapeutic agents that targets the biological mechanisms involved in depression co-morbid with obesity
- Side effects and limitations of current antidepressant therapy
- Role of serotonergic neurotransmission in depression co-morbid with obesity is still not studied
- Role of serotonergic neurotransmission on several pathological markers of depression co-morbid with obesity such as HPA axis hyperactivity, oxidative stress, leptin & insulin resistance, reduced BDNF level, and hippocampal neuronal damage are not explored till date
- Role of 5-HT_3 receptors and 5-HT_3 receptor antagonists in depression co-morbid with obesity is still not studied

Considering the above awaited studies in the neuropsychopharmacological research dealing mainly depression co-morbid with obesity, following specific objectives were set for the present study;

- To address the biological mechanisms linking depression and obesity
- To standardize suitable in-vivo rodent model that mimic behavioral, biochemical and molecular aspects of depression co-morbid with obesity
- To evaluate the effect of standard and novel 5-HT_3 receptor antagonists (in-house synthesized) on depression co-morbid with obesity
- To study the plausible role of 5-HT_3 receptors in mediating antidepressant-like effect in depression co-morbid with obesity

3.1. Plan of work

The present research work was designed to study the biological mechanisms linking depression co-morbid with obesity. Several mechanisms involved in depression associated with obesity as mentioned earlier such as HPA axis hyperactivity, oxidative stress, leptin resistance, insulin resistance, reduced BDNF and neuronal damage were taken into consideration. Role of serotonergic neurotransmission on the biological mechanisms involved in depression co-morbid with obesity was studied. The effect of standard and novel serotonergic modulators, 5-HT_3 receptor antagonists for depression co-morbid with obesity was evaluated as a novel therapeutic alternative.

3.1.1. Selection of 5-HT_3 receptor antagonists and standard antidepressant

Based on the molecular modeling and pharmacophoric design, several novel 5-HT_3 receptor antagonists were synthesized in-house by the Medicinal Chemistry Group of the Department. Using in-vitro approach, 5-HT_3 receptor antagonistic action of these novel compounds were evaluated in terms of pA2 value. Selection of novel 5-HT_3 receptor antagonists for extensive pharmacological screening was carried out based on the pA2 value of the novel compounds (comparable to that of standard Ondansetron).

- Ondansetron **(OND)**
- 3-methoxy-*N*-*p*-tolylquinoxalin-2-carboxamide **(QCM-4)**
- (4-phenylpiperazin-1-yl) (quinoxalin-2-yl) methanone **(4a)**
- Escitalopram **(ESC)**

3.1.2. Preliminary work

3.1.2.1. Dose response studies

Dose response studies were performed initially for selected 5-HT_3 receptor antagonists **QCM-4** and **4a** using preliminary assays for depression such as forced swim test (FST), tail suspension test (TST), and anxiety including elevated plus maze (EPM), hole board test (HBT) and light/dark (L/D) test.

3.1.2.2. Screening of 5-HT_3 receptor antagonists in chronic models of depression

Depression is a neuropsychiatric disorder that requires chronic treatment in clinics. In order to mimic the neurobehavioral features of clinical depression and to evaluate the effect of repetitive treatment of selected novel 5-HT_3 receptor antagonists **QCM-4** and **4a**, chronic rodent models of depression such as olfactory balbectomy (OBX) and CUMS were standardized in-house and used.

3.1.2.3. Behavioral test procedures and parameters measured in OBX and CUMS model

Chronic rodent model(s) of any neuropsychiatric disorder need a proper behavioral evaluation tests. Behavioral assays indicate a brief idea about the functioning of the standardized model for the respective neuropsychiatric disorder. Following behavioral assays/tests were employed in OBX (**Table 3.1)** and CUMS (**Table 3.2**) models of depression after chronic treatment with 5-HT_3 receptor antagonists **QCM-4** and **4a**.

Table 3.1: Behavioral tests and parameters measured in OBX model

Sr. No.	Behavioral test(s)	Parameters/observations
1.	Sucrose preference test (SPT)	Volume of sucrose solution consumed
2.	Open field test (OFT)	Ambulatory, rearing and fecal pellet scores
3.	Elevated plus maze (EPM)	% open arm entries (OAE) and % time spent in open arm time (OAT)

Table 3.2: Behavioral tests and parameters measured in CUMS model

Sr. No.	Behavioral test(s)	Parameters/observations
1.	SPT	Volume of sucrose solution consumed
2.	FST	Duration of immobility (s)
3.	TST	Duration of immobility (s)
4.	EPM	% OAE and % OAT

Our first attempt in the research work was to design and standardize laboratory model that mimic most of the clinical signs of depression co-morbid with obesity. Based on the extensive literature review, the approach was to design rodent models for studying the neurobehavioral, biochemical and molecular aspects involved in depression co-morbid with obesity (as mentioned in **Table 3.3**).

5-HT_3 receptor antagonists were screened in experimental models of depression co-morbid with obesity and their mechanisms were studied using behavioral, biochemical and molecular investigations.

Table 3.3: Animals models to study to study depression co-morbid with obesity

Sr. No.	Animal model	Sequence
1.	HFD induced depressive phenotypes	i) HFD feeding ii)Treatment with 5-HT_3 receptor antagonists iii) Behavioral assays iv) Biochemical assays v) Molecular assays
2.	HFD fed mice subjected to chronic unpredictable mild stress (CUMS)	i) HFD feeding ii) Chronic stress procedure iii)Treatment with 5-HT_3 receptor antagonists iv) Behavioral assays v) Biochemical assays vi) Molecular assays

3.1.3. Standardization of animal models for depression co-morbid with obesity

To study the neuropsychiatric disorders such as depression and anxiety, it is essential to develop and standardize laboratory animal models that resemble the behavioral, biochemical and physiological features, as close as possible, to that of humans.

In the present research work, it was aimed to standardize the animals models to study behavioral, biochemical and molecular aspects involved in depression co-morbid with obesity and to screen potential 5-HT_3 receptor antagonist **OND**, **QCM-4** and **4a** in the management of such co-morbid disorders.

3.1.3.1. HFD induced depressive phenotypes

Animals (mice) fed with high fat diet (HFD), were screened for depressive and anxiety-like behaviors. The effect of 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** on depression co-morbid with obesity was evaluated in HFD fed mice using behavioral (**Table 3.4**), biochemical (**Table 3.5**) and molecular (**Table 3.6**) assays.

Table 3.4: Behavioral assays of depression and anxiety in HFD fed mice

Sr. No.	Behavioral test(s)	Parameters/observations
1.	SPT	Volume of sucrose solution consumed
2.	FST	Duration of Immobility (s)
3.	TST	Duration of Immobility (s)
4.	EPM	% OAE and % OAT
5.	HBT	Head dip and transition scores
6.	L/D test	Time in light chamber and transition score

Table 3.5: Biochemical assays of depression co-morbid with obesity in HFD fed mice

Sr. No.	Biochemical measurement	Marker
1.	Glucose	Glycemic control
2.	Total cholesterol	Lipid profile
3.	Triglycerides	
4.	Lipid peroxidation marker malonaldehyde (MDA)	Oxidative stress
5.	Anti-oxidant reduced glutathione (GSH)	
6.	Oral glucose tolerance test (OGTT)	Glucose sensitivity
7.	Corticosterone	Endocrinological changes
8.	Leptin	
9.	Insulin	

Table 3.6: Molecular assays of depression co-morbid with obesity in HFD fed mice

Sr. No.	Molecular assays	Marker
1.	Serotonin (5-HT) level in hippocampus	Neurotransmitter
2.	Cyclic adenosine monophosphate (cAMP) level in hippocampus	Neurotrophic factors involved in neurogenesis
3.	Brain derived neurotrophic factor (BDNF) level in hippocampus	
4.	Histological studies in hippocampal dentate gyrus (DG) region	Neuronal morphology
5.	Immunohistochemistry (IHC) assay of p53 in hippocampal DG region	p53 is involved in neuronal damage

3.1.3.2. HFD fed mice subjected to CUMS as a model to study depression co-morbid with obesity

In order to reflect the symptomlogy of depression in obesity, and to evaluate the effect of chronic stress procedure on obese animals, HFD fed mice were subjected to CUMS. CUMS model reflects most of the clinical signs of depressive behavior. Besides, this model also represents the severity of depressive-phenotypes due to CUMS procedure. The 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** were screened for depression co-morbid with obesity in HFD fed mice subjected to CUMS using behavioral (**Table 3.7**), biochemical (**Table 3.8**) and molecular assays (**Table 3.9**).

Table 3.7: Behavioral assays of depression and anxiety in HFD fed mice subjected to CUMS

Sr. No.	Behavioral test(s)	Parameters/observations
1.	SPT	Volume of sucrose solution consumed
2.	FST	Duration of Immobility (s)
3.	TST	Duration of Immobility (s)
4.	EPM	% OAE and % OAT
6.	L/D test	Time in light chamber and transition score

Table 3.8: Biochemical assays of depression co-morbid with obesity in HFD fed mice subjected to CUMS

Sr. No.	Biochemical measurement	Marker
1.	Glucose	Glycemic control
2.	Total cholesterol	Lipid profile
3.	Triglycerides	
4.	Lipid peroxidation marker MDA	Oxidative stress and anti-oxidant
5.	Anti-oxidant GSH	
6.	Corticosterone	Endocrinological changes
7.	Leptin	
8.	Insulin	

Table 3.9: Molecular assays of depression co-morbid with obesity in HFD fed mice subjected to CUMS

Sr. No.	Molecular assays	Marker
1.	Serotonin (5-HT) level in hippocampus	Neurotransmitter
2.	Cyclic adenosine monophosphate (cAMP) level in hippocampus	Neurotrophic factors involved in neurogenesis
3.	Brain derived neurotrophic factor (BDNF) level in hippocampus	
4.	Histological studies in hippocampal dentate gyrus (DG) region	Neuronal morphology

3.1.4. Confirmatory study: Role of 5-HT$_3$ receptors in mediating the antidepressant effect of OND, QCM-4 and 4a in HFD fed mice

Effect of 5-HT$_3$ receptor antagonists OND, QCM-4 and 4a on depressive behavior in 1-(m-chlorophenyl)-biguanide (mCPBG) pre-treated HFD fed mice

Screening of 5-HT$_3$ receptor antagonists **OND**, **QCM-4** and **4a** for depression co-morbid obesity in mCPBG pre-treated mice. mCPBG is potent 5-HT$_3$ receptor agonists that has high affinity for 5-HT$_3$ receptors and does not have any effect on depressive phenotypes. This model provides the mechanistic instincts of 5-HT$_3$ receptor antagonism by standard drugs and novel compounds, as in presence of mCPBG, they will not be able to bind and block 5-HT$_3$ receptors and produce antidepressant effect. Following assays (**Table 3.10**) were performed to assess the effect of 5-HT$_3$ receptor antagonists **OND**, **QCM-4** and **4a** in HFD fed mice pre-treated with mCPBG.

Table 3.10: Assessments of depressive-phenotypes in mCPBG pre-treated model

Sr. No.	Behavioral test(s)	Parameters/observations
1.	FST	Duration of Immobility (s)
2.	TST	Duration of Immobility (s)
3.	EPM	% OAE and % OAT
4.	L/D test	Time in light chamber and transition score
5.	5-HT measurement	Neurotransmitter

4. EXPERIMENTAL METHODOLOGY

4.1. Animals

Male Swiss albino mice (20-25 g) and Wistar rats (230-250 g) were procured from Chaudhary Charan Singh Hisar Agricultural University, Hisar, Haryana, India (Reg. No. 417/01/a/ CPCSEA). Animals were housed in cages [mice (24 x 17 x 14 cm), rats (36 x 23 x 17 cm)] and maintained at standard laboratory conditions temperature 22 ± 2 ^{0}C and room humidity 60 ± 10 %, 12:12 h of light/dark cycle and had free access to food (standard pellet chow food for laboratory animals) and water in the Central Animal Facility of the Institute. After two weeks of quarantine period, animals were randomized on the basis of body weight for different assay protocols.

4.2. Ethical Approval

In India, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) is established under "Prevention of Cruelty to Animals Act 1960". CPCSEA has a representative body at Institute level named as Institutional Animal Ethics Committee (IAEC). All the experimental procedures performed on animals, were in compliance with the protocols, approved by IAEC, of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/16/06; IAEC/RES/16/06/REV/1/20; IAEC/RES/18/09; IAEC/RES/18/09/REV-1/19/29; IAEC/RES/18/09/REV-2/21/12).

4.3. Drugs and Chemicals

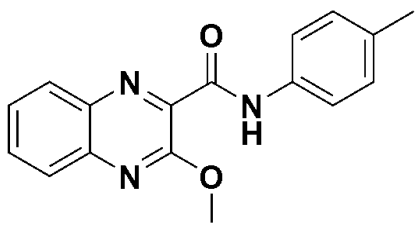
4.3.1. Drugs used for *In vivo* behavioral assays

OND was procured as a generous gift sample from Akums Drugs and Pharmaceuticals Limited, India. ESC was kindly provided by Ranbaxy Research Laboratories, India as a gift. Diazepam (DZM) was purchased from Cipla Ltd, India. mCPBG was obtained from Tocris Chemicals, United Kingdom. All the other chemicals used in various assays were of analytical grade. All the drugs were freshly prepared before administration by intraperitoneally (i.p.) or per-oral (p.o.) route.

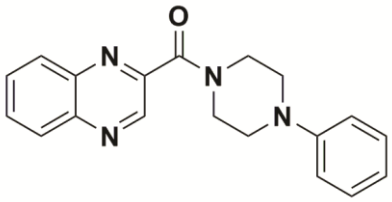
4.3.2. New chemical entities (NCE's)

Novel 5-HT$_3$ receptor antagonists were selected based on their high pA2 and log p values. 3-methoxy-N-p-tolylquinoxalin-2-carboxamide (**QCM-4**) and (4-phenylpiperazin-1-yl)(quinoxalin-2-yl) methanone (**4a**) were selected based on their features mentioned in **Table 4.1** (Mahesh et al., 2010, 2011, 2012).

QCM-4 and **4a** were synthesized (structures mentioned in **Fig 4.1**) in the Medicinal Chemistry Laboratory, Birla Institute of Technology & Science, Pilani, Pilani Campus and were used as test drugs. **QCM-4** and **4a** were freshly prepared for administration in distilled water (for i.p. administration) and 0.25% sodium carboxymethyl cellulose (CMC) (for p.o. administration). Their relation with 5-HT_3 receptor was established through different studies and they were among the few primary compounds synthesized.



QCM-4



4a

Fig 4.1: Structures of in-house synthesized 5-HT_3 receptor antagonists **QCM-4** and **4a**

Table 4.1: Specifications of novel 5-HT_3 receptor antagonists QCM-4 and 4a

Features	QCM-4	4a
IUPAC name	3-methoxy-N-p-tolylquinoxalin-2-carboxamide	(4-phenylpiperazin-1-yl)(quinoxalin-2-yl) methanone
pA2 value	7.3	6.8
Log P value	3.64	2.43
Melting point	102-104 ºC	128-130 ºC

4.3.3. Composition of HFD

Powdered normal chow used in the preparation of HFD was obtained from Ratatouile Solutions, Rajasthan, India. Lard was purchased from local vendor, casein, cholesterol, vitamin and mineral mix, dl-methionine, yeast powder and sodium chloride were procured from Hi-Media.

4.3.4. Requirements for glucose and lipid profile estimations

Diagnostic assay kits were procured from Spinreact, Girona, Spain for the estimation of glucose, total cholesterol and triglycerides.

4.3.5. Requirements for oxidative stress parameters estimation

The chemicals and reagents used in the estimations of oxidative stress parameters and were of laboratory grade. Sodium dodecyl sulphate, glacial acetic acid, thiobarbituric acid, sulfosalicyclic acid, 5,5'-dithiobis (2-nitrobenzoic acid), sulphanilamide, naphthyl ethylenediamine dihydrochloride, phosphoric acid, potassium dichromate, hydrochloric acid, hydrogen peroxide, etc, were purchased from various companies such as Hi-Media, SD Fine, Spectrochem Chemicals, India.

4.3.6. Requirements for corticosterone (CORT) estimations

CORT was procured from Sigma Chemicals, United States. Laboratory grade reagents such as chloroform, sulfuric acid, sodium hydroxide and methanol were obtained from Sisco Research Labs, SD fine and Hi-Media, India.

4.3.7. Enzyme linked immunosorbent assay (ELISA) and antibody kits

ELISA kit for insulin was obtained from Crystal Chem Inc, United States. Leptin kit was purchased from Aviscera Bioscience, Inc., United States. ELISA kit for cAMP was purchased from Enzo Life Science Ltd, United States. BDNF Elisa kit was procured from Boster Biological Technology Co. Ltd, United States. Antibodies required for Immunohistochemical assays were procured from Santa Cruz Biotechnology, Inc, United States.

4.3.8. Requirements for neurotransmitter estimation

Standard 5-HT was purchased from Lancaster Chemicals, United Kingdom. Analytical and laboratory grade chemicals and reagents such as sodium metabisulfite, perchloric acid, ethylenediaminetetraacetic acid (EDTA), methanol, contents of phosphate buffer were purchased from SD Fine and Hi-Media.

4.3.9. Requirements for histological studies

Requirements for block preparation such as paraffin wax, heamotoxylin-eosin (H & E) was procured from Sigma Chemicals United States. Laboratory grade reagents such as methanol, xylene and contents of phosphate buffer were purchased from Hi-Media and SD Fine.

4.4. Surgicals

4.4.1. Sterile suture

Ethicon 4-0, Non-absorbable surgical sutures USP, Ethicon 4-0, Absorbable surgical sutures, USP (Catgut), were purchased from Johnson and Johnson, India and Mersilk (Braided silk black).

4.4.2. Haemostatic sponge

AbGel, Absorbable gelatin sponge USP, were obtained from Srikrishna Laboratories, Mumbai, India.

4.4.3. Surgical needles

Curved surgical needles were procured from Pricon Surgicals, New Delhi, India.

4.5. Equipments

- ✓ Stereotaxic Frame: Inco Ambala, India
- ✓ Digital actophotometer: Inco Ambala, India
- ✓ Digital EPM: SN Scientific, India
- ✓ Centrifuge: Eppendorf refrigerated centrifuge, 5702-R, Eppendorf AG, Germany
- ✓ Tissue Homogenizer: Kinematica^{TM} Polytron^{TM} Homogenizers, Germany
- ✓ Elisa Pate reader and washer: Ark Diagnostic, India
- ✓ Spectrophotometer: UV-1800, Shimadzhu, Japan
- ✓ Spectrofluorophotometer: Shimadzu RF 5301 PC Spectrofluorophotometer, Shimadzu, Japan
- ✓ Optical Microscope: Optika Microscope, Italy
- ✓ Deep freeze (-80 ºC): OPR-DFC-300CE, Operon Co. Ltd., Korea
- ✓ Equipments designed according to laboratory standards for: FST, TST, EPM, HBT, L/D, OFT
- ✓ Microtome: Leica Biosystems, Wetzlar, Germany.

4.6. Pharmacological Screening

4.6.1. General considerations for behavioral assays

In the preliminary behavioral assays, separate sets of animals were used to avoid habituation effects with experimental situations. All the solutions of standard drugs and novel compounds were freshly prepared and administered by i.p./p.o. route, at a constant dose volume of 10 ml/kg for mice and 2 ml/kg for rats. Experimental animals were acclimatized to the test room at least one h prior to the experiment and behavioral assays were conducted between 0900 and 1500 h.

4.7. Pharmacological preliminary behavioral assays for depression

4.7.1. Spontaneous locomotor activity (SLA) score

Rationale

The animal models used in the assessments of antidepressive or anxiolytic activities of any known or unknown compounds/drugs are based on the principle of despair and exploratory behavior. It is very essential to take into consideration the basal locomotor scores of test animals in order to rule out the possibilities of false positive or false negative effect of the test compounds/drugs. Hence, the doses of compounds/drugs that do not alter the basal locomotor score are more preferred for the behavioral antidepressant and anxiolytic activities in rodents.

Fig 4.2: Pictorial representation of actophotometer

Different sets of mice were used for each dose of **QCM-4** and **4a** as mentioned in **Table 4.2**.

Table 4.2: Groups used for screening QCM-4 and 4a for SLA score

Sr. No.	Groups used for QCM-4 in SLA score	Groups used for 4a in SLA score
1.	Control	Control
2.	**QCM-4** (0.5 mg/kg, i.p.)	**4a** (0.5 mg/kg, i.p.)
3.	**QCM-4** (1 mg/kg, i.p.)	**4a** (1 mg/kg, i.p.)
4.	**QCM-4** (2 mg/kg, i.p.)	**4a** (2 mg/kg, i.p.)
5.	**QCM-4** (4 mg/kg, i.p.)	**4a** (4 mg/kg, i.p.)
6.	……	**4a** (8 mg/kg, i.p.)

(n=6/group)

Procedure

SLA score was measured by using the digital actophotometer (30 cm x 30 cm) with inside walls painted black (Boissier and Simon, 1965) (as shown in **Fig 4.2**). 30 min post administration of drug/compound/vehicle the animals were placed in the centre of the apparatus and allowed to access the area for 10 min consisting initial 2 min of acclimatization and remaining 8 min for measuring the locomotor activity score. After each test, the floor was cleaned thoroughly with 70% volume/volume (v/v) alcohol solution to eliminate possible bias due to odors left by previous mice.

4.7.2. Forced swim test (FST)

Rationale

FST is a high predictive validity model used to assess the antidepressant activity of any known and unknown drugs in the laboratory (Cryan et al., 2002). FST reflects high sensitivity towards monoamine alterations (Holmes, 2003) which provide a model for studying neurobiological and genetic mechanisms involved in antidepressant response of drugs (Porsolt, 2000, Nestler et al., 2002).

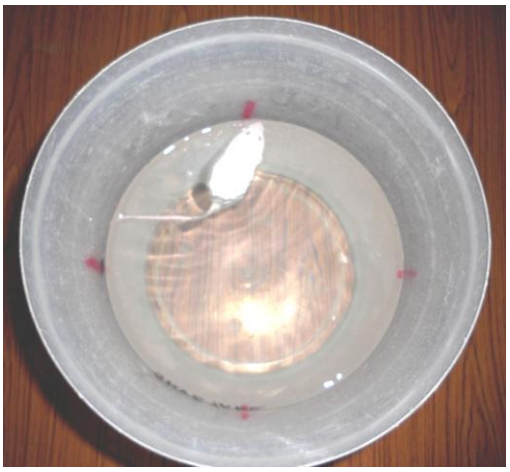


Fig 4.3: Pictorial representation of FST

Procedure

FST was performed as described previously (Porsolt et al., 1977) with specific modification. Briefly, the animals were individually forced to swim in a glass cylinder (diameter: 22.5 cm, height: 30 cm) filled with water (23 ± 2 °C) (**Fig 4.3**) up to a height of 15 cm for 6 min. Initial 2 min was for adaptation and the duration of immobility in sec (s) was recorded in remaining 4 min. Animals were given training of 15 min for swimming 24 h before the commencement of test. The animals were considered immobile when they were passive, completely motionless and did not show any body movement. Groups used for screening **QCM-4** and **4a** in FST were as per **Table 4.3.**

Table 4.3: Groups used for screening QCM-4 and 4a in FST and TST

Sr. No.	Groups used for QCM-4 in FST and TST	Groups used for 4a in FST and TST
1.	Control	Control
2.	**QCM-4** (0.5 mg/kg, i.p.)	**4a** (0.5 mg/kg, i.p.)
3.	**QCM-4** (1 mg/kg, i.p.)	**4a** (1 mg/kg, i.p.)
4.	**QCM-4** (2 mg/kg, i.p.)	**4a** (2 mg/kg, i.p.)
5.	……	**4a** (4 mg/kg, i.p.)
6.	ESC (10 mg/kg, i.p.)	ESC (10 mg/kg, i.p.)

(n=6/group/assay)

4.7.3. Tail suspension test (TST)

Rationale

TST is a high predictive validity behavioral assay commonly used for the assessment of antidepressant-like effect of novel or standard drugs at experimental laboratory settings. TST represents the immobility of the animal due to its inability to maintain the escape effort and animals are capable to adapt to this posture rather quickly. Drugs that inhibits this immobility posture are supposed to show antidepressant effect (Cryan et al., 2005). The immobility is considered as analogous to clinical observations, where depressed patients often lack the efforts to escape, as observed in prominent psychomotor impairments (Weingartner and Silberman, 1982).

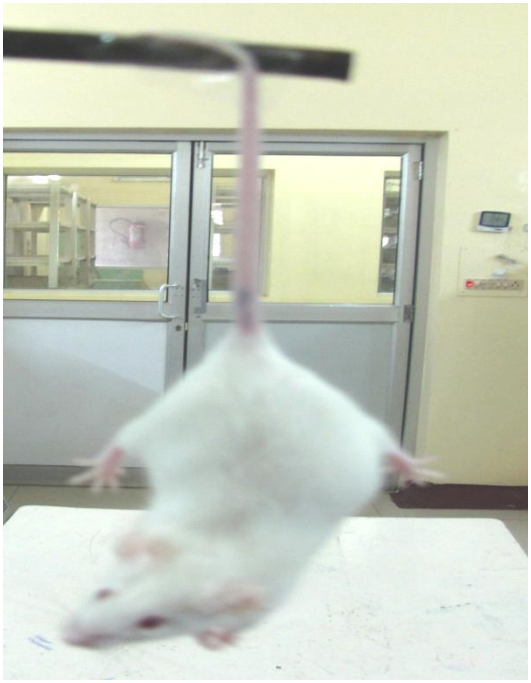


Fig 4.4: Pictorial representation of TST

Procedure

TST was performed as described earlier (Steru et al., 1985). From the horizontal bar mice were suspended by tail at a distance of 50 cm from floor (distance from tip of the tail = 2 cm) (as shown in **Fig 4.4**). Test consists of 6 min duration in which the immobility time was recorded. A mouse was considered immobile when it remained passive, completely motionless and did not show any body movements. Groups used for screening **QCM-4** and **4a** in TST are mentioned in **Table 4.3.**

4.8. Pharmacological preliminary behavioral assays for anxiety

In the preliminary screening of **QCM-4** and **4a** compounds for anxiety, the behavioral assay paradigms used were elevated plus maze (EPM), hole board test (HBT) and light/dark (L/D) test. The groups of **QCM-4** and **4a** used in all these anxiety tests were as following **(Table 4.4)**.

Table 4.4: Groups of animals used in screening of QCM-4 and 4a in EPM, HBT, L/D

Sr. No.	Groups used for QCM-4 in anxiety tests	Groups used for 4a in anxiety tests
1.	Control	Control
2.	**QCM-4** (1 mg/kg, i.p.)	**4a** (2 mg/kg, i.p.)
3.	**QCM-4** (2 mg/kg, i.p.)	**4a** (4 mg/kg, i.p.)
4.	Diazepam (2 mg/kg, i.p.)	Diazepam (2 mg/kg, i.p.)

(n=6/group/assay)

4.8.1. Elevated plus maze (EPM)

Rationale

EPM, the behavioral paradigm is widely used at laboratory level assessment of anxiolytic effect of known or unknown compounds/drugs (Hogg, 1996). EPM reflects the psychomotor and emotional aspects in rodent which correlates with unconditioned anxiety. Moreover, the compounds/drugs showing anxiolytic activity improve the open arm entries and time (Dawson and Tricklebank, 1995, Rodgers and Dalvi, 1997) and vice-versa is true with anxiogenic agents.

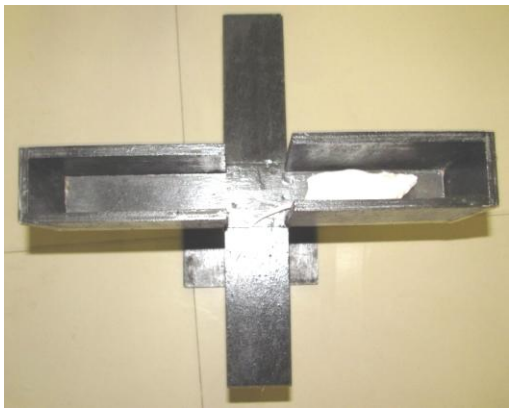


Fig 4.5: Pictorial representation of EPM test

Procedure

The EPM was performed using the method mentioned elsewhere (Adeyemi et al., 2006). Briefly, it consisted of two open and two closed arms (all arms: 20×4×12 cm) made of wooden blocks, elevated at a height of 25 cm from floor (shown in **Fig 4.5**), which was lighten with 60 W bulb through a height of 100 cm. Each mouse was placed in the central square (5 cm×5 cm) facing one of the open arm and allowed to explore the maze for 5 min of test period. The parameter measured was percent open arm entries (% OAE) and percent open arm time (% OAT). The maze was cleaned with 70 % v/v alcohol solution in between two test sessions to get rid of residual odor.

4.8.2. Hole board test (HBT)

Rationale

The HBT provide a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety, and/or responses to stress in animals (Takeda et al., 1998). The head dip behavior reflects the sensitivity towards the changes in emotional state of the animal and provide the information that a fearless state in animals may be reflected by the improved head dipping behavior (Nolan and Parkes, 1973).

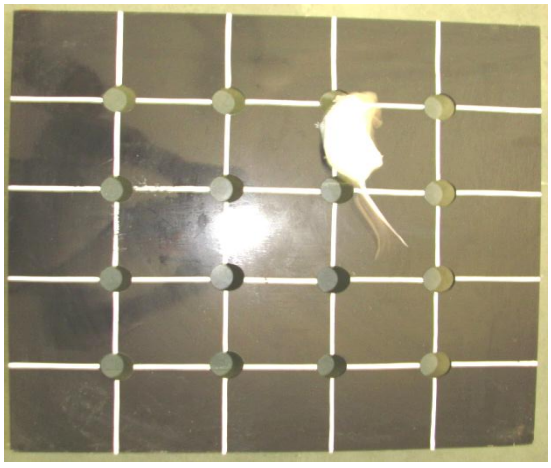


Fig 4.6: Pictorial representation of HBT

Procedure

The HBT was performed according to the method described earlier (Nolan and Parkes, 1973), with slight modifications. Briefly, the hole board apparatus consisted of a gray plexiglas chamber (40 cm × 40 cm) raised to a height of 15 cm from the floor of a wooden gray box (40 cm × 40 cm × 40 cm) having 16 equidistant holes, each of 3 cm in diameter distributed on the floor as shown in **Fig 4.6**. Animals were individually placed in the center of the hole board apparatus and allowed to freely explore the apparatus for 5 min. The numbers of squares crossed, the head dip score were recorded. A head dip was scored, if both eyes of mice were disappeared into the hole. After each test, the apparatus was sprayed with 70% v/v alcohol and wiped thoroughly to eliminate the residual odor.

4.8.3. Light/dark (L/D) test

Rationale

L/D test represents one of the highly practiced laboratory animal models for anxiety. It is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, a novel environment and light. It is reported that an animal spending more time in light chamber along with increased transition number is considered to show anxiolytic activity (Imaizumi et al., 1994).

Fig 4.7: Pictorial representation of L/D test

Procedure

The method developed by Crawley and Goodwin (1980), based on the natural aversion of animal for brightly lit places, was adopted. Briefly, in a two compartment box, one dark and one brightly lit (shown in **Fig 4.7**), the time spent in the light compartment and the crossings between the light and dark compartment was recorded for 5 min. After each test, the apparatus was sprayed with 70% v/v alcohol and wiped thoroughly to eliminate the residual odor. Test was performed in a temperature, noise, and light controlled room.

4.9. Pharmacological chronic models of depression

4.9.1. Olfactory balbectomy (OBX) surgical model in rats

Rationale

OBX model is one of most reliable model for assaying antidepressant activity of a drugs or novel compounds. OBX results in the altered behavioral and neurochemical signaling circuits contributing to the clinical symptoms of depression (Lumia et al., 1992, Kelly et al., 1997). OBX animals develop a specific abnormal features characterized by hyperactivity, increase in ambulation, rearing and fecal contents in various behavioral assays (Song and Leonard, 2005).

Procedure

Male Wistar rats were grouped as mentioned in **Table 4.5** and bulbectomized at the age of 7-8 weeks. Bilateral olfactory bulbectomy was performed according to the previously described method (Tasset et al., 2010). Briefly, rats were anaesthetized with the cocktail of xylazine and ketamine (5 and 75 mg/kg i.p. respectively). The animals were fixed in a stereotactic frame (INCO, India), and the skull was exposed by a midline incision.

The burr holes (2 mm 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 an eye (as showed in **Fig 4.8**). The olfactory bulbs were removed by suction, the holes were then filled with haemostatic sponge to control excessive bleeding and the scalp was sutured. Sham-operated rats were subjected to the same surgical procedure, including piercing of the dura mater, but their bulbs were left intact (schematic presentation of OBX surgical procedure is showed in **Fig 4.9**). Following a rehabilitation period of 14 days, OBX/sham operated rats were treated orally with QCM-4 (1 and 2 mg/kg)/4a (2 and 4 mg/kg)/EST (10 mg/kg)/vehicle (2 ml/kg) once daily between 09:00 - 11.00 a.m. for 14 days from 15th onwards (Time-line mentioned in **Table 4.6**).

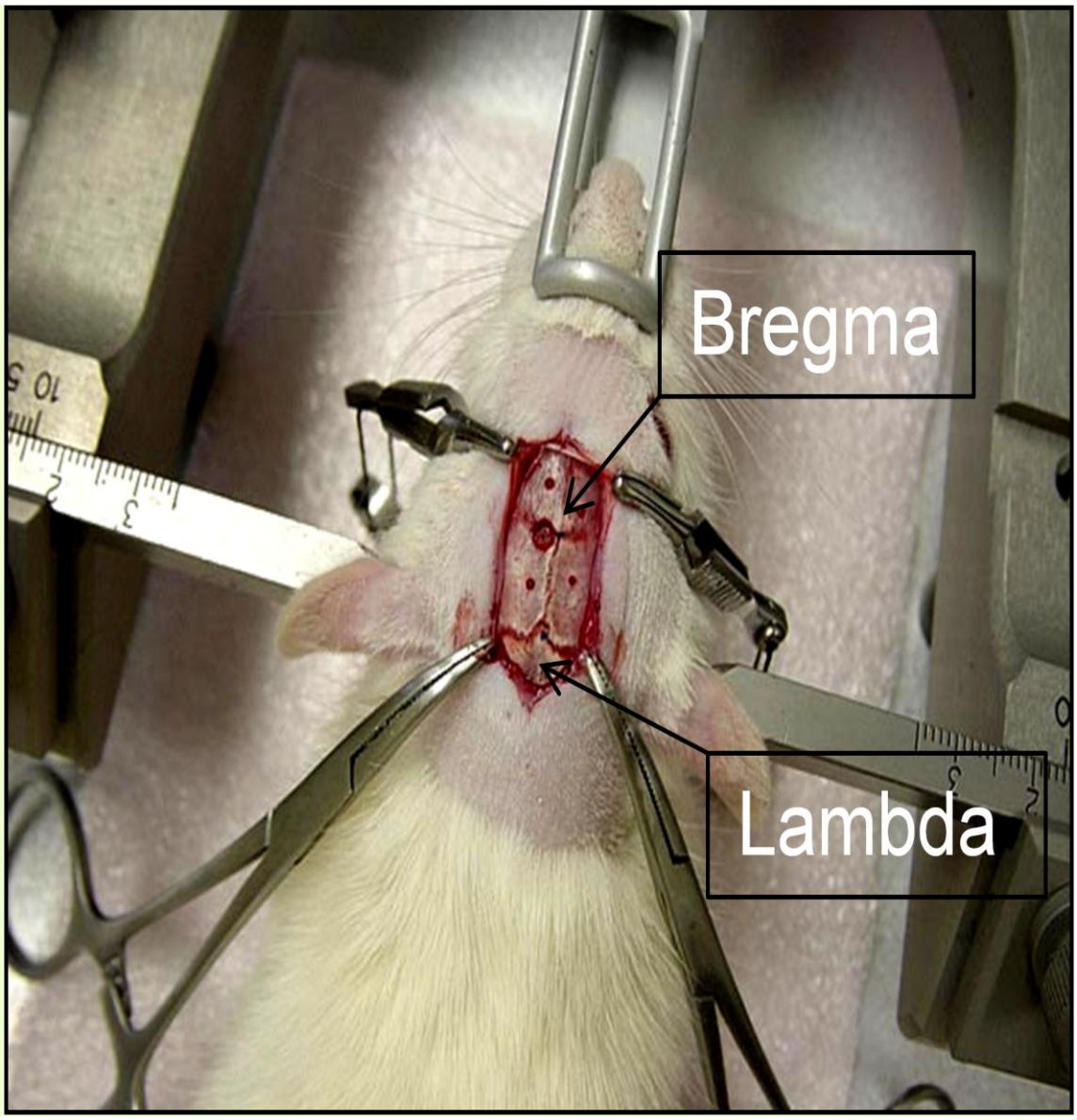


Fig 4.8: Bregma region for OBX surgery in rats

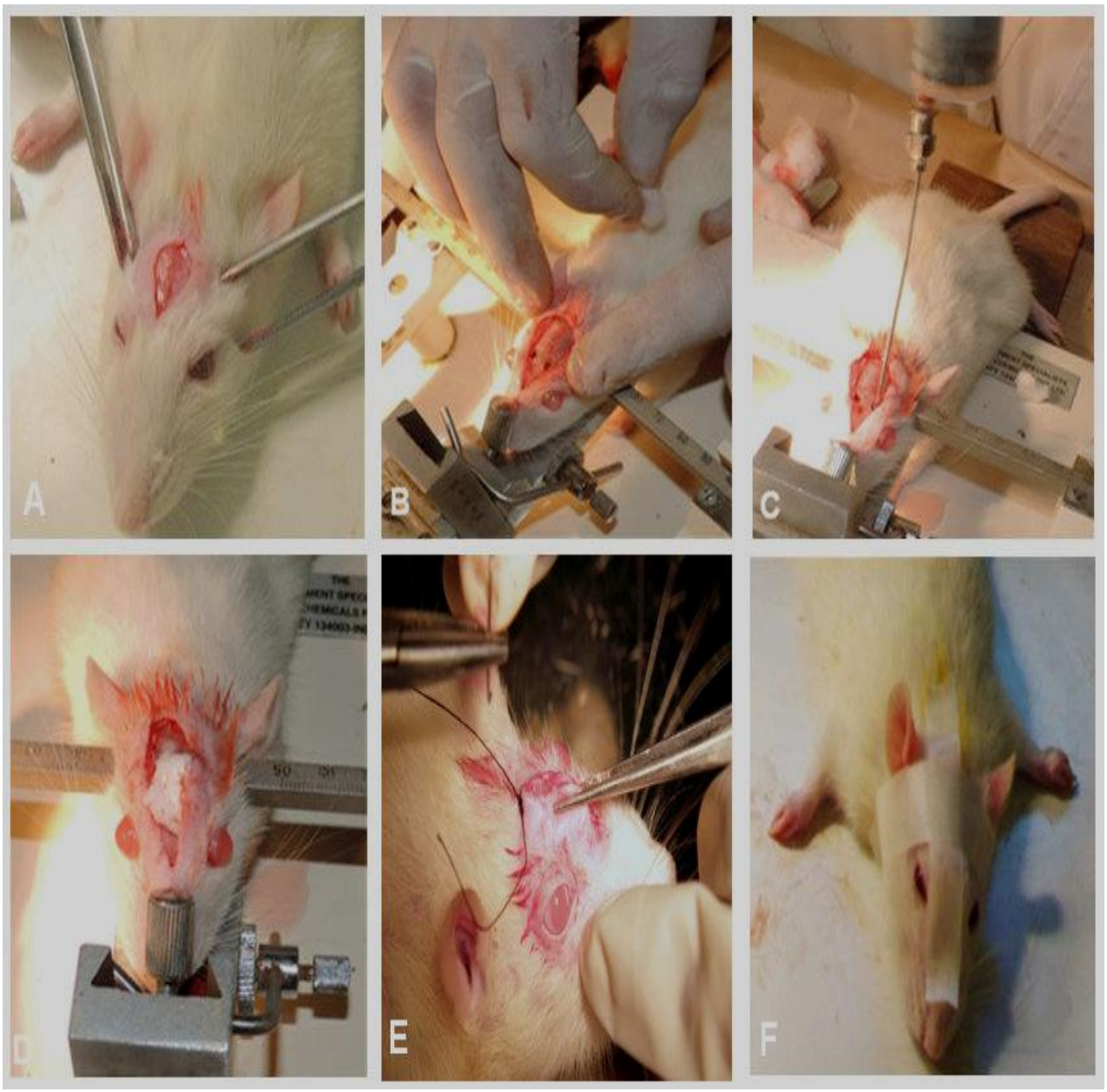


Fig 4.9: OBX surgical procedure in rats (A) positioning of rats, (B) burr holes, (C) removal of olfactory bulbs, (D) hemostatic sponge insertion, (E) suturing of incision site and (F) healing process

Table 4.5: Groups used for screening QCM-4 and 4a in OBX model of depression

Sr. No.	Groups used for QCM-4 in OBX model of depression	Groups used for 4a in OBX model of depression
1.	Sham control	Sham control
2.	Sham + **QCM-4** (1 mg/kg, p.o.)	Sham + **4a** (2 mg/kg, p.o.)
3.	Sham + **QCM-4** (2 mg/kg, p.o.)	Sham + **4a** (4 mg/kg, p.o.)
4.	Sham + ESC (10 mg/kg, p.o.)	Sham + ESC (10 mg/kg, p.o.)
5.	OBX control	OBX control
6.	OBX + **QCM-4** (1 mg/kg, p.o.)	OBX + **4a** (2 mg/kg, p.o.)
7.	OBX + **QCM-4** (2 mg/kg, p.o.)	OBX + **4a** (4 mg/kg, p.o.)
8.	OBX + ESC (10 mg/kg, p.o.)	OBX + ESC (10 mg/kg, p.o.)

(n =6/group)

Table 4.6: Time-line for OBX model of depression

Day 0	Day 0 – 1	Day 1 – 14	Day 15 – 28	Day 29 – 31 (Treatment was continued)		
				Behavioral assay on day		
				25-29	30	31
OBX surgery	Post surgery recovery (continuous care)	Post surgery rehabilitation period (daily handling and observation)	Oral treatment with **OND/QCM-4/ 4a/**ESC/vehicle	SPT	EPM	OFT

4.9.1.2. Behavioral assays performed post OBX

4.9.1.2.1. Sucrose preference test (SPT)

Rationale

Sucrose preference, acts as a reward and reduced sucrose consumption, is termed as anhedonia. Anhedonia represents the core symptoms of human major depression showing reduced responsiveness to the rewards measured by decreased sucrose preference (Garza et al., 2012).

Procedure

SPT was performed as per the method described by (Willner, 1997, Casarotto and Andreatini, 2007) with specific modification. All the animals had free access for two bottles containing sucrose solution (1%, w/v) and water, respectively for 5 days. The position of both bottles was switch each day to avoid the chances of side preference. On the test day animals were presented two bottles containing sucrose solution and water, respectively in the morning (9:30 am). Volume consumed after 24 h was measured and percent sucrose preference was calculated using the following formula;

% sucrose preference = [(sucrose consumption (ml)/water + sucrose consumption (ml)] × 100]

4.9.1.2.2. EPM

Rationale

OBX animal shows hyperactivity, natural aversion to open area, preference of protected and unprotected area that is evaluated in EPM test.

Fig 4.10: Pictorial representation of EPM exploration for OBX rats

Procedure

EPM test was performed as per the method of (Pellow et al., 1985). The apparatus used for EPM test consisted of two open arms (50 cm x 50 cm) and two closed arms (50 cm x 50 cm) with 30 cm high walls. All the four arms were joined in the centre with platform space (10 cm x 10 cm) as shown in **Fig 4.10**. The apparatus was elevated at a height of 60 cm from the floor and illuminated with ceiling lamp (60 W) that was suspended 90 cm above the maze.

During test, animals were individually introduced in the centre platform facing one of the open arms and subsequently number of entries and time spent in open arm were recorded for 5 min and % OAE and % OAT were calculated. The apparatus was cleaned with 70% ethyl alcohol after each test.

4.9.1.2.3. Modified open field test (OFT)

Rationale

Measurement of hyperactivity reflects the psychomotor behavior. OBX surgery develops specific abnormal features characterized by hyperactivity that is measured in terms of increased ambulation, rearing and fecal contents in behavioral assay such as OFT (Song and Leonard, 2005).

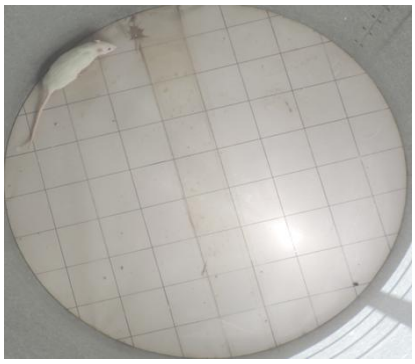


Fig 4.11: Pictorial representation of modified open-field exploration for OBX rats

Procedure

The OFT was conducted as described previously (Kelly et al., 1997, Ramamoorthy et al., 2008). The apparatus used for OFT consisted of a circular (diameter: 90 cm) arena (shown in **Fig 4.11**) with 75 cm high aluminum walls and floor equally divided into 10 cm squares. 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. On the day of experiment, each animal was individually placed in the center of the open field apparatus and the ambulation scores (number of squares crossed), rearing and fecal pellets were recorded for 5 min. After each test, the apparatus was cleaned with 70% (v/v) alcohol to get rid of the odor of previous animal and avoid bias observations.

4.9.2. Chronic unpredictable mild stress (CUMS) model of depression

Rationale

Stress has been one of the most important pathogenic factors in the neuropsychiatric disorders such as depression and anxiety (García-Bueno et al., 2008). In rodents, the chronic unpredictable mild stress (CUMS) model is mostly used for assessing the pathophysiology of depression and the associated therapeutic intervention (Willner, 2005). Furthermore, CUMS leads to various long-term behavioral, neurochemical, neuroimmune and neuroendocrine alterations that resemble those observed in patients with depression, where symptoms are reversed only by chronic, but not acute treatment with antidepressants (Cryan and Holmes, 2005). The mechanism for CUMS induced depression is still not clearly exposed.

Procedure

Male Swiss albino mice were grouped as mentioned in **Table 4.7** for CUMS model of depression. The CUMS was performed as described earlier by (Ducottet et al., 2003, Mao et al., 2009, Jindal et al., 2013). In brief, the CUMS protocol consisted of the sequential application of a variety of mild stressors. These stressors were randomly scheduled over a 1-week period and repeated throughout the 4-week experiment (**Table 4.8 and Fig 4.12**). Non stressed animals were left undisturbed in their home cages except during housekeeping procedures such as cage cleaning.

Oral treatment with **QCM-4** (1 and 2 mg/kg)/**4a** (2 and 4 mg/kg)/ESC (10 mg/kg)/vehicle (10 ml/kg) was started from day 15 to 28 of the stress procedure (last two weeks) (time-line mentioned in **Table 4.9**). From day 29 onwards behavioral assays were performed followed by biochemical estimations.

Table 4.7: Groups used for screening QCM-4 and 4a in CUMS model of depression

Sr. No.	Groups used for QCM-4 in CUMS model of depression	Groups used for 4a in CUMS model of depression
1.	Normal control	Normal control
2.	CUMS control	CUMS control
3.	CUMS + **QCM-4** (1 mg/kg, p.o.)	CUMS + **4a** (2 mg/kg, p.o.)
4.	CUMS + **QCM-4** (2 mg/kg, p.o.)	CUMS + **4a** (4 mg/kg, p.o.)
5.	CUMS + ESC (10 mg/kg, p.o.)	CUMS + ESC (10 mg/kg, p.o.)

(n=6/group)

Table 4.8: Schedule for CUMS procedure

Sr. No.	Day	Type of stress procedure	Time
1.	Monday	Food and water deprivation	24 h (9 am to 9 am)
2.	Tuesday	Empty bottle Foreign object	1 h (9 am to 10 am) 24 h (9 am to 9 am)
3.	Wednesday	Overnight illumination Forced swimming	10 h (8 pm to 6 am) 1 h (4 min/ animal) (9 am to 10 am)
4.	Thursday	Cage tilt Restraint	5 h (12 pm to 5 pm) 1 h (10 am to 11 am)
5.	Friday	Food deprivation Soiled cage (with water)	12 h (9 am to 9 pm) 12 h (9 pm to 9 am)
6.	Saturday	Water deprivation Overnight illumination	12 h (10 am to 10 pm) 12 h (7 pm to 7 am)
7.	Sunday	Empty bottles Cage tilt (25º angle)	2 h (10 am to 12 am) 5 h (12 am to 5 pm)

Fig 4.12: Various stressors used in CUMS protocol (A) food and water deprivation, (B) empty bottles, (C) FST, (D) cage tilt, (E) bed wet, (F) water withdrawal, (G) food withdrawal and (H) immobilization stress

Table 4.9: Time-line for CUMS model of depression

Day 1 -14	**Day 15 – 28**	**Day 29 - 34 Behavioral assays**				
CUMS	CUMS+ oral treatment with **QCM-4**/**4a**/ESC/vehicle	Day 25-29	Day 30	Day 31	Day 32	Day 33
		SPT	SLA	FST	TST	EPM

4.9.2.1. Behavioral assays performed on animals subjected to CUMS

The behavioral assays were performed according to the procedures mentioned in earlier sections as following;

SLA- 4.7.1

SPT- 4.9.1.2.1

FST- 4.7.2

TST- 4.7.3

EPM- 4.8.1

4.10. Method for induction of obesity

Rationale

High fat diet (HFD) induced obesity model was standardized in the laboratory for performing various assays of depression. HFD leads to dysregulation of glucose homeostasis, hyperglycemia, hyperleptenemia, hyperinsulinemia, and hypertiglycerides due to disturbances of Arc nucleus present in the hypothalamus region of brain controlling the leptin signaling for energy balace (Goyal, 2012).

Procedure

The composition of HFD used for induction of obesity was; powdered normal chow 365 g, lard 310 g, casein 250 g, cholesterol 10 g, vitamin and mineral mix 60 g, dl-methionine 03 g, yeast powder 01 g and NaCl 01 g for 1.0 kg of diet (Srinivasan et al., 2005) as showed in **Fig 4.13**.

Male Swiss albino mice were fed with HFD for 14 weeks to induce obesity and weekly body weight was measured. At the end of the 14 weeks of HFD feeding, body weights were measured and animals tested for biochemical parameters namely plasma glucose, total cholesterol and triglycerides. Mice fed with HFD and normal pellet diet (NPD) for 14 weeks, are shown in **Fig 4.14 (a,b)**. Higher body weight animals were selected for various behavioral assays.

Fig 4.13: HFD prepared in the laboratory

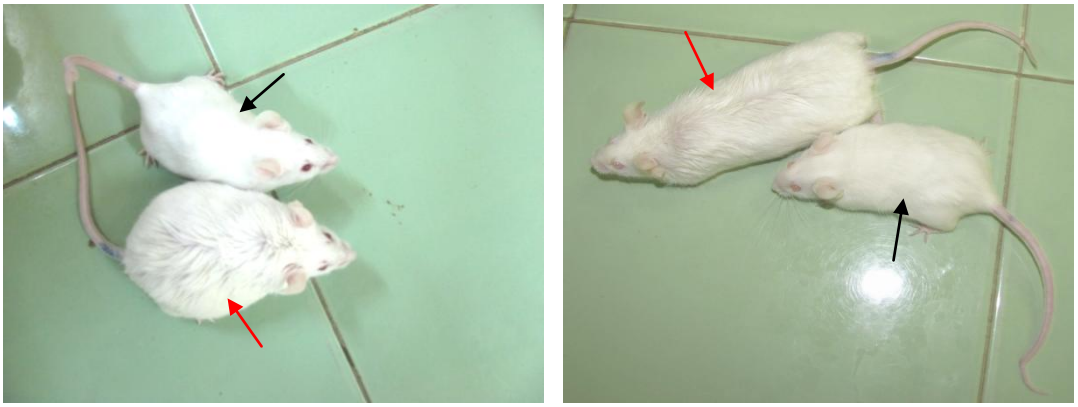


Fig 4.14 (a, b): HFD (red arrow) and NPD (black arrow) fed mice

4.10.1. Measurement of body weight in HFD fed mice

Groups (n=10/group)

1. NPD control
2. HFD control

Body weight (g) was recorded once in a week for 14 weeks of NPD and HFD fed animals.

4.10.2. Estimations of plasma biochemical parameters

Groups (n=10/group)

1. NPD control
2. HFD control

4.10.2.1. Estimation of plasma glucose

Rationale

Glucose is the main source of energy for most cells of the body and insulin helps in the entry of glucose into various body cells. Glucose level increases severely in metabolic disorders such as obesity and diabetes mellitus. Estimation of glucose is very essential in order to have proper endocrine functions (According to Spinreact, Ref: 41012).

The Principle of glucose oxidase-peroxidase (GOD-POD) method is involved in glucose estimation as shown in **Fig 4.15**. Glucose is catalyzed to gluconic acid by GOD and hydrogen peroxide is (H_2O_2) formed which is detected in presence of POD by chromogenic oxygen acceptor, phenol and 4-aminophenazone (4-AP). The color intensity is directly proportional to the concentration of glucose in the sample (Trinder, 1969).

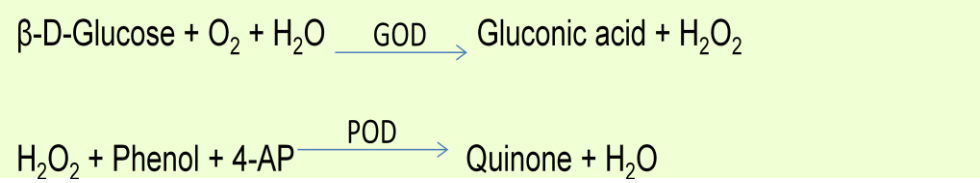


Fig 4.15: Principle of glucose estimation

Procedure

Procedure followed for estimation of glucose was according to the information brochure provided by the kit supplier (Spinreact, Ref: 41012).

(i) Assay conditions:

- Wavelength: 505 nm (490-550 nm)
- Cuvette: 1 cm light path
- Temperature: 37ºC (15-25 ºC)

(ii) Instrument was adjusted to zero with distilled water.

(iii) Dilutions were made as shown in **Table 4.10**

Table 4.10: Dilutions for plasma glucose estimation

	Blank	**Standard**	**Sample**
Reagent (R) (ml)	1.0	1.0	1.0
Standard (µl)	---	10	---
Samples (µl)	---	---	10

(iv) Sample/standard were mixed with reagent and incubated for 10 min at 37ºC.

(v) Absorbance (A) was measured for samples and standard against blank.

(vi) Calculations: (A) Sample / (A) Standard x 100 (conc. of standard) = mg/dl glucose in the samples.

4.10.2.2. Estimation of plasma total cholesterol

Rationale

Cholesterol is a fat-like substance known as lipid which is found in all body cells. Liver is the main producer of cholesterol that leads to the formation of cell membranes and certain hormones. Cholesterol is one of the important markers in metabolic studies such as obesity, diabetes mellitus, lipemia etc. (According to Spinreact, Ref: 41022).

The principle of cholesterol oxidase-peroxidase (CHOD-POD) is involved in the estimation of cholesterol (**Fig 4.16**). Cholesterol esters with water molecule forms cholesterol and fatty acids in presence of cholesterol esterase (CHE). Cholesterol with oxygen (O_2) in presence of cholesterol oxidase (CHOD) gives 4-Cholestenona and hydrogen peroxide (H_2O_2). In the final step H_2O_2 in combination of phenol and 4-AP produce Quinonimine.

The color intensity is directly proportional to the cholesterol concentration in the sample (Meiattini et al., 1978).

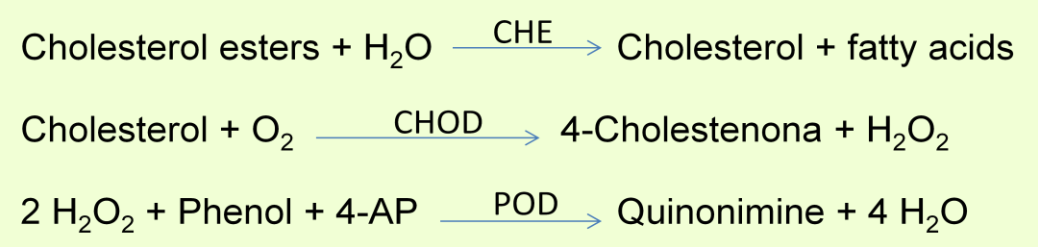


Fig 4.16: Principle of total cholesterol estimation

Procedure

Procedure followed for estimation of glucose was according to the information broacher provided by the kit supplier (Spinreact, Ref: 41022).

(i) Assay conditions:

- Wavelength: 505 nm (500-550 nm)
- Cuvette: 1 cm light path
- Temperature: 37ºC (15-25 ºC)

(ii) Instrument was adjusted to zero with distilled water.

(iii) Dilutions were made as shown in **Table 4.11.**

Table 4.11: Dilutions for plasma total cholesterol estimation

	Blank	**Standard**	**Sample**
Reagent (R) (ml)	1.0	1.0	1.0
Standard (µl)	---	10	---
Samples (µl)	---	---	10

(iv) Sample/standard were mixed with reagent and incubated for 5 min at 37ºC.

(v) Absorbance (A) were measured for samples and standard against blank.

(vi) Calculations: (A) Sample / (A) Standard x 200 (conc. of standard) = mg/dl cholesterol in the samples.

4.10.2.3. Estimation of plasma triglycerides

Rationale

Triglycerides are delivered to the body's cells by lipoproteins in the blood. Diet rich in saturated fats or carbohydrates increases the triglyceride levels, often observed with liver cirrhosis, hepatitis, diabetes mellitus, and obesity (According to Spinreact, Ref: 41031).

The Principle of glycerol phosphate dehydrogenase-peroxidase (GPO-POD) involved in triglyceride estimation, mentioned in **Fig 4.17**.

Triglycerides incubated with lipoprotein lipase (LPL), liberate glycerol and free fatty acids. Glycerol kinase and ATP converts glycerol into glycerol phosphate (G3P) and adenosine-5-diphosphate (ADP). GPO converts G3P into dihydroxyacetone phosphate (DAP) and p-chlorophenol in the presence of POD, that gives colored dyes. The intensity of color formed is proportional to the concentration of triglycerides in the sample (Bucolo and David, 1973).

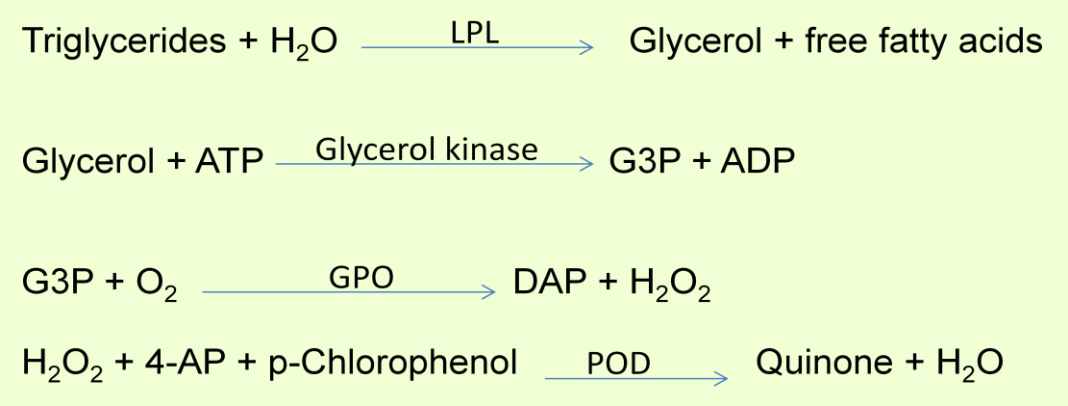


Fig 4.17: Principle of triglyceride estimation

Procedure

Procedure followed for estimation of glucose was according to the information broacher provided by the kit supplier (Spinreact, Ref: 41031).

(i) Assay conditions:

- Wavelength: 505 nm (490-550 nm)
- Cuvette: 1 cm light path
- Temperature: 37ºC (15-25 ºC)

(ii) Instrument was adjusted to zero with distilled water.

(iii) Dilutions were made as shown in **Table 4.12**.

Table 4.12: Dilutions for plasma triglycerides estimation

	Blank	**Standard**	**Sample**
Reagent (R) (ml)	1.0	1.0	1.0
Standard (µl)	---	10	---
Samples (µl)	---	---	10

(iv) Sample/standard were mixed with reagent and incubated for 10 min at 37ºC.

(v) Absorbance (A) was measured for samples and standard against blank.

(vi) Calculations: (A) Sample / (A) Standard x 200 (conc. of standard) = mg/dl cholesterol in the samples.

4.11. Screening of HFD fed mice for depressive behavior using various behavioral assays

Groups (n=6/group)

1. NPD control
2. HFD control

The procedures for behavioral assays of depression were followed as mentioned in earlier section;

SPT- 4.9.1.2.1

SLA- 4.7.1

FST- 4.7.2

TST- 4.7.3

4.12. Screening of HFD fed mice for anxiety-like behavior using various behavioral assays

Groups (n=6/group)

1. NPD control
2. HFD control

The procedures for behavioral assays of anxiety were followed as mentioned in earlier section;

EPM- 4.8.1

HBT- 4.8.2

LDT- 4.8.3

4.13. Effect of 5-HT_3 receptor antagonists OND, QCM-4 and 4a with chronic dosing on behavioral models of depression and anxiety in HFD fed mice

Grouping: HFD fed mice for screening of **OND**, **QCM-4** and **4a** in depression co-morbid with obesity were divided according to **Table 4.13, 4.14, and 4.15, respectively**.

Table 4.13: Groups used for screening OND for depression co-morbid with obesity in HFD fed mice

Sr. No.	Groups for screening OND in HFD fed mice
1.	Normal control
2.	HFD control
3.	HFD + **OND** (0.5 mg/kg, p.o.)
4.	HFD + **OND** (1 mg/kg, p.o.)
5.	HFD + ESC (10 mg/kg, p.o.)

(n=6/group)

Table 4.14: Groups used for screening QCM-4 for depression co-morbid with obesity in HFD fed mice

Sr. No.	Groups for screening QCM-4 in HFD fed mice
1.	Normal control
2.	HFD control
3.	HFD + **QCM-4** (1 mg/kg, p.o.)
4.	HFD + **QCM-4** (2 mg/kg, p.o.)
5.	HFD + ESC (10 mg/kg, p.o.)

(n=6/group)

Table 4.15: Groups used for screening 4a for depression co-morbid with obesity in HFD fed mice

Sr. No.	Groups for screening 4a in HFD fed mice
1.	Normal control
2.	HFD control
3.	HFD + **4a** (2 mg/kg, p.o.)
4.	HFD + **4a** (4 mg/kg, p.o.)
5.	HFD + ESC (10 mg/kg, p.o.)

(n=6/group)

Experimental design

Mice were fed HFD for 14 weeks. HFD fed mice were administered once daily with **OND**/**QCM-4**/**4a**/ESC/vehicle for 28 days and further screened for various behavioral, biochemical and molecular assays of depression, as mentioned in **Table 4.16**.

Table 4.16: Time-line for screening 5-HT$_3$ receptor antagonists for depression co-morbid with obesity in HFD fed mice

0 - 14 weeks	**Day 0 – 28**	**Behavioral assays**						
		25-29	**30**	**31**	**32**	**33**	**34**	**35**
HFD feeding	Treatment with **OND**/**QCM-4**/**4a**/ESC/vehicle once daily	SPT	SLA	FST	TST	EPM	HBT	L/D

Day 37 OGTT
Day 40 blood and brain tissue collection

Biochemical assays

- Plasma glucose, total cholesterol, triglycerides
- Hippocampal MDA, GSH
- Plasma CORT, leptin, insulin

Molecular assays

- Hippocampal 5-HT, cAMP, BDNF
- Histological assay
- IHC p53 assay

4.14. Effect of 5-HT_3 receptor antagonists OND, QCM-4 and 4a on CUMS induced depression and anxiety-like behavior in HFD fed mice using behavioral assays

Groupings: HFD fed mice were subjected to CUMS for screening of **OND**, **QCM-4** and **4a** in depression co-morbid with obesity. Animals were divided according to **Table 4.17, 4.18 and 4.19, respectively**.

Table 4.17: Groups used for screening OND for depression co-morbid with obesity in HFD fed mice subjected to CUMS

Sr. No.	Groups for screening OND in HFD+CUMS model
1.	Normal control
2.	NPD + CUMS control
3.	NPD + CUMS + **OND** (0.5 mg/kg, p.o.)
4.	NPD + CUMS + **OND** (1 mg/kg, p.o.)
5.	NPD + CUMS + ESC (10 mg/kg, p.o.)
6.	HFD control
7.	HFD + CUMS control
8.	HFD + CUMS + **OND** (0.5 mg/kg, p.o.)
9.	HFD + CUMS + **OND** (1 mg/kg, p.o.)
10.	HFD + CUMS + ESC (10 mg/kg, p.o.)

(n=6/group)

Table 4.18: Groups used for screening QCM-4 for depression co-morbid with obesity in HFD fed mice subjected to CUMS

Sr. No.	Groups for screening QCM-4 in HFD+CUMS model
1.	Normal control
2.	NPD + CUMS control
3.	NPD + CUMS+ **QCM-4** (1 mg/kg, p.o.)
4.	NPD + CUMS + **QCM-4** (2 mg/kg, p.o.)
5.	NPD + CUMS + ESC (10 mg/kg, p.o.)
6.	HFD control
7.	HFD + CUMS control
8.	HFD + CUMS + **QCM-4** (1 mg/kg, p.o.)
9.	HFD + CUMS + **QCM-4** (2 mg/kg, p.o.)
10.	HFD + CUMS + ESC (10 mg/kg, p.o.)

(n=6/group)

Table 4.19: Groups used for screening 4a for depression co-morbid with obesity in HFD fed mice subjected to CUMS

Sr. No.	Groups for screening 4a in HFD+CUMS model
1.	Normal control
2.	NPD + CUMS control
3.	NPD + CUMS + **4a** (2 mg/kg, p.o.)
4.	NPD + CUMS + **4a** (4 mg/kg, p.o.)
5.	NPD + CUMS+ ESC (10 mg/kg, p.o.)
6.	HFD control
7.	HFD + CUMS control
8.	HFD + CUMS + **4a** (2 mg/kg, p.o.)
9.	HFD + CUMS + **4a** (4 mg/kg, p.o.)
10.	HFD + CUMS+ ESC (10 mg/kg, p.o.)

(n=6/group)

Experimental design

Mice were fed with HFD for 14 weeks to induce obesity. HFD fed mice were further subjected to CUMS procedure for 4 weeks (as mentioned in **Table 4.8**) and treatment with 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** was started from 2nd week of CUMS procedure. Furthermore, various behavioral, biochemical and molecular assays of depression were performed as mentioned in **Table 4.20**.

Table 4.20: Time-line for screening 5-HT_3 receptor antagonists for depression co-morbid with obesity in HFD fed mice subjected to CUMS

0 - 14 weeks	**Day 0-7**	**Day 8 – 28**	**Behavioral assays**					
			25-29	**30**	**31**	**32**	**33**	**34**
HFD feeding	CUMS procedure	CUMS procedure + Treatment with **OND**/**QCM-4**/ **4a**/ESC/vehicle once daily	SPT	SLA	FST	TST	EPM	L/D

Day 36 blood and brain tissue collection

Biochemical assays

- Plasma glucose, total cholesterol, triglycerides
- Hippocampal MDA and GSH
- Plasma CORT, leptin and insulin

Molecular assays

- Hippocampal 5-HT, cAMP, BDNF
- Histological assay

4.15. Effect of 5-HT_3 receptor antagonists OND, QCM-4 and 4a with chronic dosing on behavioral assays of depression in;

- HFD fed mice
- HFD mice subjected to CUMS model of depression

The procedures for behavioral assays of depression were followed as mentioned in earlier section;

SPT- 4.9.1.2.1

SLA- 4.7.1

FST- 4.7.2

TST- 4.7.3

4.16. Effect of 5-HT_3 receptor antagonists OND, QCM-4 and 4a with chronic dosing on behavioral assays of anxiety in;

- HFD fed mice
- HFD mice subjected to CUMS model of depression

The procedures for behavioral assays of anxiety were followed as mentioned in earlier section;

EPM- 4.8.1

HBT- 4.8.2

LDT- 4.8.3

4.17. Biochemical assays in;

- HFD fed
- HFD mice subjected to CUMS model of depression

4.17.1. Collection of blood samples and separation of plasma

The bleeding techniques and procedure was followed as per the standard guidelines (Mitruka and Rawnsley, 1977). Two days post behavioral tests, in each study, blood samples were collected (0.2-0.3 ml) for each animal from sinus retro-orbital route using fine capillary tube in a eppendorf tube containing 10% EDTA solution (100 µl/ ml) and centrifuged at 10000 revolutions per minute (rpm) for 10 min. Plasma layer was collected in the fresh eppendorf tube and stored at -80ºC until the assay performance.

4.17.2. Estimations of plasma biochemical parameters

The procedures for plasma glucose, total cholesterol and triglycerides were followed as mentioned in earlier section;

Plasma glucose- 4.10.2.1

Plasma total cholesterol- 4.10.2.2

Plasma triglycerides- 4.10.2.3

4.17.3. Oral glucose tolerance test (OGTT)

Rationale

OGTT is usually used as test for diabetes, insulin resistance and impaired beta pancreatic cells function. OGTT is performed in metabolic studies, where post glucose loading how quickly glucose is cleared of the body or how well body is able to break down glucose is monitored.

Procedure

OGTT was performed as described elsewhere (Fraulob et al., 2010). From the overnight fasted animals, blood samples were collected by tail vein puncture and basal blood glucose were measured by Accu-Check glucometer. This was followed by gastric administration of glucose (2 g/kg, p.o.). Blood samples were collected at 30, 60 and 120 min post glucose loading and blood glucose was measured by glucometer and reported.

4.17.4. Estimation of brain oxidative stress markers

4.17.4.1. Collection of brain samples and isolation of hippocampus

At the end of each study, animals were sacrificed by cervical dislocation and brain samples were collected and subsequently hippocampus was isolated for oxidative stress analysis.

4.17.4.2. Preparation of brain homogenates for estimation of oxidative stress parameters

Brain hippocampus was used for estimation of oxidative stress parameters. The tissues were rinsed with ice cold saline (0.9% sodium chloride) and were homogenized in 10% (w/v) chilled phosphate buffer solution (pH 7.4), further subjected to centrifugation at 12000 rpm for 15 min at 4°C and supernatant was used for estimation MDA and GSH concentrations.

4.17.4.3. Estimation of oxidative stress marker MDA concentrations

Rationale

MDA is an important pro-oxidant known as marker of lipid peroxidation and become worst by releasing free radicals with increased stress that damage the DNA, and leads to neurodegeneration (Bruce-Keller et al., 2010).

Preparation of reagents

- 8% sodium dodecyl sulfate (SDS): 2.025g (SDS) in 25 ml phosphate buffer saline (PBS pH 7.4)
- 20% glacial acetic acid (GAA): 5 ml GAA in 20 ml distilled water
- 08% Thiobarbituric acid (TBA): 200 mg in 25 ml PBS

Procedure

MDA is known as a biomarker for lipid peroxidation in the process of oxidative stress, which was measured according to Ohkawa et al., (1979). Brain MDA content was expressed as microgram (µg) per mg protein. 50 µl sodium dodecyl sulfate (8%), 375 µl glacial acetic acid (20%) pH 3.5 and 375 µl thiobarbituric acid (0.8%) were added to 50 µl of brain homogenate (supernatant) and 150 µl of distilled water. The mixture was incubated at 90 °C for 60 min. Then the mixture was cooled under tap water and centrifuged at 1000 rpm for 10 min. Supernatant was collected and the absorbance was measured at 532 nm using Perkin Elmer lambda 20 spectrophotometer (Shimadzu, Kyoto, Japan).

4.17.4.4. Estimation of anti-oxidant enzyme GSH concentrations

Rationale

GSH is a thiol and redox buffers present in the brain cells exhibit protective action against reactive oxygen species (ROS) induced oxidative damage (Wang and Ballatori, 1998). Elevated ROS, results in the oxidation of GSH to glutathione disulfide (GSSG) that decreases the GSH concentration, resulting in neuropsychiatric disorders like depression, anxiety and metabolic disorders such as obesity, diabetes mellitus.

Preparation of reagents

- 5% Sulfosalicylic acid: 105 g in 30 ml PBS
- 0.1mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB): 4 mg in 100 ml PBS

Procedure

GSH level was measured according to the method of Ellaman, (1959). Briefly, 250 µl of brain homogenate (supernatant) was added to 250 µl chilled sulfosalicylic acid (5%) and centrifuged at 12000 rpm for 10 min. 100 µl of supernatant was added to 150 µl phosphate buffer (pH 7.4), vortex and added 3 times 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent). The mixture was incubated at 37 °C for 10 min and the absorbance was measured using Perkin Elmer lambda 20 spectrophotometer (Shimadzu, Kyoto, Japan) at 412 nm and expressed as µg of GSH per mg of protein.

4.17.4.5. Estimation of brain proteins by Lowry method

Rationale

The oxidative and antioxidant markers calculations are based on the protein content of brain tissue. Protein content was measured in the brain samples following the method of (Lowry et al., 1951), with bovine serum albumin (BSA), as standard.

Preparation of reagents

- Reagent D: 500 mg sodium carbonate (Na_2CO_3) + 100 mg sodium hydroxide (NaOH) in 25 ml distilled water,

  ↓ Discard 1 ml

  To remaining 24 ml add 0.5 ml sodium tartarate + 0.5 ml copper sulfate ($CuSO_4$),

  ↓

  Gives reagent D
- Follin’s reagent: Prepared with distilled water (1:1)
- Standard BSA: Prepared concentration as 1 mg/ml

Procedure

The procedure of Lowrey et al.,) 1951) was followed for estimation of protein content. 145 µl of distilled water was added with 750 µl reagent D. To this, 5 µl brain homogenate (supernatant)/standard was added and incubated at 37ºC for 15 min. To this, 75 µl of Follin’s reagent was added and incubated at 37ºC for 30 min, and absorbance was measured at 650 nm.

4.18. Estimations of plasma endocrinological parameters in;

- HFD fed
- HFD mice subjected to CUMS model of depression

4.18.1. Estimation of plasma corticosterone (CORT)

Rationale

HPA axis hyperactivity leads to excess CORT in circulation that plays an important role in the pathophysiology of co-morbid disorders such as depression and anxiety (Vreeburg et al., 2009, Vreeburg et al., 2010). Several mechanisms play a central role in depression associated with obesity. HPA axis dysregulation in obesity, leads to secretion of excess CORT, that further cause insulin resistance, altered plasma glucose, hyperlipidemia (Ferris and Kahn, 2012), which triggers the development of severe depression, in obese individuals.

Hence, estimation of plasma CORT is very important while studying depression co-morbid with obesity. Therefore, in the present study, effect of 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a**, on HPA axis hyperactivity, involved in depression co-morbid with obesity, was measured through estimation of plasma CORT.

Procedure

CORT assay was performed by the method of Katyare and Pandya, (2005). Briefly, 0.2 ml plasma sample was mixed with 0.2 ml of chloroform:methanol mixture (2:1 v/v). 3 ml chloroform was added and samples were vortexed, and centrifuged at 2000 rpm for 10 min. The chloroform layer was separated and treated with 0.3 ml of sodium hydroxide (0.1 N) followed by 3 ml of 30 N sulfuric acid. The sulfuric acid layer was separated and kept in the dark for 30-60 min. Fluorescence was measured in an SL-174-spectrofluorometer with 472 nm excitation and 523 nm emission wavelengths, respectively.

4.18.2. Estimation of plasma leptin

Rationale

The assay principle of sandwich assay is involved in the estimation of leptin by using ELISA kit, where antigens are quantified between two layers of antibodies (capture and detection antibodies). Among the several important pathogenic links for depression co-morbid with obesity, leptin is very important causative factor as its dysregulation results in depression and anxiety disorders (Guo and Lu, 2014), and leptin resistance is very common with obesity (Kloiber et al., 2013). Estimation of plasma leptin concentration becomes very important from the mechanistic point of view of such co-morbid disorders. Hence, in the present study, treatment effect of 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** on leptin regulation in depression co-morbid with depression were studied.

Plasma sample collection and preparations

Plasma collection: Plasma samples were collected as per the information provided on the manual of ELISA kit. Plasma samples were collected by using EDTA as anticoagulant, centrifuged at 10000 rpm for 10 min and stored at -20 ºC.

Plasma sample preparation: Mouse plasma samples were diluted by 5 folds (50 µl plasma sample + 250 µl dilution buffer).

Preparations of standard curve

Leptin estimation was performed according to the ELISA kit by Aviscera Bioscience, Inc. (catalog no. SK00050-08; lot no. 201111107) and all the samples were analyzed in duplicate. Standard leptin was reconstituted with 0.5 ml dilution buffer that gave stock solution of 16000 pg/ml. In a eppendorf tubes #1 to #7, 250 µl of dilution buffer was added and dilutions were made as per following **Table 4.21**.

Table 4.21: Preparation of standard curve for leptin estimation

Tube No.	Standard	Dilution Buffer	Concentration (pg/ml)
Stock	Powder	500 µl	16000
1	250 µl of stock	250 µl	8000
2	250 µl of 1	250 µl	4000
3	250 µl of 2	250 µl	2000
4	250 µl of 3	250 µl	1000
5	250 µl of 4	250 µl	500
6	250 µl of 5	250 µl	250
7	250 µl of 6	250 µl	125

Procedure

- 100 µl of standard, samples, positive control were added to each well and incubated for 2 h on plate shaker at room temperature.
- Then the wells were aspirated and washed 4 times.
- 100 µl of Detection Antibody working solution was added to each well and incubated for 2 h on plate shaker at room temperature.
- All the wells were aspirated and washed 4 times.
- 100 µl Streptavidin-HRP conjugate working solution was added to each well and incubated at 60 min on plate shaker at room temperature. This reaction was protected from light.
- Wells were aspirated and washed 4 times.
- 100 µl of Substrate solution was added to each well and incubated for 15-25 min on plate shaker at room temperature. This reaction was protected from light.
- 100 µl of Stop solution was added to each well and absorbance was recorded at 450 nm within 15 min.

4.18.3. Insulin

Rationale

Assay principle of sandwich assays is involved in the insulin estimation by ELISA kit. An important factor involved in the pathogenesis of depression co-morbid with obesity, is insulin resistance. Insulin concentrations, have been found to be abnormal, in depression and obesity (Adriaanse et al., 2006, Qatanani and Lazar, 2007). Hence, the estimation of insulin level, as a pathogenic link for depression co-morbid with obesity, becomes very essential. In the present study, treatment effects of **OND**, **QCM-4** and **4a** was studied on insulin resistance.

Preparation of plasma sample

Blood was collected in a tube containing 10% EDTA (100 µl/ml) and centrifuged at 10000 rpm for 10 min at 4ºC, plasma was separated and stored at -20ºC till assay performance.

Preparation of standard curve

Plasma insulin assessment was performed in duplicate as per guidelines mentioned in ELISA kit for insulin by Crystal Chem, Inc. (catalog no. # 90080).

Insulin standard was reconstituted by adding 100 µl of distilled water to the vial that gave stock solution of 25.6 ng/ml concentration. In series of eppendorf tubes from #2 to #8, 50 µl of sample diluent was added and dilutions were made, as per following **Table 4.22**.

Table 4.22: Preparation of standard curve for insulin estimation

Tube No.	Sample diluents	Standard	Concentration (ng/ml)
Stock	Ready to use stock of standard insulin of concentration 25.6 ng/ml		
1	150 µl	50 µl stock	6.4
2	50 µl	50 µl of 1	3.2
3	50 µl	50 µl of 2	1.6
4	50 µl	50 µl of 3	0.8
5	50 µl	50 µl of 4	0.4
6	50 µl	50 µl of 5	0.2
7	50 µl	50 µl of 6	0.1
8	50 µl	---------	0

Assay procedure

- Antibody- coated microplate was fixed to the frame.
- 95 µl of sample diluents was added to each well.
- 5 µl of sample or standards from 0 to 6.4 ng/ml was added to the wells in duplicate and microplate was incubated for 2 h at 4 ºC.
- Each well was washed with wash buffer (300 µl / well each time).
- Then 100 µl of anti-insulin enzyme conjugate was added to each well.
- Microplate was incubated at room temperature for 30 min.
- Then, each microplate well was washed seven times with wash buffer (300 µl / well each time).
- Each well was added with 100 µl of enzyme substrate solution.
- Microplate was incubated at room temperature for 40 min.
- Reaction was stopped by adding 100 µl of stop solution to each well, absorbance was measured and insulin concentration was calculated in each sample using standard curve.

4.19. Molecular assays in;

- HFD fed
- HFD mice subjected to CUMS model of depression

4.19.1. Estimation of brain hippocampal neurotransmitter 5-HT concentrations

Rationale

Among the brain neurotransmitters, 5-HT plays a significant role in the disease pathophysiology of depression (Owens and Nemeroff, 1994). Reduced 5-HT, has been known, since long time, describing monoamine theory of depression. 5-HT also plays an important role in regulation of appetite (Lam et al., 2010).

Reduced levels of 5-HT has been observed in obesity, that may potentially lead to disturbances in the hippocampus mediated neurotransmission and signalling resulting in, overeating or continuous eating. As 5-HT, is involved in regulation of mood and appetite, the effect of **OND**, **QCM-4** and **4a** on hippocampal 5-HT concentration were studied.

Preparation of brain homogenates for estimation of 5-HT

Hippocampus was separately homogenized in 0.5 ml solution, containing a mixture of 0.4 mM sodium metabisulfite, 0.5 M perchloric acid and 1 mM EDTA and centrifuged at 12000 rpm (Eppendorf centrifuge 5702R) at 4 °C, and the resulting supernatant was separated and used for estimation of 5-HT, by high pressure liquid chromatography (HPLC).

Chromatographic separation and analysis

Instrumentation

Liquid chromatograph LC-2010C_{HT} (Shimadzu, Tokyo, Japan) was used. 5-HT was monitored using electrochemical detector (791 VA Detector, Metrohm, Switzerland) coupled to liquid chromatograph. Electrochemical detector consisted of glassy carbon as a working electrode, silver/silver chloride as reference electrode and gold electrode as auxiliary electrode. LC solution software was used for data collection and integration.

Chromatographic conditions

Chromatographic separation of the 5-HT was achieved using C_{18} column (BDS Hypersil endcapped; 250 mm x 4.6 mm, 5 µ, Thermo Scientific, India). The optimized mobile phase used was a mixture of 10 mM phosphate buffer (pH 3.0, adjusted using 0.1 M orthophosphoric acid) and methanol in the ratio of 90:10 (v/v).

The buffer solution was filtered through a 0.22 µm Millipore® (Milford, MA, USA) filtration membrane. The ionization potential applied at electrochemical cell was + 800 mV. Estimation was carried out at 25.0 ± 0.2°C and mobile phase flow rate was set at 1.0 ml/min. The volume of sample injected into liquid chromatography system was 20 µl.

4.19.2. Estimation of brain hippocampus BDNF concentrations

Rationale

BDNF estimation was carried out by using ELISA kit having assay principle of sandwich assay. BDNF is a neurotrophic factor that is very essential for neurogenesis. In neuropsychiatric disorders such as depression, anxiety and metabolic diseases like diabetes mellitus and obesity, altered BDNF concentrations in the brain have been observed that leads to neuronal degeneration in co-morbid disorders including depression and obesity (Bocchio-Chiavetto et al., 2010, Rios, 2011). Clinical findings also reported the reduced brain BDNF concentrations in depressed patients. Various chronic studies for depression results in reduced level of BDNF mimicking the clinical pathogenesis and altering the hippocampal brain morphology. Hence, estimation of brain hippocampal BDNF remains very important aspect from the molecular point of view in neurodegenerative disorders. In the present study, effect of **OND**, **QCM-4** and **4a** were studied on hippocampal BDNF concentration from molecular mechanistic point of view.

Brain sample preparation

Brain hippocampus was isolated and homogenized in lysis buffer and centrifuged 3500 rpm for 15 min, supernatant was collected and stored at -20 ºC until assay performance.

Preparation of standard curve

BDNF concentrations were estimated using commercially available ELISA kit (Code: EK0309, Boster Biological Technology Co., LTD., CA, USA) as per the manual.

- Standard BDNF was reconstituted by adding 1 ml sample diluent buffer, vortex and mixed thoroughly to give 10000 pg/ml concentration of BDNF standard solution.
- 0.2 ml of above BDNF solution was added to 0.8 ml of sample diluent buffer, vortexed and mixed thoroughly to give 2000 pg/ml concentration of BDNF standard solution.

- For preparing a series of different concentrations ranging from 1000 – 500 – 250 – 125 – 62.5 – 31.2 pg/ml of standard BDNF, a serial dilutions were made as mentioned in **Table 4.23.**

Table 4.23: Preparation of standard curve for BDNF estimation

Tube No.	Sample diluent buffer	Standard	Concentration (pg/ml)
Stock	Stock of standard BDNF 2000 pg/ml		
1	0.3 ml	0.3 ml of stock	1000
2	0.3 ml	0.3 ml of 1	500
3	0.3 ml	0.3 ml of 2	250
4	0.3 ml	0.3 ml of 3	125
5	0.3 ml	0.3 ml of 4	62.5
6	0.3 ml	0.3 ml of 5	31.25

Procedure

Principle of sandwich ELISA assay is applicable for BDNF estimation. The concentration of BDNF is proportional to the density of yellow colour produced.

- Microplate wells were loaded with samples (prepared with dilution buffer as 1:10 ratio) and standard concentrations (31.25 – 2000 pg/ml) for 90 min.
- Microplate wells were aspirated and washed with wash buffer.
- Biotinylated anti-rat/mouse BDNF antibody was added to the wells and incubated at room temperature for 1 h.
- Wells were aspirated and washed thrice with wash buffer solution.
- Then, wells were incubated at room temperature for 30 min after adding Avidin-Biotin-Peroxidase working solution.
- Wells were aspirated and washed five times with wash buffer.
- TMB colour developing agent was added to each well and the plate was incubated in dark for 20 min at room temperature.
- Stop solution was added to each well to stop the reaction and the absorbance was measured at 450 nm.
- The calculations were done from the standard curve which suggests the direct relationship between optical density and BDNF concentrations.

4.19.3. Estimation of brain hippocampus cAMP concentration

Rationale

Sandwich assay principle is involved in the estimation of cAMP by using ELISA kit. cAMP acts as a second messenger system, involved in the signal transduction mechanisms, stimulating CREB and finally increases the neurotrophic factor responsible for neurogenesis, BDNF (Nakagawa et al., 2002, Mällo et al., 2008). Delayed clinical antidepressant efficacy allowed the researchers to study the downstream mechanisms involved in antidepressant effect. BDNF is an important neurotrophin that is widely distributed throughout the brain and involved in the regulation of neuronal survival, synapse/neurite formation, migration, axonal and dentrite growth along with the key role of synaptic plasticity and behavior (Martinowich and Lu, 2008). Extensive studies later confirmed the involvement of neurogenesis in the antidepressant effect through increased BDNF (Malberg and Blendy, 2005). Hence, in the present study, estimation of cAMP was performed, to assess the effect of **OND**, **QCM-4** and **4a** in depression co-morbid with obesity.

Tissue homogenate preparation

The brain hippocampus samples were homogenized according to the information provided by ELISA kit manufacturer broacher using the calibration buffer provided by the manufacturer. Brain homogenates were centrifuged to 5000 rpm for 10 min at 4ºC and the supernatant was used collected and stored at -80 ºC for the assay.

Preparation of standard curve

The standard curve for the assay of cAMP was prepared as per the kit instructions (Enzo Life Sciences, Cat No. ADI-900-067, USA). Standards ranging from 200, 50, 12.5, 3.12, 0.78 pmol/ml were prepared as per **Table 4.24**.

Table 4.24: Preparation of standard curve for cAMP estimation

Tube No.	Sample diluent buffer	Standard	Concentration (pmol/ml)
Stock	Stock of standard BDNF 2000 pmol/ml		
1	900 µl	100 µl of stock	200
2	750 µl	250 µl of 1	50
3	750 µl	250 µl of 2	12.5
4	750 µl	250 µl of 3	3.13
5	750 µl	250 µl of 4	0.78

Procedure

- Sufficient number of microtiter plate well was arranged on the frame provided by kit to accommodate samples and standards in duplicates.
- 100 µl of standard diluent (Assay buffer 2) was added to the non-specific binding (NSB) and Bo (0 pmol/ml standard) wells. Then, 50 µl of assay buffer 2 was added to NSB wells.
- 100 µl of the standard concentrations (200, 50, 12.5, 3.12, 0.78 pmol/ml) and unknown brain samples were dispensed at the bottom of the wells.
- 50 µl of blue conjugate was added to each well except the TA and blank wells.
- Then, 50 µl of yellow antibody was added to each well except the TA, blank and NSB wells and incubated for 2 h on plate shaker (500 rpm) at room temperature.
- Then, the contents of the plate were discarded and wells were washed with wash buffer (400 µl/well) thrice. 5 µl of blue conjugate was added to the TA wells.
- 200 µl of substrate solution was added to each well and incubated for 1 h at room temperature without shaking. Then, 50 µl stop solution was added to each well and the optical density was measured at 405 nm.
- In order to normalize the protein content, the obtained concentrations of cAMP (pmol/ml) was divided by total proteins (mg/ml) and the cAMP contents of was expressed as cAMP pmol/mg of total proteins.

4.20. Neuro-anatomical studies (Histological examination of brain samples) in HFD fed mice

Rationale

Neurodegenerative disease such as depression, shows morphological changes especially in the dentate gyrus (DG) region of hippocampus. Neuronal degeneration and altered size, shape of hippocampal DG region are mostly observed in patients with depression. Role of 5-HT in regulation of mood and appetite are well established. Alteration in 5-HT signaling, in the brain, significantly affects the synaptic plasticity and hippocampal neurogenesis (Ferrés-Coy et al., 2013, Mahar et al., 2014). 5-HT, plays a crucial role in the hippocampal neurogenesis through improving the survival, neurite outgrowth and synaptogenesis by reversing the HPA axis hyperactivity (Ferrés-Coy et al., 2013). Obesity is a stressful inflammatory state with several biological mechanisms that can lead to hippocampal damage and depressive disorder.

Hence, effect of **OND**, **QCM-4** and **4a** on brain hippocampal DG neuronal morphology in HFD fed mice was undertaken in the present study.

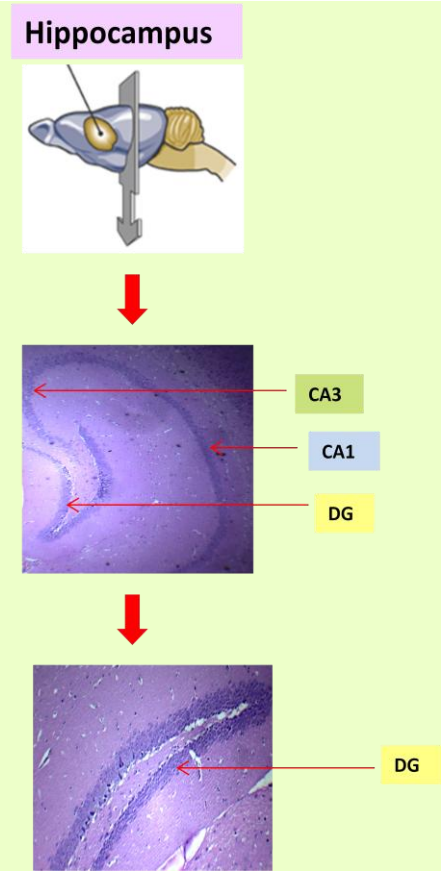


Fig 4.18: Schematic representation of mice hippocampus

Procedure

The brain samples were collected and fixed in 10% (v/v) formalin in phosphate buffered saline and embedded in paraffin. 5 µm sections of the hippocampus region were stained with H & E to study glomerular damage and observed for any histological changes. Histopathological images were captured by using Optika microscope (Model no. 4083.B5, Italy) connected to digital camera and Optika View 7 Software. The morphology of both healthy and pyknotic neuronal cells were examined in the hippocampal DG region using Image J software (US National Institutes of Health, Bethesda, USA) (Hill et al., 2005, Gaikwad et al., 2010).

4.21. Immunohistochemistry (IHC) studies p53 in HFD fed mice

Rationale

IHC is a widely used technique to detect/locate the presence of abnormal or defective or disease causing proteins/antigens in the specific regions of the tissues, in diseased or normal conditions. IHC process involved staining which is accomplished with specific antibodies that detect the target protein (Ramos-Vara and Miller, 2014). Obesity is a pro-inflammatory state that results in neuroinflammation, further causing degeneration of the neuronal cells (Cai, 2013, Aguilar-Valles et al., 2015). The p53 mediated apoptosis is well evident in literature and hence, it was hypothesized that p53 could play an important role in obesity associated neuroinflammation, that further results in neurodegenerative disorders such as depression (Bogazzi et al., 2013, Hwang et al., 2013, Ghavami et al., 2014). Therefore, studies on the effect of **OND**, **QCM-4** and **4a** on p53 mediated hippocampal DG region apoptosis in HFD fed mice, were undertaken.

Procedure

Animals were sacrificed and brains were post-fixed overnight in paraformaldehyde and stored in 30% sucrose at 4 ºC. Serial sections (5 µm) of the hippocampus were taken from paraffin blocks and deparaffinized with xylene, followed by antigen retrieval by heating in citrate buffer (10 mmol/l). The following primary antibodies were used: anti-p53 (mouse, 1:200 dilution; Santa Cruz Biotechnology) and anti-mouse Horse Radish Peroxidase conjugated secondary antibody were used followed by detection with diaminobenzidine (DAB) as a chromogen. The slides were counterstained with hematoxylin, dehydrated with alcohol and xylene, and mounted in DPX (Sigma-Aldrich). Images of DG region were taken and analyzed using Image J software for calculating DAB positive area (Pandey et al., 2015).

4.22. Confirmatory studies: Role of 5-HT_3 receptors in antidepressant-like effect of OND, QCM-4 and 4a in HFD fed mice

4.22.1. Effect of 5-HT_3 receptor antagonists OND, QCM-4 and 4a in HFD fed mice pre-treated with mCPBG

Rationale

To assess the possible involvement of 5-HT_3 receptor through serotonergic neurotransmission in the antidepressant-like effect, mCPBG model is followed (Nakagawa et al., 1998) in research laboratories. mCPBG is a 5-HT_3 receptor agonist, that compete with 5-HT_3 receptor antagonists for receptor occupancy and inhibit the antidepressant-like effect of 5-HT_3 receptor antagonists (Machado et al., 2009).

Procedure

Mice were fed with HFD for 14 weeks to induce obesity. Then, the animals were injected with mCPBG (10 mg/kg, i.p.) and, 30 min later, administered with **OND** (0.5 and 1 mg/kg, p.o.)/**QCM-4** (1 and 2 mg/kg, p.o.)/**4a** (2 and 4 mg/kg, p.o.)/vehicle (10 mg/kg, p.o.), once daily for 28 days. 30 min post last dose of **OND**/**QCM-4**/**4a**/vehicle, animals were subjected for behavioral assays including, SPT, FST, TST, EPM and L/D (Jindal et al., 2013) followed by the estimation of hippocampal 5-HT concentrations as mentioned in **Table 4.28**. Grouping was done as mentioned in **Tables 4.25, 4.26, and 4.27**, respectively.

Table 4.25: Groups used for screening OND for depression co-morbid with obesity in HFD fed mice pre-treated with mCPBG

Sr. No.	Groups for screening OND in HFD fed mice pre-treated with mCPBG for depression and anxiety
1.	Normal control
2.	HFD + mCPBG (10 mg/kg, i.p.)
3.	HFD + **OND** (0.5 mg/kg, p.o.)
4.	HFD + mCPBG + **OND** (0.5 mg/kg, p.o.)
5.	HFD + **OND** (1 mg/kg, p.o.)
6.	HFD + mCPBG + **OND** (1 mg/kg, p.o.)

(n=6/group)

Table 4.26: Groups used for screening QCM-4 for depression co-morbid with obesity in HFD fed mice pre-treated with mCPBG

Sr. No.	Groups for screening QCM-4 in HFD fed mice pre-treated with mCPBG for depression and anxiety
1.	Normal control
2.	HFD + mCPBG (10 mg/kg, i.p.)
3.	HFD + **QCM-4** (1 mg/kg, p.o.)
4.	HFD + mCPBG + **QCM-4** (1 mg/kg, p.o.)
5.	HFD + **QCM-4** (2 mg/kg, p.o.)
6.	HFD + mCPBG + **QCM-4** (2 mg/kg, p.o.)

(n=6/group)

Table 4.27: Groups used for screening 4a for depression co-morbid with obesity in HFD fed mice pre-treated with mCPBG

Sr. No.	Groups for screening 4a in HFD fed mice pre-treated with mCPBG for depression and anxiety
1.	Normal control
2.	HFD + mCPBG (10 mg/kg, i.p.)
3.	HFD + **4a** (2 mg/kg, p.o.)
4.	HFD + mCPBG + **4a** (2 mg/kg, p.o.)
5.	HFD + **4a** (4 mg/kg, p.o.)
6.	HFD + mCPBG + **4a** (4 mg/kg, p.o.)

(n=6/group)

Table 4.28: Time-line for screening 5-HT_3 receptor antagonists for depression co-morbid with obesity in mCPBG injected HFD fed mice

0 - 14 weeks	**Day 0 – 28**	**Behavioral assays**					
		25-29	**30**	**31**	**32**	**33**	**34**
HFD feeding	Injected with mCPBG (10 mg/kg, i.p.) and Treatment with **OND**/**QCM-4**/ **4a**/ESC/vehicle once daily	SPT	SLA	FST	TST	EPM	L/D

Day 36 brain tissue collection

Estimation of hippocampal 5-HT

4.22.2. Behavioral assays mCPBG injected obese mice

Behavioral assays of depression and anxiety were performed as per the procedures described in earlier sections;

SPT- 4.9.1.2.1

SLA- 4.7.1

FST- 4.7.2

TST- 4.7.3

EPM- 4.8.1

L/D- 4.8.3

4.22.3. Estimation of hippocampal 5-HT concentrations in mCPBG injected HFD fed mice

The protocol was followed according to section 4.19.1

4.23. Statistical analysis

Graph Pad PRISM software version 2.01 (Graph Pad Software, La Jolla, USA) was used for data analysis. Values were expressed as mean ± standard error of the mean (S.E.M.).

Preliminary studies for **QCM-4** and **4a** were analyzed by one way analysis of variance (ANOVA) followed by Bonferroni post test for analysis of differences between various groups, where $P<0.05$, was considered statistically significant.

In the standardization of HFD fed obesity model for depressive phenotypes, Student t-test was used for statistatical analysis and $P<0.05$, was considered statistically significant.

The data obtained from screening of **OND**, **QCM-4** and **4a** in HFD fed mice for depressive behavior was analyzed by using one way ANOVA followed by Bonferroni post test and $P<0.05$, was considered statistically significant.

The data obtained from screening of **OND**, **QCM-4** and **4a** in HFD fed mice subjected to CUMS was analyzed by using two way ANOVA followed by Bonferroni post test. $P<0.05$, was considered statistically significant.

5. RESULTS

5.1. Evaluation of QCM-4 in behavioral rodent models of depression

5.1.1. Effect of QCM-4 on SLA score in mice

QCM-4 was administered at different doses (0.5, 1, 2 and 4 mg/kg, i.p.) to mice. **QCM-4** did not show any significant [F (4, 25) = 4.47, P>0.05] alterations in the basal locomotor score of mice at 0.5, 1 and 2 mg/kg as compared to control group. However, **QCM-4** showed significant (P<0.05) reduction in basal locomotor score at 4 mg/kg, as compared to control group (**Fig 5.1**).

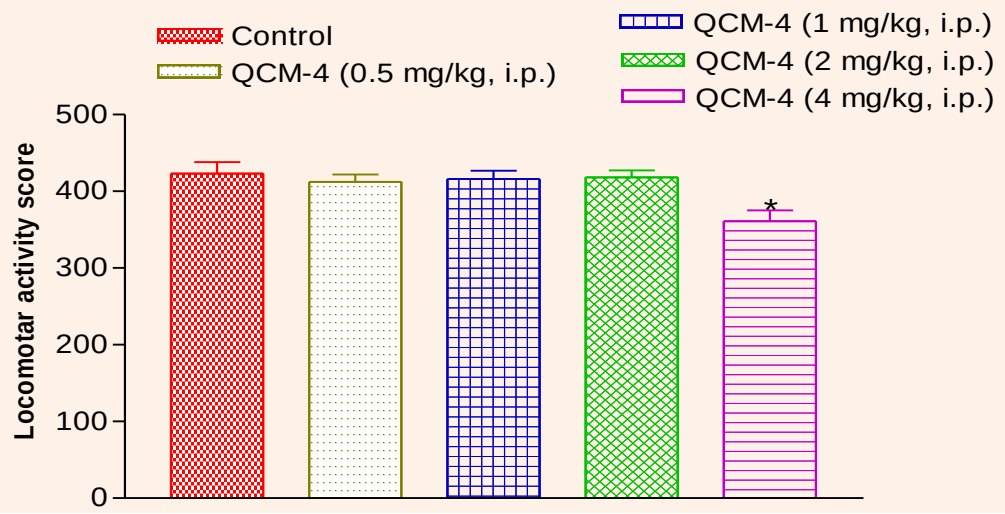


Fig 5.1: Effect of **QCM-4** on basal locomotor score in mice. The values are expressed as mean ± S.E.M., *P<0.05 vs control group, n=6/group.

5.1.2. Effect of QCM-4 on duration of immobility in mice using FST

QCM-4 at (0.5, 1 and 2 mg/kg, i.p.) and standard ESC at (10 mg/kg, i.p.) was administered to mice and duration of immobility time recorded, as showed in **Fig 5.2**. **QCM-4** (1 and 2 mg/kg, i.p.) and ESC (10 mg/kg, i.p.) showed significant [F (4, 25) = 32.77, P<0.01] reduction in duration of immobility of mice in FST, compared to control group. However, **QCM-4** at 0.5 mg/kg, did not showed significant (P>0.05) change in the immobility time, compared to control group, possibly due to sub-therapeutic dose.

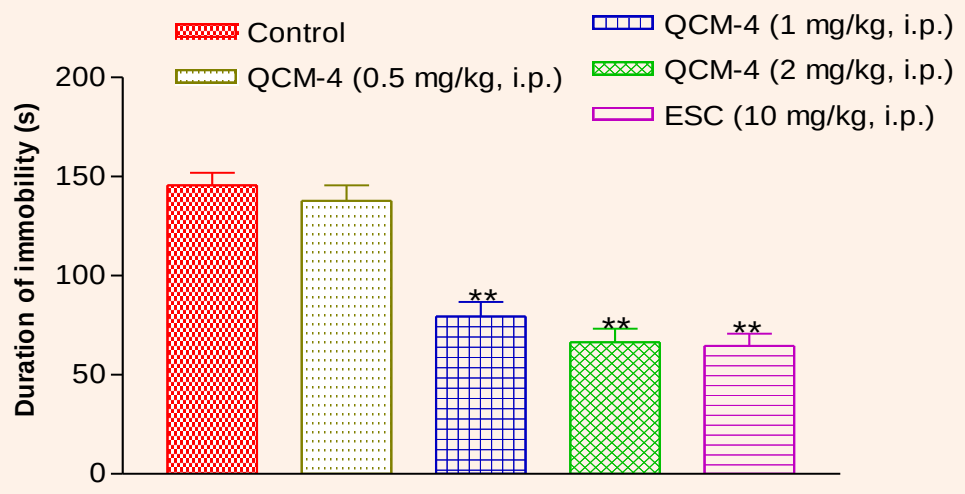


Fig 5.2: Effect of **QCM-4** on duration of immobility in mice using FST. The values are expressed as mean ± S.E.M., **P<0.01 vs control group, n=6/group.

5.1.3. Effect of QCM-4 on duration of immobility in mice using TST

QCM-4 at (0.5, 1 and 2 mg/kg, i.p.) and standard antidepressant ESC at (10 mg/kg, i.p.) were administered to mice and duration of immobility was recorded in TST. **QCM-4** (1 and 2 mg/kg, i.p.) and ESC (10 mg/kg, i.p.) showed significant [F (4, 25) = 44.55, P<0.01] inhibition of duration of immobility in mice, compared to control group. However, **QCM-4** at 0.5 mg/kg, did not show any significant (P>0.05) alteration in the duration of immobility, compared to control group (**Fig 5.3**).

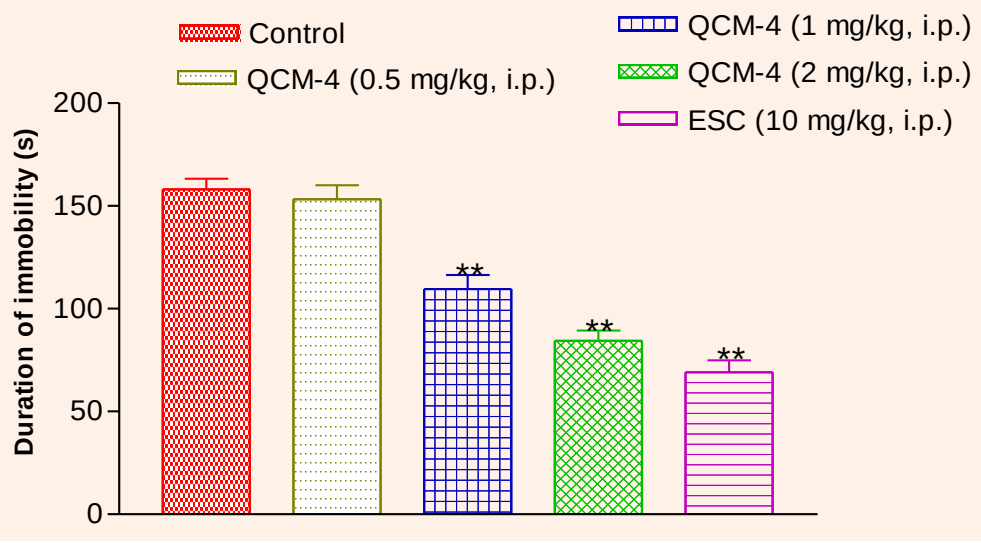


Fig 5.3: Effect of **QCM-4** on duration of immobility using TST in mice. The values are expressed as mean ± S.E.M., **P<0.01 vs control group, n=6/group.

5.2. Evaluation of QCM-4 in behavioral rodent models of anxiety

5.2.1. Effect of QCM-4 on mice behavior in EPM

QCM-4 (1 and 2 mg/kg, i.p.) and standard diazepam (2 mg/kg, i.p.) injected groups showed significantly increased % OAE [F (3, 20) = 15.12, P<0.05] and % OAT [F (3, 20) = 107.8, P<0.01] as compared to control group (**Table 5.1**).

Table 5.1: Effect of QCM-4 on mice in EPM

Groups	% OAE	% OAT (s)
Control	25.56 ± 3.51	22.39 ± 1.31
QCM-4 (1 mg/kg, i.p.)	46.29 ± 4.49*	33.44 ± 1.77**
QCM-4 (2 mg/kg, i.p.)	53.90 ± 4.28**	44.67 ± 1.15**
Diazepam (2 mg/kg, i.p.)	62.63 ± 3.94**	57.72 ± 1.54**

The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs control group, n = 6/group.

5.2.2. Effect of QCM-4 on mice behavior in HBT

QCM-4 (1 and 2 mg/kg, i.p.) and standard diazepam (2 mg/kg, i.p.) significantly increased the head dip [F (3, 20) = 15.98, P<0.05] and crossing scores [F (3, 20) = 31.31, P<0.05] as compared to control group as showed in **Table 5.2**.

Table 5.2: Effect of QCM-4 on mice in HBT

Groups	Number of head dips	Number of Crossings
Control	30.50 ± 3.89	38.67 ± 3.24
QCM-4 (1 mg/kg, i.p.)	46.50 ± 2.83*	57.33 ± 4.23*
QCM-4 (2 mg/kg, i.p.)	53.83 ± 4.21**	69.17 ± 4.03**
Diazepam (2 mg/kg, i.p.)	66.67 ± 4.03**	88.67 ± 3.40**

The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs control group, n = 6/group.

5.2.3. Effect of QCM-4 on mice behavior in L/D test

Acute administration of **QCM-4** (1 and 2 mg/kg, i.p.) and standard diazepam (2 mg/kg, i.p.) significantly increased the time in light chamber [F (3, 20) = 13.08, P<0.05] and transition score [F (3, 20) = 25.35, P<0.01] as compared to control group (**Table 5.3**).

Table 5.3: Effect of QCM-4 on mice in L/D test

Groups	Time in light chamber (s)	Transition Score
Control	31.50 ± 3.80	20.50 ± 3.18
QCM-4 (1 mg/kg, i.p.)	48.67 ± 3.50*	38.83 ± 3.70**
QCM-4 (2 mg/kg, i.p.)	59.33 ± 4.50**	46.67 ± 5.55**
Diazepam (2 mg/kg, i.p.)	65.83 ± 4.67**	60.67 ± 3.40**

The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs control group, n = 6/group.

5.3. Evaluation of QCM-4 on co-morbid depression and anxiety using OBX model

5.3.1. Effect of QCM-4 on body weight (g) of OBX rats

Initial body weight was not significantly different between sham and OBX control groups [F (7, 40) = 0.195, P>0.05]. OBX control rats showed significantly [F (7, 40) = 5.40, P<0.01] reduced final body weight compared to sham control group. **QCM-4** (1 and 2 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) did not altered the body weight in sham as well as OBX rats as showed in **Table 5.4**.

Table 5.4: Effect of QCM-4 on body weight changes in OBX rats

Groups	Initial body weight (g)	Final body weight (g)
Sham control	255.56 ± 4.17	293.89 ± 5.91
Sham + QCM-4 (1 mg/kg, p.o.)	251.12 ± 5.13	287.96 ± 5.78
Sham + QCM-4 (2 mg/kg, p.o.)	254.14 ± 4.52	289.72 ± 6.19
Sham + ESC (10 mg/kg, p.o.)	252.32 ± 5.60	287.29 ± 5.16
OBX control	252.74 ± 4.09	257.52 ± 6.58$^{\#\#}$
OBX + QCM-4 (1 mg/kg, p.o.)	250.04 ± 4.72	264.95 ± 4.92
OBX + QCM-4 (2 mg/kg, p.o.)	254.69 ± 5.29	275.58 ± 4.34
OBX + ESC (10 mg/kg, p.o.)	256.19 ± 5.37	272.36 ± 5.84

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs control group, n = 6/group.

5.3.2. Effect of QCM-4 treatment on SPT in OBX rats

Result of the SPT showed that, OBX control group exhibited significantly (P<0.01) reduced sucrose solution consumption compared to sham control group. Treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (7, 40) = 43.19, P<0.01] increased the sucrose consumption in OBX rats as compared to OBX control group, as showed in **Fig 5.4**.

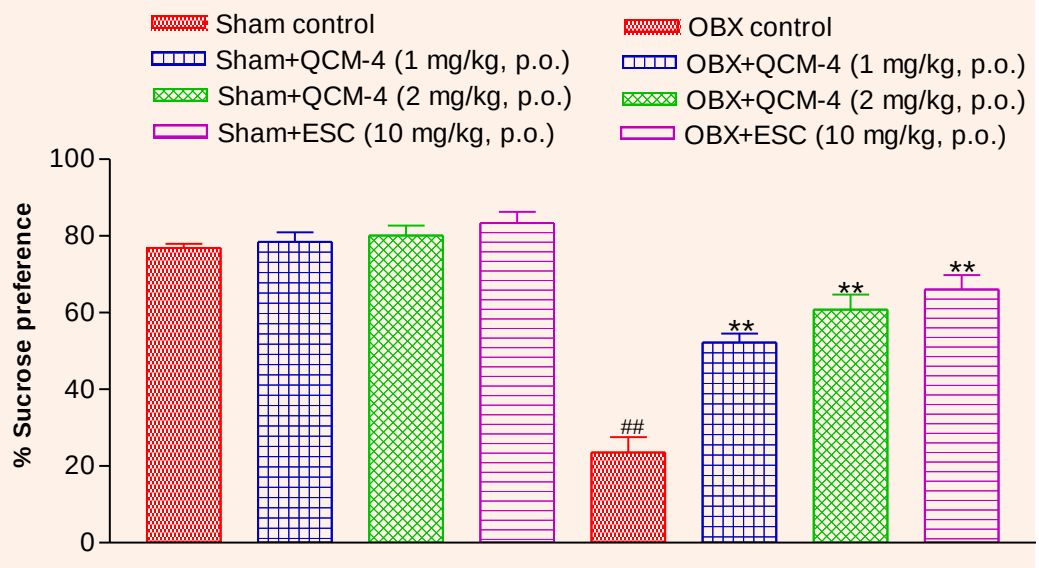


Fig 5.4: Effect of **QCM-4** on SPT in OBX rats. The values are expressed as mean ± S.E.M., ##P<0.01 vs sham control group, **P<0.01 vs OBX control group, n=6/group.

5.3.3. Effect of QCM-4 treatment on EPM in OBX rats

OBX control group showed significantly (P<0.01) elevated % OAE and % OAT (s) in EPM, compared to sham control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.), significantly inhibited the % OAE [F (7, 40) = 4.03, P<0.05] and % OAT [F (7, 40) = 8.38, P<0.05] in OBX rats as compared to OBX control group (**Table 5.5**).

Table 5.5: Effect of QCM-4 on EPM in OBX rat

Groups	% OAE	% OAT (s)
Sham control	38.22 ± 3.13	25.06 ± 1.86
Sham + QCM-4 (1 mg/kg, p.o.)	40.87 ± 7.04	32.67 ± 2.12
Sham + QCM-4 (2 mg/kg, p.o.)	51.62 ± 5.76	37.78 ± 2.64
Sham + ESC (10 mg/kg, p.o.)	49.10 ± 8.34	39.83 ± 3.18
OBX control	71.58 ± 2.39##	55.17 ± 3.13##
OBX + QCM-4 (1 mg/kg, p.o.)	44.68 ± 4.55*	39.44 ± 1.46**
OBX + QCM-4 (2 mg/kg, p.o.)	42.93 ± 3.52*	32.33 ± 3.63**
OBX + ESC (10 mg/kg, p.o.)	40.52 ± 5.25**	31.50 ± 5.08**

The values are expressed as mean ± S.E.M., ##P<0.01 vs sham control group, *P<0.05; **P<0.01 vs OBX control group, n = 6/group.

5.3.4. Effect of QCM-4 treatment on modified OFT in OBX rats

Results of OFT (**Table 5.6**) showed that, OBX control group significantly (P<0.01) increased the ambulations, rearings and fecal pellets compared to sham control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) significantly reduced the ambulatory [F (7, 40) = 136.1, P<0.01], rearing scores [F (7, 40) = 44.03, P<0.01] and fecal pellets [F (7, 40) = 12.51, P<0.01] in OBX rats as compared to OBX control group.

Table 5.6: Effect of QCM-4 on OFT in OBX rats

Groups	Ambulatory score	Rearing Score	Fecal pellets score
Sham control	98.33 ± 2.89	12.67 ± 0.76	2.50 ± 0.43
Sham + QCM-4 (1 mg/kg, p.o.)	100.83 ± 2.61	9.83 ± 0.48	2.33 ± 0.49
Sham + QCM-4 (2 mg/kg, p.o.)	107.00 ± 4.72	8.67 ± 0.76	2.00 ± 0.26
Sham + ESC (10 mg/kg, p.o.)	109.83 ± 4.56	8.33 ± 0.67	1.83 ± 0.31
OBX control	227.83 ± 3.86$^{\#\#}$	35.50 ± 2.51$^{\#\#}$	6.50 ± 0.43$^{\#\#}$
OBX + QCM-4 (1 mg/kg, p.o.)	156.33 ± 4.08**	26.67 ± 1.82**	3.83 ± 0.48**
OBX + QCM-4 (2 mg/kg, p.o.)	125.00 ± 3.06**	17.17 ± 1.58**	3.17 ± 0.48**
OBX + ESC (10 mg/kg, p.o.)	113.50 ± 3.39**	15.50 ± 1.73**	2.83 ± 0.48**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs sham control group,
**P<0.01 vs OBX control group, n = 6/group.

5.4. Evaluation of QCM-4 on CUMS induced depressive behavior in mice using behavioral and biochemical assessments

5.4.1. Effect of QCM-4 on body weight (g) of mice subjected to CUMS

CUMS control group (**Table 5.7**) showed significantly [F (4, 25) = 3.40, P<0.05] reduced final body weight, compared to normal control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) to mice subjected to CUMS, showed no alteration in body weight, compared to CUMS control group.

Table 5.7: Effect of QCM-4 on body weight of mice exposed to CUMS

Groups	Initial body weight (g)	Final body weight (g)
Normal control	24.50 ± 0.85	26.50 ± 0.62
CUMS control	25.50 ± 1.09	21.40 ± 1.03$^{\#}$
CUMS + QCM-4 (1 mg/kg, p.o.)	24.00 ± 0.63	24.00 ± 0.67
CUMS + QCM-4 (2 mg/kg, p.o.)	25.17 ± 0.83	25.50 ± 1.41
CUMS + ESC (10 mg/kg, p.o.)	25.33 ± 1.38	25.33 ± 1.44

The values are expressed as mean ± S.E.M., $^{\#}$P<0.05 vs normal control group, n = 6/group.

5.4.2. Effect of QCM-4 on SPT in mice subjected to CUMS

Result of SPT showed (**Fig 5.5**) that, CUMS control group exhibited significant (P<0.01) decrease in consumption sucrose solution (1%), compared to normal control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) significantly [F (4, 25) = 27.81, P<0.01] improved the sucrose consumption in CUMS animals as compared to CUMS control group.

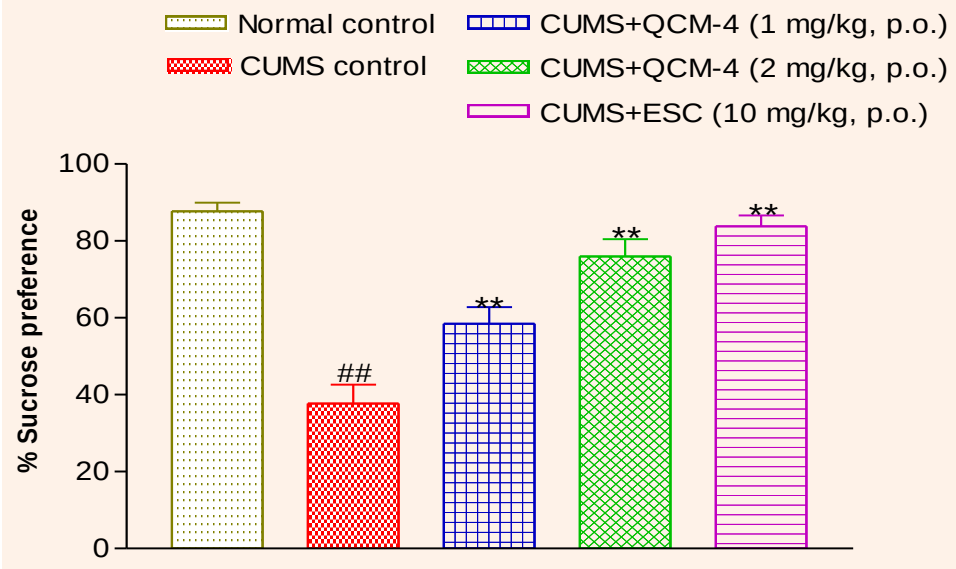


Fig 5.5: Effect of **QCM-4** on SPT in mice subjected to CUMS. The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs normal control group, **P<0.01 vs CUMS control group, n=6/group.

5.4.3. Effect of QCM-4 on SLA score in mice subjected to CUMS

The basal locomotor activity score was not significantly (P>0.05) changed in mice exposed to CUMS, compared to normal control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.), and standard ESC (10 mg/kg, p.o.) in CUMS mice, did not show any significant [F (4, 25) = 0.098, P>0.05] alteration in locomotor scores as compared to CUMS control group (**Fig 5.6**).

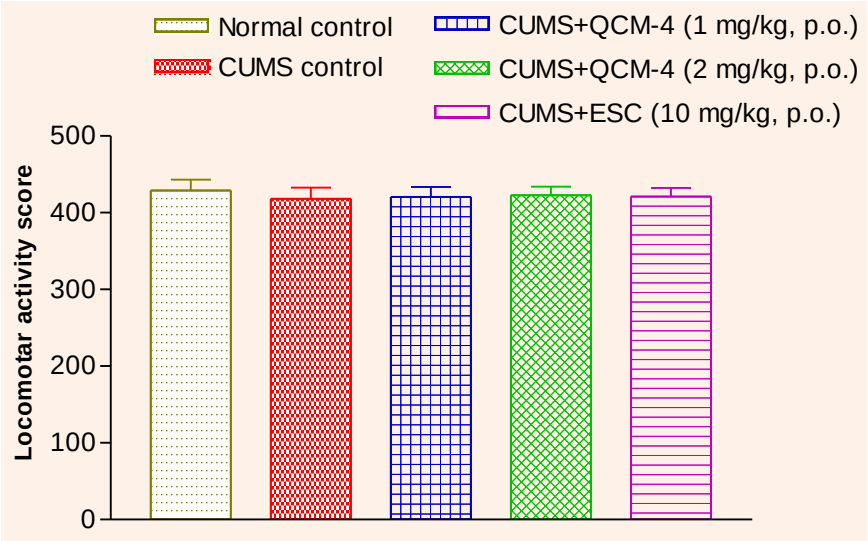


Fig 5.6: Effect of **QCM-4** on locomotor activity score in mice exposed to CUMS. The values are expressed as mean ± S.E.M., n=6/group.

5.4.4. Effect of QCM-4 on duration of immobility in FST in mice subjected to CUMS

Results of the FST (**Fig 5.7**), showed that CUMS exposed mice showed significantly (P<0.05) increased immobility time in FST compared to normal control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) significantly [F (4, 25) = 83.11, P<0.01] reduced the immobility time in CUMS exposed mice, compared to CUMS control group.

5.4.5. Effect of QCM-4 on duration of immobility in TST in mice subjected to CUMS

Results of TST (**Fig 5.8**), represented that CUMS exposed mice showed significantly (P<0.05) increased immobility time in TST compared to normal control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 25) = 103.5, P<0.01] reduced the immobility time in CUMS exposed mice, compared to CUMS control group.

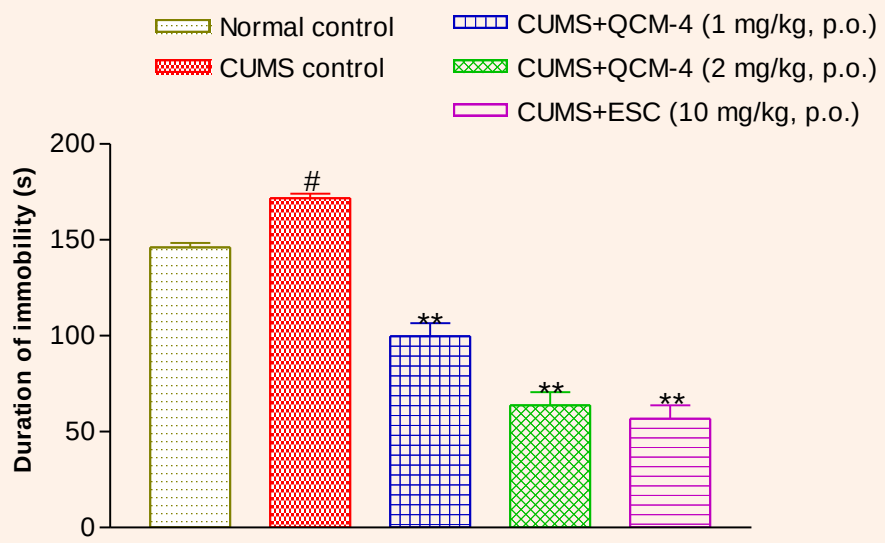


Fig 5.7: Effect of **QCM-4** on duration of immobility in FST in mice exposed to CUMS. The values are expressed as mean ± S.E.M., #P<0.05 vs normal control group, **P<0.01 vs CUMS control, n=6/group.

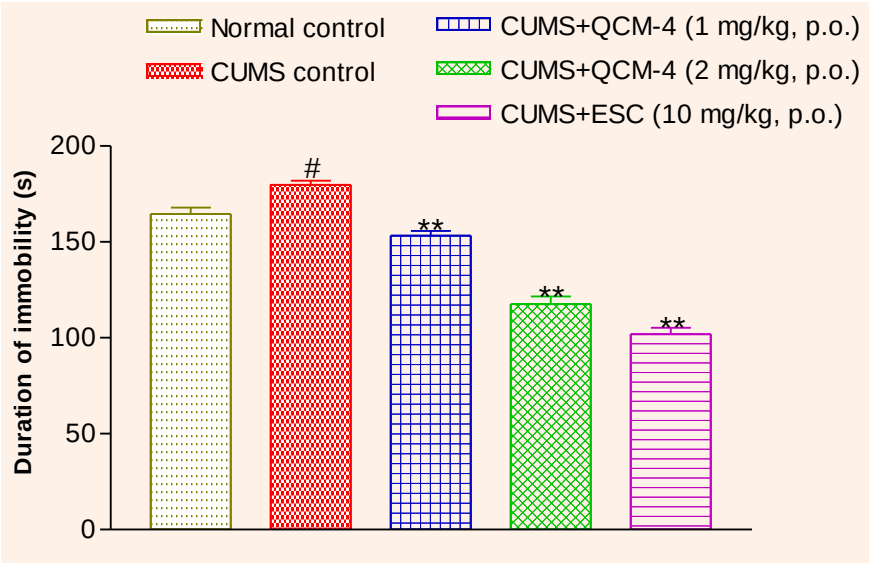


Fig 5.8: Effect of **QCM-4** on duration of immobility in TST in mice exposed to CUMS. The values are expressed as mean ± S.E.M., #P<0.05 vs normal control group, **P<0.01 vs CUMS control group, n=6/group.

5.4.6. Effect of QCM-4 on behavior of mice subjected to CUMS in EPM

Result of EPM **(Table 5.8)** represented that, CUMS control mice exhibited significantly (P<0.05) reduced % OAE and % OAT as compared to normal control. Repetitive treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) significantly increased the % OAE [F (4, 25) = 86.04, P<0.01] and % OAT [F (4, 25) = 159.7, P<0.01] in CUMS mice as compared to CUMS control group.

Table 5.8: Effect of QCM-4 on EPM in CUMS exposed mice

Groups	% OAE	% OAT (s)
Normal control	52.73 ± 0.48	20.90± 0.51
CUMS control	10.67 ± 0.19$^{\#}$	10.07 ± 0.61$^{\#\#}$
CUMS + QCM-4 (1 mg/kg, p.o.)	48.39 ± 0.54**	26.67 ± 0.50**
CUMS + QCM-4 (2 mg/kg, p.o.)	63.95 ± 0.57**	39.28 ± 0.54**
CUMS + ESC (10 mg/kg, p.o.)	80.00 ± 0.81**	51.52 ± 0.45**

The values are expressed as mean ± S.E.M., $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs normal control group, **P<0.01 vs CUMS control group, n = 6/group.

5.5. Evaluation of 4a in behavioral rodent models of depression

5.5.1. Effect of 4a on SLA score in mice

4a was injected to mice at various doses (0.5, 1, 2, 4 and 8 mg/kg, i.p.). No significant [F (5, 30) = 3.55, P>0.05] alteration in basal locomotor score activity was observed, with **4a** administrations at 0.5, 1, 2, and 4 mg/kg, where as administration of **4a** at 8 mg/kg, reported significant (P<0.05) hypolocomotion as compared to control group, as showed in **Fig 5.9**.

5.5.2. Effect of 4a on duration of immobility in mice using FST

4a (0.5, 1, 2 and 4 mg/kg, i.p.) and standard ESC at (10 mg/kg, i.p.) were administered and immobility time in FST, were recorded. **4a** (2 and 4 mg/kg, i.p.) and standard antidepressant ESC (10 mg/kg, i.p.) showed significant [F (5, 30) = 18.34, P<0.05] reduction in immobility time in mice, as compared with control group. However, **4a** at 0.5 and 1 mg/kg, did not show significant (P>0.05) change in the immobility time, as compared to control group (**Fig 5.10**).

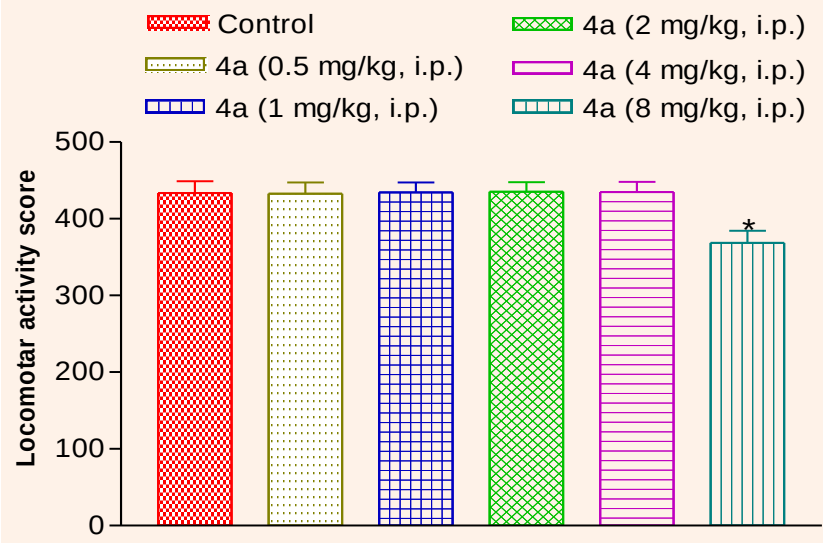


Fig 5.9: Effect of **4a** on basal locomotor score in mice. The values are expressed as mean ± S.E.M., *P<0.05 vs control group, n=6/group.

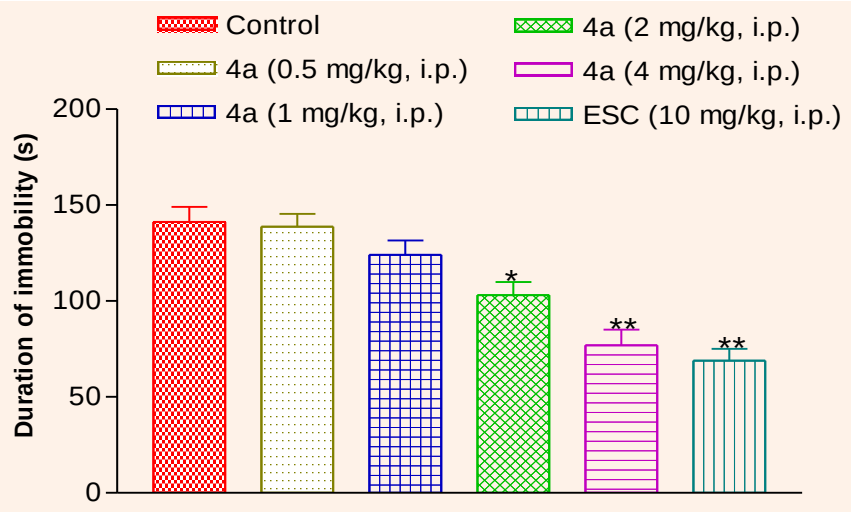


Fig 5.10: Effect of **4a** on duration of immobility in mice using FST. The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs control group, n=6/group.

5.5.3. Effect of 4a on duration of immobility in mice using TST

Results of TST (**Fig 5.11**), showed that **4a** at (0.5, 1, 2 and 4 mg/kg, i.p.) and standard antidepressant ESC at (10 mg/kg, i.p.) were injected to mice and immobility time was recorded. **4a** (2 and 4 mg/kg, i.p.) and standard ESC (10 mg/kg, i.p.) showed significant [F (5, 30) = 24.23, P<0.05] inhibition of immobility time in mice as compared to control group. However, **4a** at 0.5 and 1 mg/kg, did not show any significant (P>0.05) change in the immobility time compared to control group.

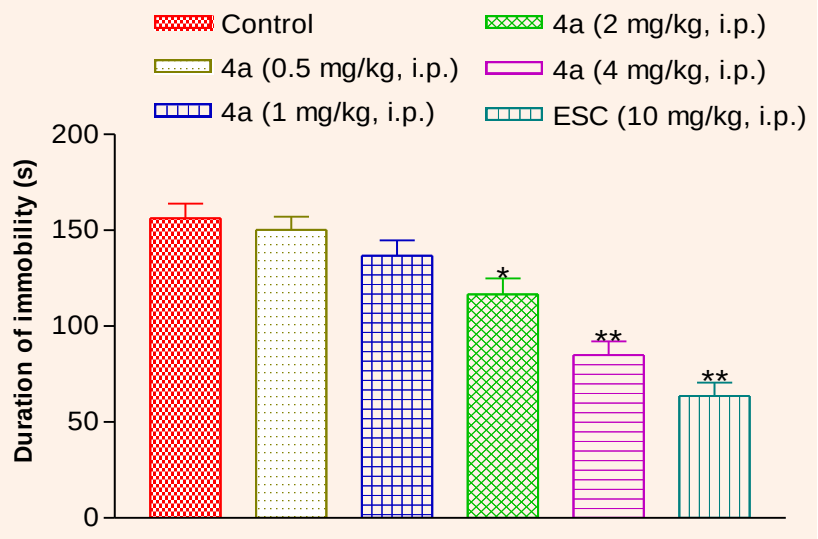


Fig 5.11: Effect of **4a** on duration of immobility in mice using TST. The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs control group, n=6/group.

5.6. Evaluation of 4a in behavioral rodent models of anxiety

5.6.1. Effect of 4a on mice behavior in EPM

Results of EPM (**Table 5.9**), showed that **4a** (2 and 4 mg/kg, i.p.) and standard diazepam (2 mg/kg, i.p.) injected groups, significantly increased % OAE [F (3, 20) = 13.45, P<0.01] and % OAT [F (3, 20) = 44.57, P<0.01], compared to control group.

Table 5.9: Effect of 4a on mice in EPM

Groups	% OAE	% OAT (s)
Control	31.44 ± 3.97	19.22 ± 1.46
4a (2 mg/kg, i.p.)	56.49 ± 4.53**	28.28 ± 1.38**
4a (4 mg/kg, i.p.)	62.16 ± 4.56**	35.72 ± 1.75**
Diazepam (2 mg/kg, i.p.)	70.63 ± 5.27**	45.00 ± 1.91**

The values are expressed as mean ± S.E.M., **P<0.01 vs control group, n = 6/group

5.6.2. Effect of 4a on mice behavior in HBT

Result of HBT (**Table 5.10**), showed that **4a** (2 and 4 mg/kg, i.p.) and standard diazepam (2 mg/kg, i.p.), significantly increased the head dip [F (3, 20) = 16.05, P<0.05] and crossing scores [F (3, 20) = 14.03, P<0.05], compared to control group.

Table 5.10: Effect of 4a on mice in HBT

Groups	Number of head dips	Number of crossings
Control	25.17 ± 3.13	34.83 ± 3.65
4a (2 mg/kg, i.p.)	41.83 ± 3.19*	52.33 ± 4.43*
4a (4 mg/kg, i.p.)	48.33 ± 3.15**	60.17 ± 3.65**
Diazepam (2 mg/kg, i.p.)	62.50 ± 5.47**	71.83 ± 4.75**

The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs control group, n = 6/group.

5.6.3. Effect of 4a on mice behavior in L/D test

4a (2 and 4 mg/kg, i.p.) and standard diazepam (2 mg/kg, i.p.), significantly increased the time in light chamber [F (3, 20) = 14.71, P<0.05] and transition score [F (3, 20) = 10.67, P<0.01], compared to control group (**Table 5.11**).

Table 5.11: Effect of 4a on mice in L/D test

Groups	Time in light chamber (s)	Number of transitions
Control	34.83 ± 5.49	21.17 ± 4.38
4a (2 mg/kg, i.p.)	55.00 ± 3.28*	40.67 ± 3.12*
4a (4 mg/kg, i.p.)	63.50 ± 3.54**	46.50 ± 4.40**
Diazepam (2 mg/kg, i.p.)	70.50 ± 3.25**	54.00 ± 5.03**

The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs control group, n = 6/group.

5.7. Evaluation of 4a on co-morbid depression and anxiety using OBX model

5.7.1. Effect of 4a on body weight (g) of OBX rats

No significant difference were observed in the initial body weights between sham and OBX control groups (**Table 5.12**) [F (7, 40) = 0.019, P>0.05], whereas, final body weight was significantly [F (7, 40) = 7.85, P<0.01] reduced in OBX rats as compared to sham control group. Sub-chronic administration of **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) did not significantly (P>0.05) altered the body weight in sham as well as OBX rats.

Table 5.12: Effect of 4a on body weight in OBX rats

Groups	Initial body weight (g)	Final body weight (g)
Sham control	256.12 ± 5.47	296.87 ± 5.25
Sham + 4a (2 mg/kg, p.o.)	255.53 ± 5.18	291.85 ± 4.47
Sham + 4a (4 mg/kg, p.o.)	254.42 ± 4.30	293.32 ± 4.16
Sham + ESC (10 mg/kg, p.o.)	255.35 ± 5.29	291.00 ± 4.74
OBX control	256.36 ± 5.49	260.77 ± 4.59$^{\#\#}$
OBX + 4a (2 mg/kg, p.o.)	254.67 ± 6.05	268.02 ± 6.14
OBX + 4a (4 mg/kg, p.o.)	256.37 ± 5.11	276.14 ± 4.18
OBX + ESC (10 mg/kg, p.o.)	255.78 ± 5.03	274.28 ± 4.56

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs control group, n = 6/group.

5.7.2. Effect of 4a treatment on SPT in OBX rats

OBX control rats showed significant (P<0.01) reduction in sucrose (1%) consumption compared to sham control group (**Fig 5.12**). Treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.), significantly [F (7, 40) = 23.07, P<0.01] increased the sucrose solution consumption in OBX rats as compared to OBX control group.

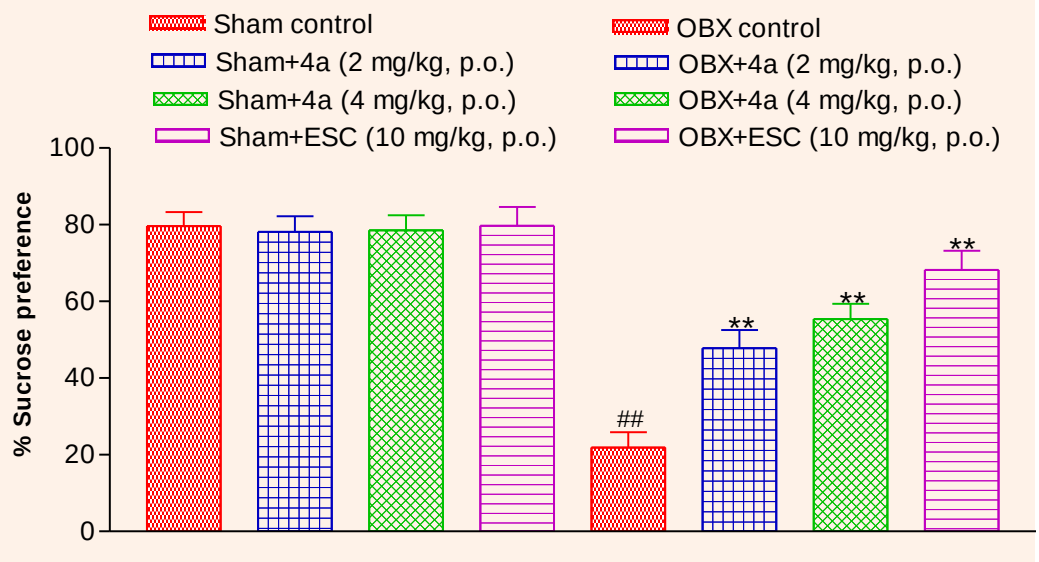


Fig 12: Effect of **4a** on SPT in OBX rats. The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs sham control group, **P<0.01 vs OBX control group, n=6/group.

5.7.3. Effect of 4a treatment on EPM in OBX rats

OBX control group showed significantly (P<0.01) increased % OAE and % OAT in EPM (**Table 5.13**) as compared to sham control group. Treatment with **4a** (2 and 4 mg/kg, p.o.) and ESC (10 mg/kg, p.o.) significantly reduced the % OAE [F (7, 40) = 7.15, P<0.05] and % OAT [F (7, 40) = 14.78, P<0.05] in OBX rats as compared to OBX control group.

Table 5.13: Effect of 4a on EPM in OBX rat

Groups	% OAE	% OAT (s)
Sham control	35.09 ± 4.29	23.89 ± 2.03
Sham + 4a (2 mg/kg, p.o.)	37.74 ± 3.89	29.56 ± 2.82
Sham + 4a (4 mg/kg, p.o.)	41.84 ± 5.60	35.50 ± 2.59
Sham + ESC (10 mg/kg, p.o.)	45.06 ± 4.22	36.72 ± 2.35
OBX control	73.23 ± 2.49$^{\#\#}$	57.61 ± 4.20$^{\#\#}$
OBX + 4a (2 mg/kg, p.o.)	51.43 ± 2.19*	44.28 ± 2.07*
OBX + 4a (4 mg/kg, p.o.)	48.69 ± 4.84**	42.39 ± 1.16**
OBX + ESC (10 mg/kg, p.o.)	44.07 ± 6.24**	36.94 ± 2.88**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs sham control group,
*P<0.05; **P<0.01 vs OBX control group, n = 6/group.

5.7.4. Effect of 4a treatment on modified OFT in OBX rats

In OFT, OBX control group showed significantly (P<0.01) increased ambulation, rearing score and fecal pellets as compared to sham control group. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) significantly reduced the ambulatory [F (7, 40) = 55.32, P<0.01] and rearing scores [F (7, 40) = 5.76, P<0.05], and fecal pellets [F (7, 40) = 3.44, P<0.05] in OBX rats as compared to OBX control group as represented in **Table 5.14**.

Table 5.14: Effect of 4a on OFT in OBX rats

Groups	Ambulatory score	Rearing score	Fecal pellets Score
Sham control	108.00 ± 6.59	3.00 ± 0.97	15.17 ± 3.27
Sham + 4a (2 mg/kg, p.o.)	110.00 ± 5.70	2.83 ± 0.70	14.33 ± 3.60
Sham + 4a (4 mg/kg, p.o.)	114.00 ± 6.84	2.67 ± 0.61	13.83 ± 4.20
Sham + ESC (10 mg/kg, p.o.)	115.83 ± 5.62	2.50 ± 0.56	13.67 ± 3.88
OBX control	235.67 ± 5.39$^{\#\#}$	6.00 ± 0.52$^{\#\#}$	40.33 ± 4.51$^{\#\#}$
OBX + 4a (2 mg/kg, p.o.)	173.33 ± 5.75**	3.67 ± 0.33	28.00 ± 3.39
OBX + 4a (4 mg/kg, p.o.)	152.00 ± 6.11**	3.00 ± 0.52*	21.33 ± 3.77*
OBX + ESC (10 mg/kg, p.o.)	126.83 ± 5.47**	3.00 ± 0.45**	19.50 ± 4.26**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs sham control group, *P<0.05; **P<0.01 vs OBX control group, n = 6/group.

5.8. Evaluation of 4a on CUMS induced depressive behavior in mice using behavioral and biochemical assessments

5.8.1. Effect of 4a on body weight (g) of mice subjected to CUMS

CUMS control group exhibited marked [F (4, 25) = 2.87, P<0.05] reduction in final body weight (**Table 5.15**), as compared to normal control group. Sub-chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in mice subjected to CUMS showed no significant (P>0.05) alteration in body weight as compared to CUMS control group.

Table 5.15: Effect of 4a on body weight (g) of mice exposed to CUMS

Groups	Initial body weight (g)	Final body weight (g)
Normal control	24.00 ± 0.77	25.50 ± 0.43
CUMS control	24.50 ± 1.26	21.17 ± 0.87$^{\#}$
CUMS + 4a (2 mg/kg, p.o.)	24.67 ± 0.49	24.00 ± 1.03
CUMS + 4a (4 mg/kg, p.o.)	24.83 ± 0.60	24.17 ± 0.70
CUMS + ESC (10 mg/kg, p.o.)	25.33 ± 1.41	25.00 ± 1.55

The values are expressed as mean ± S.E.M., $^{\#}$P<0.05 vs normal control group, n = 6/group.

5.8.2. Effect of 4a on SPT in mice subjected to CUMS

CUMS control group showed remarkable (P<0.01) reduction in consumption of sucrose solution (**Fig 5.13**) as compared with normal control group. Chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.), significantly [F (4, 25) = 36.61, P<0.05] improved the sucrose consumption in CUMS animals as compared to CUMS control group.

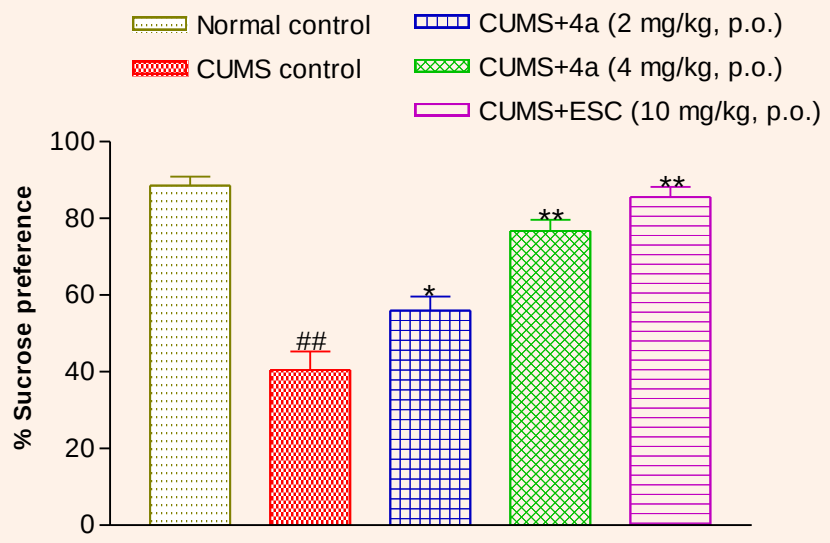


Fig 5.13: Effect of **4a** on SPT in mice subjected to CUMS. The values are expressed as mean ± S.E.M., ##P<0.01 vs normal control group, *P<0.05; **P<0.01 vs CUMS control group, n=6/group.

5.8.3. Effect of 4a on SLA score in mice subjected to CUMS

The basal locomotor activity score was not significantly (P>0.05) changed in mice exposed to CUMS as compared to normal control group (**Fig 5.14**). Sub-chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) in CUMS mice did not showed any significant [F (4, 25) = 0.098, P>0.05] change in locomotor activity score as compared to CUMS control group.

5.8.4. Effect of 4a on duration of immobility in FST in mice subjected to CUMS

CUMS exposed mice, exhibited significantly (P<0.01) increased immobility time, in FST, as compared to normal control group (**Fig 5.15**). Sub-chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 25) = 124.3, P<0.01] decreased the immobility time in CUMS mice as compared to CUMS control group.

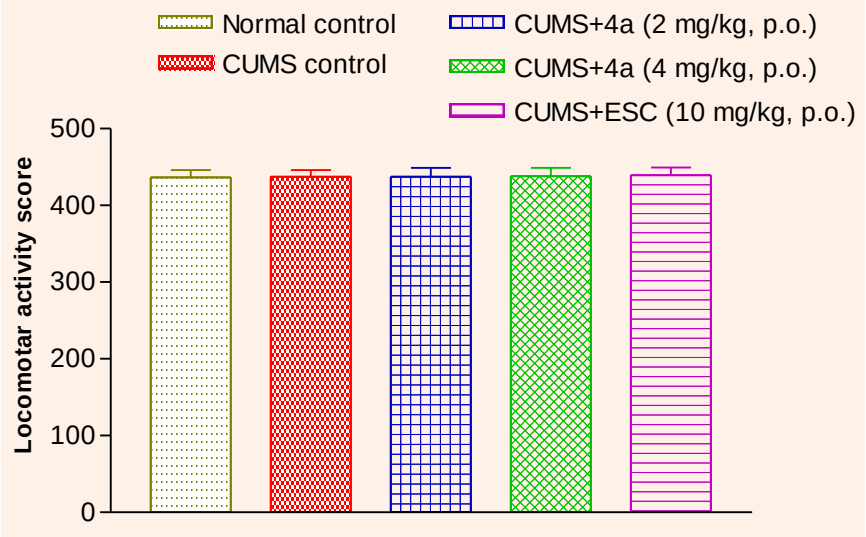


Fig 5.14: Effect of **4a** on locomotor activity score in mice exposed to CUMS. The values are expressed as mean ± S.E.M., n=6/group.

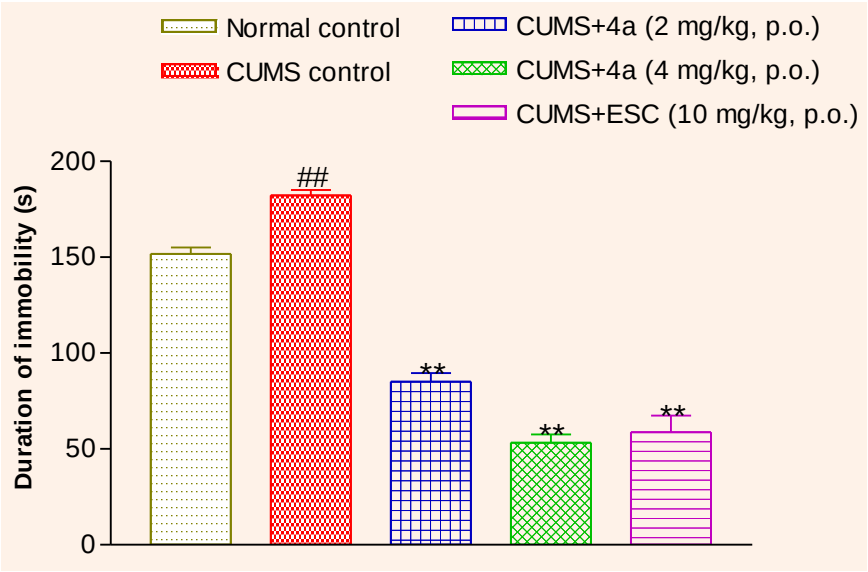


Fig 5.15: Effect of **4a** on duration of immobility in FST in mice exposed to CUMS. The values are expressed as mean ± S.E.M., ##P<0.01 vs normal control group, **P<0.01 vs CUMS control, n=6/group.

5.8.5. Effect of 4a on duration of immobility in TST in mice subjected to CUMS

CUMS exposed mice showed (**Fig 5.16**) significantly (P<0.05) increased immobility time in TST as compared to normal control group. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) significantly [F (4, 25) = 79.30, P<0.01] decreased the immobility time in CUMS exposed mice as compared to CUMS control group.

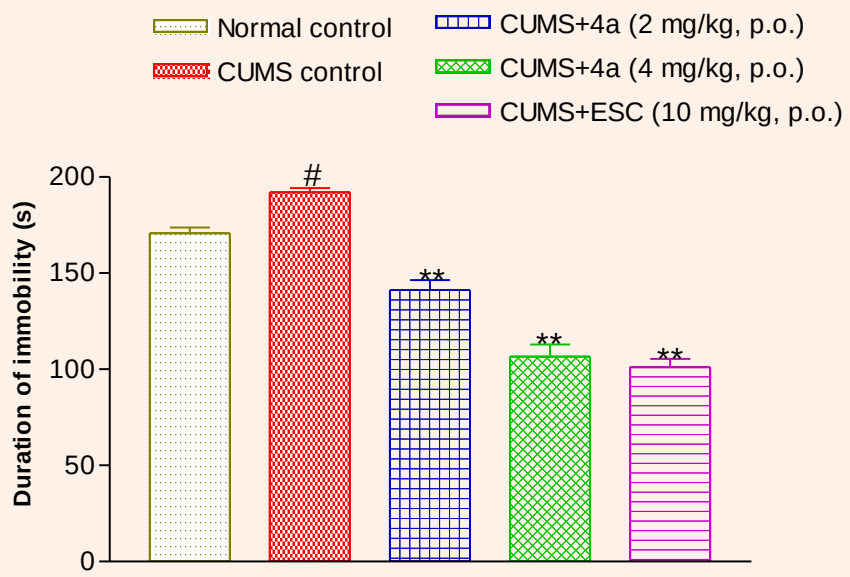


Fig 5.16: Effect of **4a** on duration of immobility in TST in mice exposed to CUMS. The values are expressed as mean ± S.E.M., #P<0.05 vs normal control group, **P<0.01 vs CUMS control group, n=6/group.

5.8.6. Effect of 4a on behavior of mice subjected to CUMS in EPM

Result of 4a treatment on EPM in CUMS exposed mice (**Table 5.16**), represented that, CUMS control mice exhibited significantly (P<0.01) reduced % OAE and % OAT compared to normal control. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the % OAE [F (4, 25) = 117.6, P<0.01] and % OAT [F (4, 25) = 156.5, P<0.01] in CUMS mice as compared to CUMS control group.

Table 5.16: Effect of 4a on EPM in CUMS exposed mice

Groups	% OAE	% OAT (s)
Normal control	54.94 ± 4.32	21.44 ± 1.07
CUMS control	10.07 ± 1.06##	9.78 ± 1.39##
CUMS + 4a (2 mg/kg, p.o.)	60.75 ± 2.69**	32.44 ± 1.57**
CUMS + 4a (4 mg/kg, p.o.)	70.67 ± 1.95**	45.67 ± 1.65**
CUMS + ESC (10 mg/kg, p.o.)	84.64 ± 1.68**	54.44 ± 1.44**

The values are expressed as mean ± S.E.M., ##P<0.01 vs normal control group, **P<0.01 vs CUMS control group, n = 6/group.

5.9. Standardization of HFD induced obesity model

5.9.1. Measurement of body weight (g) in HFD fed mice

Body weight profile of the mice fed with HFD for 14 weeks is represented in **Fig 5.17**. Body weight was recorded once every week. HFD fed group showed significantly increased body weight [F (1, 18) = 17.11, P<0.01] as compared to NPD group.

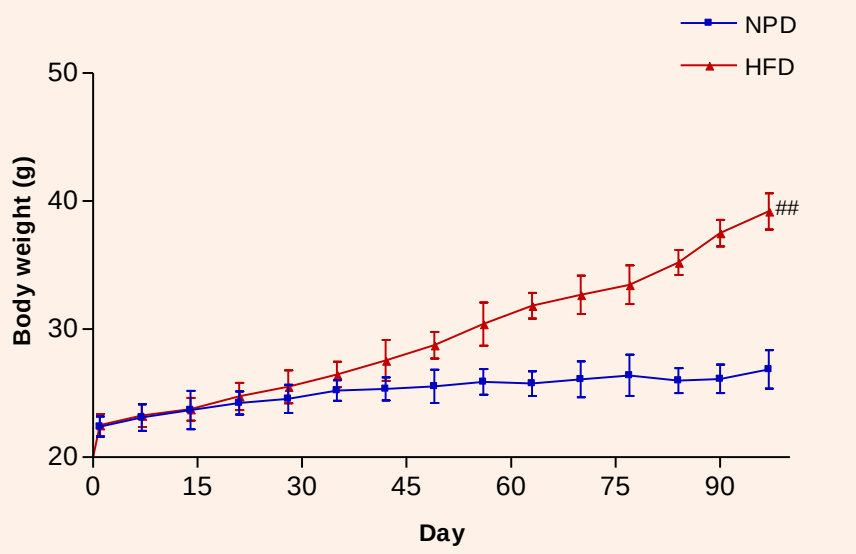


Fig 5.17: Effect of HFD on body weight in mice. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD group, n=10/group.

5.9.2. Biochemical estimations in HFD fed mice

5.9.2.1. Estimation of plasma glucose in HFD fed mice

HFD fed mice showed significantly [F (1, 18) = 2.10, P<0.01] increased plasma glucose as compared to NPD group, as represented in **Fig 5.18**.

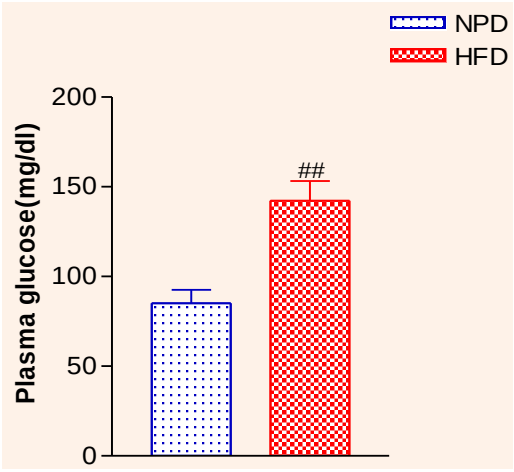


Fig 5.18: Effect of HFD on plasma glucose in mice. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD group, n=10/group.

5.9.2.2. Estimation of plasma total cholesterol in HFD fed mice

HFD fed mice showed significantly [F (1, 18) = 1.17, P<0.01] increased plasma total cholesterol level as compared to NPD group, (**Fig 5.19**).

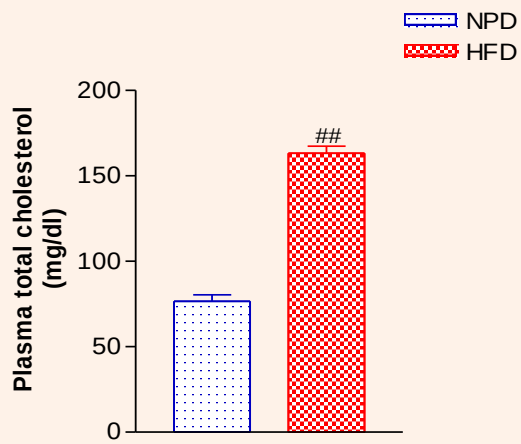


Fig 5.19: Effect of HFD on plasma total cholesterol in mice.

The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD group, n=10/group.

5.9.2.3. Estimation of plasma triglycerides in HFD fed mice

Plasma triglycerides were significantly [F (1, 18) = 3.01, P<0.01] higher in HFD group as compared to NPD group, as represented in **Fig 5.20**.

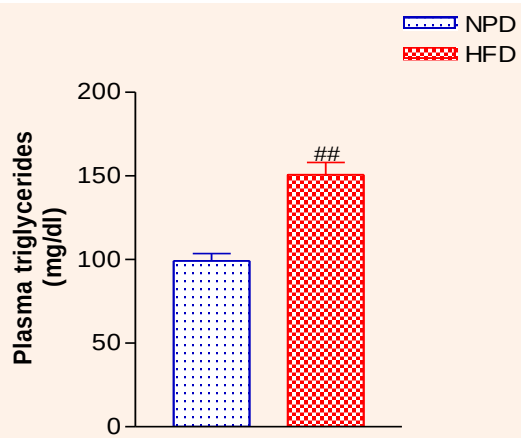


Fig 5.20: Effect of HFD on plasma triglycerides in mice.

The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD group, n=10/group.

5.10. Screening of HFD fed mice for depressive phenotypes

5.10.1. Screening of HFD fed mice for depressive behavior using behavioral assays

5.10.1.1. Behavioral assay of HFD fed mice on SPT

HFD group exhibited significantly [F (1, 10) = 2.24, P<0.01] reduced sucrose solution consumption in SPT as compared to NPD group, (**Fig 5.21**).

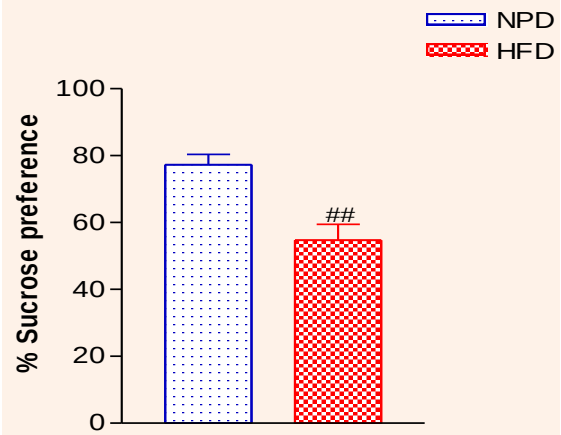


Fig 5.21: Effect of HFD fed mice on SPT. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD group, n=6/group.

5.10.1.2. Behavioral assay of HFD fed mice on SLA score

Locomotor activity score was not significantly [F (1, 10) = 1.10, P>0.05] altered in HFD group as compared to NPD group as shown in **Fig 5.22**.

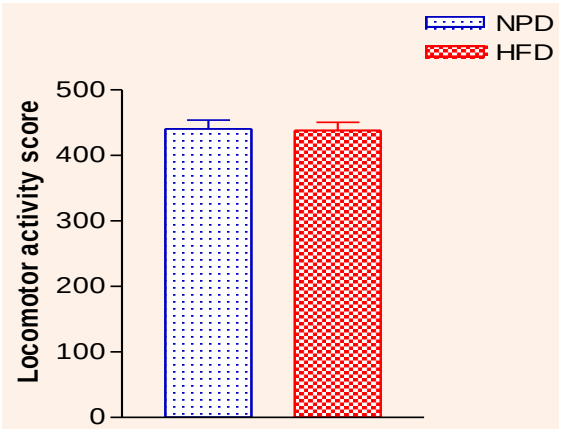


Fig 5.22: Effect of HFD fed mice on SLA using actophotometer. The values are expressed as mean ± S.E.M., n=6/group.

5.10.1.3. Behavioral assay of HFD fed mice on duration of immobility in FST

Effect of HFD fed animals on FST is represented in **Fig 5.23**. HFD group showed significantly [F (1, 10) = 1.20, P<0.01] higher immobility time in FST as compared to NPD group.

5.10.1.4. Behavioral assay of HFD fed mice on duration of immobility in TST

Effect of HFD fed animals on TST is represented in **Fig 5.24** represented the behavioral assay of HFD fed mice on immobility time in TST. HFD group showed significantly [F (1, 10) = 1.31, P<0.01] higher immobility time in TST as compared to NPD group.

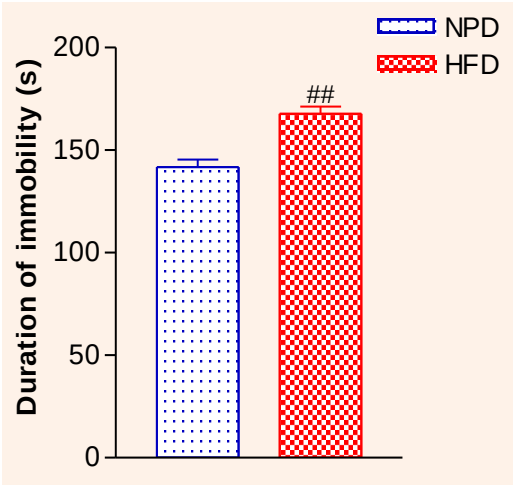


Fig 5.23: Effect of HFD fed mice on duration of immobility in FST. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD group, n=6/group.

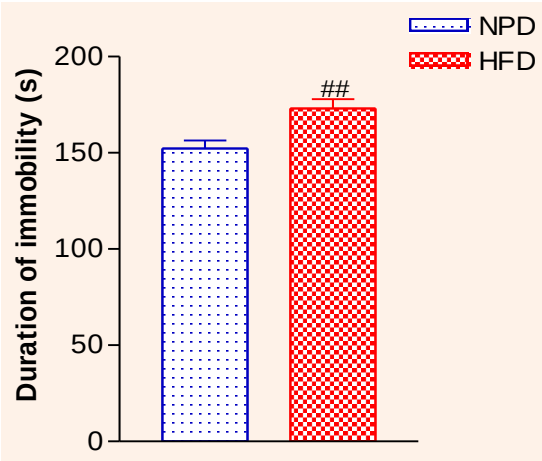


Fig 5.24: Effect of HFD fed mice on duration of immobility in TST. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD group, n=6/group.

5.10.2. Screening of HFD fed mice for anxiety-like behavior

5.10.2.1. Behavioral assay of HFD fed mice on EPM

HFD group showed significantly reduced % OAE [F (1, 10) = 1.23, P<0.01] and % OAT (s) [F (1, 10) = 2.72, P<0.01] as compared to NPD group in EPM, as represented in **Table 5.17**.

Table 5.17: Effect of HFD fed mice on EPM

Groups	% OAE	% OAT (s)
NPD	31.85 ± 3.40	15.56 ± 1.72
HFD	14.16 ± 3.78$^{\#\#}$	8.11 ± 1.05$^{\#\#}$

The values are expressed as mean ± S.E.M.,

$^{\#\#}$P<0.01 vs NPD group, n = 6/group.

5.10.2.2. Behavioral assay of HFD fed mice on HBT

HFD group exhibited significantly reduced number of head dips [F (1, 10) = 1.49, P<0.05] and crossing score [F (1, 10) = 1.34, P<0.01] in HBT as compared to NPD group (**Table 5.18**)

Table 5.18: Effect of HFD fed mice on HBT

Groups	Number of head dips	Number of crossings
NPD	29.00 ± 4.18	38.00 ± 3.85
HFD	13.67 ± 3.42$^{\#}$	16.83 ± 3.32$^{\#\#}$

The values are expressed as mean ± S.E.M.,

$^{\#}$P<0.05; $^{\#\#}$P<0.01 vs NPD group, n = 6/group.

5.10.2.3. Behavioral assay of HFD fed mice on L/D test

HFD group exhibited significantly decreased time spent in light chamber [F (1, 10) = 1.32, P<0.01] and transition score [F (1, 10) = 2.00, P<0.05] as compared to NPD group in L/D, as represented in **Table 5.19**.

Table 5.19: Effect of HFD fed mice on L/D test

Groups	Time in light chamber (s)	Number of transitions
NPD	31.50 ± 3.63	22.00 ± 3.15
HFD	14.00 ± 3.15$^{\#\#}$	9.83 ± 2.23$^{\#}$

The values are expressed as mean ± S.E.M.,

$^{\#}$P<0.05; $^{\#\#}$P<0.01 vs NPD group, n = 6/group.

5.11. Effect of 5-HT_3 receptor antagonist OND on depression co-morbid with obesity in HFD fed mice

5.11.1 Effect of 5-HT_3 receptor antagonist OND on behavioral models of depression in HFD fed mice

5.11.1.1. Effect of OND on body weight (g) in HFD fed mice

The effect of **OND** on body weight in HFD mice is shown in **Fig 5.25**. HFD animals showed significantly (P<0.01) higher body weight as compared to NPD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) did not significantly [F (4, 35) = 4473, P>0.05] alter the body weight in HFD mice as compared to HFD control group.

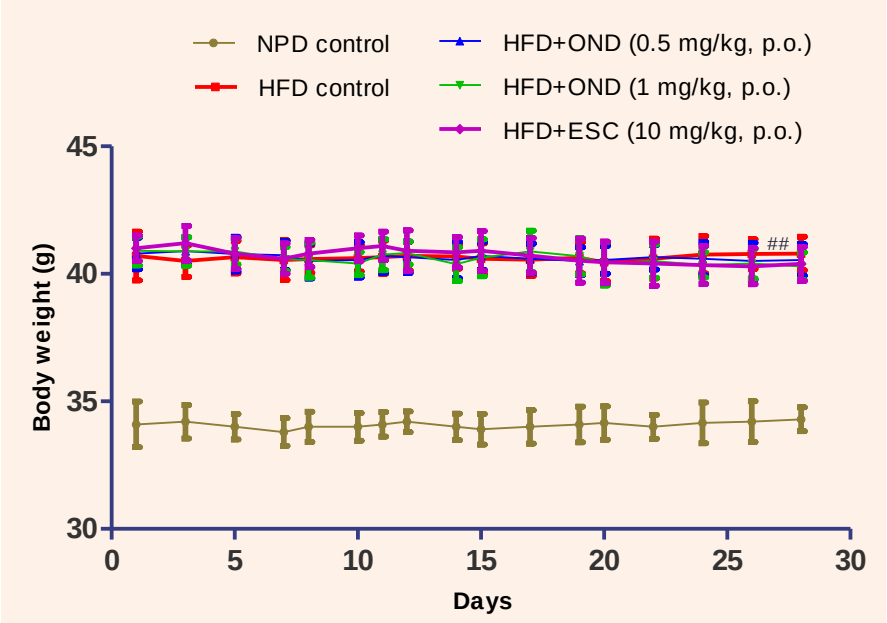


Fig 5.25: Effect of **OND** on body weight of HFD fed mice. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, n=6/group.

5.11.1.2. Effect of OND on SPT in HFD fed mice

HFD group showed significantly (P<0.01) reduced sucrose consumption as compared to NPD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 6.21, P<0.05] increased the sucrose consumption in HFD mice as compared to HFD control group, as represented in **Fig 5.26**.

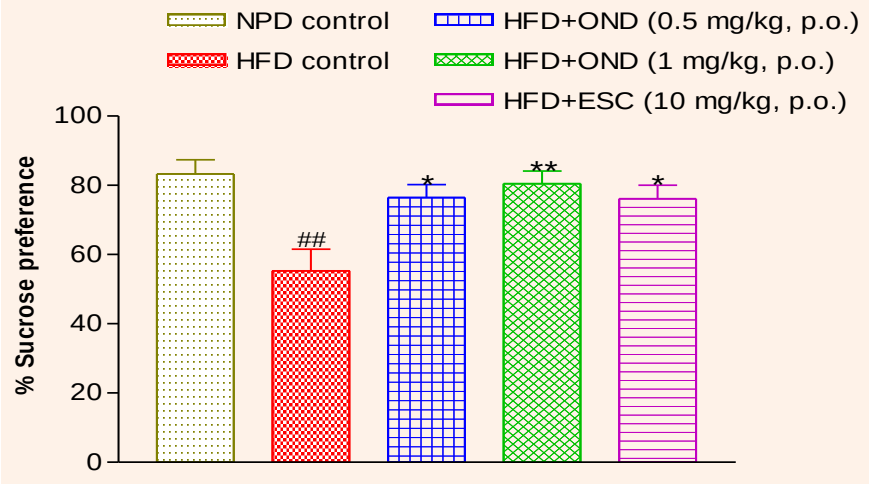


Fig 5.26: Effect of **OND** on SPT in HFD fed mice. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, *P<0.05;**P<0.01 vs HFD control group, n=6/group.

5.11.1.3. Effect of OND on SLA score in HFD fed mice

OND (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) did not significantly [F (4, 35) = 0.029, P>0.05] alter the basal locomotor activity score, in HFD mice, compared to HFD control group (**Fig 5.27**).

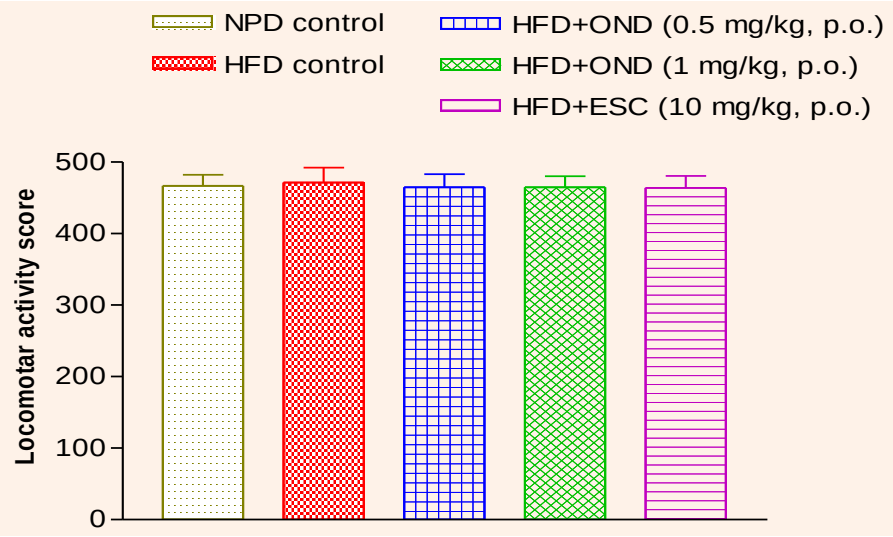


Fig 5.27: Effect of **OND** on SLA score in HFD fed mice. The values are expressed as mean ± S.E.M., n=6/group.

5.11.1.4. Effect of OND on FST in HFD fed mice

HFD control group showed significantly (P<0.01) increased immobility time as compared to NPD control. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 13.35, P<0.01] reduced the immobility time of HFD mice as compared to HFD control group in FST, as represented in **Fig 5.28**.

5.11.1.5. Effect of OND on TST in HFD fed mice

HFD control group showed significantly (P<0.01) increased immobility time compared to NPD control. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 9.41, P<0.01] decreased the immobility time of HFD mice as compared to HFD control group in TST, as shown in **Fig 5.29**.

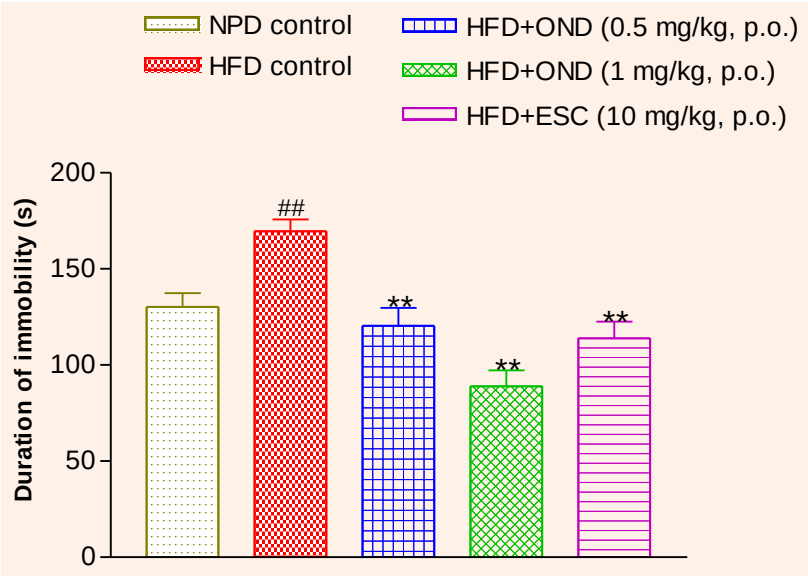


Fig 5.28: Effect of **OND** on duration of immobility in HFD fed mice in FST. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, **P<0.01 vs HFD control group, n=6/group.

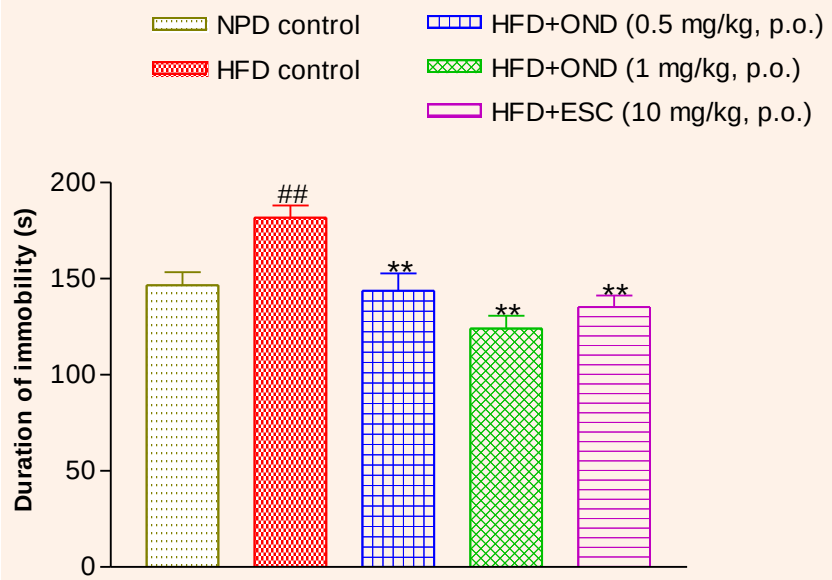


Fig 5.29: Effect of **OND** on duration of immobility in HFD fed mice in TST. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, **P<0.01 vs HFD control group, n=6/group.

5.11.2. Effect of OND on behavioral models of anxiety in HFD fed mice

5.11.2.1. Effect of OND on EPM in HFD fed mice

HFD control group showed significantly (P<0.05) decreased % OAE and % OAT in EPM as compared to NPD control group in EPM (**Table 5.20**). Repetitive treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the % OAE [F (4, 35) = 7.18, P<0.05] and % OAT [F (4, 35) = 5.20, P<0.05] in HFD fed mice as compared to HFD control group.

Table 5.20: Effect of OND on behavior of HFD fed mice in EPM

Groups	% OAE	% OAT (s)
NPD control	35.14 ± 4.39	15.39 ± 1.65
HFD control	13.23 ± 2.99$^{\#}$	8.50 ± 1.15$^{\#}$
HFD + OND (0.5 mg/kg, p.o.)	37.09 ± 5.40*	14.56 ± 1.32*
HFD + OND (1 mg/kg, p.o.)	48.69 ± 5.93**	16.33 ± 1.57**
HFD + ESC (10 mg/kg, p.o.)	38.94 ± 5.09*	15.06 ± 1.05*

The values are expressed as mean ± S.E.M., $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs NPD control, *P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.11.2.2. Effect of OND on HBT in HFD fed mice

HFD group showed observable (P<0.05) reduced number of head dips and crossings as compared to NPD control group in HBT. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and antidepressant ESC (10 mg/kg, p.o.) significantly increased the number of head dips [F (4, 35) = 5.62, P<0.05] and crossing scores [F (4, 35) = 6.62, P<0.05] in HFD fed mice as compared to HFD control group, (**Table 5.21**).

Table 5.21: Effect of OND on behavior of HFD fed mice in HBT

Groups	Number of head dips	Number of crossings
NPD control	31.00 ± 3.81	40.50 ± 3.81
HFD control	13.33 ± 2.54$^{\#}$	16.33 ± 3.01$^{\#\#}$
HFD + OND (0.5 mg/kg, p.o.)	29.83 ± 3.62*	34.83 ± 3.54*
HFD + OND (1 mg/kg, p.o.)	35.67 ± 4.00**	41.67 ± 4.75**
HFD + ESC (10 mg/kg, p.o.)	31.83 ± 4.09*	36.50 ± 4.54*

The values are expressed as mean ± S.E.M., $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs NPD control, *P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.11.2.3. Effect of OND on L/D test in HFD fed mice

HFD group showed marked decreased time in light chamber and transition score (P<0.05) as compared to NPD control group in L/D test. Repetitive treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the time in light chamber [F (4, 35) = 8.26, P<0.05] and transition score [F (4, 35) = 5.28, P<0.05] in HFD fed mice as compared to HFD control group, as represented in **Table 5.22**.

Table 5.22: Effect of OND on behavior of HFD fed mice in L/D test

Groups	Time in light chamber (s)	Number of transitions
NPD control	30.33 ± 3.21	23.50 ± 3.17
HFD control	14.00 ± 3.34$^{\#\#}$	10.17 ± 2.56$^{\#}$
HFD + OND (0.5 mg/kg, p.o.)	29.50 ± 3.02*	22.83 ± 2.40*
HFD + OND (1 mg/kg, p.o.)	37.00 ± 2.67**	27.50 ± 2.85**
HFD + ESC (10 mg/kg, p.o.)	33.17 ± 2.98*	24.00 ± 3.36*

The values are expressed as mean ± S.E.M., $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs NPD control, *P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.11.3. Effect of OND on biochemical estimations in HFD fed mice

5.11.3.1. Effect of OND on plasma glucose, total cholesterol and triglycerides in HFD fed mice

HFD group exhibited remarkable (P<0.01) higher plasma glucose, total cholesterol and triglycerides as compared to NPD control group, (**Table 5.23**). Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly reduced the abnormally elevated plasma glucose [F (4, 35) = 9.13, P<0.05], total cholesterol [F (4, 35) = 13.40, P<0.01] and triglycerides [F (4, 35) = 8.72, P<0.05] towards the basal values in HFD fed mice as compared to HFD control group.

Table 5.23: Effect of OND on plasma glucose, total cholesterol and triglycerides in HFD fed mice

Groups	Plasma glucose (mg/dl)	Plasma total cholesterol (mg/dl)	Plasma triglycerides (mg/dl)
NPD control	93.60 ± 7.27	108.07 ± 5.84	96.89 ± 9.34
HFD control	153.36 ± 7.01$^{\#\#}$	194.63 ± 9.34$^{\#\#}$	157.55 ± 4.82$^{\#\#}$
HFD + OND (0.5 mg/kg, p.o.)	120.66 ± 7.24*	140.98 ± 6.56**	112.64 ± 5.79*
HFD + OND (1 mg/kg, p.o.)	105.02 ± 6.21**	122.11 ± 11.40**	92.58 ± 12.42**
HFD + ESC (10 mg/kg, p.o.)	118.47 ± 9.15*	141.62 ± 10.40**	107.53 ± 11.18**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.11.3.2. Effect of OND on OGTT in HFD fed mice

The effect of **OND** on OGTT in HFD mice is represented in **Fig 5.30**. HFD control group showed observable (P<0.05) elevated blood glucose at 30, 60, 90 and 120 min post glucose (2 g/kg, p.o.) loading as compared to NPD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.), reduced the blood glucose at 30,60,90 and 120 min, post glucose loading, but changes were insignificant [F (4, 35) = 2.49, P>0.05] in HFD fed mice as compared to HFD control group.

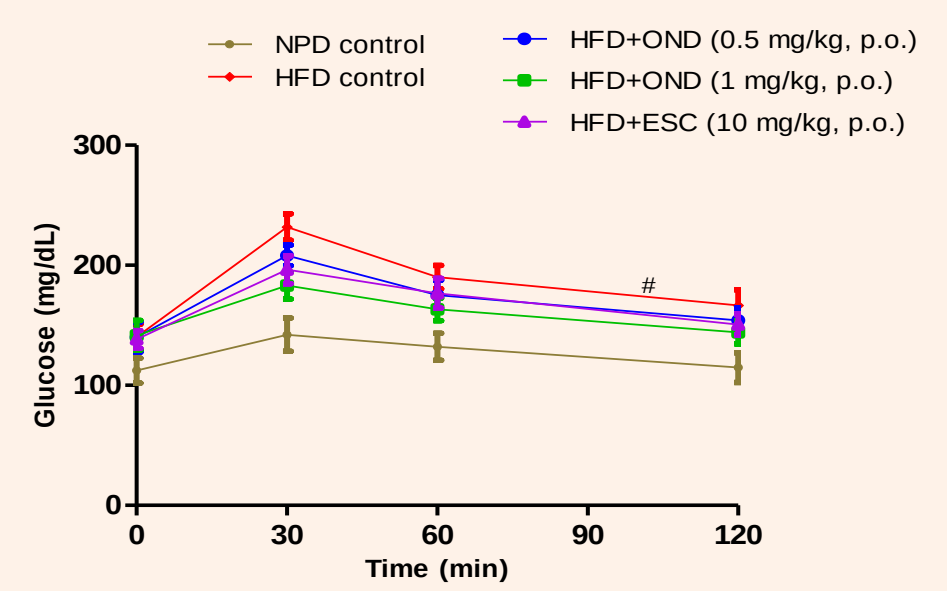


Fig 5.30: Effect of **OND** on OGTT in HFD fed mice. The values are expressed as mean ± S.E.M., $^{\#}$P<0.05 vs NPD control group, n=6/group.

5.11.3.3. Effect of OND on brain hippocampus oxidative stress parameters in HFD fed mice

HFD group exhibited remarkable (P<0.01) higher brain hippocampal lipid peroxidation marker MDA and reduced anti-oxidant GSH concentrations, compared to NPD control group (**Table 5.24**). Repetitive treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.), significantly reduced the MDA [F (4, 35) = 7.00, P<0.05] and increased the GSH [F (4, 35) = 13.97, P<0.05] concentrations in HFD fed mice as compared to HFD control group.

Table 5.24: Effect of OND on brain hippocampus MDA and GSH concentrations in HFD mice

Groups	MDA (µg/mg of proteins)	GSH (µg/mg of proteins)
NPD control	1.63 ± 0.19	0.64 ± 0.08
HFD control	4.25 ± 0.67$^{\#\#}$	0.08 ± 0.02$^{\#\#}$
HFD + OND (0.5 mg/kg, p.o.)	2.51 ± 0.30*	0.34 ± 0.03*
HFD + OND (1 mg/kg, p.o.)	2.42 ± 0.21**	0.46 ± 0.08**
HFD + ESC (10 mg/kg, p.o.)	2.46 ± 0.18*	0.35 ± 0.03*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,

*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.11.4. Effect of OND on neuroendocrinological changes: plasma CORT, leptin and insulin in HFD fed mice

The effect of **OND** on plasma CORT, leptin and insulin concentrations in HFD animals is shown in **Table 5.25**. HFD group showed marked (P<0.01) elevated plasma CORT, leptin and insulin concentrations in HFD fed mice as compared to NPD control group. Repetitive treatment with **OND** (0.5 and 1 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.), significantly inhibited the abnormally elevated plasma CORT [F (4, 35) = 240.5, P<0.01], leptin [F (4, 35) = 75.45, P<0.01] and insulin [F (4, 35) = 18.04, P<0.05] in HFD mice as compared to HFD control group.

Table 5.25: Effect of OND on plasma CORT, leptin and insulin concentrations in HFD fed mice

Groups	Plasma CORT (ng/ml)	Plasma leptin (pg/ml)	Plasma insulin (ng/ml)
NPD control	118.47 ± 13.29	953.63 ± 61.25	0.543 ± 0.070
HFD control	863.20 ± 23.94$^{\#\#}$	1729.05 ± 85.47$^{\#\#}$	1.669 ± 0.131$^{\#\#}$
HFD + OND (0.5 mg/kg, p.o.)	220.33 ± 26.86**	1309.88 ± 114.63**	1.240 ± 0.085*
HFD + OND (1 mg/kg, p.o.)	166.94 ± 15.15**	1018.49 ± 110.54**	1.131 ± 0.075**
HFD + ESC (10 mg/kg, p.o.)	201.79 ± 17.00**	1214.13 ± 54.65**	1.219 ± 0.101*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.11.5. Effect of OND on molecular mechanisms: 5-HT, cAMP and BDNF in HFD fed mice

The effect of **OND** on brain hippocampal 5-HT, cAMP and BDNF concentrations in HFD mice is represented in **Table 5.26**. HFD animals showed marked (P<0.01) reduced 5-HT, cAMP and BDNF concentrations as compared to NPD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the hippocampus 5-HT [F (4, 35) = 23.18, P<0.01], cAMP [F (4, 35) = 37.79, P<0.01] and BDNF [F (4, 35) = 63.91, P<0.05] concentrations in HFD mice as compared to HFD control group.

Table 5.26: Effect of OND on brain hippocampal 5-HT, cAMP and BDNF concentrations in HFD fed mice

Groups	5-HT (ng/g)	cAMP (pmol/mg of proteins)	BDNF (ng/mg of proteins)
NPD control	298.78 ± 13.09	5.61 ± 0.12	3.63 ± 0.23
HFD control	102.01 ± 7.37$^{\#\#}$	2.58 ± 0.12$^{\#\#}$	0.56 ± 0.05$^{\#\#}$
HFD + OND (0.5 mg/kg, p.o.)	225.96 ± 16.96**	3.83 ± 0.10**	1.31 ± 0.09*
HFD + OND (1 mg/kg, p.o.)	290.61 ± 23.04**	4.83 ± 0.14**	2.54 ± 0.12**
HFD + ESC (10 mg/kg, p.o.)	275.01 ± 19.57**	4.06 ± 0.32**	1.84 ± 0.16*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.12 Effect of 5-HT$_3$ receptor antagonist QCM-4 on depression co-morbid with obesity in HFD fed mice

5.12.1 Effect of QCM-4 on behavioral models of depression in HFD fed mice

5.12.1.1. Effect of QCM-4 on body weight (g) in HFD fed mice

HFD animals exhibited observable (P<0.01) higher body weight compared to NPD control animals. No significant changes in the body weight was observed with repetitive treatment of **QCM-4** (1 and 2 mg/kg, p.o.) and standard reference antidepressant ESC (10 mg/kg, p.o.) [F (4, 35) = 804.6, P>0.05] in HFD mice compared to HFD control group, as shown in **Fig 5.31**.

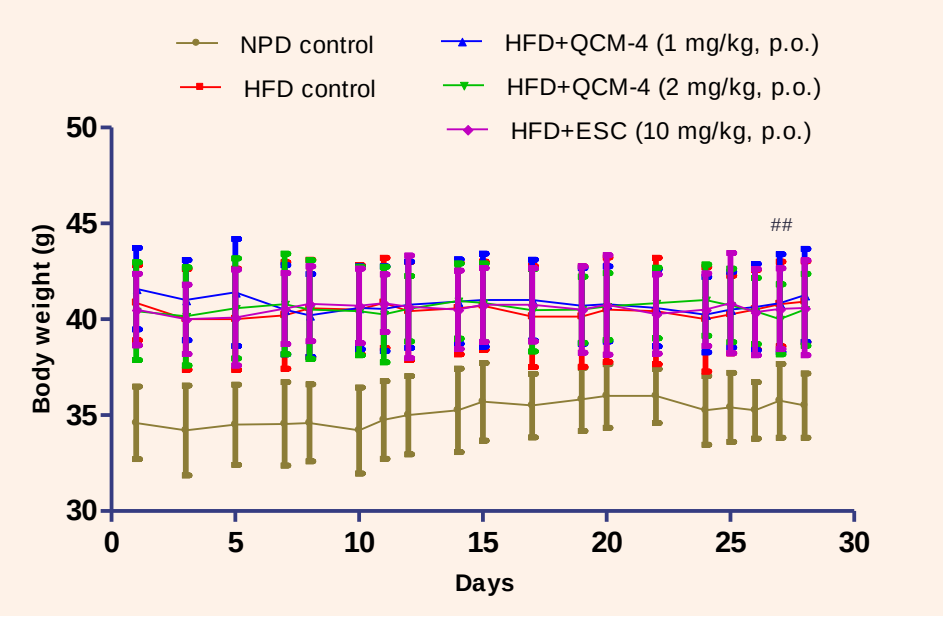


Fig 5.31: Effect of **QCM-4** on body weight of HFD fed mice. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, n=6/group.

5.12.1.2. Effect of QCM-4 on SPT in HFD fed mice

The effect of **QCM-4** on sucrose consumption in SPT is represented in **Fig 5.32**. HFD animals showed remarkable (P<0.01) reduced sucrose consumption compared to NPD control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 7.00, P<0.01] increased the sucrose consumption in HFD mice as compared to HFD control group.

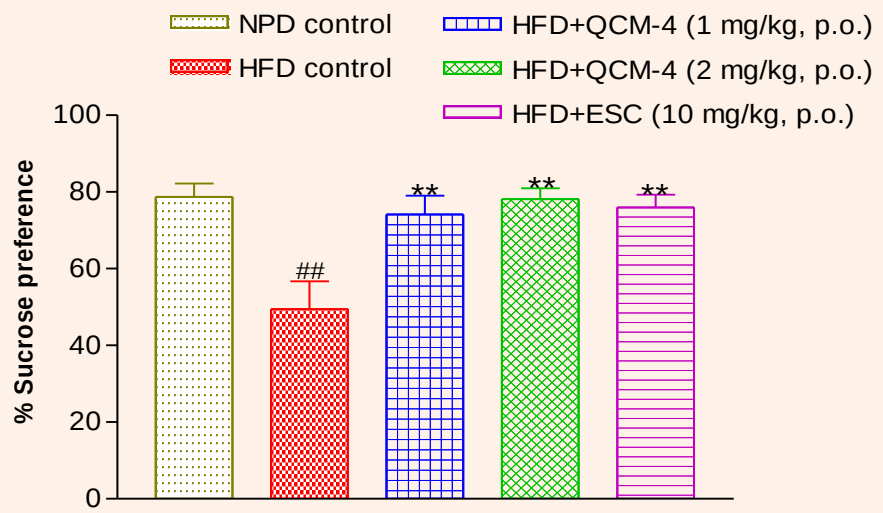


Fig 5.32: Effect of **QCM-4** on SPT in HFD fed mice. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, **P<0.01 vs HFD control group, n=6/group.

5.12.1.3. Effect of QCM-4 on SLA score in HFD fed mice

QCM-4 (1 and 2 mg/kg, p.o.) and standard reference antidepressant ESC (10 mg/kg, p.o.) did not significantly [F (4, 35) = 0.029, P>0.05] alter the basal locomotor activity score in HFD mice as compared to HFD control group, as represented in **Fig 5.33.**

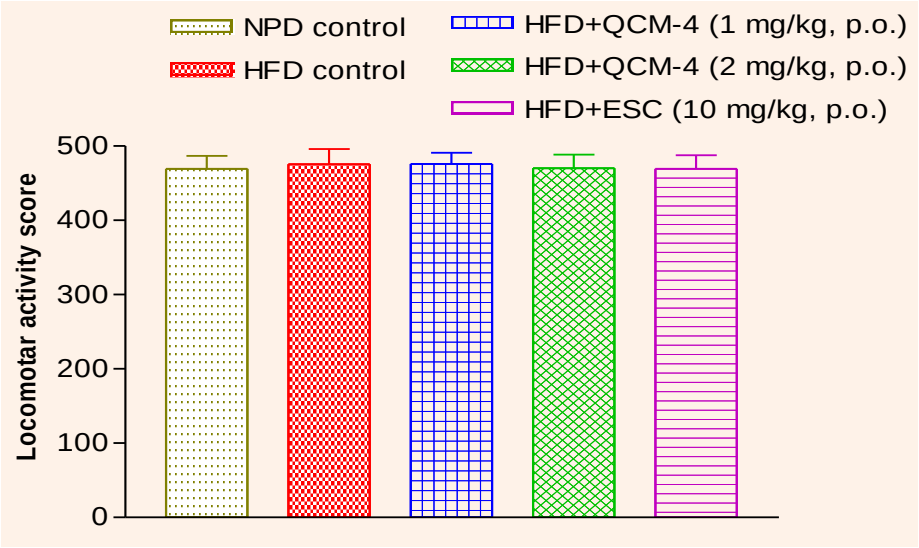


Fig 5.33: Effect of **QCM-4** on SLA score in HFD fed mice. The values are expressed as mean ± S.E.M., n=6/group.

5.12.1.4. Effect of QCM-4 on FST in HFD fed mice

The effect of **QCM-4** on immobility time of HFD fed mice in FST is represented in **Fig 5.34**. HFD control group showed marked (P<0.01) increased immobility time as compared to NPD control group.

Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 18.72, P<0.01] reduced the immobility time in HFD mice as compared to HFD control group.

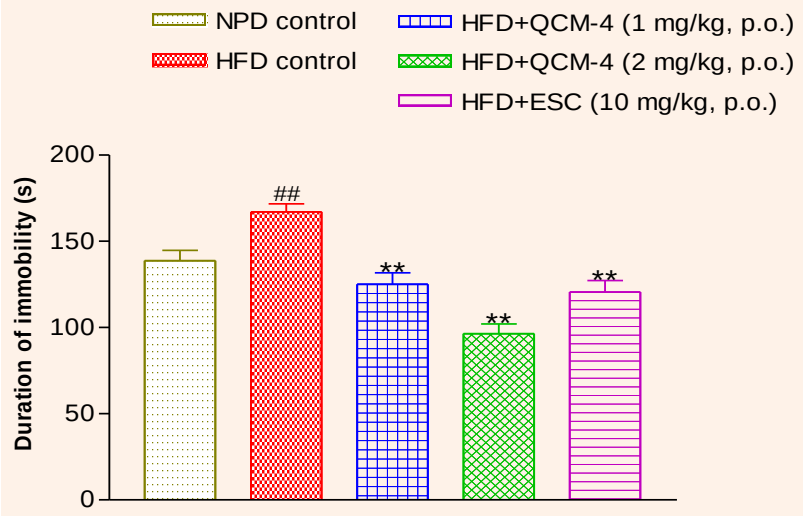


Fig 5.34: Effect of **QCM-4** on duration of immobility in HFD fed mice in FST. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, **P<0.01 vs HFD control group, n=6/group.

5.12.1.5. Effect of QCM-4 on TST in HFD fed mice

HFD control group showed marked (P<0.01) increased immobility time as compared to NPD control in TST (**Fig 5.35**). Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 8.29, P<0.05] decreased the immobility time in HFD mice as compared to HFD control group.

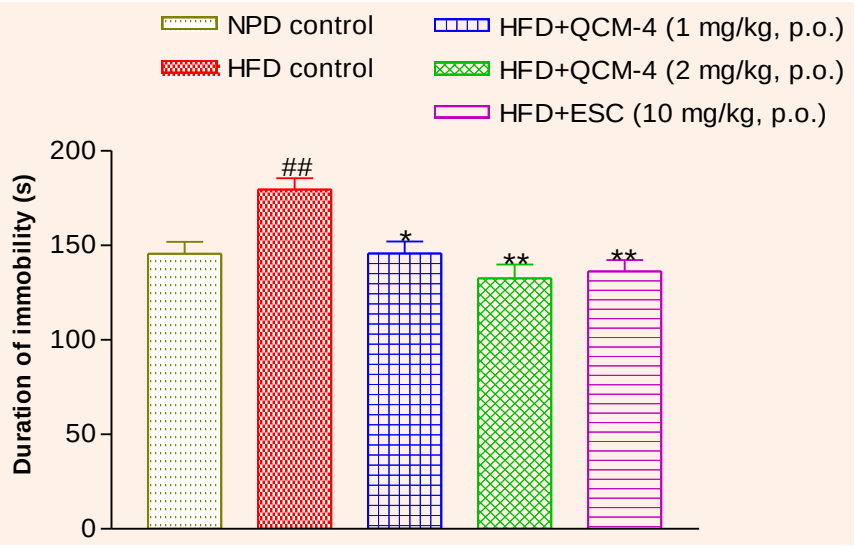


Fig 5.35: Effect of **QCM-4** on duration of immobility in HFD fed mice in TST. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.12.2. Effect of QCM-4 on behavioral models of anxiety in HFD fed mice

5.12.2.1. Effect of QCM-4 on EPM in HFD fed mice

HFD control group exhibited marked (P<0.01) decreased % OAE and % OAT as compared to NPD control group in EPM (**Table 5.27**). Repetitative treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) significantly increased the % OAE [F (4, 35) = 9.70, P<0.05] and % OAT [F (4, 35) = 6.00, P<0.05] in HFD fed animals as compared to HFD control group.

Table 5.27: Effect of QCM-4 on behavior of HFD fed mice in EPM

Groups	% OAE	% OAT (s)
NPD control	33.84 ± 3.33	16.00 ± 1.61
HFD control	13.01 ± 2.78$^{\#\#}$	7.78 ± 1.12$^{\#\#}$
HFD + QCM-4 (1 mg/kg, p.o.)	29.56 ± 4.14*	14.00 ± 1.09*
HFD + QCM-4 (2 mg/kg, p.o.)	44.34 ± 4.94**	15.17 ± 1.45**
HFD + ESC (10 mg/kg, p.o.)	37.11 ± 3.19**	14.33 ± 1.32*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.12.2.2. Effect of QCM-4 on HBT in HFD fed mice

HFD animals showed remarkable decreased number of head dips and crossings (P<0.01) as compared to NPD control group in HBT. Chronic administration of **QCM-4** (1 and 2 mg/kg, p.o.) and antidepressant ESC (10 mg/kg, p.o.) significantly increased the head dips [F (4, 35) = 5.90, P<0.05] and crossing scores [F (4, 35) = 8.04, P<0.05] in HFD mice as compared to HFD control group, as represented in **Table 5.28**.

Table 5.28: Effect of QCM-4 on behavior of HFD fed mice in HBT

Groups	Number of head dips	Number of crossings
NPD control	32.17 ± 3.34	39.17 ± 3.98
HFD control	14.00 ± 2.63$^{\#\#}$	15.67 ± 2.70$^{\#\#}$
HFD + QCM-4 (1 mg/kg, p.o.)	28.67 ± 3.66*	30.83 ± 3.28*
HFD + QCM-4 (2 mg/kg, p.o.)	33.83 ± 3.97**	37.83 ± 3.50**
HFD + ESC (10 mg/kg, p.o.)	31.33 ± 2.75*	34.00 ± 3.04*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.12.2.3. Effect of QCM-4 on L/D test in HFD fed mice

HFD fed mice exhibited marked (P<0.01) reduced time in light chamber and transition score as compared to NPD control group in L/D test is shown in **Table 5.29**. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) significantly improved the time in light chamber [F (4, 35) = 7.08, P<0.05] and transition score [F (4, 35) = 8.78, P<0.05] in HFD mice as compared to HFD control group.

Table 5.29: Effect of QCM-4 on behavior of HFD fed mice in L/D test

Groups	Time in light chamber (s)	Number of transitions
NPD control	25.50 ± 2.40	29.50 ± 3.13
HFD control	8.50 ± 1.82$^{\#\#}$	13.50 ± 1.77$^{\#\#}$
HFD + QCM-4 (1 mg/kg, p.o.)	20.67 ± 2.80*	27.00 ± 2.85*
HFD + QCM-4 (2 mg/kg, p.o.)	25.33 ± 3.20**	34.67 ± 3.28**
HFD + ESC (10 mg/kg, p.o.)	23.00 ± 2.80*	32.33 ± 2.70*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.12.3. Effect of QCM-4 on biochemical estimations in HFD fed mice

5.12.3.1. Effect of QCM-4 on plasma glucose, total cholesterol and triglycerides in HFD fed mice

HFD fed mice exhibited significantly (P<0.01) higher plasma glucose, total cholesterol and triglycerides as compared to NPD control animals (**Table 5.30**). Repetitative treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) significantly decreased the elevated plasma glucose [F (4, 35) = 9.30, P<0.05], total cholesterol [F (4, 35) = 16.50, P<0.01] and triglycerides [F (4, 35) = 7.69, P<0.05] in HFD mice as compared to HFD control group.

Table 5.30: Effect of QCM-4 on plasma glucose, total cholesterol and triglycerides in HFD fed mice

Groups	Plasma glucose (mg/dl)	Plasma total cholesterol (mg/dl)	Plasma triglycerides (mg/dl)
NPD control	93.30 ± 7.14	106.30 ± 6.23	95.23 ± 6.96
HFD control	155.03 ± 6.47$^{\#\#}$	192.64 ± 7.89$^{\#\#}$	151.66 ± 7.68$^{\#\#}$
HFD + QCM-4 (1 mg/kg, p.o.)	121.69 ± 6.92*	138.18 ± 8.52**	117.04 ± 6.83*
HFD + QCM-4 (2 mg/kg, p.o.)	115.29 ± 8.88**	124.35 ± 8.56**	104.93 ± 7.23**
HFD + ESC (10 mg/kg, p.o.)	120.14 ± 6.61*	138.30 ± 8.26**	108.70 ± 9.94**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.12.3.2. Effect of QCM-4 on OGTT in HFD fed mice

The effect of **QCM-4** on OGTT in HFD mice is represented in **Fig 5.36**. HFD animals exhibited marked (P<0.05) elevated blood glucose compared to NPD control group at 30, 60, 90 and 120 min post glucose loading (2 g/kg, p.o.). Chronic dosing with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) reduced the blood glucose at 30, 60, 90 and 120 min post glucose loading, but changes were not significant [F (4, 35) = 2.57, P>0.05] in HFD mice as compared to HFD control group.

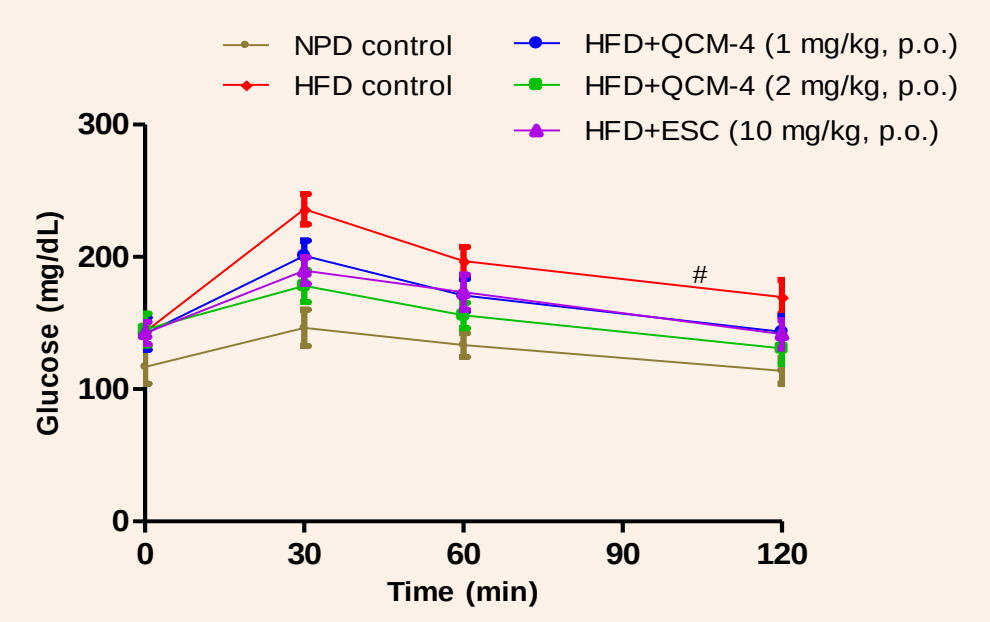


Fig 5.36: Effect of **QCM-4** on OGTT in HFD mice. The values are expressed as mean ± S.E.M., $^{\#}$P<0.05 vs NPD control group, n=6/group.

5.12.3.3. Effect of QCM-4 on brain hippocampal oxidative stress parameters in HFD fed mice

HFD animals exhibited significantly (P<0.01) higher hippocampal lipid peroxidation marker MDA and reduced anti-oxidant enzyme GSH concentrations as compared to NPD control group. Multiple dosing with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly reduced the hippocampus MDA [F (4, 35) = 5.96, P<0.05] and increased GSH [F (4, 35) = 11.28, P<0.05] concentrations in HFD mice as compared to HFD control group, as represented in **Table 5.31**.

Table 5.31: Effect of QCM-4 on brain hippocampus MDA and GSH concentrations in HFD fed mice

Groups	MDA (µg/mg of proteins)	GSH (µg/mg of proteins)
NPD control	1.71 ± 0.19	0.62 ± 0.07
HFD control	4.29 ± 0.60$^{\#\#}$	0.09 ± 0.01$^{\#\#}$
HFD + QCM-4 (1 mg/kg, p.o.)	2.55 ± 0.26*	0.36 ± 0.03*
HFD + QCM-4 (2 mg/kg, p.o.)	2.44 ± 0.30*	0.44 ± 0.07**
HFD + ESC (10 mg/kg, p.o.)	2.49 ± 0.45*	0.37 ± 0.07*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.12.4. Effect of QCM-4 on neuroendocrinological changes: plasma CORT, leptin and insulin in HFD fed mice

The effect of **QCM-4** on plasma CORT, leptin and insulin concentrations in HFD mice is shown in **Table 5.32**. HFD group showed marked (P<0.01) elevated plasma CORT, leptin and insulin concentrations as compared to NPD control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly attenuated plasma CORT [F (4, 35) = 194.2, P<0.01], leptin [F (4, 35) = 103.5, P<0.01] and insulin [F (4, 35) = 26.24, P<0.05] in HFD mice as compared to HFD control group.

Table 5.32: Effect of QCM-4 on plasma CORT, leptin and insulin concentrations in HFD fed mice

Groups	Plasma CORT (ng/ml)	Plasma leptin (pg/ml)	Plasma insulin (ng/ml)
NPD control	120.38 ± 14.19	969.46 ± 58.59	0.543 ± 0.062
HFD control	868.51 ± 23.99$^{\#\#}$	1762.38 ± 101.31$^{\#\#}$	1.754 ± 0.107$^{\#\#}$
HFD + QCM-4 (1 mg/kg, p.o.)	228.26 ± 29.86**	1399.26 ± 76.22**	1.357 ± 0.104*
HFD + QCM-4 (2 mg/kg, p.o.)	184.56 ± 22.28**	1105.28 ± 81.32**	1.150 ± 0.070**
HFD + ESC (10 mg/kg, p.o.)	200.73 ± 17.07**	1222.46 ± 54.29**	1.331 ± 0.081*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.12.5. Effect of QCM-4 on molecular mechanisms: 5-HT, cAMP and BDNF in HFD fed mice

The effect of **QCM-4** on hippocampal 5-HT, cAMP and BDNF in HFD mice is shown in **Table 5.33**. HFD fed animals showed remarkable (P<0.01) reduced 5-HT, cAMP and BDNF levels compared to NPD control group. Multiple dosing with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) significantly increased the hippocampus 5-HT [F (4, 35) = 29.53, P<0.01], cAMP [F (4, 35) = 8.54, P<0.05] and BDNF [F (4, 35) = 27.38, P<0.05] concentrations in HFD fed mice as compared to HFD control group.

Table 5.33: Effect of QCM-4 on brain hippocampus 5-HT, cAMP and BDNF concentrations in HFD fed mice

Groups	5-HT (ng/g)	cAMP (pmol/mg of proteins)	BDNF (ng/mg of proteins)
NPD control	291.28 ± 17.46	5.79 ± 0.22	3.76 ± 0.32
HFD control	102.68 ± 8.17$^{\#\#}$	2.50 ± 0.14$^{\#\#}$	0.53 ± 0.08$^{\#\#}$
HFD + QCM-4 (1 mg/kg, p.o.)	211.14 ± 11.83**	4.27 ± 0.41*	1.54 ± 0.07*
HFD + QCM-4 (2 mg/kg, p.o.)	281.67 ± 15.39**	4.72 ± 0.57**	2.32 ± 0.31**
HFD + ESC (10 mg/kg, p.o.)	270.35 ± 17.13**	4.36 ± 0.50*	1.85 ± 0.19**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.13. Effect of 5-HT_3 receptor antagonist 4a on depression co-morbid with obesity in HFD fed mice

5.13.1 Effect of 5-HT_3 receptor antagonist 4a on behavioral models of depression in HFD fed mice

5.13.1.1. Effect of 4a on body weight (g) in HFD fed mice

Body weight in HFD animals was remarkably (P<0.01) higher as compared to NPD control mice (**Fig 5.37**). Multiple dosing with **4a** (2 and 4 mg/kg, p.o.) and standard reference drug ESC (10 mg/kg, p.o.) does not significantly [F (4, 35) = 1869, P>0.05] changed the body weight in HFD mice as compared to HFD control group.

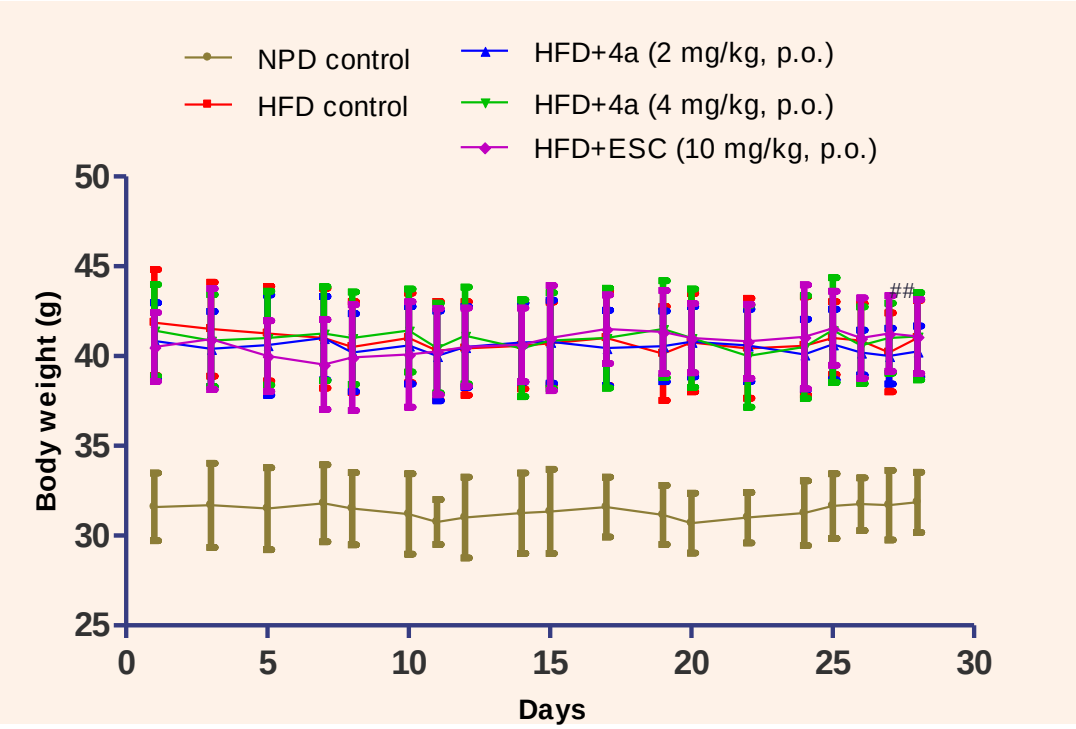


Fig 5.37: Effect of **4a** on body weight of HFD fed mice. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, n=6/group.

5.13.1.2. Effect of 4a on SPT in HFD fed mice

The effect of **4a** on sucrose consumption in SPT is represented in **Fig 5.38**. HFD animals consumed marked (P<0.01) reduced sucrose solution as compared to NPD control animals. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 8.39, P<0.05] improved the sucrose solution consumption in HFD fed mice as compared to HFD control group.

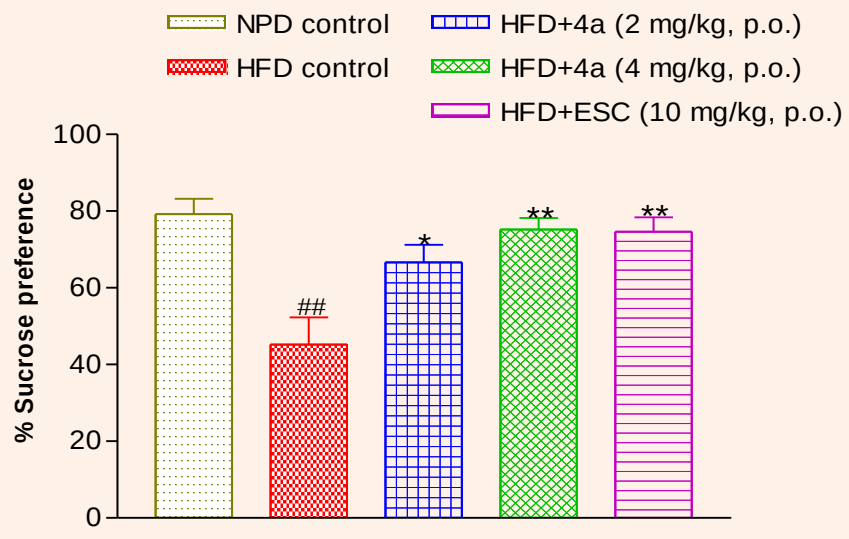


Fig 5.38: Effect of **4a** on SPT in HFD fed mice. The values are expressed as mean ± S.E.M., $^{##}$P<0.01 vs NPD control group, *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.13.1.3. Effect of 4a on SLA score in HFD fed mice

The effect of **4a** on locomotor activity score of HFD fed mice is represented in **Fig 5.39**. Chronic administration of **4a** (2 and 4 mg/kg, p.o.) and standard ESC (10 mg/kg, p.o.) did not significantly [F (4, 35) = 0.009, P>0.05] changed the basal locomotor activity score in HFD fed animals as compared to HFD control group.

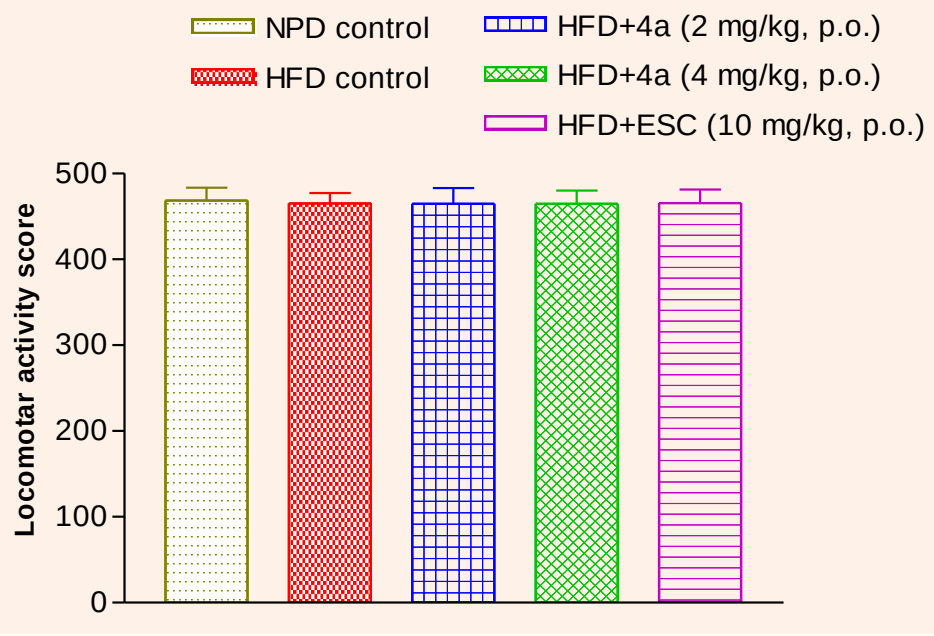


Fig 5.39: Effect of **4a** on SLA score in HFD fed mice. The values are expressed as mean ± S.E.M., n=6/group.

5.13.1.4. Effect of 4a on FST in HFD fed mice

HFD control group showed marked (P<0.05) increased immobility time as compared to NPD control group. Multiple dosing with **4a** (2 and 4 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 13.24, P<0.05] reduced the immobility time in HFD fed animals as compared to HFD control group (**Fig 5.40**).

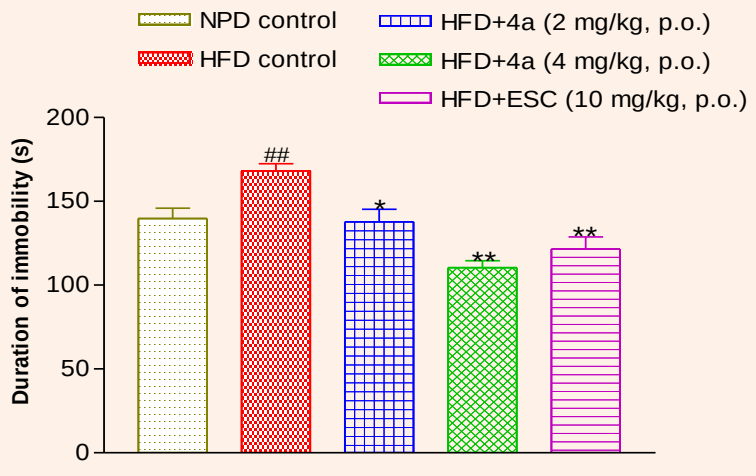


Fig 5.40: Effect of **4a** on duration of immobility in HFD fed mice in FST. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.13.1.5. Effect of 4a on TST in HFD fed mice

HFD mice showed remarkably (P<0.01) higher immobility time in TST as compared to NPD control group (**Fig 5.41)**. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and standard drug ESC (10 mg/kg, p.o.) significantly [F (4, 35) = 10.27, P<0.01] reduced the immobility time in HFD mice as compared to HFD control group.

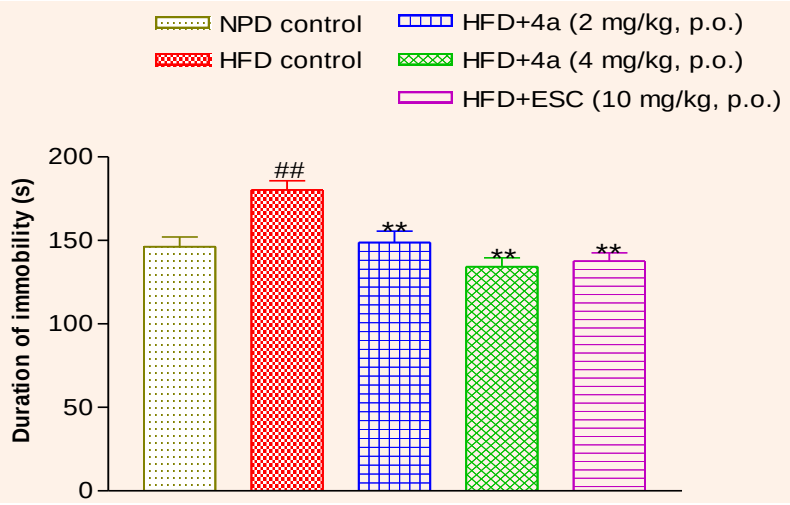


Fig 5.41: Effect of **4a** on duration of immobility in HFD fed mice in TST. The values are expressed as mean ± S.E.M., ##P<0.01 vs NPD control group, **P<0.01 vs HFD control group, n=6/group.

5.13.2. Effect of 4a on behavioral models of anxiety in HFD fed mice

5.13.2.1. Effect of 4a on EPM in HFD fed mice

HFD control group exhibited remarkable (P<0.05) reduced % OAE and % OAT in EPM as compared to NPD control group. Multiple dosing with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly improved the % OAE [F (4, 35) = 6.41, P<0.05] and % OAT [F (4, 35) = 6.06, P<0.05] in HFD mice as compared to HFD control group, as represented in **Table 5.34**.

Table 5.34: Effect of 4a on behavior of HFD fed mice in EPM test

Groups	% OAE	% OAT (s)
NPD control	29.57 ± 3.24	14.67 ± 1.68
HFD control	12.13 ± 2.63$^{\#}$	7.44 ± 1.12$^{\#\#}$
HFD + 4a (2 mg/kg, p.o.)	23.02 ± 3.27*	14.11 ± 1.22*
HFD + 4a (4 mg/kg, p.o.)	39.51 ± 5.94**	15.56 ± 1.38**
HFD + ESC (10 mg/kg, p.o.)	37.65 ± 3.89**	14.67 ± 1.24**

The values are expressed as mean ± S.E.M., $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.13.2.2. Effect of 4a on HBT in HFD fed mice

HFD mice exhibited remarkably (P<0.05) decreased number of head dips and crossings as compared to NPD control group in HBT. Chronic dosing with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the head dips [F (4, 35) = 7.23, P<0.05] and crossing scores [F (4, 35) = 6.81, P<0.05] in HFD fed animals as compared to HFD control group (**Table 5.35**)

Table 5.35: Effect of 4a on behavior of HFD fed mice in HBT

Groups	Number of head dips	Number of crossings
NPD control	33.00 ± 3.76	38.67 ± 3.99
HFD control	13.00 ± 2.59$^{\#\#}$	15.00 ± 2.78$^{\#\#}$
HFD + 4a (2 mg/kg, p.o.)	28.00 ± 3.16*	30.33 ± 3.29*
HFD + 4a (4 mg/kg, p.o.)	34.33 ± 3.31**	34.00 ± 3.89**
HFD + ESC (10 mg/kg, p.o.)	30.33 ± 3.27**	32.67 ± 3.12*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.13.2.3. Effect of 4a on L/D test in HFD fed mice

The effect of **4a** on L/D test in HFD mice is represented in **Table 5.36**. HFD mice showed marked (P<0.01) reduced time in light chamber and transition score as compared to NPD control group. Chronic dosing with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the time spend in light chamber [F (4, 35) = 5.62, P<0.05] and transition score [F (4, 35) = 6.58, P<0.05] in HFD fed mice as compared to HFD control group.

Table 5.36: Effect of 4a on behavior of HFD fed mice in L/D test

Groups	Time in light chamber (s)	Number of transitions
NPD control	26.00 ± 2.35	30.83 ± 2.98
HFD control	7.67 ± 1.80$^{\#\#}$	13.50 ± 1.77$^{\#\#}$
HFD + 4a (2 mg/kg, p.o.)	21.50 ± 3.21*	28.33 ± 3.32*
HFD + 4a (4 mg/kg, p.o.)	24.33 ± 3.66**	33.50 ± 3.91**
HFD + ESC (10 mg/kg, p.o.)	23.17 ± 3.13*	31.17 ± 3.24**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.13.3. Effect of 4a on biochemical estimations in HFD fed mice

5.13.3.1. Effect of 4a on plasma glucose, total cholesterol and triglycerides in HFD fed mice

HFD fed mice showed marked (P<0.01) higher plasma glucose, total cholesterol and triglycerides as compared to NPD control group. Chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly reduced the abnormally elevated plasma glucose [F (4, 35) = 12.62, P<0.05], total cholesterol [F (4, 35) = 28.27, P<0.01] and triglycerides [F (4, 35) = 9.30, P<0.05] in HFD fed mice as compared to HFD control group, as shown in **Table 5.37**.

Table 5.37: Effect of 4a on plasma glucose, total cholesterol and triglycerides in HFD fed mice

Groups	Plasma glucose (mg/dl)	Plasma total cholesterol (mg/dl)	Plasma triglycerides (mg/dl)
NPD control	94.60 ± 7.29	105.58 ± 6.72	95.23 ± 6.96
HFD control	156.59 ± 5.37$^{\#\#}$	195.47 ± 4.91$^{\#\#}$	151.66 ± 7.68$^{\#\#}$
HFD + 4a (2 mg/kg, p.o.)	128.50 ± 5.88*	141.32 ± 7.64**	117.04 ± 6.83*
HFD + 4a (4 mg/kg, p.o.)	119.10 ± 6.16**	125.62 ± 4.32**	104.93 ± 7.23**
HFD + ESC (10 mg/kg, p.o.)	123.31 ± 6.37*	140.26 ± 7.13**	108.70 ± 9.94**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,

*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.13.3.2. Effect of 4a on OGTT in HFD fed mice

The effect of **4a** on OGTT in HFD fed mice is represented in **Fig 5.42**. HFD mice exhibited observable (P<0.05) higher blood glucose compared to NPD control group at 30, 60, 90 and 120 min post glucose loading (2 g/kg, p.o.). Chronic dosing with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) reduced the blood glucose at 30, 60, 90 and 120 min post glucose loading in HFD fed animals compared to as HFD control group, but changes were not significant [F (4, 35) = 2.55, P>0.05].

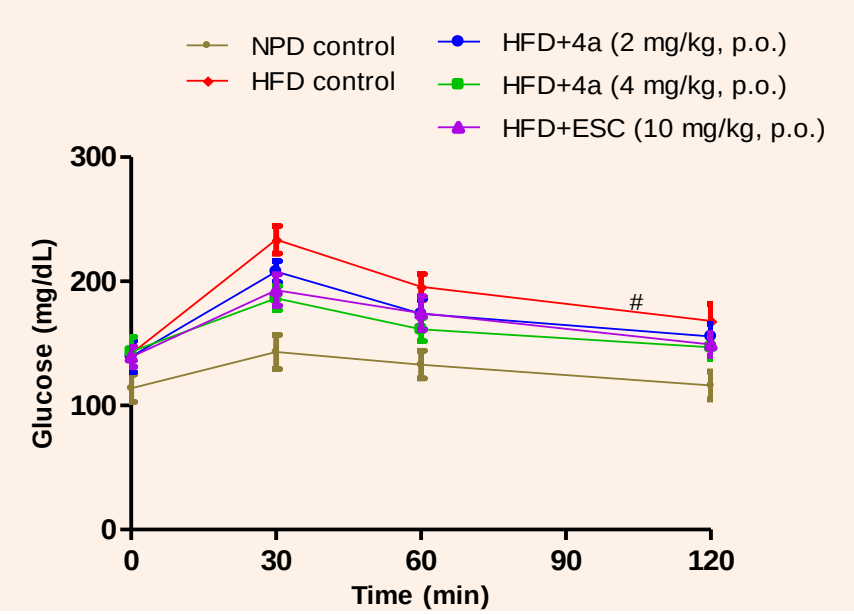


Fig 5.42: Effect of **4a** on OGTT in HFD mice. The values are expressed as mean ± S.E.M., $^{\#}$P<0.05 vs NPD control group, n=6/group.

5.13.3.3. Effect of 4a on brain hippocampus oxidative stress parameters in HFD fed mice

HFD fed animals showed marked (P<0.01) elevated hippocampus lipid peroxidation marker MDA and decreased anti-oxidant enzyme GSH concentrations as compared to NPD control group. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) significantly reduced the abnormally elevated MDA [F (4, 35) = 6.81, P<0.05] and improved GSH [F (4, 35) = 9.22, P<0.05] hippocampal concentrations in HFD fed animals as compared to HFD control group (**Table 5.38)**.

Table 5.38: Effect of 4a on brain hippocampal MDA and GSH concentrations in HFD fed mice

Groups	MDA (µg/mg of proteins)	GSH (µg/mg of proteins)
NPD control	1.77 ± 0.32	0.64 ± 0.10
HFD control	4.65 ± 0.86$^{\#\#}$	0.10 ± 0.03$^{\#\#}$
HFD + 4a (2 mg/kg, p.o.)	2.41 ± 0.30*	0.39 ± 0.07*
HFD + 4a (4 mg/kg, p.o.)	1.90 ± 0.14**	0.43 ± 0.05*
HFD + ESC (10 mg/kg, p.o.)	2.08 ± 0.32**	0.41 ± 0.04*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,
*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.13.4. Effect of 4a on neuroendocrinological changes: plasma CORT, leptin and insulin in HFD fed mice

The effect of **4a** on plasma CORT, leptin and insulin concentrations in HFD fed animals is shown in **Table 5.39**. HFD fed mice exhibited remarkably (P<0.01) elevated plasma CORT, leptin and insulin concentrations as compared to NPD control group. Multiple dosing with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly inhibited elevated plasma CORT [F (4, 35) = 212.8, P<0.01], leptin [F (4, 35) = 60.61, P<0.05] and insulin [F (4, 35) = 19.88, P<0.05] in HFD fed mice as compared to HFD control group.

Table 5.39: Effect of 4a on plasma CORT, leptin and insulin concentrations in HFD fed mice

Groups	Plasma CORT (ng/ml)	Plasma leptin (pg/ml)	Plasma insulin (ng/ml)
NPD control	121.44 ± 14.27	971.96 ± 33.19	0.569 ± 0.077
HFD control	869.57 ± 23.65$^{\#\#}$	1730.92 ± 85.84$^{\#\#}$	1.710 ± 0.136$^{\#\#}$
HFD + 4a (2 mg/kg, p.o.)	239.68 ± 26.94**	1485.02 ± 121.85*	1.303 ± 0.063*
HFD + 4a (4 mg/kg, p.o.)	189.54 ± 22.02**	1151.90 ± 81.46**	1.271 ± 0.090*
HFD + ESC (10 mg/kg, p.o.)	198.61 ± 16.00**	1169.78± 132.62**	1.291 ± 0.077*

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,

*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.13.5. Effect of 4a on molecular mechanisms: 5-HT, cAMP and BDNF in HFD fed mice

The effect of **4a** on hippocampal 5-HT, cAMP and BDNF concentrations in HFD mice is represented in **Table 5.40**. HFD mice showed marked (P<0.01) decreased 5-HT, cAMP and BDNF levels as compared to NPD group. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the hippocampus 5-HT [F (4, 35) = 22.00, P<0.01], cAMP [F (4, 35) = 16.67, P<0.05] and BDNF [F (4, 35) = 49.23, P<0.05] concentrations in HFD fed animals as compared to HFD control group.

Table 5.40: Effect of 4a on brain hippocampal 5-HT, cAMP and BDNF concentrations in HFD fed mice

Groups	5-HT (ng/g)	cAMP (pmol/mg of proteins)	BDNF (ng/mg of proteins)
NPD control	293.81 ± 22.15	5.80 ± 0.20	3.69 ± 0.28
HFD control	97.39 ± 15.45$^{\#\#}$	2.60 ± 0.17$^{\#\#}$	0.54 ± 0.06$^{\#\#}$
HFD + 4a (2 mg/kg, p.o.)	199.88 ± 17.72**	3.84 ± 0.12*	1.28 ± 0.09*
HFD + 4a (4 mg/kg, p.o.)	271.94 ± 15.28**	4.17 ± 0.14**	1.82 ± 0.09**
HFD + ESC (10 mg/kg, p.o.)	268.50 ± 13.63**	4.26 ± 0.53**	1.89 ± 0.18**

The values are expressed as mean ± S.E.M., $^{\#\#}$P<0.01 vs NPD control,

*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.14. Effect of 5-HT_3 receptor antagonist OND on depression co-morbid with obesity in HFD fed mice subjected to CUMS

5.14.1. Effect of OND on behavioral models of depression and anxiety in HFD fed mice subjected to CUMS

5.14.1.1. Effect of OND on body weight (g) of HFD fed mice subjected to CUMS

The effect of **OND** treatment on body weight of HFD fed mice subjected to CUMS is shown in **Table 5.41**. HFD control group showed marked (P<0.01) higher body weight as compared to NPD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed no significant alterations in the initial [F (9, 50) = 0.287, P>0.05] as well as final [F (9, 50) = 3.120, P>0.05] body weight of HFD fed mice exposed to CUMS as compared to HFD+CUMS control group.

Table 41: Effect of OND on body weight of HFD fed mice subjected to CUMS

Groups	Initial body weight (g)	Final body weight (g)
NPD control	26.83 ± 1.56	28.33 ± 1.54
NPD + CUMS control	26.00 ± 1.39	28.00 ± 1.48
NPD + CUMS + OND (0.5 mg/kg, p.o.)	26.50 ± 1.61	28.17 ± 1.25
NPD + CUMS + OND (1 mg/kg, p.o.)	26.33 ± 1.38	27.50 ± 1.28
NPD + CUMS + ESC (10 mg/kg, p.o.)	27.00 ± 1.91	28.50 ± 1.15
HFD control	39.00 ± 2.31^{aa}	39.83 ± 3.32^{aa}
HFD + CUMS control	38.83 ± 2.15	34.00 ± 2.02
HFD + CUMS + OND (0.5 mg/kg, p.o.)	39.17 ± 1.89	37.33 ± 3.80
HFD + CUMS + OND (1 mg/kg, p.o.)	39.00 ± 2.45	37.50 ± 2.67
HFD + CUMS + ESC (10 mg/kg, p.o.)	39.50 ± 2.80	38.00 ± 1.91

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, n = 6/group.

5.14.1.2. Effect of OND on SPT in HFD fed mice subjected to CUMS

HFD control animals showed marked (P<0.01) reduced sucrose consumption as compared to NPD control group. HFD+CUMS control animals showed observable (P<0.01) decreased sucrose consumption compared to HFD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (9, 50) = 91.20, P<0.01] increased the sucrose solution consumption in HFD+CUMS mice as compared to HFD+CUMS control group, as represented in **Fig 5.43**.

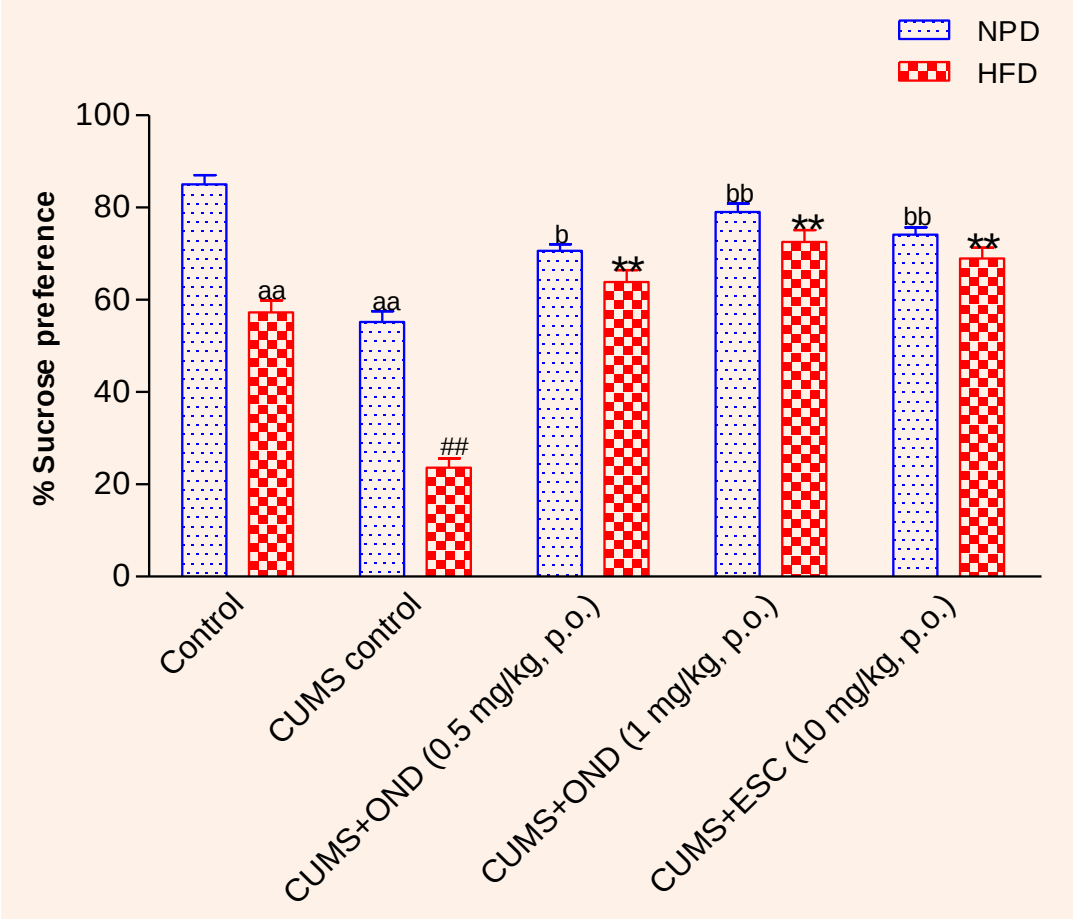


Fig 5.43: Effect of **OND** on SPT in HFD fed mice subjected to CUMS. The values are expressed as mean ± S.E.M., [aa]P<0.01 vs NPD control, [bb]P<0.01 vs NPD+CUMS control, [##]P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n=6/group.

5.14.1.3. Effect of OND on SLA score of HFD fed mice subjected to CUMS

The effect of **OND** treatment on locomotor activity score in HFD fed mice subjected to CUMS is shown in **Fig 5.44**. Locomotor activity score was not changed significantly (P>0.05) in HFD control as compared to NPD control group, and HFD+CUMS control as compared to HFD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed no significant [F (9, 50) = 2.31, P>0.05] alteration in the locomotor activity score in HFD fed mice exposed to CUMS as compared to HFD+CUMS control group.

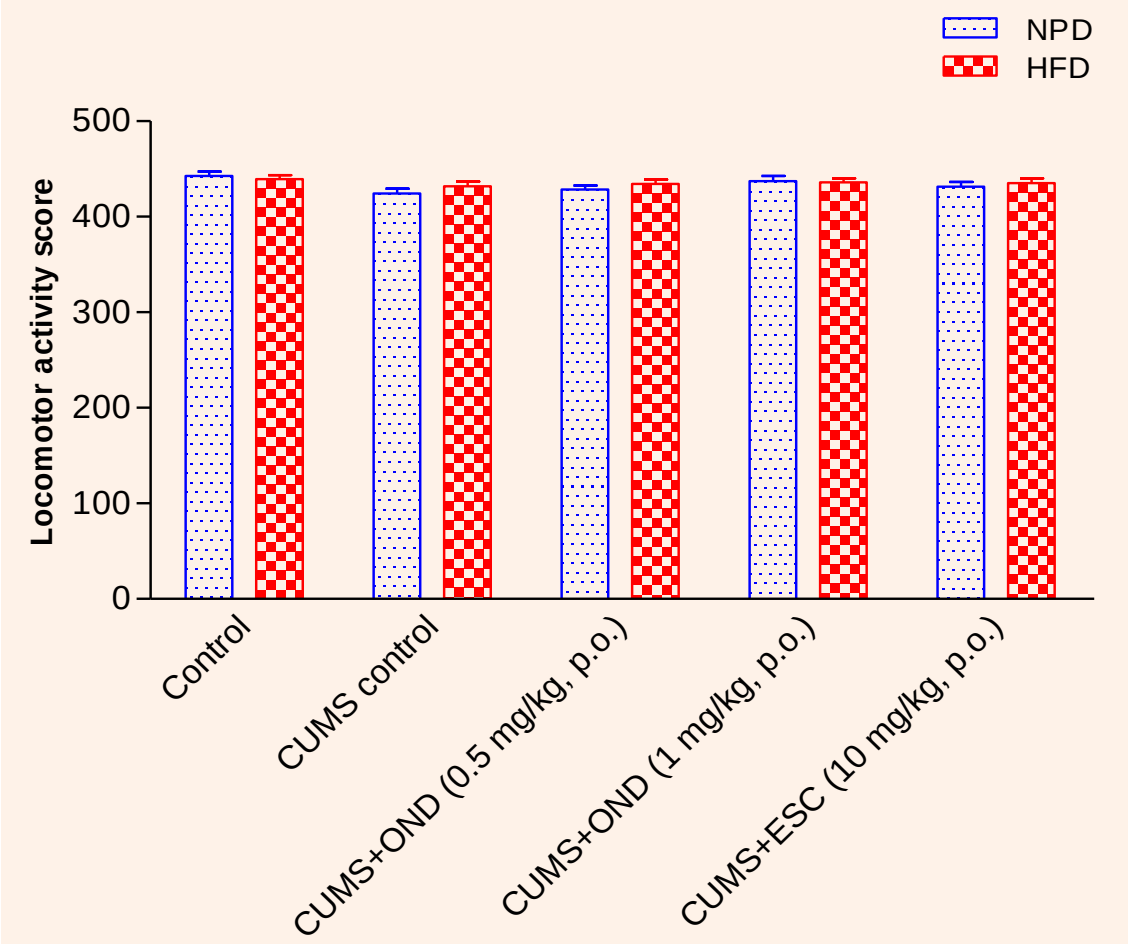


Fig 5.44: Effect of **OND** on SLA score in HFD fed mice subjected to CUMS. The values are expressed as mean ± S.E.M., n=6/group.

5.14.1.4. Effect of OND on FST in HFD fed mice subjected to CUMS

The effect of **OND** on immobility time in FST of HFD+CUMS mice is represented in **Fig 5.45**. HFD control group showed observable ($P<0.05$) higher duration of immobility as compared to NPD control group. HFD+CUMS control group exhibited significantly ($P<0.01$) increased immobility time s compared to HFD control group. Repetitive treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in HFD fed CUMS exposed mice significantly [$F(9, 50) = 206.1$, $P<0.01$] reduced the immobility time as compared to HFD+CUMS control group in FST.

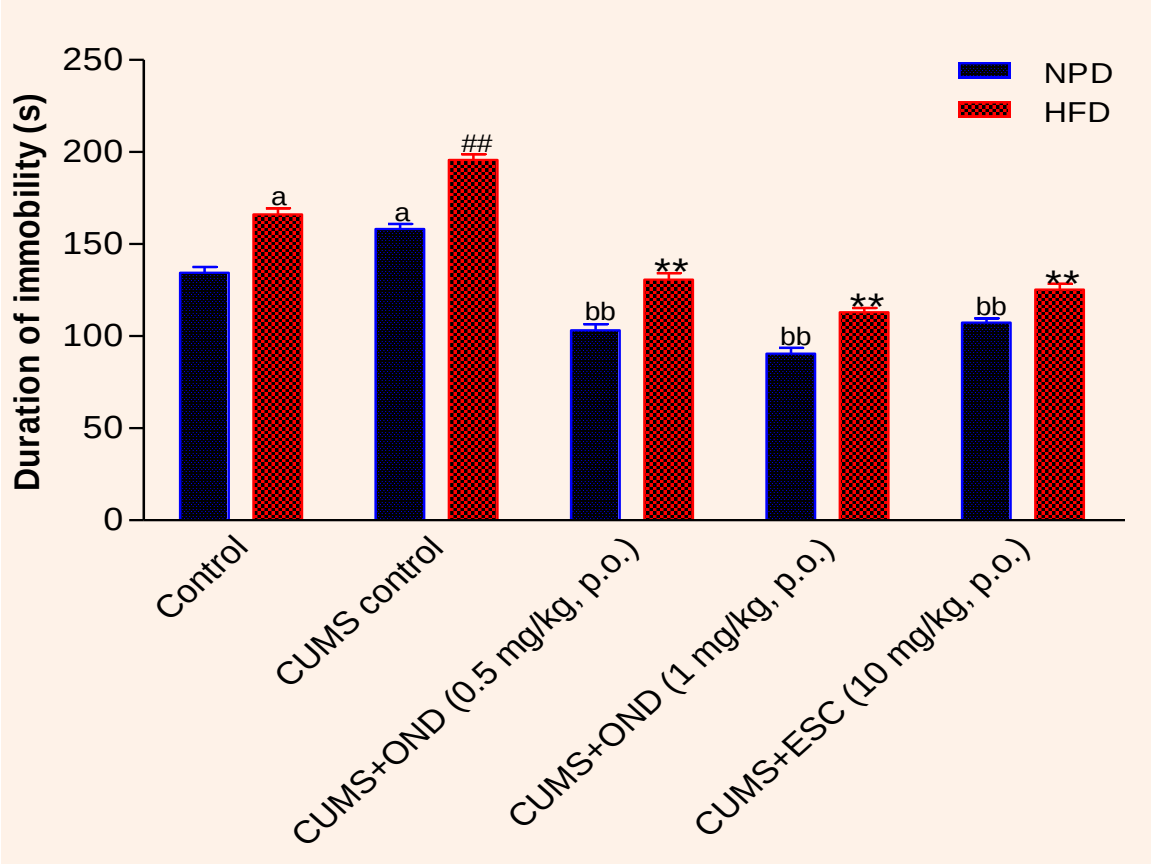


Fig 5.45: Effect of **OND** on duration of immobility in HFD fed mice subjected to CUMS in FST. The values are expressed as mean ± S.E.M., [a]P<0.05 vs NPD control, [bb]P<0.01 vs NPD+CUMS control, [##]P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n=6/group.

5.14.1.5. Effect of OND on TST in HFD fed mice subjected to CUMS

HFD control group showed observable (P<0.05) higher immobility time compared to NPD control group. HFD+CUMS control group showed marked (P<0.01) increased immobility time as compared to HFD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) in HFD+CUMS mice significantly [F (9, 50) = 192.6, P<0.01] reduced the immobility time as compared to HFD+CUMS control group in TST, as represented in **Fig 5.46**.

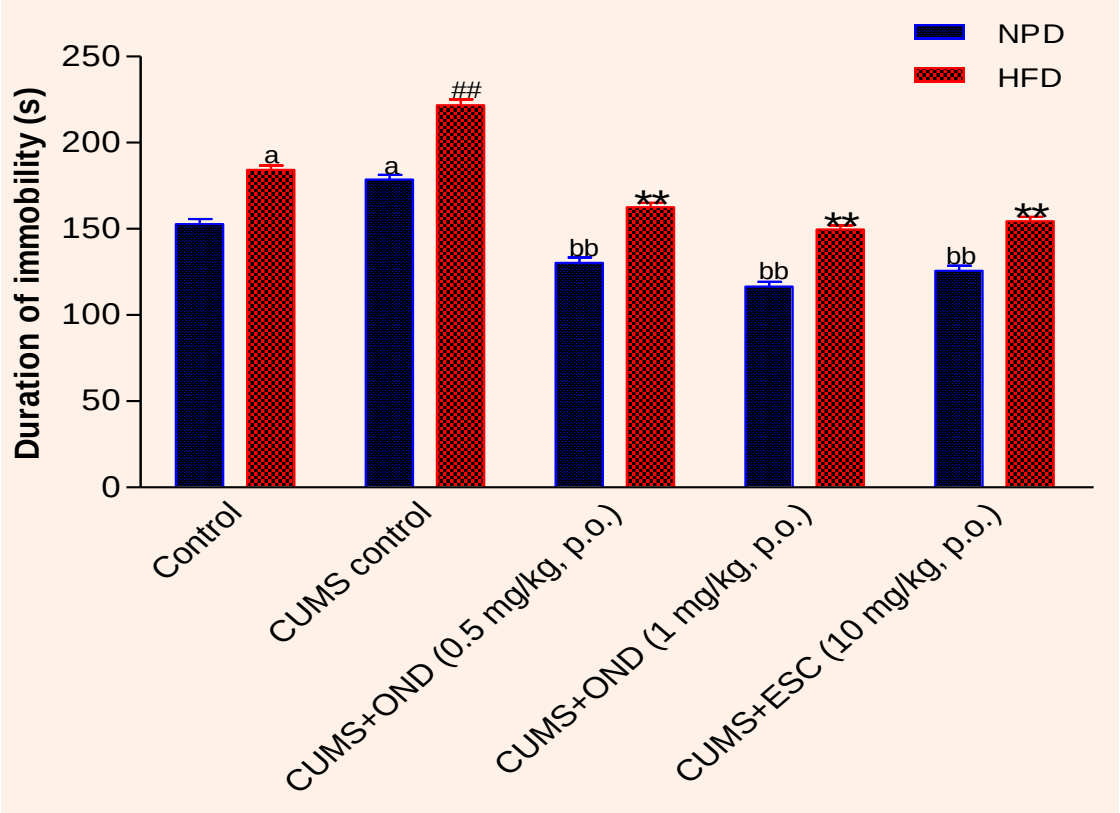


Fig 5.46: Effect of **OND** on duration of immobility in HFD fed mice subjected to CUMS in TST. The values are expressed as mean ± S.E.M., [a]P<0.05 vs NPD control, [bb]P<0.01 vs NPD+CUMS control, [##]P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n=6/group.

5.14.1.6. Effect of OND on EPM in HFD fed mice subjected to CUMS

HFD and HFD+CUMS control groups showed remarkably (P<0.05) reduced % OAE and % OAT as compared to NPD and HFD control groups, respectively in EPM (**Table 5.42)**. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the % OAE [F (9, 50) = 51.78, P<0.01] and % OAT [F (9, 50) = 74.36, P<0.01] in HFD fed mice exposed to CUMS as compared to HFD+CUMS control group.

5.14.1.7. Effect of OND on L/D test in HFD fed mice subjected to CUMS

HFD and HFD+CUMS control groups showed marked (P<0.05) reduced time in light chamber and number of transitions compared to NPD and HFD control groups, respectively in L/D test. Repetitive dosing with **OND** (0.5 and 1 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) significantly increased [F (9, 50) = 127.2, P<0.01] the time in light chamber and transition score [F (9, 50) = 79.04, P<0.01] in HFD fed mice subjected to CUMS as compared to HFD+CUMS control group (**Table 5.43**).

Table 5.42: Effect of OND on behavior of HFD fed mice subjected to CUMS in EPM

Groups	% OAE	% OAT (s)
NPD control	40.42 ± 8.96	15.72 ± 1.85
NPD + CUMS control	14.39 ± 2.81^{aa}	7.83 ± 1.25^{aa}
NPD + CUMS + OND (0.5 mg/kg, p.o.)	32.32 ± 6.05^{bb}	14.61 ± 1.70^{bb}
NPD + CUMS + OND (1 mg/kg, p.o.)	49.58 ± 6.57^{bb}	17.33 ± 1.56^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	41.11 ± 4.31^{bb}	15.72 ± 1.52^{bb}
HFD control	16.17 ± 3.95^{aa}	8.11 ± 1.23^{a}
HFD + CUMS control	6.74 ± 2.57$^{\#\#}$	4.06 ± 1.20$^{\#\#}$
HFD + CUMS + OND (0.5 mg/kg, p.o.)	21.75 ± 4.74**	10.33 ± 1.05**
HFD + CUMS + OND (1 mg/kg, p.o.)	33.61 ± 6.62**	13.50 ± 1.46**
HFD + CUMS + ESC (10 mg/kg, p.o.)	24.94 ± 4.99**	12.22 ± 1.53**

The values are expressed as mean ± S.E.M., ^{a}P<0.05; ^{aa}P<0.01 vs NPD control,
^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control,
**P<0.01vs HFD+CUMS control group, n = 6/group.

Table 5.43: Effect of OND on behavior of HFD fed mice subjected to CUMS in L/D test

Groups	Time in light chamber (s)	Number of transitions
NPD control	31.50 ± 3.66	25.33 ± 3.41
NPD + CUMS control	15.67 ± 2.59^{aa}	12.00 ± 2.39^{aa}
NPD + CUMS + OND (0.5 mg/kg, p.o.)	36.33 ± 3.04^{bb}	30.50 ± 4.04^{bb}
NPD + CUMS + OND (1 mg/kg, p.o.)	49.33 ± 4.01^{bb}	39.33 ± 3.28^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	42.67 ± 4.13^{bb}	36.67 ± 4.16^{bb}
HFD control	13.33 ± 3.01^{aa}	11.17 ± 3.19^{aa}
HFD + CUMS control	6.83 ± 2.52$^{\#\#}$	5.50 ± 2.05$^{\#\#}$
HFD + CUMS + OND (0.5 mg/kg, p.o.)	21.50 ± 3.68**	15.33 ± 2.76**
HFD + CUMS + OND (1 mg/kg, p.o.)	30.67 ± 3.46**	20.67 ± 3.53**
HFD + CUMS + ESC (10 mg/kg, p.o.)	24.00 ± 3.19**	17.00 ± 2.91**

The values are expressed as mean ± S.E.M., ^{a}P<0.05; ^{aa}P<0.01 vs NPD control,
^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control,
**P<0.01vs HFD+CUMS control group, n = 6/group.

5.14.2. Effect of OND on biochemical estimations in HFD fed mice subjected to CUMS

5.14.2.1. Effect of OND on plasma glucose, total cholesterol and triglycerides in HFD fed mice subjected to CUMS

HFD fed mice subjected to CUMS exhibited marked (P<0.01) increased plasma glucose concentration as compared to HFD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly reduced the abnormally elevated plasma glucose [F (9, 50) = 21.89, P<0.05], total cholesterol [F (9, 50) = 26.94, P<0.05] and triglycerides [F (9, 50) = 9.31, P<0.05] in HFD fed mice exposed to CUMS as compared to HFD+CUMS control group, as shown in **Table 5.44**.

Table 5.44: Effect of OND on plasma glucose, total cholesterol and triglycerides in HFD fed mice subjected to CUMS

Groups	Plasma glucose (mg/dl)	Plasma total cholesterol (mg/dl)	Plasma triglycerides (mg/dl)
NPD control	96.98 ± 5.94	107.12 ± 9.40	95.68 ± 8.30
NPD + CUMS control	98.66 ± 6.70	104.92 ± 10.24	96.91 ± 7.65
NPD + CUMS + OND (0.5 mg/kg, p.o.)	97.44 ± 5.69	106.89 ± 7.76	97.84 ± 6.70
NPD + CUMS + OND (1 mg/kg, p.o.)	96.63 ± 6.94	105.38 ± 10.21	97.36 ± 6.73
NPD + CUMS + ESC (10 mg/kg, p.o.)	97.61 ± 5.47	106.57 ± 8.52	97.12 ± 6.51
HFD control	145.74 ± 6.33^{aa}	192.28 ± 9.82^{aa}	153.80 ± 9.19^{aa}
HFD + CUMS control	166.04 ± 7.36$^{\#}$	186.84 ± 10.42	148.55 ± 9.08
HFD + CUMS + OND (0.5 mg/kg, p.o.)	132.77 ± 6.43*	144.45 ± 9.94*	128.52 ± 9.49*
HFD + CUMS + OND (1 mg/kg, p.o.)	123.92 ± 3.99**	127.44 ± 11.50**	119.64 ± 8.84**
HFD + CUMS + ESC (10 mg/kg, p.o.)	134.97 ± 6.70*	136.70 ± 11.49**	126.23 ± 7.98*

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, $^{\#}$P<0.05 vs HFD control, *P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

5.14.2.2. Effect of OND on brain hippocampus oxidative stress parameters in HFD fed mice subjected to CUMS

HFD and HFD+CUMS control groups exhibited remarkably (P<0.01) increased hippocampal lipid peroxidation marker MDA and reduced anti-oxidant enzyme GSH concentrations as compared to NPD and HFD control groups, respectively (**Table 5.45)**. Repetitive treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly reduced the brain hippocampal MDA [F (9, 50) = 130.8, P<0.01] and improved GSH [F (9, 50) = 59.83, P<0.01] concentrations in HFD animals subjected to CUMS as compared to HFD+CUMS control group.

Table 5.45: Effect of OND on brain hippocampus MDA and GSH concentrations in HFD fed mice subjected to CUMS

Groups	MDA (µg/mg protein)	GSH (µg/mg protein)
NPD control	1.45 ± 0.23	0.73 ± 0.06
NPD + CUMS control	2.49 ± 0.31^{aa}	0.21 ± 0.03^{aa}
NPD + CUMS + OND (0.5 mg/kg, p.o.)	1.31 ± 0.27^{bb}	0.53 ± 0.05^{b}
NPD + CUMS + OND (1 mg/kg, p.o.)	1.24 ± 0.16^{bb}	0.66 ± 0.13^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	1.27 ± 0.27^{bb}	0.59 ± 0.12^{bb}
HFD control	4.51 ± 0.49^{aa}	0.10 ± 0.02^{aa}
HFD + CUMS control	6.90 ± 0.30$^{\#\#}$	0.03 ± 0.01$^{\#\#}$
HFD + CUMS + OND (0.5 mg/kg, p.o.)	3.14 ± 0.36**	0.31 ± 0.07**
HFD + CUMS + OND (1 mg/kg, p.o.)	2.91 ± 0.29**	0.42 ± 0.08**
HFD + CUMS + ESC (10 mg/kg, p.o.)	3.09 ± 0.50**	0.37 ± 0.05**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control, **P<0.01vs HFD+CUMS control group, n = 6/group.

5.14.3. Effect of OND on hormonal changes: plasma CORT, leptin and insulin in HFD fed mice subjected to CUMS

HFD and HFD+CUMS groups showed marked (P<0.05) increased plasma CORT, leptin and insulin concentrations compared to NPD and HFD control groups, respectively. Chronic administration of **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly decreased the plasma CORT [F (9, 50) = 1912, P<0.01], leptin [F (9, 50) = 25.37, P<0.05] and insulin [F (9, 50) = 211.0, P<0.05] in HFD fed mice exposed to CUMS as compared to HFD+CUMS control group (**Table 5.4**6).

Table 5.46: Effect of OND on plasma CORT, leptin and insulin concentrations in HFD fed mice subjected to CUMS

Groups	Plasma CORT (ng/ml)	Plasma leptin (pg/ml)	Plasma insulin (ng/ml)
NPD control	116.07 ± 18.61	970.30 ± 34.11	0.531 ± 0.071
NPD + CUMS control	364.72 ± 23.35^{aa}	971.14 ± 50.18	1.278 ± 0.074^{a}
NPD + CUMS + OND (0.5 mg/kg, p.o.)	176.31 ± 15.65^{bb}	978.16 ± 67.30	0.871 ± 0.071^{b}
NPD + CUMS + OND (1 mg/kg, p.o.)	132.35 ± 19.57^{bb}	983.42 ± 71.68	0.673 ± 0.078^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	155.36 ± 14.69^{bb}	979.28 ± 64.49	0.798 ± 0.075^{b}
HFD control	880.25 ± 34.57^{aa}	1729.05 ± 85.47^{aa}	1.734 ± 0.117^{aa}
HFD + CUMS control	1326.88 ± 30.37$^{\#\#}$	1665.21 ± 90.34	2.710 ± 0.158$^{\#}$
HFD + CUMS + OND (0.5 mg/kg, p.o.)	265.94 ± 25.03**	1396.42 ± 78.08*	1.641 ± 0.080*
HFD + CUMS + OND (1 mg/kg, p.o.)	213.97 ± 20.42**	1108.11 ± 118.74**	1.403 ± 0.075**
HFD + CUMS + ESC (10 mg/kg, p.o.)	239.25 ± 17.74**	1328.42 ± 141.34*	1.526 ± 0.084*

The values are expressed as mean ± S.E.M., ^{a}P<0.05; ^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs HFD control, *P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

5.14.4. Effect of OND on molecular mechanisms: hippocampal 5-HT, cAMP and BDNF in HFD fed mice subjected to CUMS

HFD and HFD+CUMS groups showed remarkably (P<0.05) decreased brain hippocampal 5-HT, cAMP and BDNF levels as compared to NPD and HFD groups, respectively as shown in **Table 5.47**. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the hippocampal 5-HT [F (9, 50) = 221.0, P<0.01], cAMP [F (9, 50) = 379.4, P<0.01] and BDNF [F (9, 50) = 494.8, P<0.05] concentrations in HFD fed animals subjected to CUMS as compared to HFD+CUMS control group.

Table 5.47: Effect of OND on brain hippocampus 5-HT, cAMP and BDNF concentrations in HFD fed mice subjected to CUMS

Groups	5-HT (ng/g)	cAMP (pmol/mg protein)	BDNF (ng/mg protein)
NPD control	291.65 ± 19.27	5.650 ± 0.183	3.634 ± 0.057
NPD + CUMS control	132.55 ± 10.48^{aa}	3.469 ± 0.117^{a}	1.051 ± 0.079^{aa}
NPD + CUMS + OND (0.5 mg/kg, p.o.)	214.73 ± 14.94^{bb}	4.300 ± 0.139^{b}	1.962 ± 0.098^{b}
NPD + CUMS + OND (1 mg/kg, p.o.)	277.71 ± 11.05^{bb}	4.812 ± 0.078^{bb}	2.833 ± 0.153^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	285.20 ± 23.01^{bb}	4.474 ± 1.147^{b}	2.173 ± 0.145^{b}
HFD control	104.18 ± 8.75^{aa}	2.695 ± 0.100^{aa}	0.594 ± 0.076^{aa}
HFD + CUMS control	56.00 ± 5.30$^{\#\#}$	1.704 ± 0.045$^{\#}$	0.168 ± 0.032$^{\#\#}$
HFD + CUMS + OND (0.5 mg/kg, p.o.)	158.06 ± 15.81**	3.278 ± 0.105**	0.792 ± 0.086*
HFD + CUMS + OND (1 mg/kg, p.o.)	229.77 ± 16.65**	3.486 ± 0.088**	1.614 ± 0.115**
HFD + CUMS + ESC (10 mg/kg, p.o.)	210.26 ± 15.69**	3.388 ± 0.106**	1.172 ± 0.107**

The values are expressed as mean ± S.E.M., ^{a}P<0.05; ^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs HFD control, *P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group

5.15. Effect of 5-HT_3 receptor antagonist QCM-4 on depression co-morbid with obesity in HFD fed mice subjected to CUMS

5.15.1. Effect of QCM-4 on behavioral models of depression and anxiety in HFD fed mice subjected to CUMS

5.15.1.1. Effect of QCM-4 on body weight (g) of HFD fed mice subjected to CUMS

HFD control group showed observable (P<0.01) higher body weight as compared to NPD control group. Repetitive treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) showed no significant change in the initial [F (9, 50) = 0.272, P>0.05] and final [F (9, 50) = 5.521, P>0.05] body weight of HFD fed mice subjected to CUMS as compared to HFD+CUMS control group (**Table 5.48**).

Table 5.48: Effect of QCM-4 on body weight of HFD fed mice subjected to CUMS

Groups	Initial body weight (g)	Final body weight (g)
NPD control	24.33 ± 1.76	27.83 ± 1.47
NPD + CUMS control	24.50 ± 1.84	27.33 ± 1.76
NPD + CUMS + QCM-4 (1 mg/kg, p.o.)	24.83 ± 1.64	27.00 ± 1.03
NPD + CUMS + QCM-4 (2 mg/kg, p.o.)	24.17 ± 1.60	26.67 ± 0.95
NPD + CUMS + ESC (10 mg/kg, p.o.)	25.17 ± 1.72	27.33 ± 2.38
HFD control	36.33 ± 2.32^{aa}	37.17 ± 2.48^{aa}
HFD + CUMS control	36.50 ± 2.35	31.17 ± 1.58
HFD + CUMS + QCM-4 (1 mg/kg, p.o.)	37.17 ± 2.15	35.17 ± 2.29
HFD + CUMS + QCM-4 (2 mg/kg, p.o.)	37.50 ± 3.00	34.83 ± 1.76
HFD + CUMS + ESC (10 mg/kg, p.o.)	37.00 ± 2.67	33.00 ± 1.21

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, n = 6/group.

5.15.1.2. Effect of QCM-4 on SPT in HFD fed mice subjected to CUMS

The effect of **QCM-4** administration on sucrose consumption by HFD fed mice subjected to CUMS is represented in **Fig 5.47**. HFD and HFD+CUMS control animals showed marked (P<0.01) decreased sucrose consumption as compared to NPD and HFD control groups, respectively. Chronic dosing with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (9, 50) = 186.4, P<0.01] improved the sucrose consumption in HFD mice exposed to CUMS compared to HFD+CUMS control group.

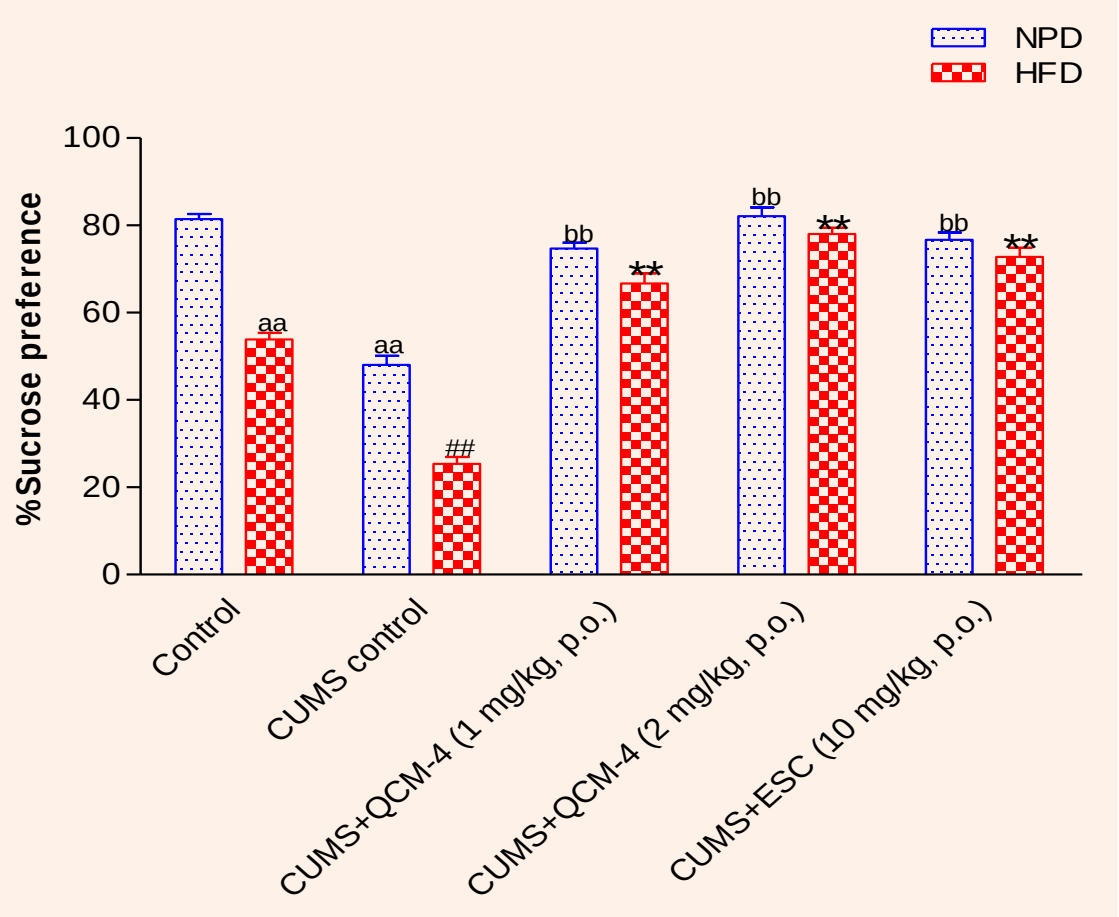


Fig 5.47: Effect of **QCM-4** on SPT in HFD fed mice subjected to CUMS. The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n=6/group.

5.15.1.3. Effect of QCM-4 on SLA score of HFD fed mice subjected to CUMS

The effect of **QCM-4** on basal locomotor activity score in HFD fed mice exposed to CUMS is shown in **Fig 5.48**. Locomotor activity score was not changed significantly (P>0.05) in HFD and HFD+CUMS control groups compared to NPD and HFD control group, respectively. Repetitive dosing with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) showed no significant [F (9, 50) = 2.49, P>0.05] alteration in the locomotor activity score in HFD fed mice subjected to CUMS as compared to HFD+CUMS control group.

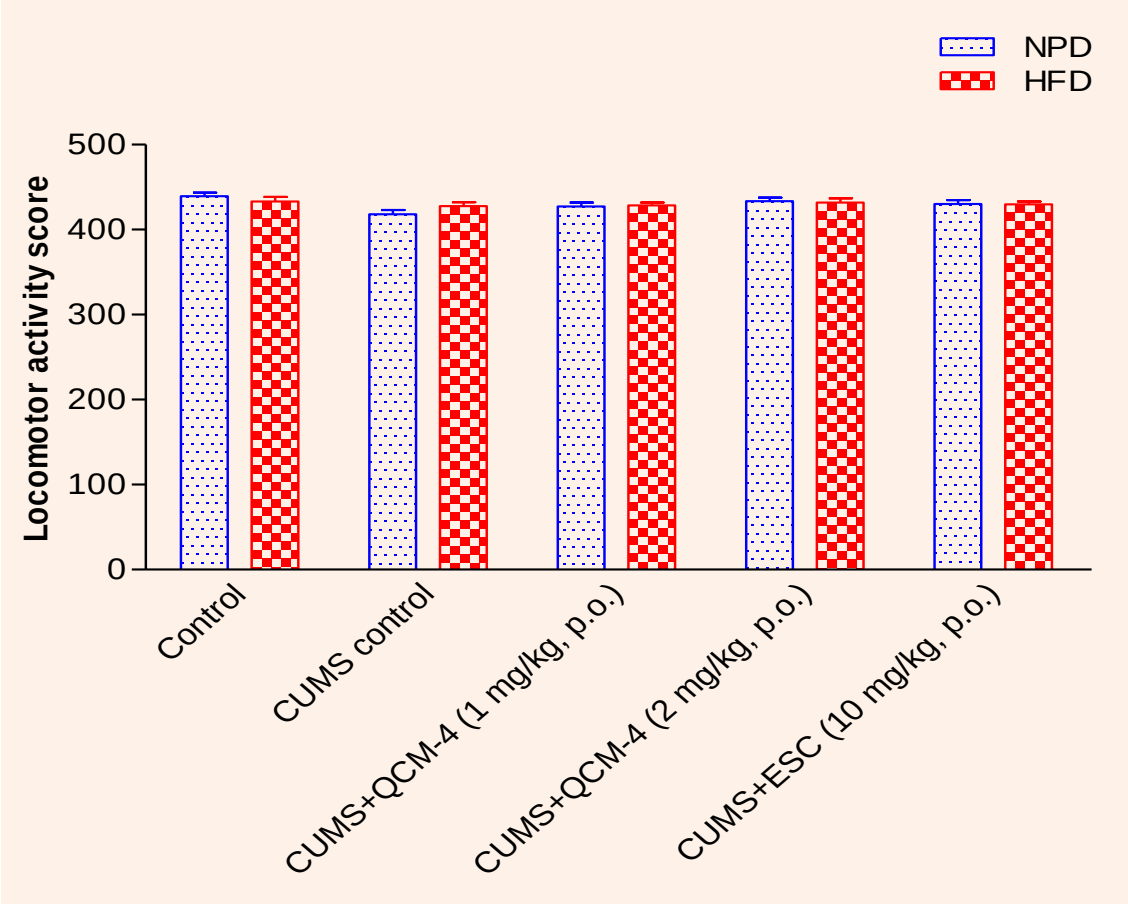


Fig 5.48: Effect of **QCM-4** on SLA score in HFD fed mice subjected to CUMS. The values are expressed as mean ± S.E.M., n=6/group.

5.15.1.4. Effect of QCM-4 on FST in HFD fed mice subjected to CUMS

The effect of chronic administration of **QCM-4** in CUMS exposed HFD fed mice on immobility time in FST is represented in **Fig 5.49**. HFD and HFD+CUMS control groups showed marked ($P<0.05$) higher immobility time as compared to NPD and HFD control groups, respectively. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in HFD fed mice subjected to CUMS significantly [$F(9, 50) = 175.6$, $P<0.01$] reduced the immobility time in FST as compared to HFD+CUMS control group.

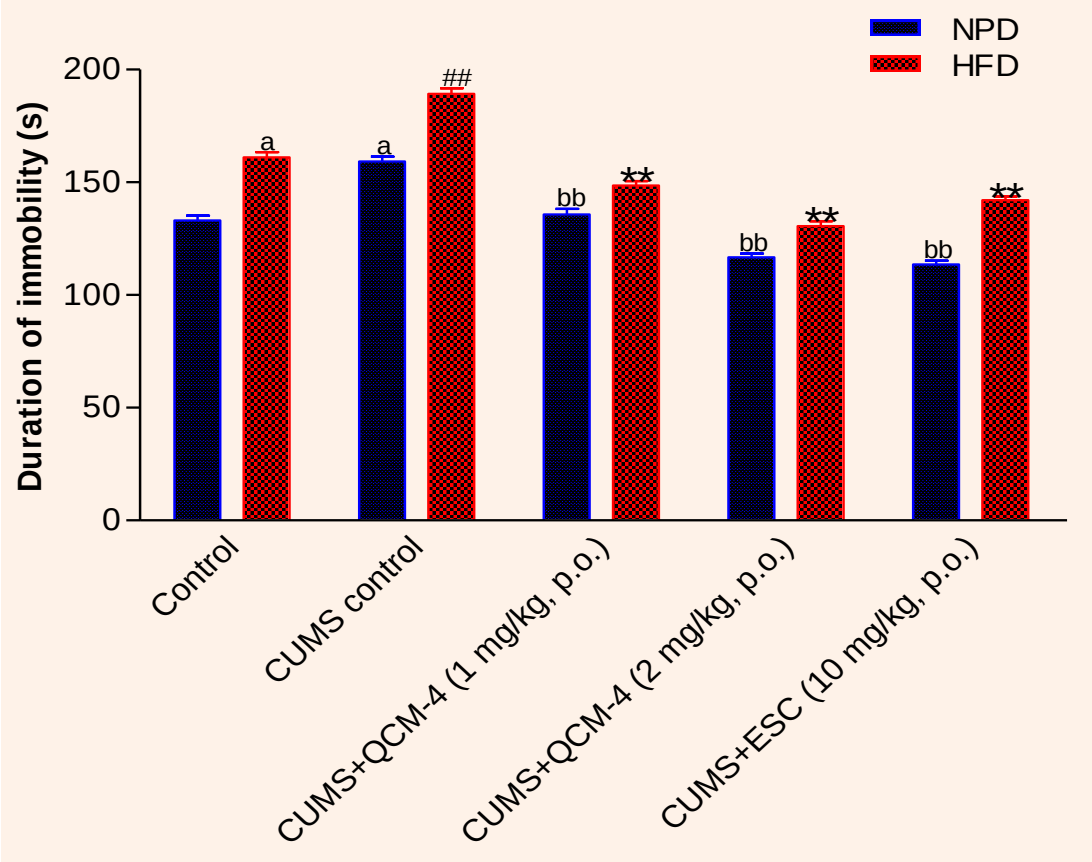


Fig 5.49: Effect of **QCM-4** on duration of immobility in HFD fed mice subjected to CUMS in FST. The values are expressed as mean ± S.E.M., [a]P<0.05 vs NPD control, [bb]P<0.01 vs NPD+CUMS control, [##]P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n=6/group.

5.15.1.5. Effect of QCM-4 on TST in HFD fed mice subjected to CUMS

The effect of **QCM-4** treatment in CUMS exposed HFD fed mice on immobility time in TST is shown in **Fig 5.50**. HFD and HFD+CUMS control groups showed remarkable (P<0.05) higher immobility time compared to NPD and HFD control groups, respectively. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) in HFD fed CUMS exposed mice significantly [F (9, 50) = 201.5, P<0.01] reduced the immobility time in TST as compared to HFD+CUMS control group.

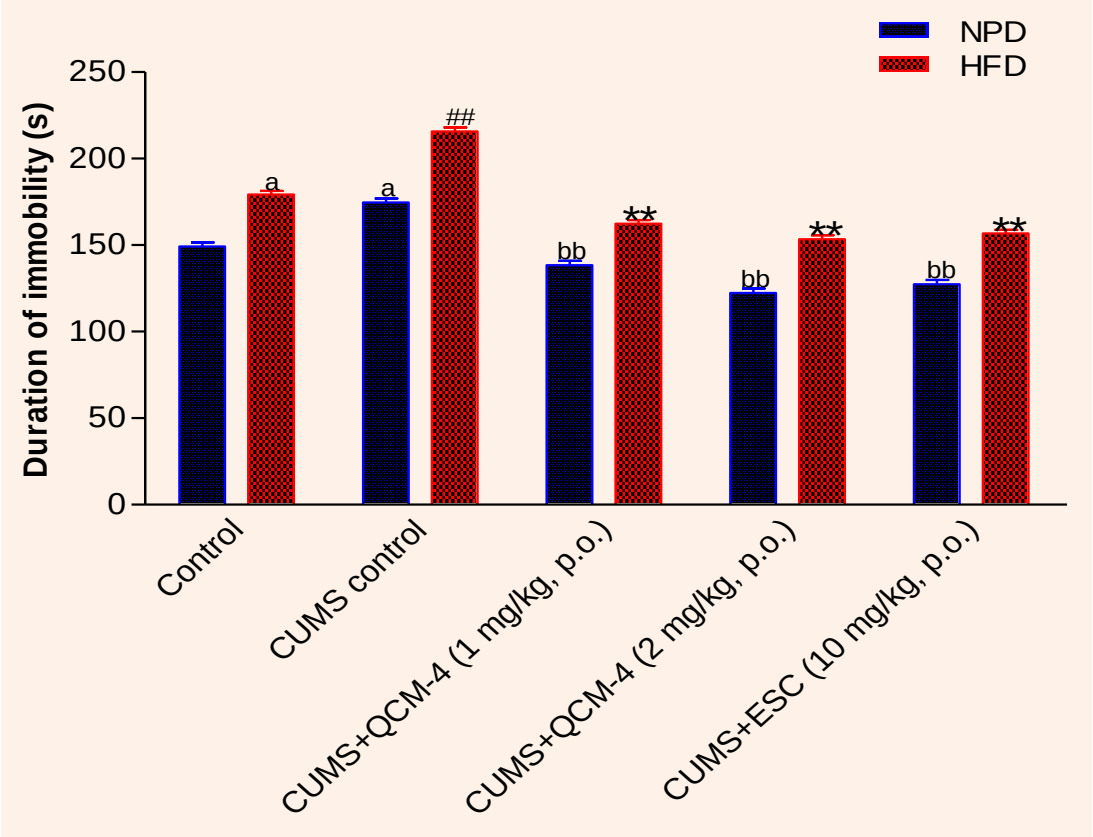


Fig 5.50: Effect of **QCM-4** on duration of immobility in HFD fed mice subjected to CUMS in TST. The values are expressed as mean ± S.E.M., [a]P<0.05 vs NPD control, [bb]P<0.01 vs NPD+CUMS control, [##]P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n=6/group.

5.15.1.6. Effect of QCM-4 on EPM in HFD fed mice subjected to CUMS

HFD and HFD+CUMS control groups exhibited remarkably (P<0.05) reduced % OAE and % OAT as compared to NPD and HFD control groups, respectively. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the % OAE [F (9, 50) = 75.42, P<0.01] and % OAT [F (9, 50) = 68.03, P<0.01] in HFD fed mice subjected to CUMS as compared to HFD+CUMS control group, as represented in **Table 5.49.**

5.15.1.7. Effect of QCM-4 on L/D test in HFD fed mice subjected to CUMS

HFD and HFD+CUMS control groups showed marked (P<0.01) decreased time in light chamber and transition score as compared to NPD and HFD control groups, respectively. Repetitive treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased [F (9, 50) = 95.28, P<0.01] the time in light chamber and transition score [F (9, 50) = 70.96, P<0.01] in HFD fed mice exposed to CUMS as compared to HFD+CUMS control group (**Table 5.50**).

Table 5.49: Effect of QCM-4 on HFD fed mice subjected to CUMS in EPM test

Groups	% OAE	% OAT (s)
NPD control	40.69 ± 4.45	16.11 ± 1.61
NPD + CUMS control	7.83 ± 1.25^{aa}	8.06 ± 1.14^{aa}
NPD + CUMS + QCM-4 (1 mg/kg, p.o.)	14.61 ± 1.70^{b}	13.44 ± 1.50^{b}
NPD + CUMS + QCM-4 (2 mg/kg, p.o.)	17.33 ± 1.56^{bb}	16.83 ± 1.85^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	15.72 ± 1.52^{bb}	15.33 ± 1.42^{bb}
HFD control	8.11 ± 1.23^{aa}	7.72 ± 1.22^{a}
HFD + CUMS control	4.06 ± 1.20$^{\#\#}$	3.83 ± 1.23$^{\#\#}$
HFD + CUMS + QCM-4 (1 mg/kg, p.o.)	10.33 ± 1.05**	9.83 ± 1.21**
HFD + CUMS + QCM-4 (2 mg/kg, p.o.)	13.50 ± 1.46**	12.83 ± 1.52**
HFD + CUMS + ESC (10 mg/kg, p.o.)	12.22 ± 1.53**	11.78 ± 1.44**

The values are expressed as mean ± S.E.M., ^{a}P<0.05; ^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control, **P<0.01vs HFD+CUMS control group, n = 6/group.

Table 5.50: Effect of QCM-4 on behavior of HFD fed mice subjected to CUMS in L/D

Groups	Time in light chamber (s)	Number of transitions
NPD control	33.00 ± 3.19	26.50 ± 3.45
NPD + CUMS control	16.50 ± 2.74^{aa}	12.33 ± 2.28^{aa}
NPD + CUMS + QCM-4 (1 mg/kg, p.o.)	32.50 ± 3.72^{bb}	29.00 ± 3.61^{bb}
NPD + CUMS + QCM-4 (2 mg/kg, p.o.)	44.50 ± 3.83^{bb}	37.50 ± 3.28^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	41.00 ± 3.75^{bb}	35.00 ± 3.71^{bb}
HFD control	14.17 ± 3.18^{aa}	12.00 ± 3.00^{aa}
HFD + CUMS control	7.00 ± 2.48$^{\#\#}$	5.00 ± 2.08$^{\#\#}$
HFD + CUMS + QCM-4 (1 mg/kg, p.o.)	18.00 ± 3.06**	12.83 ± 2.44**
HFD + CUMS + QCM-4 (2 mg/kg, p.o.)	27.33 ± 3.74**	18.17 ± 3.32**
HFD + CUMS + ESC (10 mg/kg, p.o.)	23.50 ± 3.04**	16.00 ± 2.88**

The values are expressed as mean ± S.E.M., ^{a}P<0.05; ^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control, **P<0.01vs HFD+CUMS control group, n = 6/group.

5.15.2. Effect of QCM-4 on biochemical estimations in HFD fed mice subjected to CUMS

5.15.2.1. Effect of QCM-4 on plasma glucose, total cholesterol and triglycerides in HFD fed mice subjected to CUMS

HFD fed mice exposed to CUMS showed observable (P<0.05) increased plasma glucose concentration as compared to HFD control group, as represented in **Table 5.51**. Repetitive treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly decreased the plasma glucose [F (9, 50) = 18.20, P<0.05], total cholesterol [F (9, 50) = 30.39, P<0.05] and triglycerides [F (9, 50) = 14.72, P<0.05] in HFD fed mice subjected to CUMS as compared to HFD+CUMS control group.

Table 5.51: Effect of QCM-4 on plasma glucose, total cholesterol and triglycerides in HFD obese mice subjected to CUMS

Groups	Plasma glucose (mg/dl)	Plasma total cholesterol (mg/dl)	Plasma triglycerides (mg/dl)
NPD control	94.15 ± 5.71	104.57 ± 6.84	98.10 ± 4.83
NPD + CUMS control	97.49 ± 4.89	102.75 ± 8.50	95.00 ± 5.08
NPD + CUMS + QCM-4 (1 mg/kg, p.o.)	96.75 ± 4.88	105.12 ± 7.98	96.35 ± 5.85
NPD + CUMS + QCM-4 (2 mg/kg, p.o.)	96.61 ± 4.78	106.09 ± 10.57	94.13 ± 4.93
NPD + CUMS + ESC (10 mg/kg, p.o.)	96.48 ± 5.72	106.07 ± 8.41	94.56 ± 5.17
HFD control	140.25 ± 5.20^{aa}	194.43 ± 8.04^{aa}	152.04 ± 5.60^{aa}
HFD + CUMS control	161.84 ± 5.71$^{\#}$	187.59 ± 9.92	147.98 ± 8.49
HFD + CUMS + QCM-4 (1 mg/kg, p.o.)	138.47 ± 5.68*	150.83 ± 9.27*	130.84 ± 7.05*
HFD + CUMS + QCM-4 (2 mg/kg, p.o.)	127.79 ± 5.03**	130.34 ± 9.26**	123.16 ± 7.71**
HFD + CUMS + ESC (10 mg/kg, p.o.)	135.64 ± 5.63*	135.04 ± 10.30*	127.40 ± 7.44**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control,

$^{\#}$P<0.05 vs HFD control, *P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

5.15.2.2. Effect of QCM-4 on brain hippocampus oxidative stress parameters in HFD fed mice subjected to CUMS

HFD and HFD+CUMS groups exhibited marked (P<0.01) elevated hippocampal lipid peroxidation marker MDA and decreased anti-oxidant enzyme GSH concentrations as compared to NPD and HFD control groups, respectively (**Table 5.52**). Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) significantly reduced the elevated the brain hippocampal MDA [F (9, 50) = 156.0, P<0.01] and increased the GSH [F (9, 50) = 59.83, P<0.01] concentrations in HFD fed mice exposed to CUMS as compared to HFD+CUMS control group.

Table 5.52: Effect of QCM-4 on brain hippocampus MDA and GSH concentrations in HFD fed mice subjected to CUMS

Groups	MDA (µg/mg protein)	GSH (µg/mg protein)
NPD control	1.52 ± 0.19	0.74 ± 0.07
NPD + CUMS control	2.54 ± 0.26^{aa}	0.19 ± 0.02^{aa}
NPD + CUMS + QCM-4 (1 mg/kg, p.o.)	1.34 ± 0.25^{bb}	0.54 ± 0.05^{b}
NPD + CUMS + QCM-4 (2 mg/kg, p.o.)	1.27 ± 0.23^{bb}	0.58 ± 0.06^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	1.29 ± 0.21^{bb}	0.55 ± 0.08^{b}
HFD control	4.72 ± 0.43^{aa}	0.12 ± 0.02^{aa}
HFD + CUMS control	6.98 ± 0.26$^{\#\#}$	0.04 ± 0.01$^{\#\#}$
HFD + CUMS + QCM-4 (1 mg/kg, p.o.)	3.25 ± 0.41**	0.33 ± 0.07**
HFD + CUMS + QCM-4 (2 mg/kg, p.o.)	2.94 ± 0.28**	0.45 ± 0.08**
HFD + CUMS + ESC (10 mg/kg, p.o.)	3.08 ± 0.45**	0.38 ± 0.05**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control,
^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control,
**P<0.01vs HFD+CUMS control group, n = 6/group.

5.15.3. Effect of QCM-4 on plasma hormonal changes: plasma CORT, leptin and insulin in HFD fed mice subjected to CUMS

The effect of **QCM-4** on plasma CORT, leptin and insulin concentrations in HFD fed mice exposed to CUMS is represented in **Table 5.53**. HFD and HFD+CUMS animals exhibited marked (P<0.05) higher plasma CORT, leptin and insulin concentrations as compared to NPD and HFD control groups, respectively. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly reduced the plasma CORT [F (9, 50) = 2261, P<0.01], leptin [F (9, 50) = 56.57, P<0.05] and insulin [F (9, 50) = 209.6, P<0.05] in HFD fed mice subjected to CUMS as compared to HFD+CUMS control group.

Table 5.53: Effect of QCM-4 on plasma CORT, leptin and insulin concentrations in HFD fed mice subjected to CUMS

Groups	Plasma CORT (ng/ml)	Plasma leptin (pg/ml)	Plasma insulin (ng/ml)
NPD control	119.25 ± 18.68	968.63 ± 32.83	0.54 ± 0.07
NPD + CUMS control	367.91 ± 23.27^{aa}	970.31 ± 48.95	1.31 ± 0.06^{aa}
NPD + CUMS + QCM-4 (1 mg/kg, p.o.)	182.94 ± 16.72^{bb}	979.61 ± 47.88	0.96 ± 0.09^{b}
NPD + CUMS + QCM-4 (2 mg/kg, p.o.)	136.21 ± 20.74^{bb}	986.45 ± 51.94	0.74 ± 0.08^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	157.06 ± 15.58^{bb}	984.28 ± 46.89	0.82 ± 0.07^{b}
HFD control	901.48 ± 28.97^{aa}	1747.38 ± 55.66^{aa}	1.78 ± 0.11^{aa}
HFD + CUMS control	1305.65 ± 24.75$^{\#\#}$	1692.71 ± 58.62	2.76 ± 0.14$^{\#}$
HFD + CUMS + QCM-4 (1 mg/kg, p.o.)	273.52 ± 18.06**	1433.50 ± 45.11*	1.69 ± 0.08*
HFD + CUMS + QCM-4 (2 mg/kg, p.o.)	220.31 ± 17.18**	1163.08 ± 76.80**	1.50 ± 0.10**
HFD + CUMS + ESC (10 mg/kg, p.o.)	243.50 ± 18.56**	1370.09 ± 59.76*	1.55 ± 0.09**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs HFD control, *P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

5.15.4. Effect of QCM-4 on molecular mechanisms: hippocampal 5-HT, cAMP and BDNF in HFD fed mice subjected to CUMS

HFD and HFD+CUMS mice exhibited significantly (P<0.05) reduced brain hippocampal 5-HT, cAMP and BDNF levels as compared to NPD and HFD control groups, respectively. Repetitive treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly restored the decreased hippocampal 5-HT [F (9, 50) = 174.6, P<0.01], cAMP [F (9, 50) = 316.8, P<0.01] and BDNF [F (9, 50) = 624.0, P<0.05] concentrations in HFD fed mice exposed to CUMS as compared to HFD+CUMS control group (**Table 5.54**).

Table 5.54: Effect of QCM-4 on brain hippocampus 5-HT, cAMP and BDNF concentrations in HFD fed mice subjected to CUMS

Groups	5-HT (ng/g)	cAMP (pmol/mg protein)	BDNF (ng/mg protein)
NPD control	284.45 ± 14.56	5.832 ± 0.235	3.750 ± 0.070
NPD + CUMS control	137.36 ± 9.19^{aa}	3.458 ± 0.132^{aa}	1.053 ± 0.088^{aa}
NPD + CUMS + QCM-4 (1 mg/kg, p.o.)	199.61 ± 12.98^{bb}	3.870 ± 0.067^{b}	1.599 ± 0.118^{b}
NPD + CUMS + QCM-4 (2 mg/kg, p.o.)	248.18 ± 35.12^{bb}	4.597 ± 0.127^{bb}	2.479 ± 0.069^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	274.63 ± 20.28^{bb}	4.229 ± 0.073^{bb}	2.019 ± 0.114^{b}
HFD control	103.70 ± 9.13^{aa}	2.792 ± 0.193^{aa}	0.610 ± 0.090^{aa}
HFD + CUMS control	55.28 ± 5.30$^{\#\#}$	1.734 ± 0.062$^{\#}$	0.161 ± 0.031$^{\#\#}$
HFD + CUMS + QCM-4 (1 mg/kg, p.o.)	118.69 ± 12.15**	3.076 ± 0.088**	0.615 ± 0.075*
HFD + CUMS + QCM-4 (2 mg/kg, p.o.)	217.77 ± 20.75**	3.436 ± 0.114**	1.364 ± 0.086**
HFD + CUMS + ESC (10 mg/kg, p.o.)	212.43 ± 18.50**	3.226 ± 0.047**	1.112 ± 0.101**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs HFD control, *P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

5.16. Effect of 5-HT_3 receptor antagonist 4a on depression co-morbid with obesity in HFD fed mice subjected to CUMS

5.16.1 Effect of 4a on behavioral models of depression and anxiety in HFD fed mice subjected to CUMS

5.16.1.1. Effect of 4a on body weight (g) of HFD fed mice subjected to CUMS

HFD control group showed marked (P<0.01) higher body weight as compared to NPD control group. Multiple treatment with **4a** (2 and 4 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) did not showed any significant alteration in the initial [F (9, 50) = 0.435, P>0.05] and final [F (9, 50) = 3.718, P>0.05] body weight of CUMS exposed HFD fed mice s compared to HFD+CUMS control group (**Table 5.55**).

Table 5.55: Effect of 4a on body weight of HFD fed mice subjected to CUMS

Groups	Initial body weight (g)	Final body weight (g)
NPD control	25.50 ± 1.57	27.33 ± 0.95
NPD + CUMS control	25.00 ± 0.73	26.83 ± 1.35
NPD + CUMS + 4a (2 mg/kg, p.o.)	25.17 ± 1.35	26.67 ± 0.95
NPD + CUMS + 4a (4 mg/kg, p.o.)	24.50 ± 1.09	26.17 ± 1.14
NPD + CUMS + ESC (10 mg/kg, p.o.)	26.00 ± 1.77	27.00 ± 1.48
HFD control	38.00 ± 2.29^{aa}	38.33 ± 2.99^{aa}
HFD + CUMS control	38.17 ± 1.92	33.00 ± 1.59
HFD + CUMS + 4a (2 mg/kg, p.o.)	38.67 ± 1.67	36.33 ± 2.94
HFD + CUMS + 4a (4 mg/kg, p.o.)	38.17 ± 2.44	36.50 ± 1.96
HFD + CUMS + ESC (10 mg/kg, p.o.)	38.50 ± 2.46	36.17 ± 1.89

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, n = 6/group.

5.16.1.2. Effect of 4a on SPT of HFD fed mice subjected to CUMS

HFD and HFD+CUMS mice exhibited remarkable (P<0.05) lower sucrose solution intake as compared to NPD and HFD control groups, respectively. Multiple treatment with **4a** (2 and 4 mg/kg, p.o.) and standard reference antidepressant ESC (10 mg/kg, p.o.) significantly [F (9, 50) = 147.8, P<0.01] improved the sucrose solution consumption in HFD fed animals subjected to CUMS as compared to HFD+CUMS control group, as demonstrated in **Fig 5.51**.

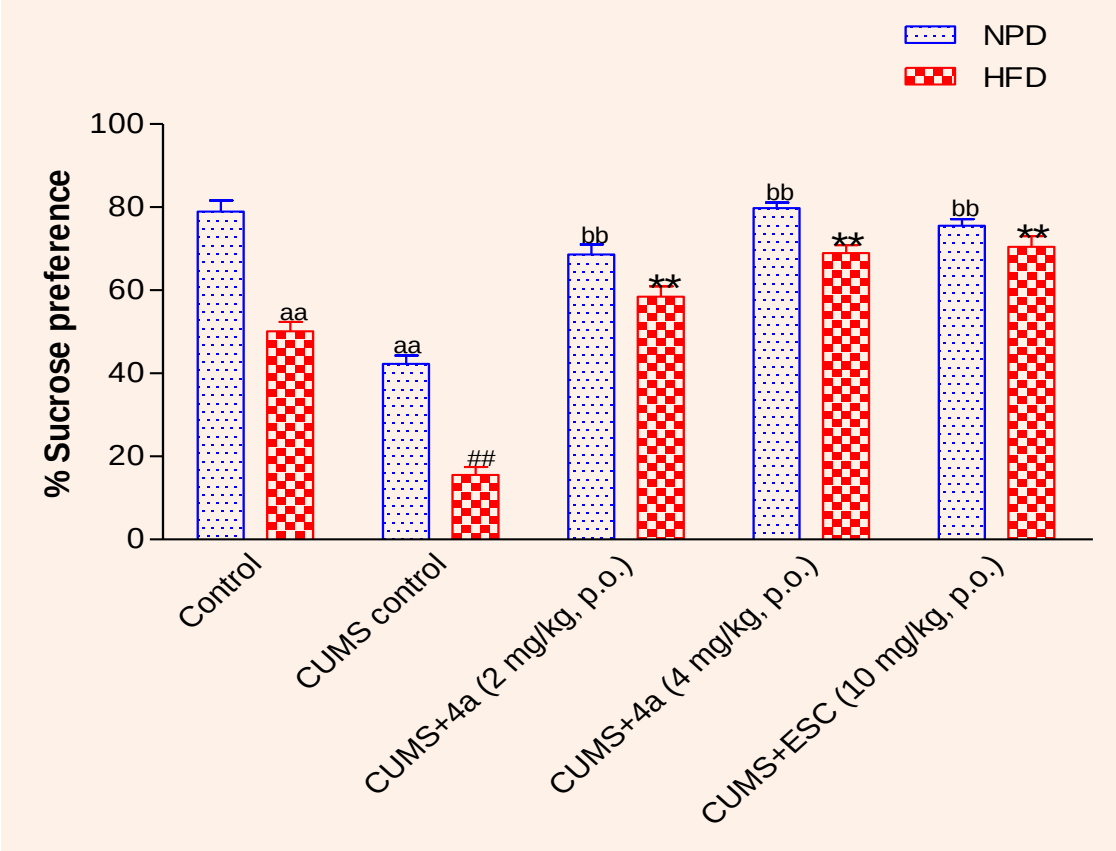


Fig 5.51: Effect of **4a** on SPT in HFD fed mice subjected to CUMS. The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n=6/group.

5.16.1.3. Effect of 4a on SLA score of HFD fed mice subjected to CUMS

The effect of **4a** treatment on basal locomotor activity score in HFD fed mice subjected to CUMS is shown in **Fig 5.52**. No marked (P>0.05) alteration in locomotor activity score was observed in HFD and HFD+CUMS control groups as compared to NPD and HFD control group, respectively. Chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed no significant [F (9, 50) = 0.674, P>0.05] change in the basal locomotor activity score in HFD fed mice subjected to CUMS as compared to HFD+CUMS control group.

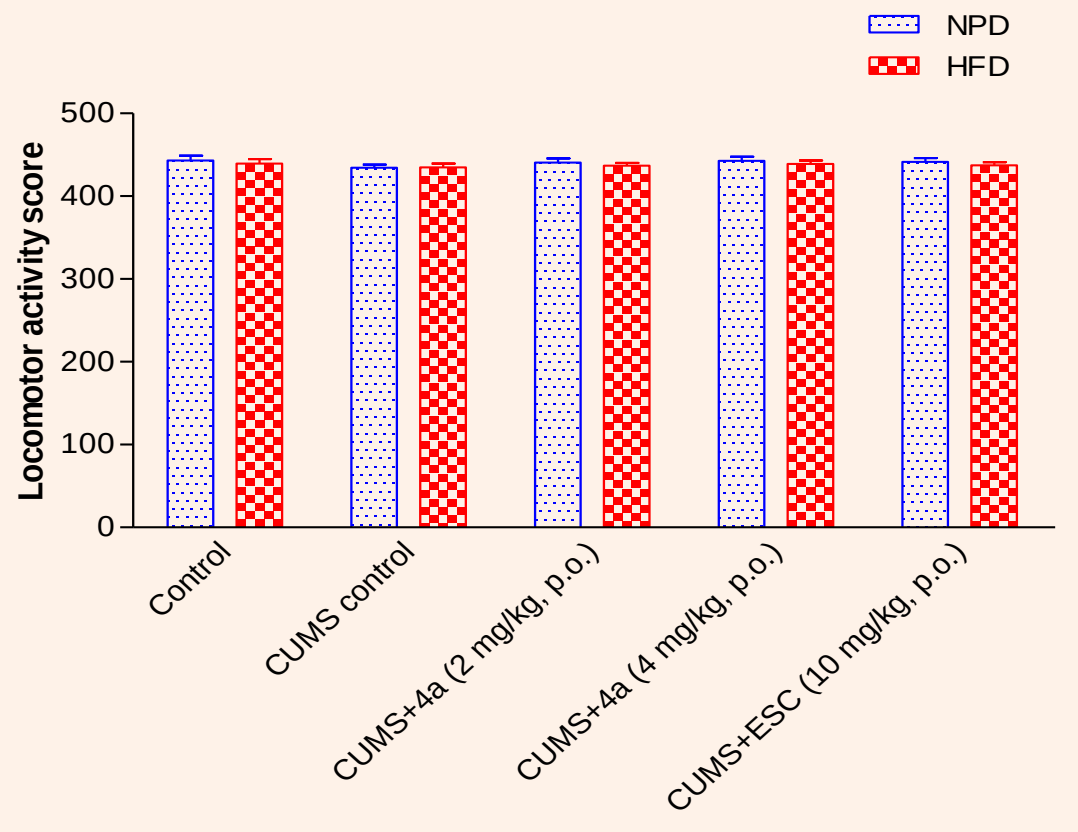


Fig 5.52: Effect of **4a** on SLA score in HFD fed mice subjected to CUMS. The values are expressed as mean ± S.E.M., n=6/group.

5.16.1.4. Effect of 4a on FST in HFD fed mice subjected to CUMS

The effect of chronic treatment of **4a** on immobility time in HFD fed mice exposed to CUMS in FST is represented in **Fig 5.53**. HFD and HFD+CUMS control animals showed remarkable ($P<0.05$) increased immobility time as compared to NPD and HFD control groups, respectively. Multiple treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in HFD fed mice subjected to CUMS significantly [$F (9, 50) = 102.9$, $P<0.05$] decreased the immobility time as compared to HFD+CUMS control group in FST.

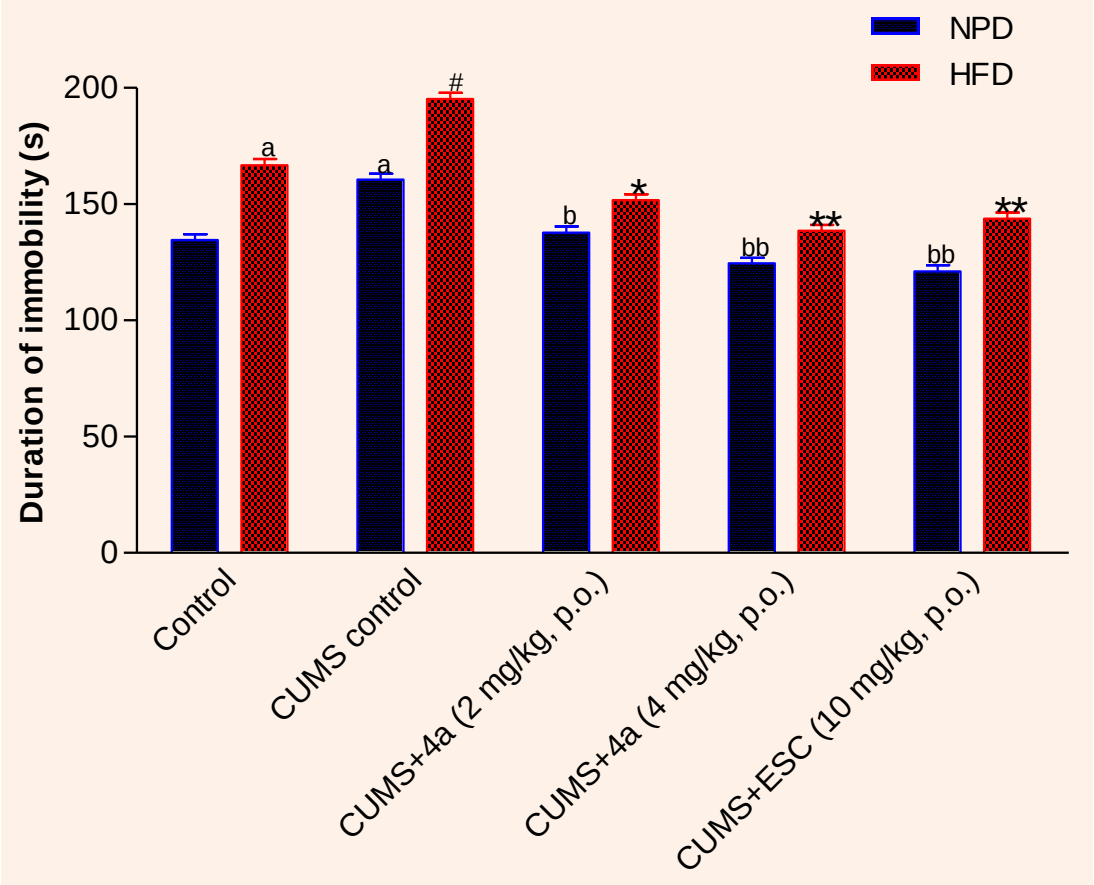


Fig 5.53: Effect of **4a** on duration of immobility in HFD fed mice subjected to CUMS in FST. The values are expressed as mean ± S.E.M., [a]P<0.05 vs NPD control, [b]P<0.05; [bb]P<0.01 vs NPD+CUMS control, [#]P<0.05 vs HFD control, *P<0.05; **P<0.01 vs HFD+CUMS control group, n=6/group.

5.16.1.5. Effect of 4a on TST in HFD fed mice subjected to CUMS

The effect of **4a** treatment in on immobility time of HFD fed CUMS exposed mice in TST is demonstrated in **Fig 5.54**. HFD and HFD+CUMS control mice exhibited remarkably (P<0.05) increased immobility time as compared to NPD and HFD control groups, respectively. Chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in HFD fed mice subjected to CUMS significantly [F (9, 50) = 161.3, P<0.01] decreased the immobility time as compared to HFD+CUMS control mice in TST.

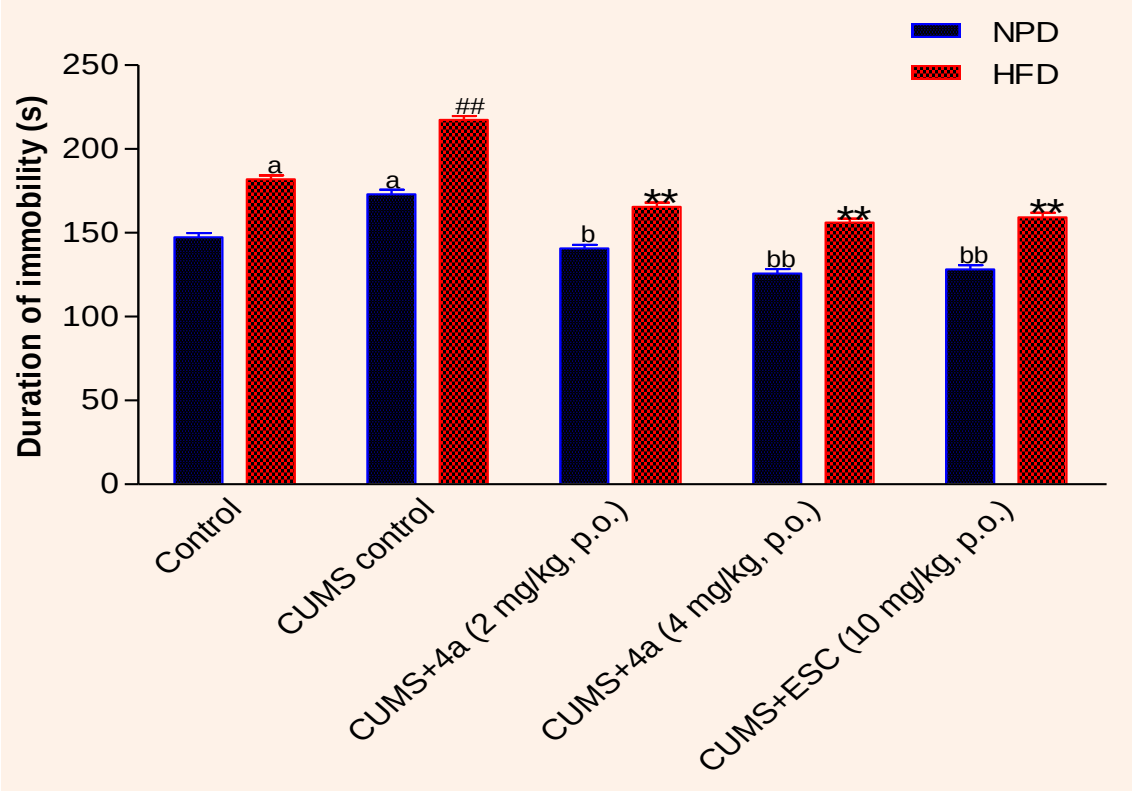


Fig 5.54: Effect of **4a** on duration of immobility in HFD fed mice subjected to CUMS in TST. The values are expressed as mean ± S.E.M., [a]P<0.05 vs NPD control, [b]P<0.05; [bb]P<0.01 vs NPD+CUMS control, [##]P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n=6/group.

5.16.1.6. Effect of 4a on EPM in HFD fed mice subjected to CUMS

The effect of **4a** treatment on EPM in HFD fed mice subjected to CUMS is shown in **Table 5.56**. HFD and HFD+CUMS mice showed marked (P<0.01) reduced % OAE and % OAT as compared to NPD and HFD control groups, respectively. Multiple treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the % OAE [F (9, 50) = 57.03, P<0.01] and % OAT [F (9, 50) = 83.76, P<0.01] in HFD fed mice exposed to CUMS as compared to HFD+CUMS control group.

5.16.1.7. Effect of 4a on L/D test in HFD fed mice subjected to CUMS

HFD and HFD+CUMS control mice exhibited remarkably (P<0.01) reduced time in light chamber and transition score as compared to NPD and HFD control groups, respectively. Chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly increased the time in light chamber [F (9, 50) = 80.81, P<0.05] and transition score [F (9, 50) = 47.78, P<0.01] in HFD fed mice subjected to CUMS as compared to HFD+CUMS control group, as demonstrated in **Table 5.57**.

Table 5.56: Effect of 4a on behavior of HFD fed mice subjected to CUMS in EPM

Groups	% OAE	% OAT (s)
NPD control	41.12 ± 5.29	17.17 ± 1.21
NPD + CUMS control	13.65 ± 4.41^{aa}	8.33 ± 1.42^{aa}
NPD + CUMS + 4a (2 mg/kg, p.o.)	29.97 ± 5.97^{bb}	12.72 ± 1.44^{b}
NPD + CUMS + 4a (4 mg/kg, p.o.)	44.27 ± 4.15^{bb}	17.44 ± 1.25^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	38.68 ± 4.44^{bb}	15.72 ± 1.25^{b}
HFD control	16.64 ± 5.14^{aa}	8.06 ± 1.15^{aa}
HFD + CUMS control	7.03 ± 3.08$^{\#\#}$	3.61 ± 1.02$^{\#\#}$
HFD + CUMS + 4a (2 mg/kg, p.o.)	20.81 ± 4.93**	8.56 ± 1.33*
HFD + CUMS + 4a (4 mg/kg, p.o.)	33.40 ± 6.03**	12.06 ± 1.54**
HFD + CUMS + ESC (10 mg/kg, p.o.)	30.66 ± 6.02**	11.89 ± 1.47**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control,
^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control,
*P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

Table 5.57: Effect of 4a on behavior of HFD fed mice subjected to CUMS in L/D test

Groups	Time in light chamber (s)	Number of transitions
NPD control	35.00 ± 3.16	27.50 ± 4.06
NPD + CUMS control	17.50 ± 3.45^{aa}	14.00 ± 2.96^{a}
NPD + CUMS + 4a (2 mg/kg, p.o.)	29.83 ± 3.46^{b}	26.83 ± 3.49^{b}
NPD + CUMS + 4a (4 mg/kg, p.o.)	42.50 ± 4.04^{bb}	35.50 ± 3.13^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	41.33 ± 3.66^{bb}	34.17 ± 3.08^{bb}
HFD control	15.00 ± 3.09^{aa}	12.67 ± 3.59^{aa}
HFD + CUMS control	7.67 ± 2.82$^{\#\#}$	5.83 ± 2.71$^{\#\#}$
HFD + CUMS + 4a (2 mg/kg, p.o.)	14.00 ± 3.27*	11.33 ± 2.88**
HFD + CUMS + 4a (4 mg/kg, p.o.)	25.50 ± 3.43**	17.17 ± 3.56**
HFD + CUMS + ESC (10 mg/kg, p.o.)	24.00 ± 3.00**	16.67 ± 3.17**

The values are expressed as mean ± S.E.M., ^{a}P<0.05; ^{aa}P<0.01 vs NPD control,
^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control,
*P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

5.16.2. Effect of 4a on biochemical estimations in HFD fed mice subjected to CUMS

5.16.2.1. Effect of 4a on plasma glucose, total cholesterol and triglycerides in HFD fed mice subjected to CUMS

HFD control group exhibited marked (P<0.05) higher plasma glucose, total cholesterol and triglycerides as compared to NPD control group as shown in **Table 5.58**. HFD fed mice subjected to CUMS showed remarkably (P<0.05) increased plasma glucose concentration as compared to HFD control group. Multiple treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly decreased the plasma glucose [F (9, 50) = 18.41, P<0.05], total cholesterol [F (9, 50) = 29.70, P<0.05] and triglycerides [F (9, 50) = 12.53, P<0.05] in HFD fed CUMS exposed mice as compared to HFD+CUMS control group.

Table 5.58: Effect of 4a on plasma glucose, total cholesterol and triglycerides in HFD obese mice subjected to CUMS

Groups	Plasma glucose (mg/dl)	Plasma total cholesterol (mg/dl)	Plasma triglycerides (mg/dl)
NPD control	95.83 ± 5.60	106.54 ± 8.75	104.40 ± 7.65
NPD + CUMS control	97.02 ± 6.38	104.43 ± 7.46	102.50 ± 8.27
NPD + CUMS + 4a (2 mg/kg, p.o.)	96.67 ± 5.71	104.17 ± 7.44	102.72 ± 5.73
NPD + CUMS + 4a (4 mg/kg, p.o.)	96.63 ± 6.16	105.66 ± 8.68	101.59 ± 6.06
NPD + CUMS + ESC (10 mg/kg, p.o.)	95.12 ± 6.07	106.56 ± 8.84	98.67 ± 7.89
HFD control	143.22 ± 7.17^{aa}	195.97 ± 9.85^{aa}	156.10 ± 7.04^{aa}
HFD + CUMS control	165.53 ± 5.98$^{\#}$	190.39 ± 9.17	151.31 ± 8.65
HFD + CUMS + 4a (2 mg/kg, p.o.)	136.00 ± 5.40*	161.82 ± 7.44*	139.69 ± 6.58*
HFD + CUMS + 4a (4 mg/kg, p.o.)	126.66 ± 6.75**	139.51 ± 8.40**	128.80 ± 7.00**
HFD + CUMS + ESC (10 mg/kg, p.o.)	133.47 ± 6.26**	139.62 ± 8.73**	126.91 ± 7.37**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, $^{\#}$P<0.05 vs HFD control, *P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

5.16.2.2. Effect of 4a on brain hippocampus oxidative stress parameters in HFD fed mice subjected to CUMS

HFD and HFD+CUMS animals exhibited marked (P<0.01) raised hippocampal lipid peroxidation marker MDA and reduced anti-oxidant enzyme GSH concentrations as compared to NPD and HFD control groups, respectively. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly inhibited the abnormally elevated the brain hippocampal MDA [F (9, 50) = 192.4, P<0.01] and increased the GSH [F (9, 50) = 92.83, P<0.01] concentrations in HFD mice subjected to CUMS as compared to HFD+CUMS control group, as represented in **Table 5.59**.

Table 5.59: Effect of 4a on brain hippocampus MDA and GSH concentrations in HFD fed mice subjected to CUMS

Groups	MDA (µg/mg protein)	GSH (µg/mg protein)
NPD control	1.49 ± 0.18	0.76 ± 0.05
NPD + CUMS control	2.57 ± 0.32^{aa}	0.19 ± 0.03^{aa}
NPD + CUMS + 4a (2 mg/kg, p.o.)	1.39 ± 0.28^{bb}	0.60 ± 0.07^{b}
NPD + CUMS + 4a (4 mg/kg, p.o.)	1.02 ± 0.15^{bb}	0.69 ± 0.10^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	1.35 ± 0.25^{bb}	0.66 ± 0.09^{bb}
HFD control	4.65 ± 0.36^{aa}	0.13 ± 0.03^{aa}
HFD + CUMS control	7.02 ± 0.29$^{\#\#}$	0.03 ± 0.01$^{\#\#}$
HFD + CUMS + 4a (2 mg/kg, p.o.)	3.33 ± 0.39**	0.21 ± 0.04**
HFD + CUMS + 4a (4 mg/kg, p.o.)	2.81 ± 0.37**	0.35 ± 0.07**
HFD + CUMS + ESC (10 mg/kg, p.o.)	3.01 ± 0.20**	0.36 ± 0.05**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#\#}$P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control group, n = 6/group.

5.16.3. Effect of 4a on plasma hormonal changes: plasma CORT, leptin and insulin in HFD fed mice subjected to CUMS

HFD and HFD fed CUMS exposed animals exhibited marked (P<0.05) higher plasma CORT, leptin and insulin concentrations as compared to NPD and HFD control groups, respectively. Multiple treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly decreased the plasma CORT [F (9, 50) = 2298, P<0.01], leptin [F (9, 50) = 42.76, P<0.05] and insulin [F (9, 50) = 235.2, P<0.05] in HFD fed CUMS exposed mice as compared to HFD+CUMS control group (**Table 5.60**).

Table 5.60: Effect of 4a on plasma CORT, leptin and insulin concentrations in HFD fed mice subjected to CUMS

Groups	Plasma CORT (ng/ml)	Plasma leptin (pg/ml)	Plasma insulin (ng/ml)
NPD control	120.85 ± 18.68	975.30 ± 45.23	0.57 ± 0.08
NPD + CUMS control	371.20 ± 21.91^{aa}	976.14 ± 41.80	1.34 ± 0.07^{a}
NPD + CUMS + 4a (2 mg/kg, p.o.)	185.76 ± 17.14^{bb}	977.30 ± 62.82	0.99 ± 0.09^{b}
NPD + CUMS + 4a (4 mg/kg, p.o.)	138.38 ± 20.78^{bb}	980.38 ± 40.32	0.79 ± 0.08^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	158.03 ± 15.08^{b}	992.61 ± 42.05	0.84 ± 0.06^{b}
HFD control	903.10 ± 28.18^{aa}	1737.38 ± 64.19^{aa}	1.80 ± 0.10^{aa}
HFD + CUMS control	1308.69 ± 27.19$^{\#\#}$	1701.04 ± 52.53	2.79 ± 0.137$^{\#}$
HFD + CUMS + 4a (2 mg/kg, p.o.)	280.85 ± 15.67**	1455.34 ± 56.74*	1.74 ± 0.07*
HFD + CUMS + 4a (4 mg/kg, p.o.)	228.18 ± 16.11**	1282.44 ± 49.70**	1.54 ± 0.08**
HFD + CUMS + ESC (10 mg/kg, p.o.)	245.86 ± 18.84**	1361.75 ± 63.19*	1.57 ± 0.08**

The values are expressed as mean ± S.E.M., ^{a}P<0.05;^{aa}P<0.01 vs NPD control, ^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs HFD control, *P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group.

5.16.4. Effect of 4a on molecular mechanisms: hippocampal 5-HT, cAMP and BDNF in HFD fed mice subjected to CUMS

HFD and HFD+CUMS mice showed remarkably (P<0.05) reduced brain hippocampus 5-HT, cAMP and BDNF concentrations compared to NPD and HFD control groups, respectively. Chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly improved the hippocampal 5-HT [F (9, 50) = 215.7, P<0.01], cAMP [F (9, 50) = 259.5, P<0.01] and BDNF [F (9, 50) = 496.9, P<0.05] concentrations in HFD fed mice subjected to CUMS s compared to HFD+CUMS control group, as shown in **Table 5.61.**

Table 5.61: Effect of 4a on brain hippocampus 5-HT, cAMP and BDNF concentrations in HFD fed mice subjected to CUMS

Groups	5-HT (ng/g)	cAMP (pmol/mg protein)	BDNF (ng/mg protein)
NPD control	279.62 ± 10.13	5.785 ± 0.217	3.650 ± 0.111
NPD + CUMS control	134.61 ± 10.18^{aa}	3.487 ± 0.131^{aa}	1.017 ± 0.086^{aa}
NPD + CUMS + 4a (2 mg/kg, p.o.)	170.80 ± 16.09^{bb}	3.780 ± 0.150^{b}	1.427 ± 0.122^{b}
NPD + CUMS + 4a (4 mg/kg, p.o.)	231.86 ± 21.71^{bb}	4.505 ± 0.191^{bb}	2.090 ± 0.112^{bb}
NPD + CUMS + ESC (10 mg/kg, p.o.)	263.11 ± 22.28^{bb}	4.356 ± 0.120^{bb}	2.030 ± 0.106^{b}
HFD control	101.06 ± 8.45^{aa}	2.772 ± 0.123^{aa}	0.601 ± 0.076^{aa}
HFD + CUMS control	50.96 ± 4.87$^{\#\#}$	1.705 ± 0.045$^{\#}$	0.165 ± 0.032$^{\#\#}$
HFD + CUMS + 4a (2 mg/kg, p.o.)	97.08 ± 10.94**	3.055 ± 0.123**	0.571 ± 0.089*
HFD + CUMS + 4a (4 mg/kg, p.o.)	214.41 ± 19.23**	3.401 ± 0.132**	1.223 ± 0.078**
HFD + CUMS + ESC (10 mg/kg, p.o.)	217.08 ± 14.34**	3.236 ± 0.071**	1.133 ± 0.088**

The values are expressed as mean ± S.E.M., ^{aa}P<0.01 vs NPD control,

^{b}P<0.05; ^{bb}P<0.01 vs NPD+CUMS control, $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs HFD control,

*P<0.05; **P<0.01vs HFD+CUMS control group, n = 6/group

5.17. Effect of 5-HT_3 receptor antagonists OND, QCM-4 and 4a on histological examination of hippocampal DG neurons in HFD fed mice

The hippocampal DG histology was performed in HFD fed mice and the effect of 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** on hippocampal neuronal morphology was measured using image J software. HFD control group showed marked ($P<0.05$) higher number of pyknotic neurons (indicated by % pyknotic/damage neurons showed in red arrows in **Fig 5.55**) and reduced number of healthy neurons (showed in yellow arrows in **Fig 5.55**) in the hippocampal DG region as compared to NPD control group **(Fig 5.60).** Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) (**Fig 5.56**), **QCM-4** (1 and 2 mg/kg, p.o.) (**Fig 5.57**), **4a** (2 and 4 mg/kg, p.o.) (**Fig 5.58**) and standard antidepressant ESC (10 mg/kg, p.o.) (**Fig 5.59**) significantly [$F (8, 45) = 422.3$, $P<0.01$] reduced the % pyknotic/damage neurons and increased the healthy neurons in the DG region of hippocampus in HFD fed mice compared to HFD control group, as represented in the **Figure 5.60**.

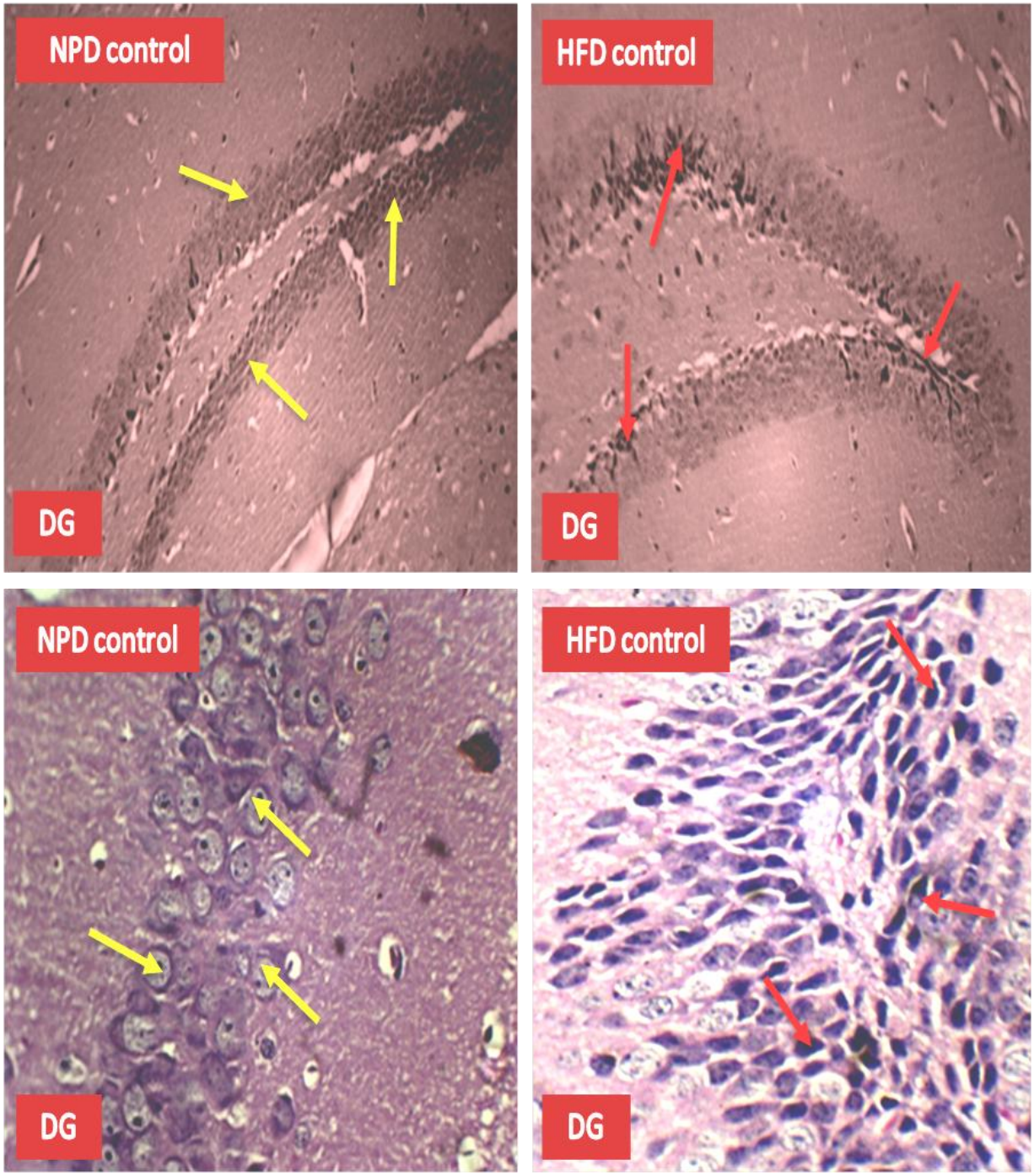


Fig 5.55: Morphological changes in the hippocampal DG region of NPD and HFD control mice (red arrow indicate pyknotic neurons and yellow arrow indicate healthy neurons) (Images taken at 100X and 400X).

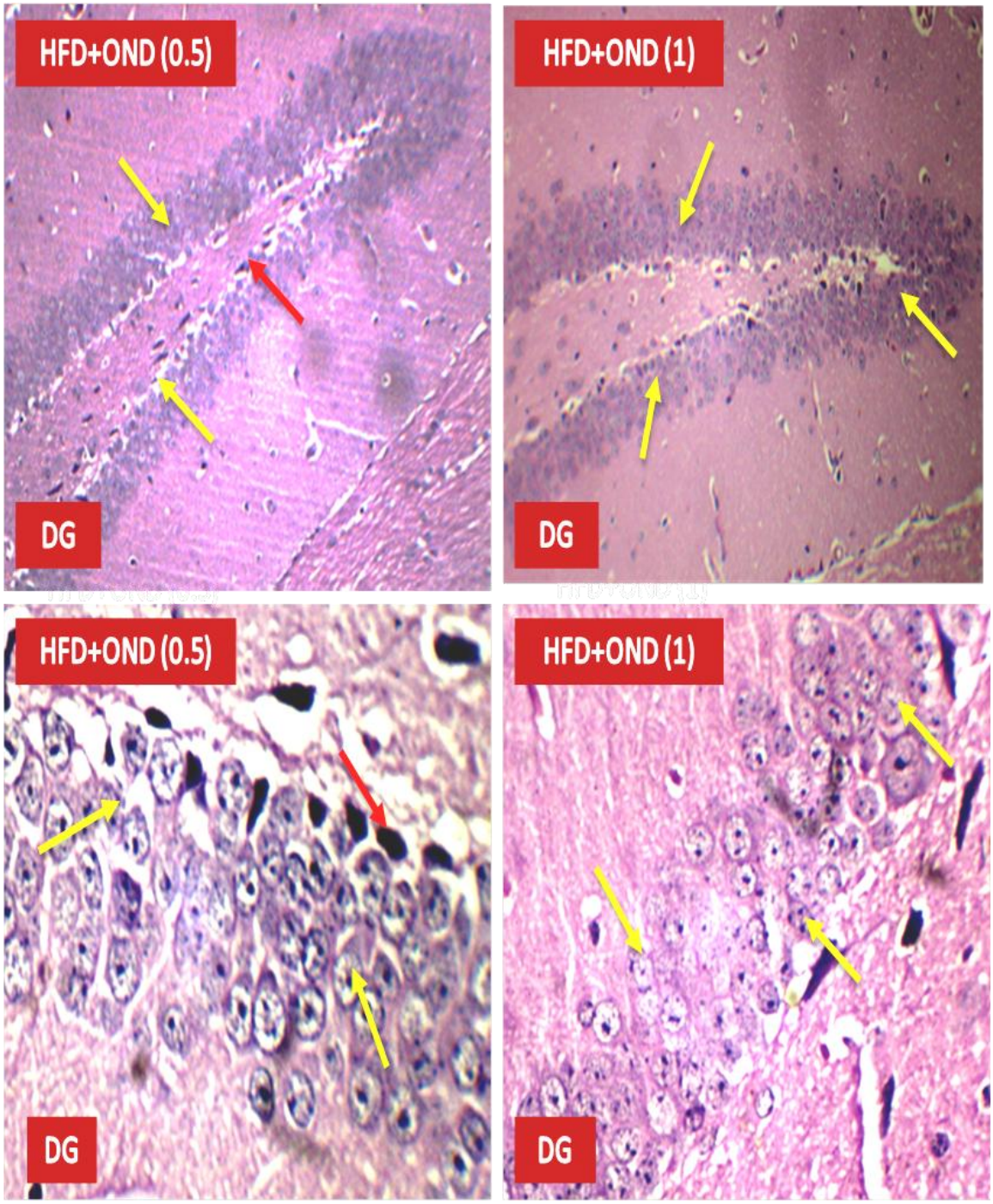


Fig 5.56: Effect of **OND** on morphological changes in the hippocampal DG region of HFD fed mice (red arrow indicate pyknotic neurons and yellow arrow indicate healthy neurons) (Images taken at 100X and 400X).

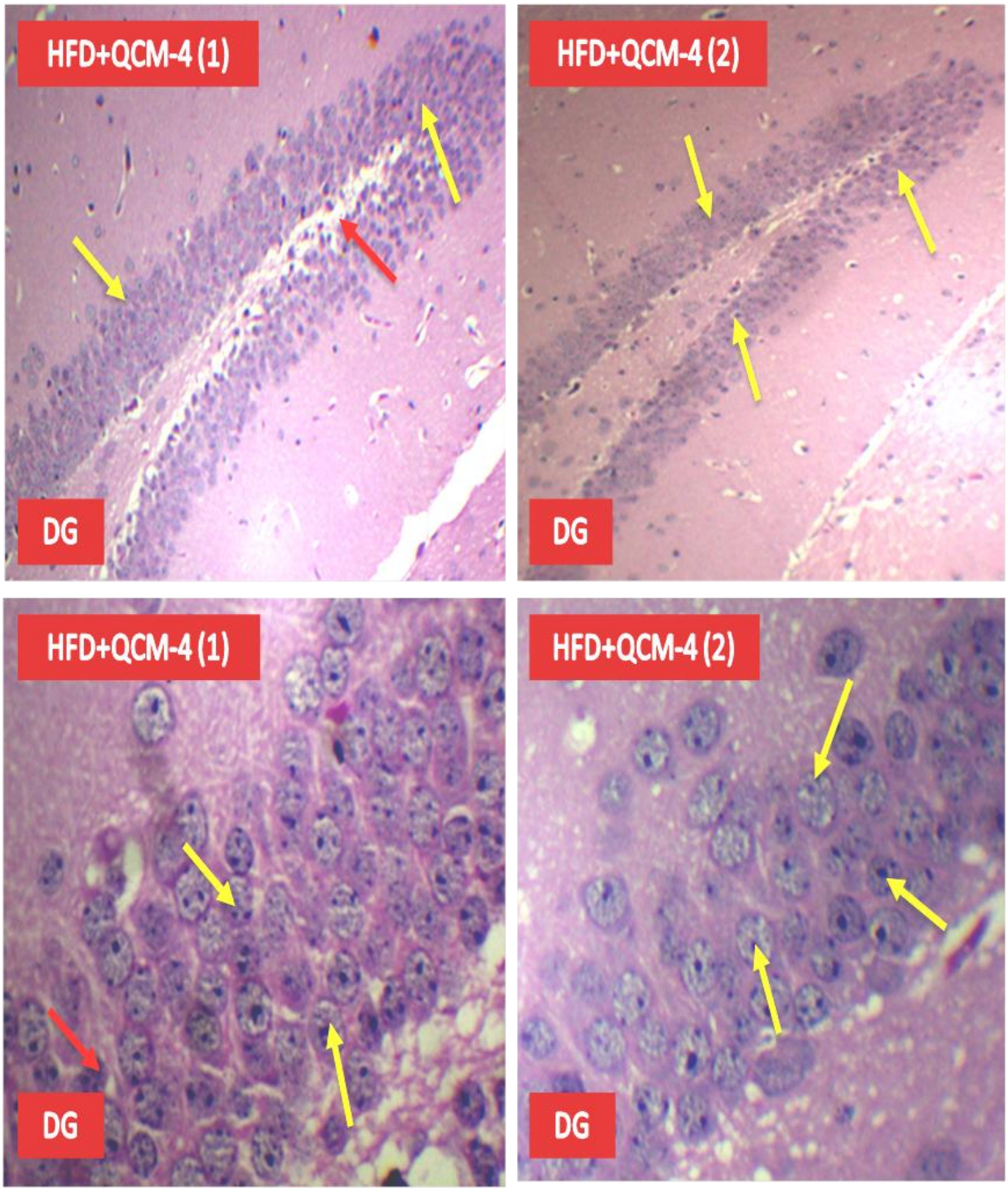


Fig 5.57: Effect of **QCM-4** on the morphological changes in the hippocampal DG region of HFD fed mice (red arrow indicate pyknotic neurons and yellow arrow indicate healthy neurons) (Images taken at 100X and 400X).

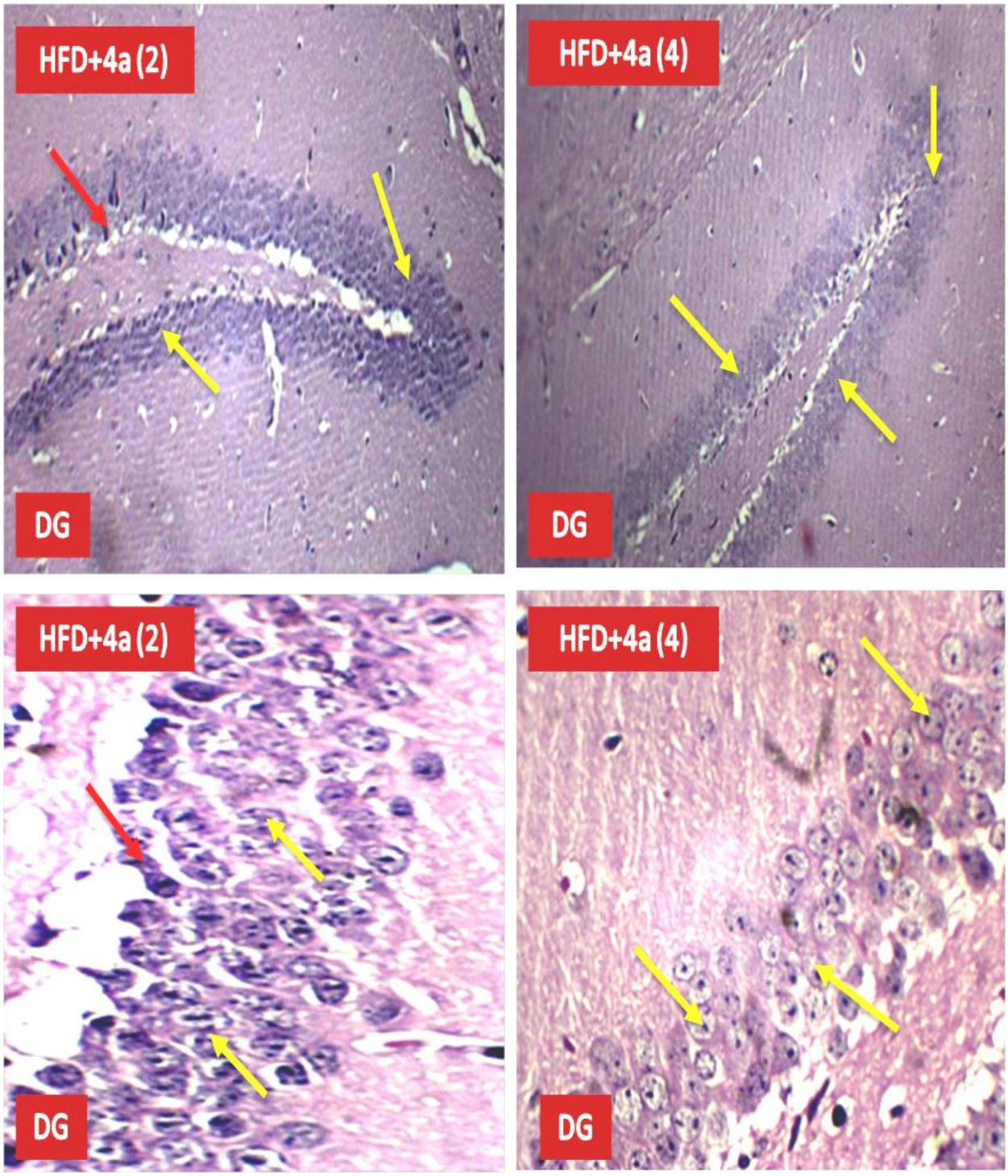


Fig 5.58: Effect of **4a** on morphological changes in the hippocampal DG region of HFD fed mice (red arrow indicate pyknotic neurons and yellow arrow indicate healthy neurons) (Images taken at 100X and 400X).

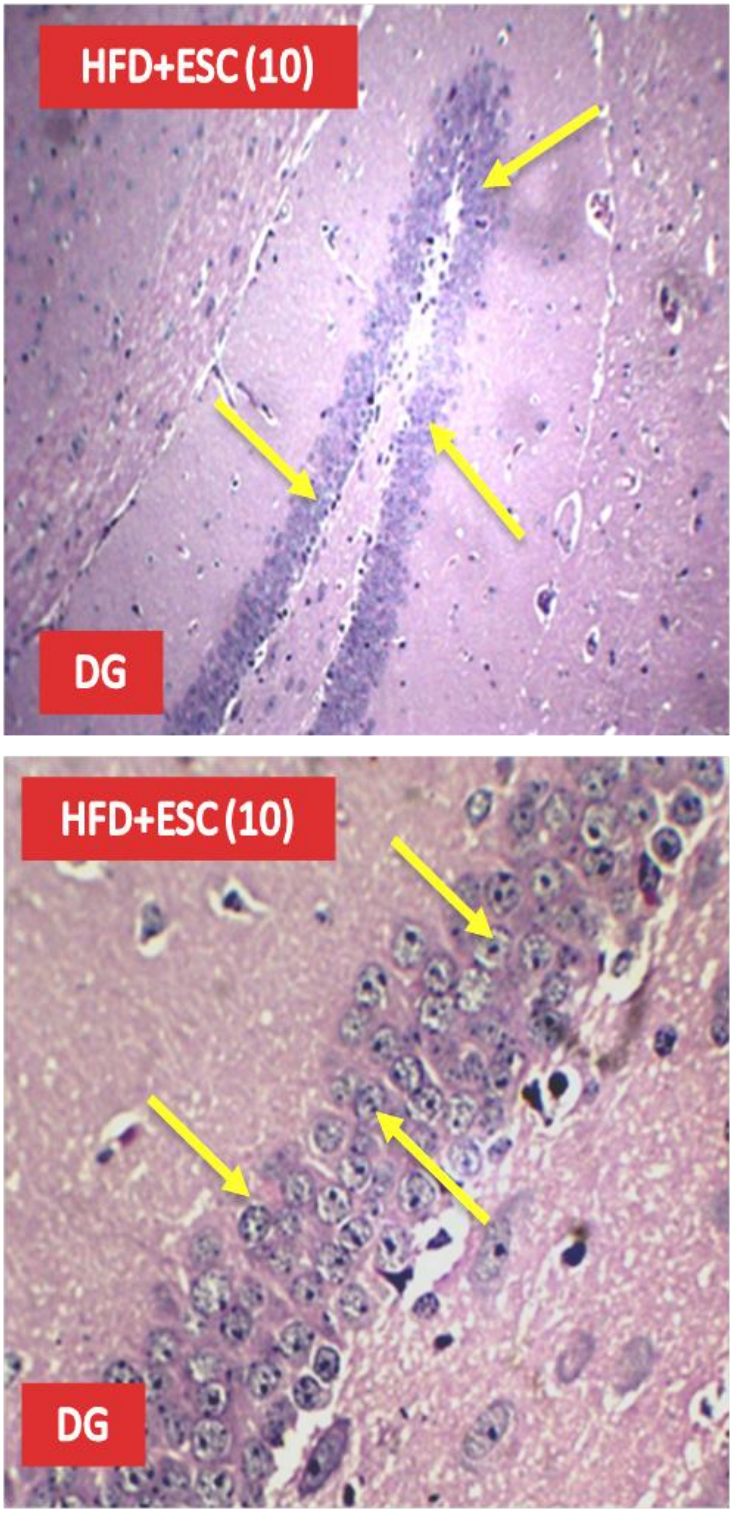


Fig 5.59: Effect of ESC on the morphological changes in the hippocampal DG region of HFD fed mice (yellow arrow indicates healthy neurons) (Images taken at 100X and 400X).

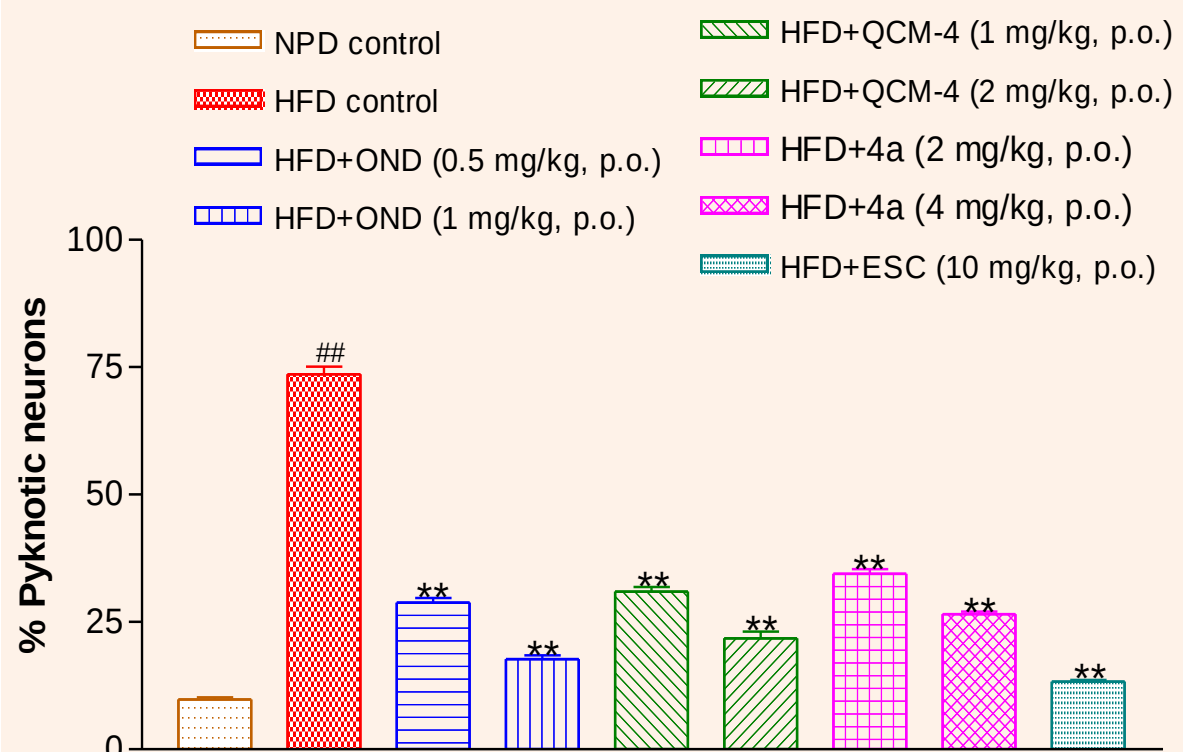


Fig 5.60: Effect of **OND**, **QCM-4** and **4a** on % pyknotic neurons in the DG region of hippocampus in HFD fed mice. ## P<0.01 vs NPD control, **P<0.01 vs HFD control

5.18. Effect of 5-HT_3 receptor antagonists OND, QCM-4 and 4a on histological examination of hippocampal neurons in HFD fed mice subjected to CUMS

The neuronal morphological changes observed in depression associated with obesity were performed in HFD fed mice subjected to CUMS model of depression. DG region of hippocampus was mainly observed for morphological changes mainly indicated by healthy neurons (yellow arrows) and pyknotic neurons (red arrows) and finally % pyknotic neurons were calculated and mentioned in **Fig 5.65.** HFD control mice showed significantly increased ($P<0.05$) hippocampal DG region neuronal damage compared to NPD group (**Fig 5.61**). HFD fed mice subjected to CUMS showed significantly ($P<0.05$) higher hippocampal DG neuronal damage compared to HFD control mice (**Fig 5.62**). Repetitive treatment with **OND** (1 mg/kg, p.o.) (**Fig 5.63**), **QCM-4** (2 mg/kg, p.o.) (**Fig 5.63**), **4a** (4 mg/kg, p.o.) (**Fig 5.64**) and standard antidepressant ESC (10 mg/kg, p.o.) (**Fig 5.64**) in HFD fed mice subjected to CUMS significantly [$F (6, 35) = 1050$, $P<0.01$] reduced the % pyknotic neurons and increased healthy neurons (**Fig 5.65**) in the DG region of hippocampus compared to HFD control group.

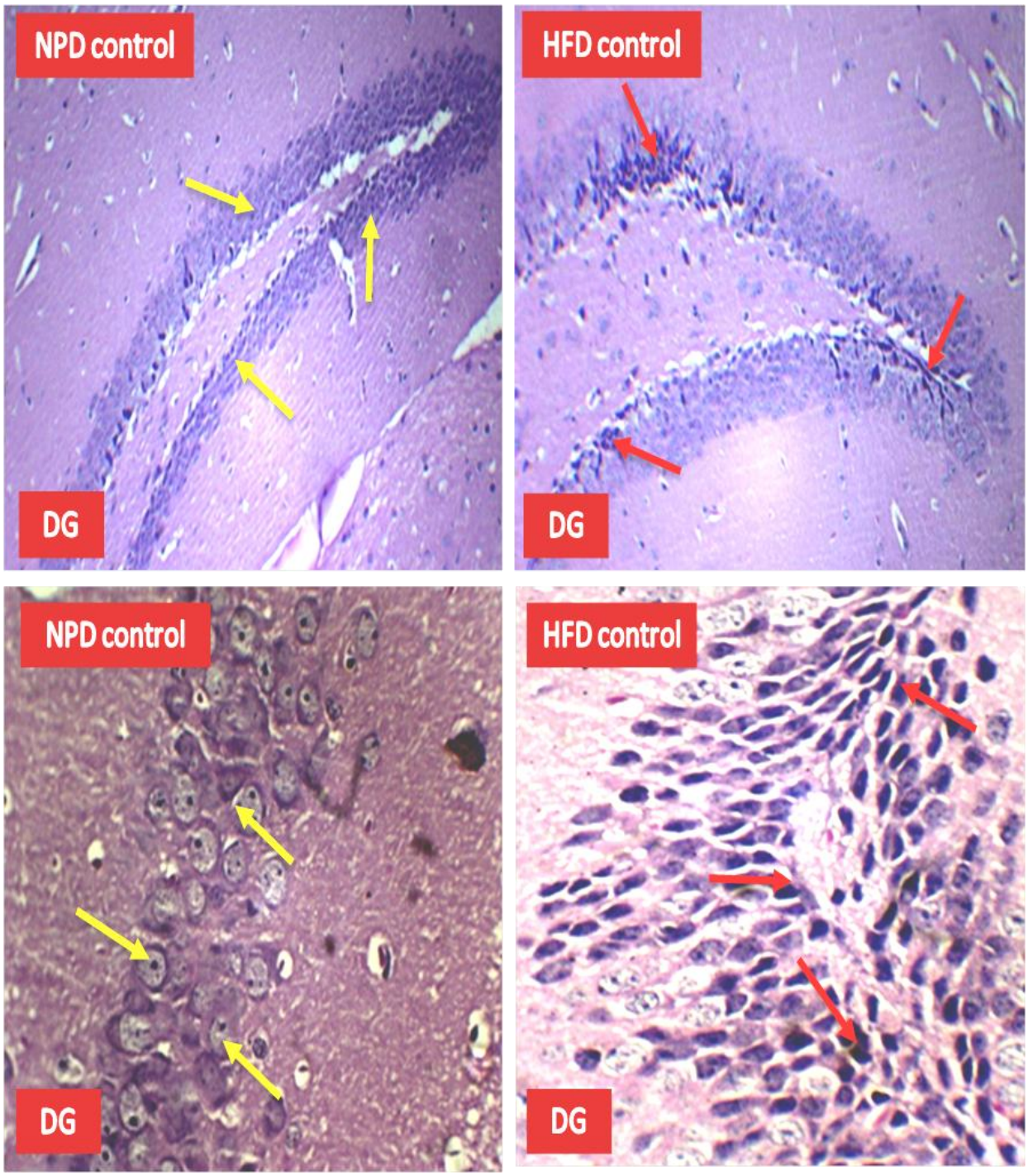


Fig 5.61: Hippocampal neuronal morphology of DG region of NPD and HFD control mice (yellow arrow and red arrow indicate healthy and pyknotic neurons, respectively) (Images taken at 100X and 400X).

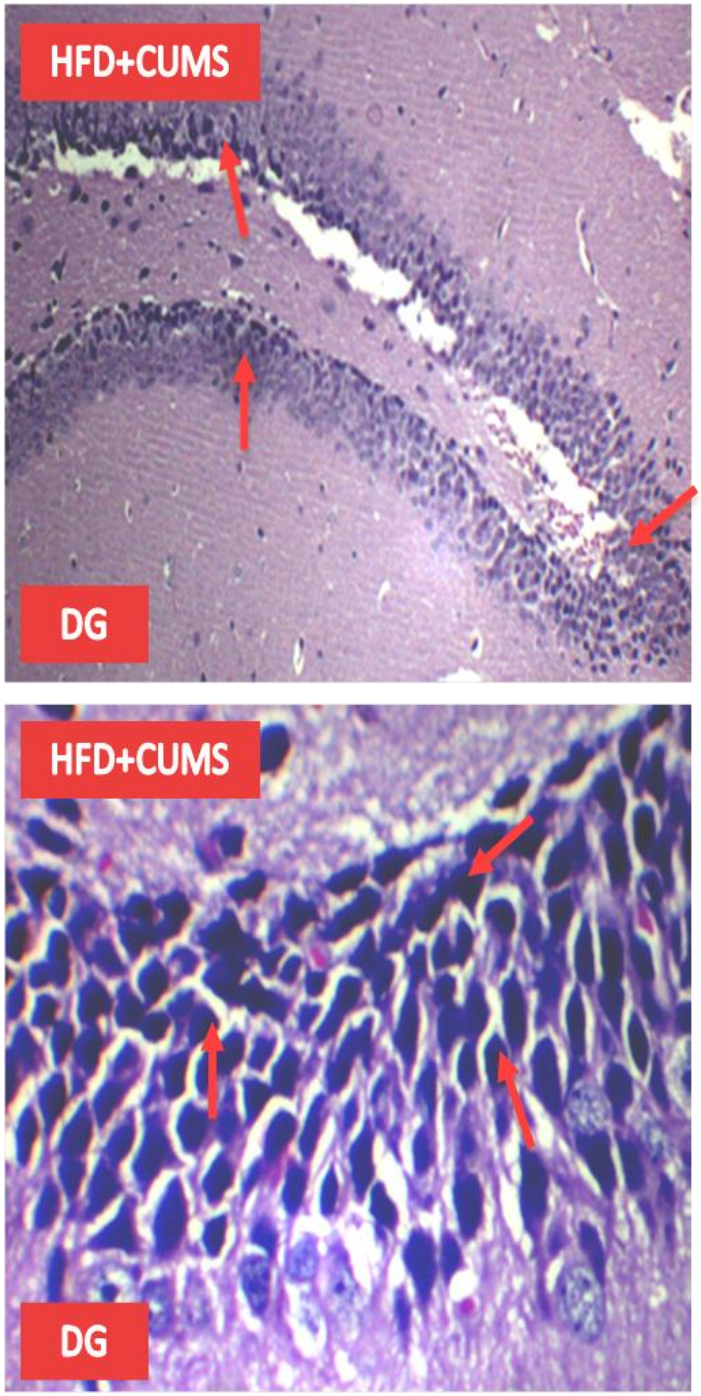


Fig 5.62: Hippocampal neuronal morphological changes in the DG region of HFD fed mice subjected to CUMS (red arrow indicate pyknotic neurons) (Images taken at 100X and 400X).

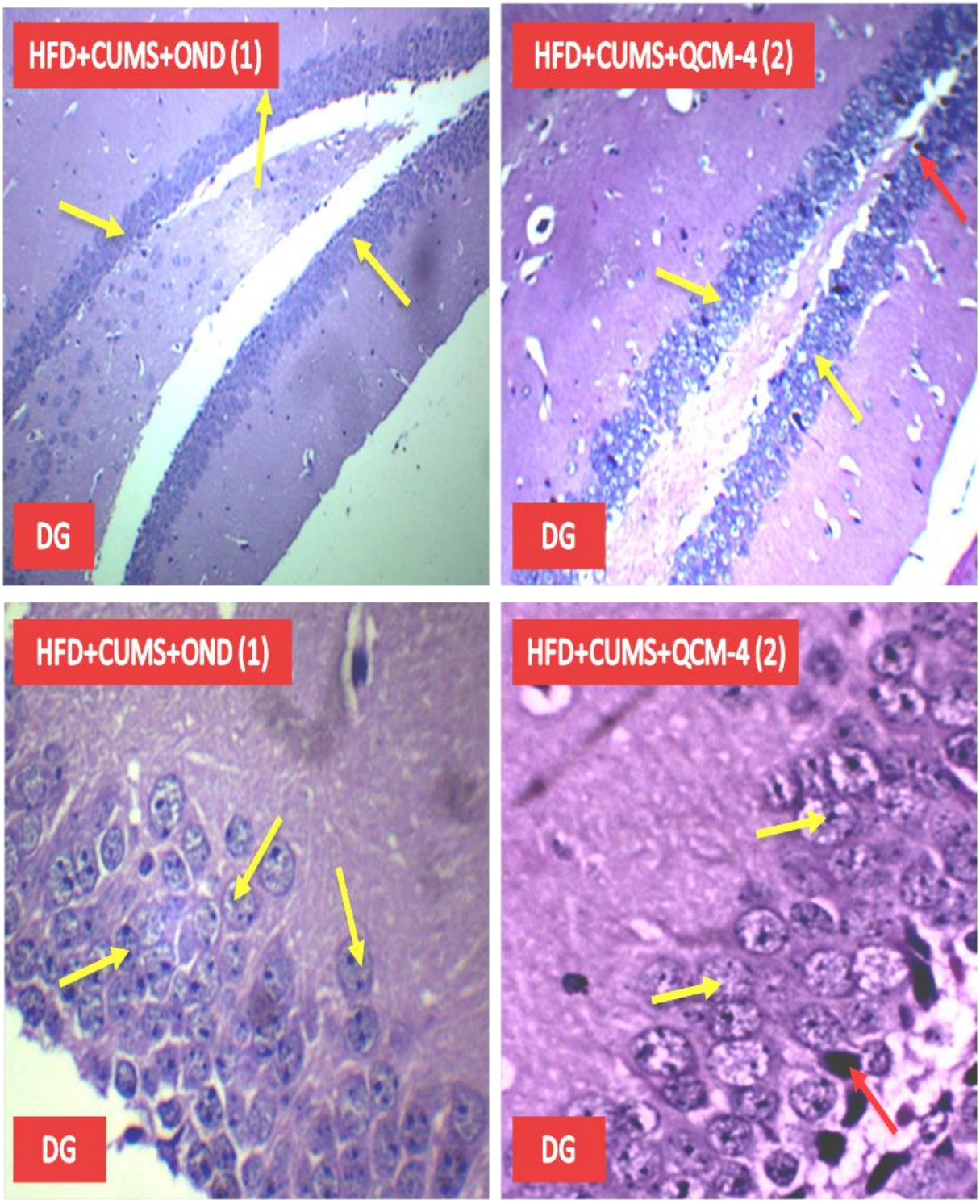


Fig 5.63: Effect of **OND** and **QCM-4** on DG region hippocampal neuronal morphology of HFD fed mice subjected to CUMS (red and yellow arrows indicated pyknotic and healthy neurons, respectively) (Images taken at 100X and 400X).

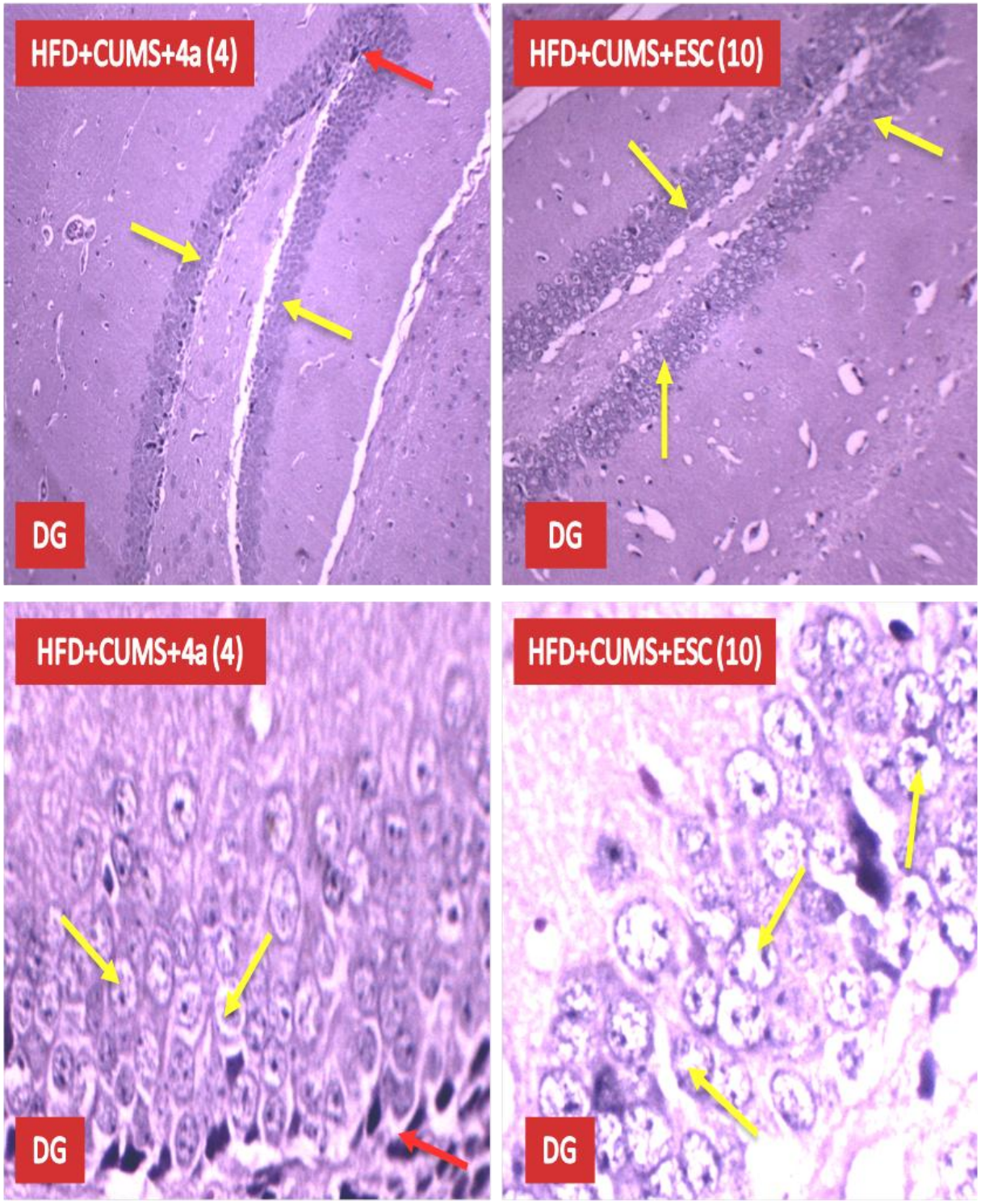


Fig 5.64: Effect of **4a** and ESC on DG region hippocampal neuronal morphology of HFD fed mice subjected to CUMS (red and yellow arrows indicates pyknotic and healthy neurons, respectively)
(Images taken at 100X and 400X).

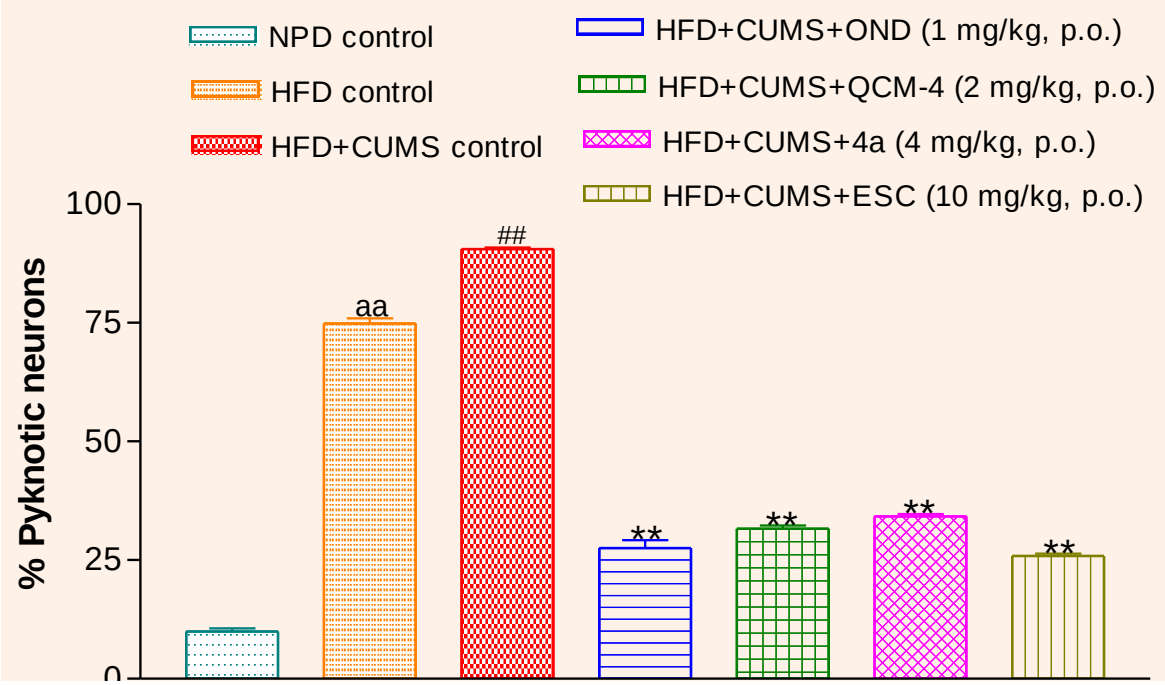


Fig 5.65: Effect of **OND**, **QCM-4** and **4a** on % pyknotic neurons in the DG region of hippocampus in HFD fed mice subjected to CUMS. aaP<0.01 vs NPD control, $^{\#\#}$P<0.01 vs HFD control, **P<0.01 vs HFD+CUMS control.

5.19. Effect of 5-HT_3 receptor antagonists OND, QCM-4 and 4a on the IHC examination of p53 in the hippocampal neurons in HFD fed mice

Depression associated with obesity shows the neuronal damage in the hippocampus region. This neuronal damage could be due to neuroinflammation or apoptosis mediated by protein such as p53. In order to locate the count of p53 in the DG region of hippocampus in HFD fed mice IHC assay was performed representing the % area of p53 as shown in **Fig 5.71.** HFD control mice demonstrated significantly ($p<0.01$) higher % area of p53 and hence neuronal damage (as shown by red arrows) compared to NPD control mice (**Fig 5.66** and **Fig 5.67**). Chronic treatment with **OND** (1 mg/kg, p.o.) (**Fig 5.68**), **QCM-4** (2 mg/kg, p.o.) (**Fig 5.69**) and **4a** (4 mg/kg, p.o.) (**Fig 5.70**) [$F(4, 25) = 126.3$, $P<0.01$] showed significantly reduced % area of p53 in the DG region of hippocampus in HFD fed mice compared to HFD control mice (red arrow indicates presence of p53).

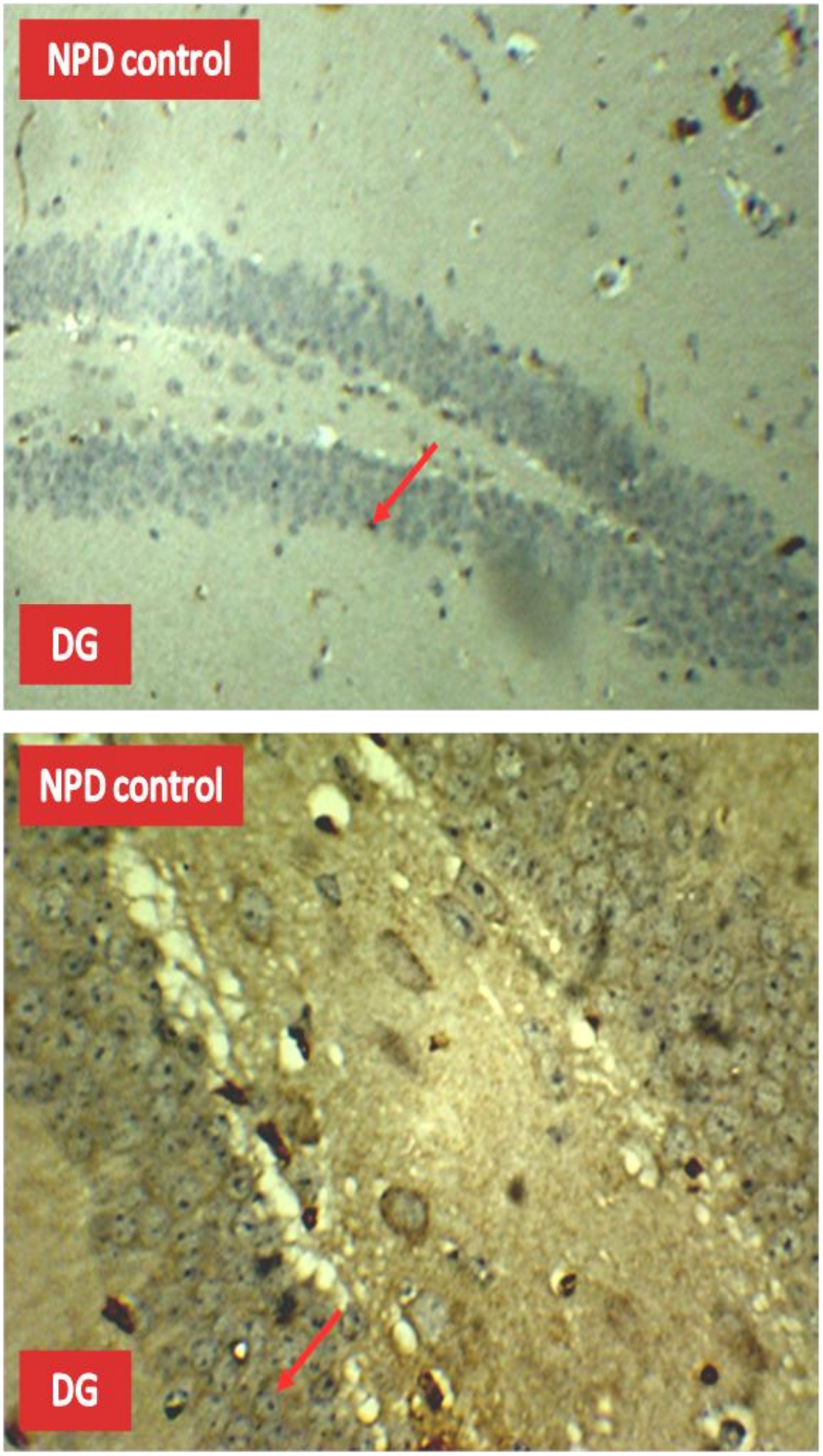


Fig 5.66: % area of p53 protein in the DG region of hippocampus in NPD control mice (red arrow indicate p53 presence) (Images taken at 100X and 400X).

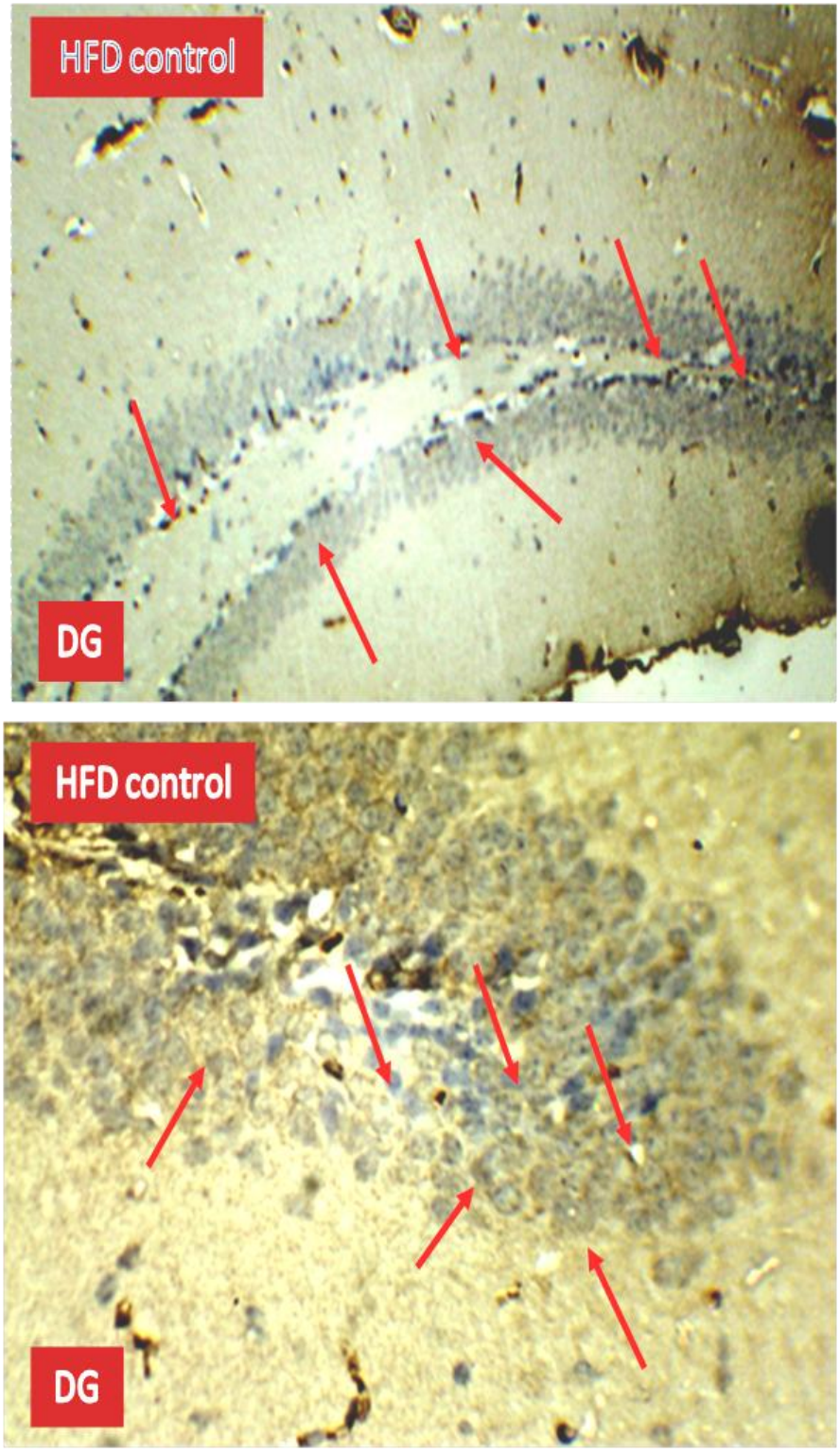


Fig 5.67: % area of p53 protein in the DG region of hippocampus in HFD control mice (red arrow indicate p53 presence) (Images taken at 100X and 400X).

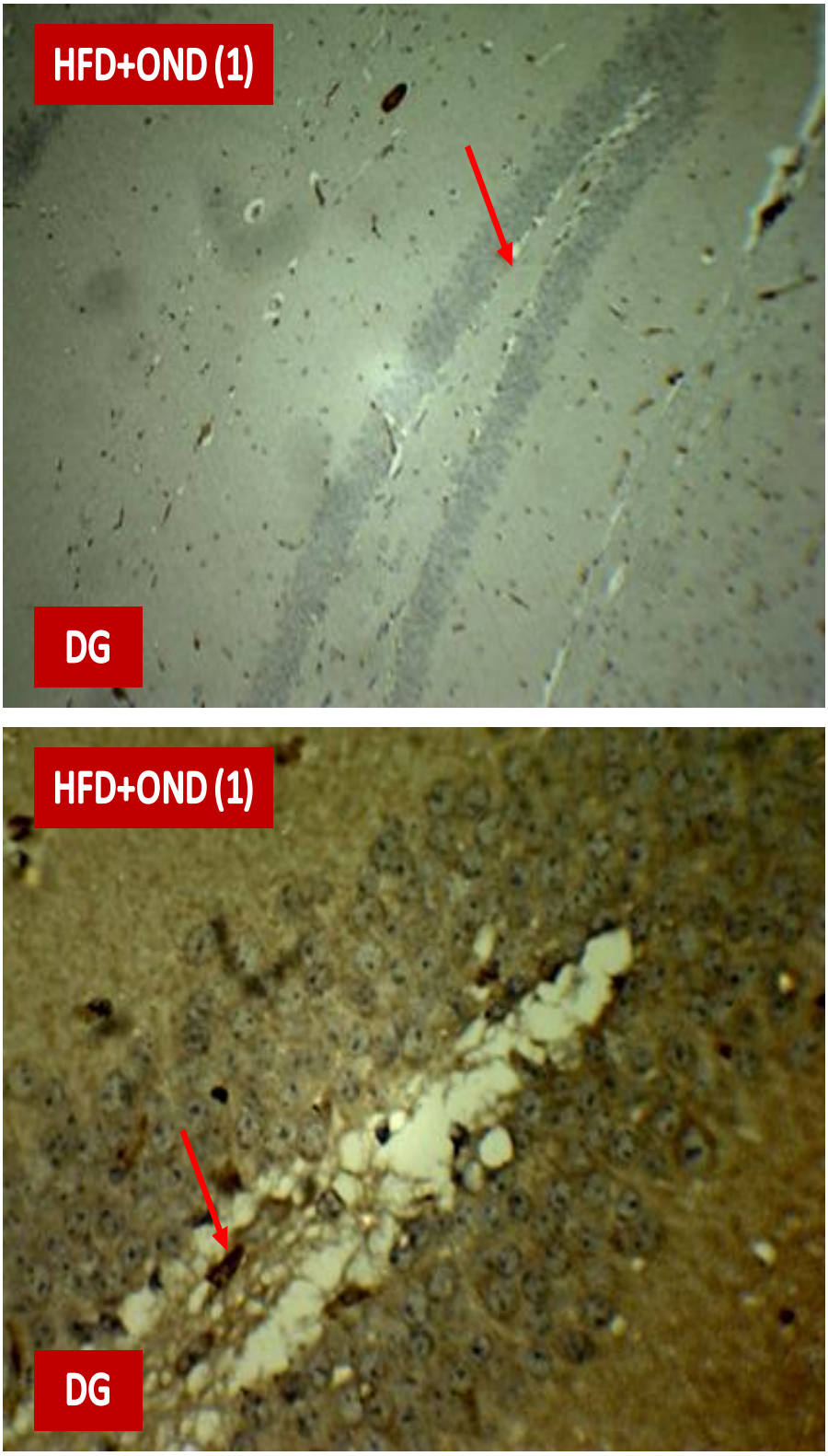


Fig 5.68: Effect of **OND** on % area of p53 in hippocampal DG region of HFD fed mice (red arrow indicate p53 presence) (Images taken at 100X and 400X).

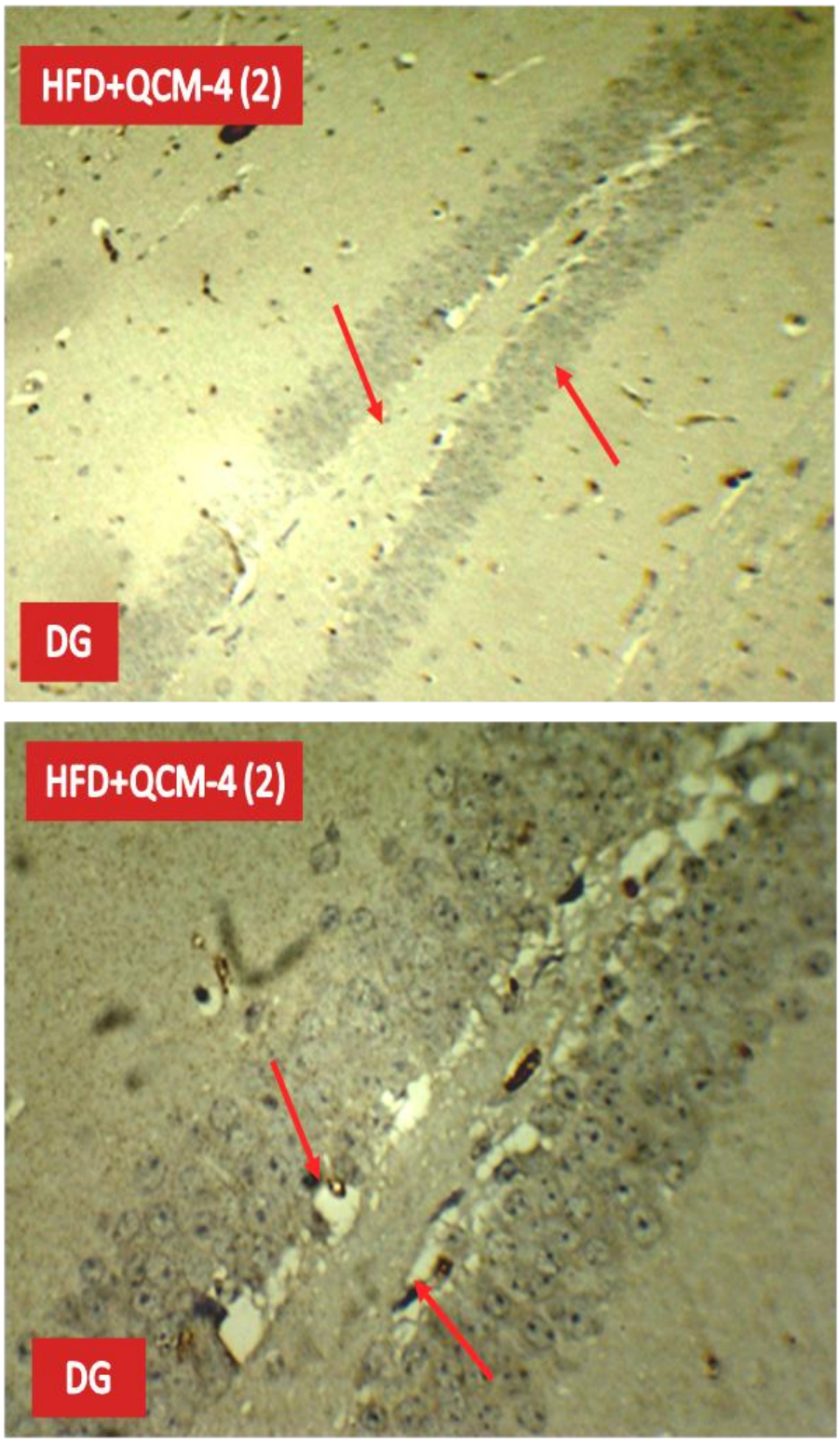


Fig 5.69: Effect of **QCM-4** on % area of p53 in hippocampal DG region of HFD fed mice (red arrow indicate p53 presence) (Images taken at 100X and 400X).

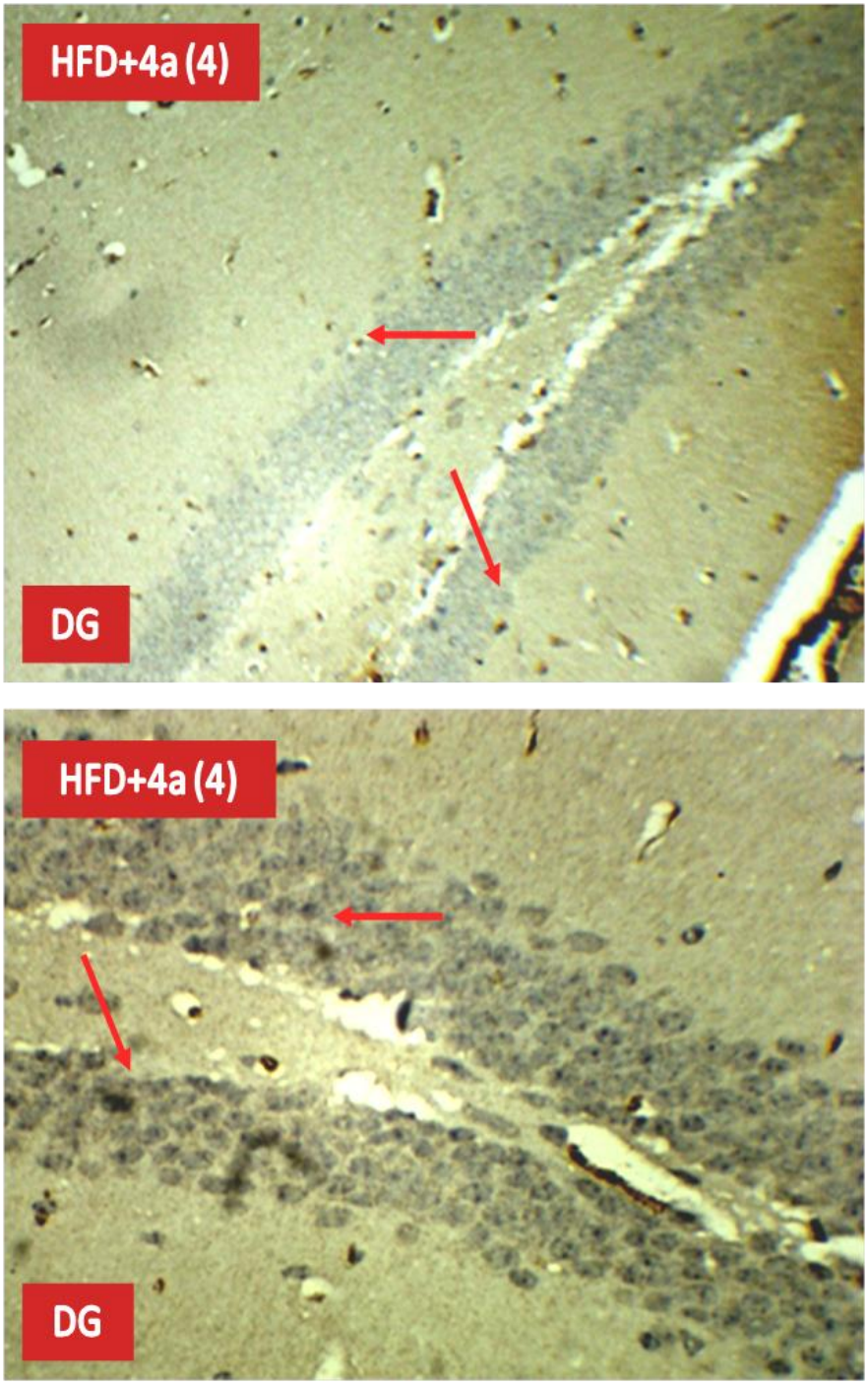


Fig 5.70: Effect of **4a** on % area of p53 in hippocampal DG region of HFD fed mice (red arrow indicates p53 presence) (Images taken at 100X and 400X).

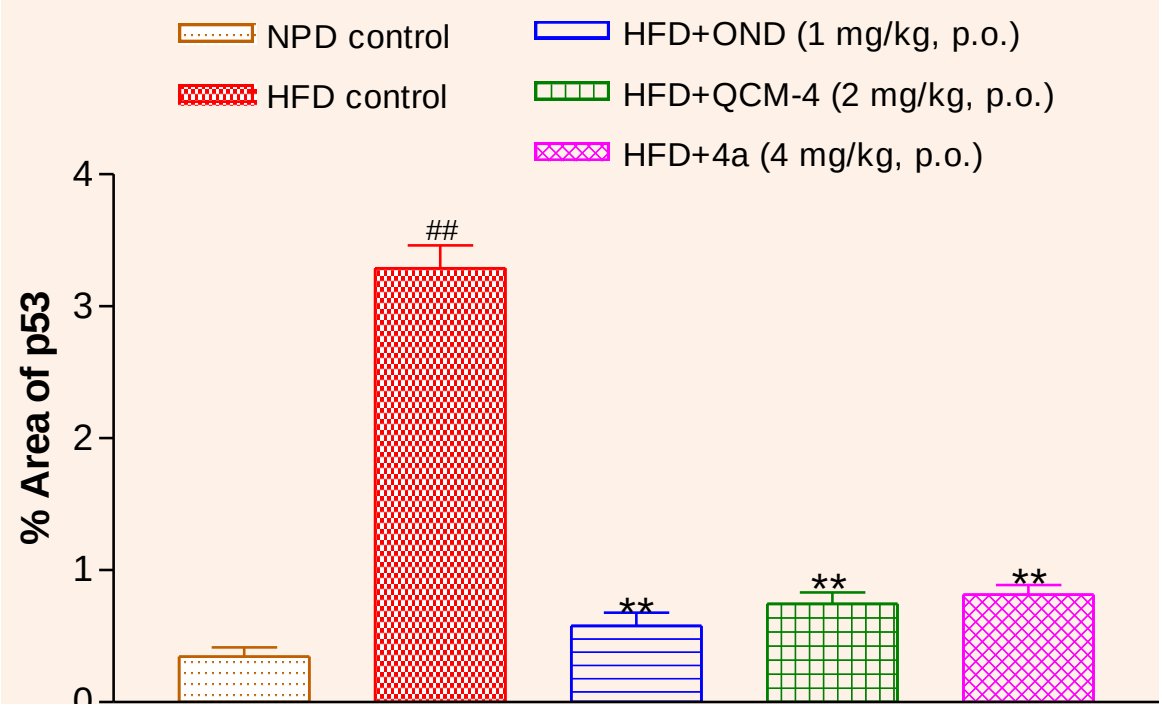


Fig 5.71: Effect of **OND**, **QCM-4** and **4a** on % area of p53 in the hippocampal DG region of HFD fed mice. ##P<0.01 vs NPD control, **P<0.01 vs HFD control.

5.20. Confirmatory study - Role of 5-HT_3 receptors in antidepressant-like effect: Effect of 5-HT_3 receptor antagonist OND on depression co-morbid with obesity in HFD mice pre-treated with 5-HT_3 receptor agonist mCPBG

5.20.1. Effect of OND on behavioral models of depression in HFD fed mice pre-treated with mCPBG

5.20.1.1. Effect of OND on body weight (g) of HFD mice pre-treated with mCPBG

The effect of **OND** on body weight of HFD fed mice injected with mCPBG is represented in **Table 5.62**. Chronic mCPBG (10 mg/kg, i.p.) injections in HFD mice exhibited no significant (P>0.05) effect on body weight as compared to HFD control group. Repetitive treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed no significant effect on the initial [F (5, 30) = 0.04, P>0.05] and final [F (5, 30) = 0.06, P>0.05] body weight of HFD and HFD+mCPBG mice, compared to HFD and HFD+mCPBG control groups, respectively.

Table 5.62: Effect of OND on body weight of HFD fed mice injected with mCPBG

Groups	Initial body weight (g)	Final body weight (g)
HFD control	40.50 ± 2.99	41.83 ± 3.24
HFD + mCPBG (10 mg/kg, i.p.)	39.50 ± 2.57	40.50 ± 2.68
HFD + OND (0.5 mg/kg, p.o.)	40.67 ± 2.99	41.83 ± 3.39
HFD + mCPBG + OND (0.5 mg/kg, p.o.)	41.33 ± 3.14	42.00 ± 2.35
HFD + OND (1 mg/kg, p.o.)	40.83 ± 2.63	41.50 ± 3.06
HFD + mCPBG + OND (1 mg/kg, p.o.)	40.50 ± 3.05	40.33 ± 2.38

The values are expressed as mean ± S.E.M., n = 6/group

5.20.1.2. Effect of OND on SPT in HFD mice pre-treated with mCPBG

The effect of **OND** treatment on preference for sucrose consumption in HFD mice injected with mCPBG is shown in **Fig 5.72**. HFD mice injected with mCPBG (10 mg/kg, i.p.) exhibited no marked (P>0.05) effect on sucrose solution consumption as compared to HFD control group. Multiple treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed significantly [F (5, 30) = 7.53, P<0.05] increased sucrose consumption in HFD mice compared to HFD control group, whereas showed no significant (P>0.05) effect on sucrose consumption in HFD mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

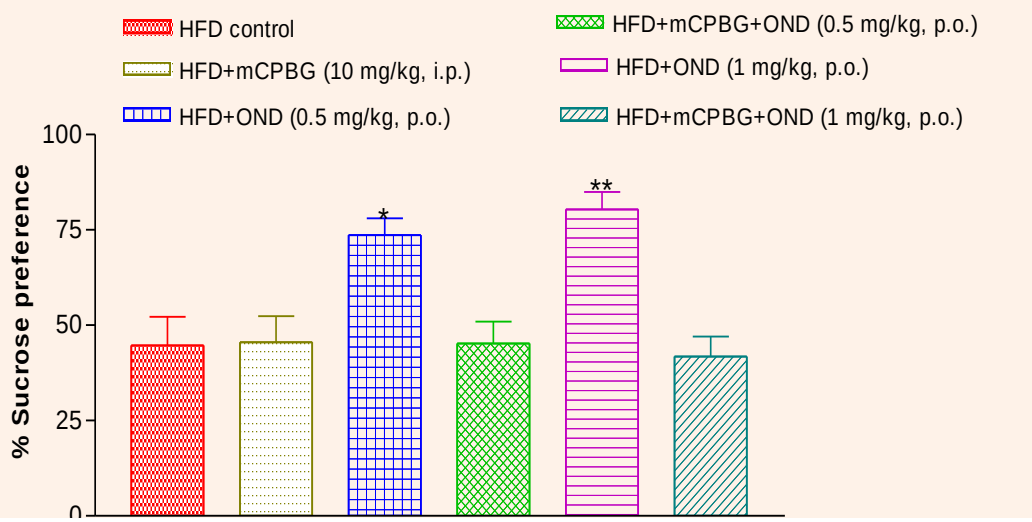


Fig 5.72: Effect of **OND** on SPT in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.20.1.3. Effect of OND on SLA score in HFD mice injected mCPBG

Repetitive injections of mCPBG (10 mg/kg, i.p.) showed no marked (P>0.05) effect on basal locomotor activity score in HFD mice as compared to HFD control group. Chronic treatment with **OND** (0.5 and 1 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) showed no observable [F (5, 30) = 0.008, P>0.05] alterations in the locomotor activity score in HFD and HFD mice pre-treated with mCPBG as compared to HFD and HFD+mCPBG control group, as represented in **Fig 5.73**.

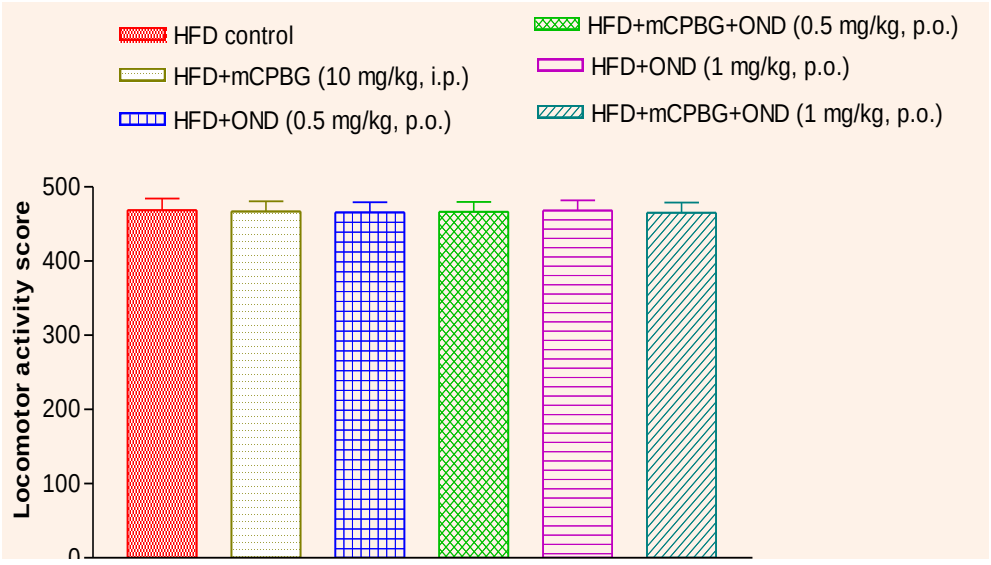


Fig 5.73: Effect of **OND** on SLA score in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., n=6/group.

5.20.1.4. Effect of OND on FST in HFD mice pre-treated with mCPBG

The effect of **OND** on duration of immobility in FST in HFD mice injected with mCPBG is demonstrated in **Fig 5.74**. Chronic injections of mCPBG (10 mg/kg, i.p.) in HFD mice showed no marked (P>0.05) effect on immobility time in FST as compared to HFD control mice. Multiple treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (5, 30) = 22.32, P<0.01] inhibited the immobility time in HFD mice compared HFD control group, whereas no significant (P>0.05) effect on immobility time was observed in HFD mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

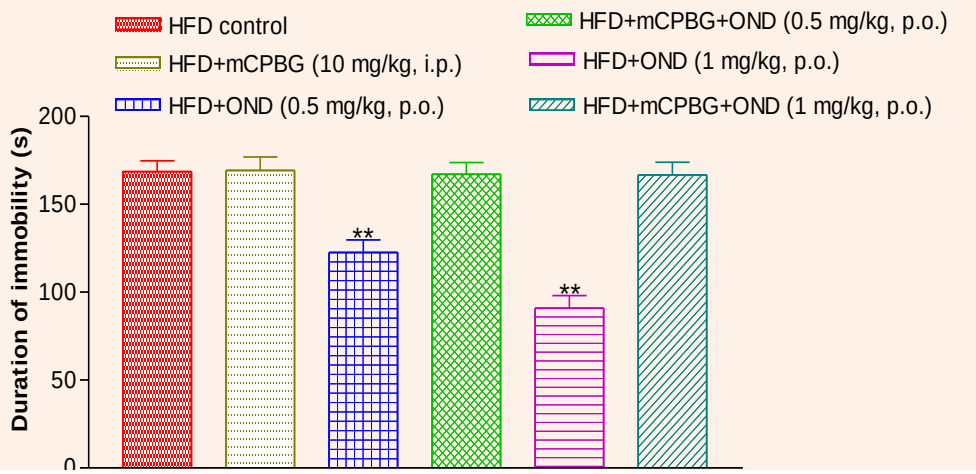


Fig 5.74: Effect of **OND** on duration of immobility in HFD fed mice injected with mCPBG in FST. The values are expressed as mean ± S.E.M., **P<0.01 vs HFD control group, n=6/group.

5.20.1.5. Effect of OND on TST in HFD mice pre-treated with mCPBG

Chronic injections of mCPBG (10 mg/kg, i.p.) in HFD mice showed no remarkable (P>0.05) effect on immobility time in TST as compared to HFD control group. Repetitive treatment with **OND** (0.5 and 1mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (5, 30) = 12.63, P<0.05] decreased the immobility time in HFD mice compared HFD control group, whereas no significant (P>0.05) effect on immobility time was observed in HFD mice pre-treated with mCPBG as compared to HFD+mCPBG control group, as represented in **Fig 5.75**.

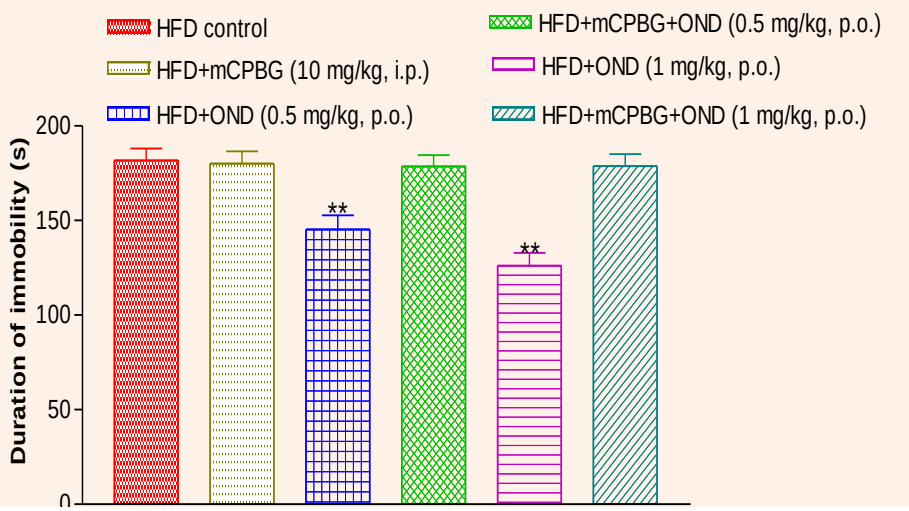


Fig 5.75: Effect of **OND** on duration of immobility in HFD fed mice injected with mCPBG in TST. The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.20.1.6. Effect of OND on EPM in HFD mice pre-treated with mCPBG

Chronic mCPBG (10 mg/kg, i.p.) injections in HFD mice showed no marked (P>0.05) effect on % OAE and % OAT compared to HFD control group. Multiple treatment with **OND** (0.5 and 1 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) in HFD mice showed significantly increased % OAE [F (5, 30) = 13.11, P<0.01] and % OAT [F (5, 30) = 7.62, P<0.05] as compared to HFD control group, whereas no significant (P>0.05) effect was observed in mCPBG injected HFD mice as compared to HFD+mCPBG control group (**Table 5.63**).

Table 5.63: Effect of OND on EPM in HFD fed mice injected with mCPBG

Groups	% OAE	% OAT
HFD control	13.75 ± 3.39	8.78 ± 1.14
HFD + mCPBG (10 mg/kg, i.p.)	13.58 ± 3.35	8.67 ± 1.30
HFD + OND (0.5 mg/kg, p.o.)	36.44 ± 5.30**	14.78 ± 1.47*
HFD + mCPBG + OND (0.5 mg/kg, p.o.)	14.28 ± 3.79	8.72 ± 1.30
HFD + OND (1 mg/kg, p.o.)	48.12 ± 5.03**	16.72 ± 1.54**
HFD + mCPBG + OND (1 mg/kg, p.o.)	15.16 ± 3.48	8.89 ± 1.15

The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n = 6/group

5.20.1.7. Effect of OND on L/D in HFD mice pre-treated with mCPBG

The effect of **OND** treatment on L/D test in mCPBG injected HFD mice is shown in **Table 5.64**. Chronic mCPBG (10 mg/kg, i.p.) injections in HFD mice showed no remarkable (P>0.05) effect on parameters of L/D test compared to HFD control group. Repetitive treatment with **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in HFD mice showed significantly increased time in light chamber [F (5, 30) = 9.82, P<0.05] and transition score [F (5, 30) = 10.02, P<0.05] as compared to HFD control group, whereas no significant (P>0.05) effect was observed in HFD mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

Table 5.64: Effect of OND on L/D in HFD fed mice injected with mCPBG

Groups	Time in light chamber (s)	Transition score
HFD control	13.50 ± 3.55	11.00 ± 2.28
HFD + mCPBG (10 mg/kg, i.p.)	13.33 ± 3.25	11.33 ± 2.56
HFD + OND (0.5 mg/kg, p.o.)	30.17 ± 3.32*	23.17 ± 2.20*
HFD + mCPBG + OND (0.5 mg/kg, p.o.)	13.83 ± 3.67	11.50 ± 1.91
HFD + OND (1 mg/kg, p.o.)	37.50 ± 3.48**	27.83 ± 2.83**
HFD + mCPBG + OND (1 mg/kg, p.o.)	14.00 ± 3.12	11.67 ± 2.22

The values are expressed as mean ± S.E.M.,*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.20.2. Effect of OND on hippocampal 5-HT concentration in HFD mice pre-treated with mCPBG

The effect of **OND** treatment on hippocampus 5-HT level in mCPBG injected HFD mice is demonstrated in **Fig 5.76**. Chronic injections of mCPBG (10 mg/kg, i.p.) in HFD mice exhibited no marked (P>0.05) change in the hippocampal 5-HT level as compared to HFD control group. Repetitive administration of **OND** (0.5 and 1 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed significantly [F (5, 30) = 47.01, P<0.01] increased 5-HT level in HFD mice compared to HFD control group, whereas showed no significant (P>0.05) effect on 5-HT concentration in mCPBG pre-treated HFD mice as compared to HFD+mCPBG control group.

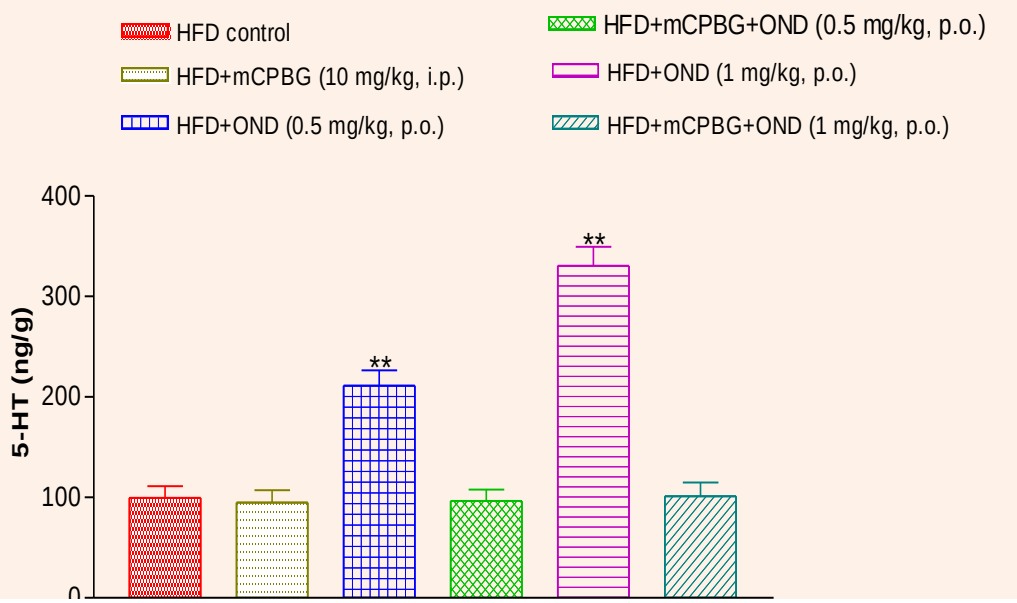


Fig 5.76: Effect of **OND** on hippocampus 5-HT concentration in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., **P<0.01 vs HFD control group, n=6/group.

5.21. Confirmatory study - Role of 5-HT_3 receptors in antidepressant-like effect: Effect of 5-HT_3 receptor antagonist QCM-4 on depression co-morbid with obesity in HFD mice pre-treated with 5-HT_3 receptor agonist mCPBG

5.21.1. Effect of QCM-4 on behavioral models of depression in HFD fed mice pre-treated with mCPBG

5.21.1.1. Effect of QCM-4 on body weight (g) of HFD mice pre-treated with mCPBG

The effect of **QCM-4** on body weight of chronic mCPBG injected HFD fed mice is shown in **Table 5.65**. Repetitive mCPBG (10 mg/kg, i.p.) injections in HFD mice showed no marked ($P>0.05$) change in the body weight as compared to HFD control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) exhibited no significant effect on the initial [$F(5, 30) = 0.04$, $P>0.05$] and final [$F(5, 30) = 0.06$, $P>0.05$] body weight of HFD and mCPBG injected HFD mice as compared to HFD and HFD+mCPBG control groups, respectively.

Table 5.65: Effect of QCM-4 on body weight of HFD fed mice injected with mCPBG

Groups	Initial body weight (g)	Final body weight (g)
HFD control	40.17 ± 2.21	41.33 ± 2.25
HFD + mCPBG (10 mg/kg, i.p.)	40.33 ± 2.59	41.83 ± 2.68
HFD + QCM-4 (1 mg/kg, p.o.)	39.67 ± 3.15	40.67 ± 2.23
HFD + mCPBG + QCM-4 (1 mg/kg, p.o.)	41.50 ± 3.00	42.17 ± 2.96
HFD + QCM-4 (2 mg/kg, p.o.)	41.17 ± 2.63	42.00 ± 2.44
HFD + mCPBG + QCM-4 (2 mg/kg, p.o.)	40.83 ± 2.75	41.50 ± 2.81

The values are expressed as mean ± S.E.M., n = 6/group

5.21.1.2. Effect of QCM-4 on SPT in HFD mice pre-treated with mCPBG

Chronic injections of mCPBG (10 mg/kg, i.p.) in HFD mice showed no remarkable ($P>0.05$) effect on consumption of sucrose solution as compared to HFD control group. Repetitive administration of **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) exhibited significantly [$F(5, 30) = 8.54$, $P<0.05$] improved sucrose consumption in HFD mice as compared to HFD control group, whereas showed no significant ($P>0.05$) effect on sucrose consumption in HFD mice pre-treated with mCPBG as compared to HFD+mCPBG control group, as represented in **Fig 5.77**.

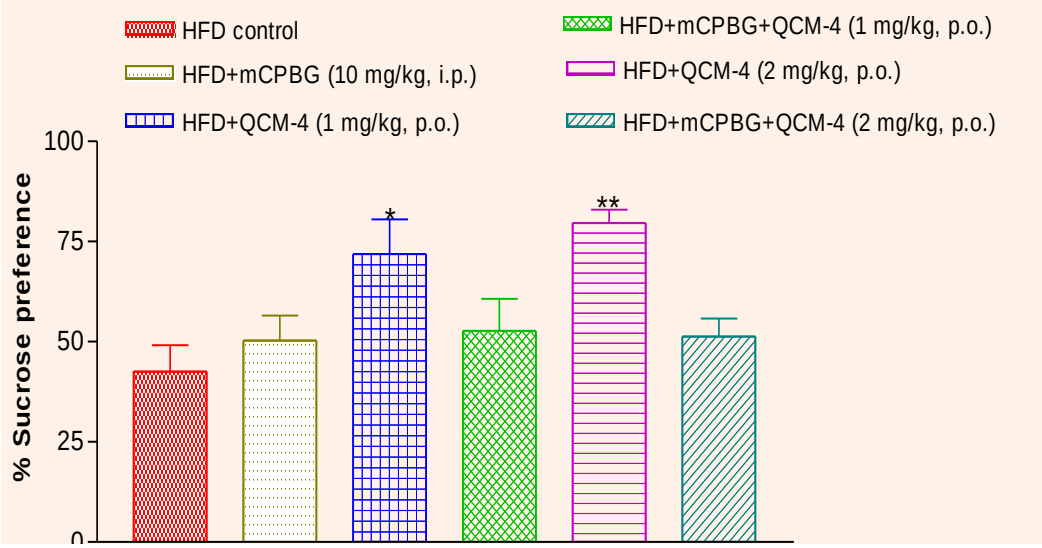


Fig 5.77: Effect of **QCM-4** on SPT in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.21.1.3. Effect of QCM-4 on SLA score in HFD mice pre-treated with mCPBG

Chronic injections of mCPBG (10 mg/kg, i.p.) showed no remarkable (P>0.05) alteration in the locomotor activity score of HFD mice as compared to HFD control group. Multiple treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed no significant [F (5, 30) = 0.017, P>0.05] change in the locomotor activity score in HFD and mCPBG injected HFD mice as compared to HFD and HFD+mCPBG control groups, respectively, as demonstrated in **Fig 5.78**.

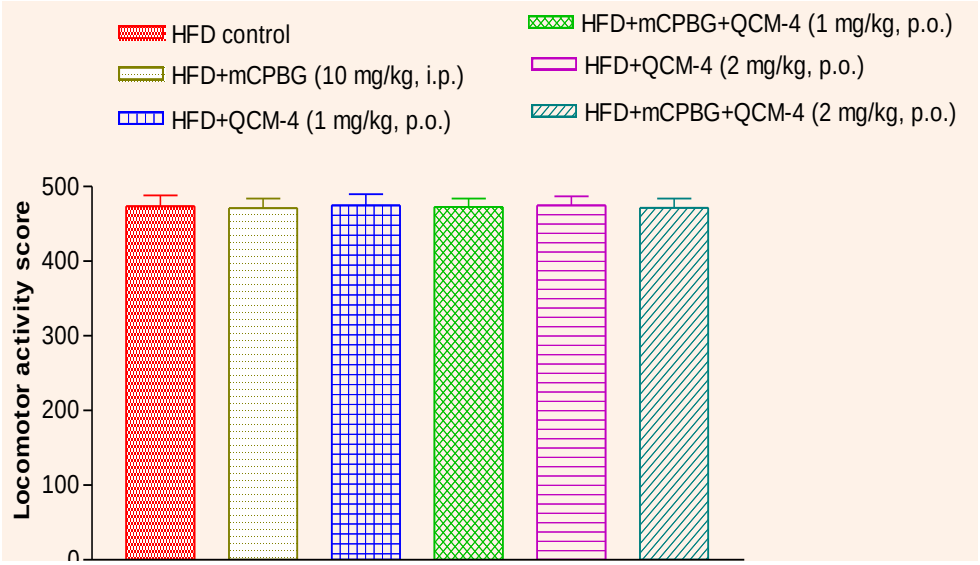


Fig 5.78: Effect of **QCM-4** on locomotor activity score in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., n=6/group.

5.21.1.4. Effect of QCM-4 on FST in HFD mice pre-treated with mCPBG

The effect of **QCM-4** on immobility time in FST in mCPBG injected HFD mice is represented in **Fig 5.79**. Repetitive injections of mCPBG (10 mg/kg, i.p.) in HFD mice exhibited no remarkable (P>0.05) change in the immobility time in FST compared to HFD control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (5, 30) = 17.46, P<0.01] decreased the immobility time in HFD mice compared HFD control group, whereas showed no significant (P>0.05) effect on immobility time in HFD mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

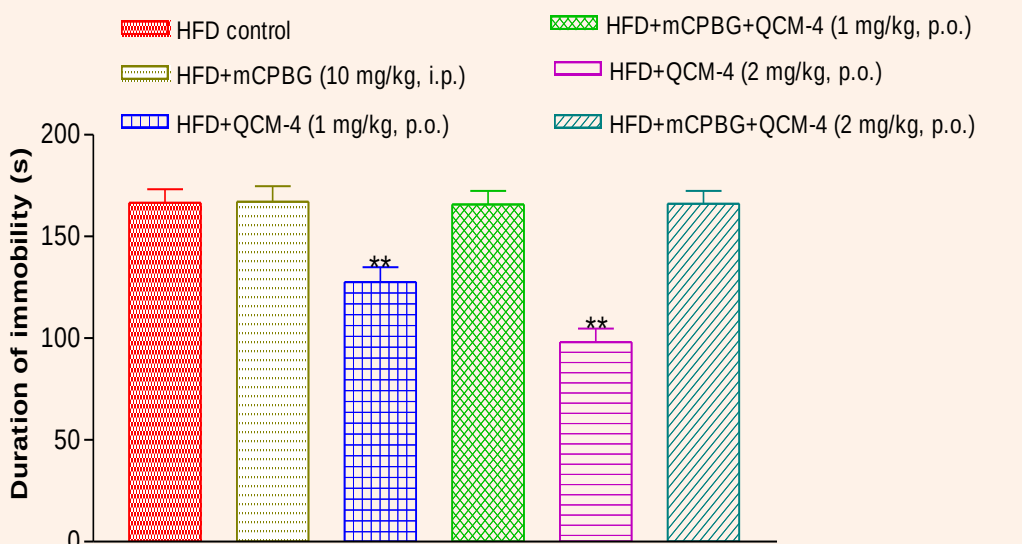


Fig 5.79: Effect of **QCM-4** on duration of immobility in HFD fed mice injected with mCPBG in FST. The values are expressed as mean ± S.E.M., **P<0.01 vs HFD control group, n=6/group.

5.21.1.5. Effect of QCM-4 on TST in HFD mice pre-treated with mCPBG

The effect of **QCM-4** on immobility time in TST in mCPBG injected HFD mice is shown in **Fig 5.80**. Chronic injections of mCPBG (10 mg/kg, i.p.) in HFD mice showed no marked (P>0.05) change in the immobility time in TST as compared to HFD control group. Multiple treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (5, 30) = 8.92, P<0.05] reduced the immobility time in HFD mice compared HFD control group, whereas showed no significant (P>0.05) effect on immobility time in HFD mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

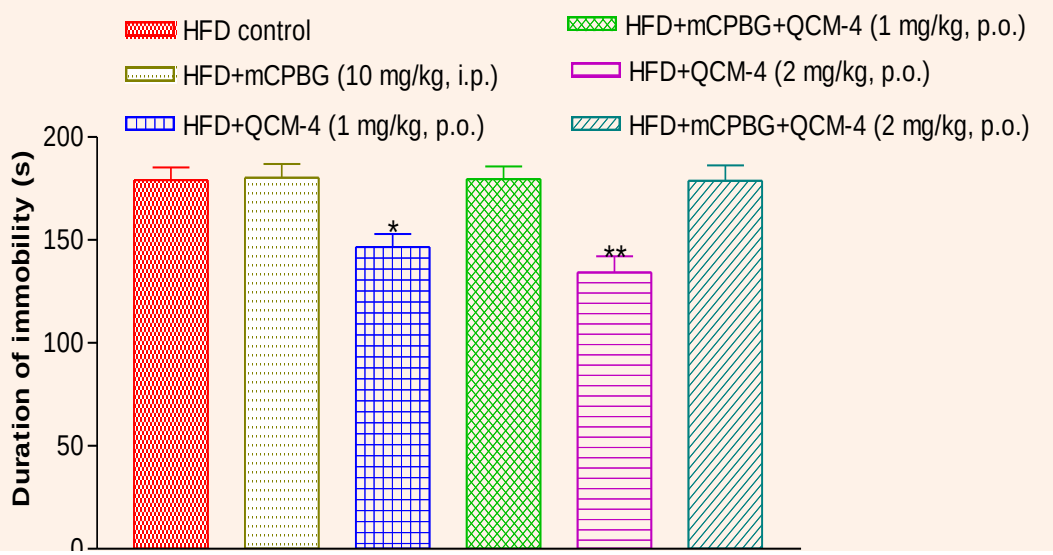


Fig 5.80: Effect of QCM-4 on duration of immobility in HFD fed mice injected with mCPBG in TST. The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.21.1.6. Effect of QCM-4 on EPM in HFD mice pre-treated with mCPBG

Chronic mCPBG (10 mg/kg, i.p.) injections in HFD mice showed no marked (P>0.05) change in % OAE and % OAT as compared to HFD control group. Repetitive treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in HFD mice showed significantly increased % OAE [F (5, 30) = 11.98, P<0.05] and % OAT [F (5, 30) = 7.15, P<0.05] as compared to HFD control group, whereas no significant (P>0.05) change was observed in HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group (**Table 5.66**).

Table 5.66: Effect of QCM-4 on EPM in HFD fed mice injected with mCPBG

Groups	% OAE	% OAT
HFD control	12.95 ± 2.80	8.50 ± 0.99
HFD + mCPBG (10 mg/kg, i.p.)	13.80 ± 2.90	8.61 ± 1.23
HFD + QCM-4 (1 mg/kg, p.o.)	32.36 ± 4.18*	14.33 ± 1.19*
HFD + mCPBG + QCM-4 (1 mg/kg, p.o.)	14.23 ± 4.35	8.44 ± 1.19
HFD + QCM-4 (2 mg/kg, p.o.)	43.61 ± 4.65**	15.44 ± 1.53**
HFD + mCPBG + QCM-4 (2 mg/kg, p.o.)	14.53 ± 3.14	8.67 ± 1.19

The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n = 6/group

5.21.1.7. Effect of QCM-4 on L/D in HFD mice pre-treated with mCPBG

Repetitive mCPBG (10 mg/kg, i.p.) injections in HFD mice exhibited no remarkable (P>0.05) change in the parameters of L/D test as compared to HFD control group. Chronic treatment with **QCM-4** (1 and 2 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) in HFD mice showed significantly increased time in light chamber [F (5, 30) = 9.08, P<0.05] and number of transitions [F (5, 30) = 6.65, P<0.05] compared to HFD control group, whereas showed no significant (P>0.05) change in HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group, as represented in **Table 5.67**.

Table 5.67: Effect of QCM-4 on L/D in HFD fed mice injected with mCPBG

Groups	Time in light chamber (s)	Transition score
HFD control	14.67 ± 2.32	10.00 ± 2.72
HFD + mCPBG (10 mg/kg, i.p.)	14.00 ± 3.25	11.33 ± 2.56
HFD + QCM-4 (1 mg/kg, p.o.)	30.00 ± 2.58*	23.33 ± 2.40*
HFD + mCPBG + QCM-4 (1 mg/kg, p.o.)	14.33 ± 4.17	11.67 ± 3.66
HFD + QCM-4 (2 mg/kg, p.o.)	35.17 ± 3.08**	26.67 ± 2.99**
HFD + mCPBG + QCM-4 (2 mg/kg, p.o.)	14.83 ± 3.20	12.00 ± 2.19

The values are expressed as mean ± S.E.M.,*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.21.2. Effect of QCM-4 on hippocampal 5-HT concentration in HFD mice pre-treated with mCPBG

The effect of **QCM-4** treatment on hippocampal 5-HT level in HFD mice injected with mCPBG is demonstrated in **Fig 5.81**. Chronic injections of mCPBG (10 mg/kg, i.p.) in HFD mice showed no marked (P>0.05) alteration in the hippocampal 5-HT level as compared to HFD control group. Multiple administration of **QCM-4** (1 and 2 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed significantly [F (5, 30) = 30.12, P<0.01] increased 5-HT concentration in HFD mice as compared to HFD control group, whereas showed no significant (P>0.05) effect on 5-HT level in HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

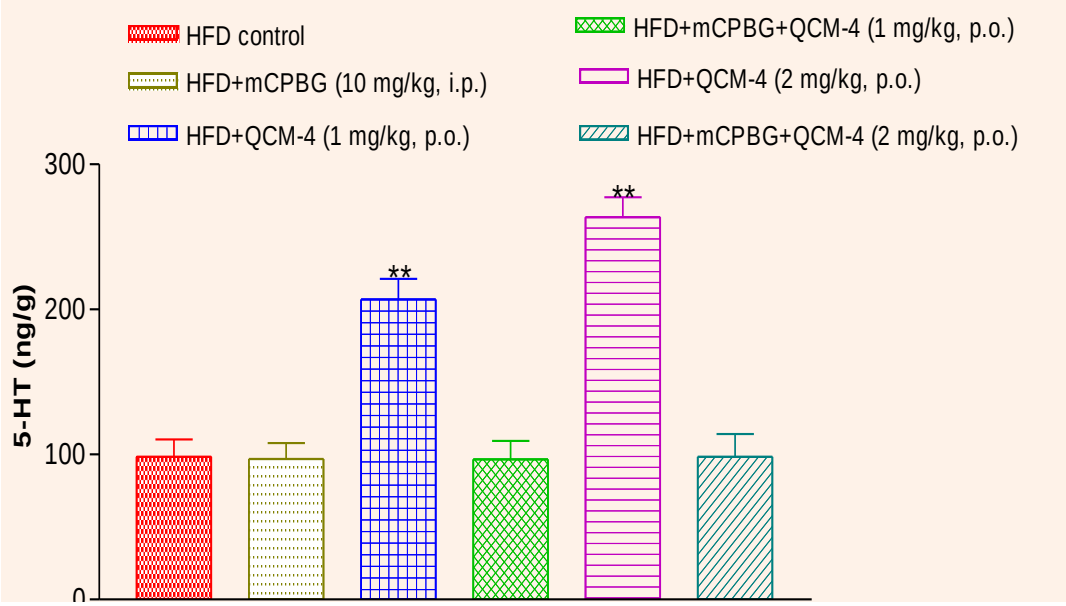


Fig 5.81: Effect of **QCM-4** on hippocampus 5-HT concentration in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., **P<0.01 vs HFD control group, n=6/group.

5.22. Confirmatory study - Role of 5-HT$_3$ receptors in antidepressant-like effect: Effect of 5-HT$_3$ receptor antagonist 4a on depression co-morbid with obesity in HFD mice pre-treated with 5-HT$_3$ receptor agonist mCPBG

5.22.1. Effect of 4a on behavioral models of depression in HFD fed mice pre-treated with mCPBG

5.22.1.1. Effect of 4a on body weight (g) of HFD mice pre-treated with mCPBG

The effect of **4a** on body weight of mCPBG injected HFD fed mice is demonstrated in **Table 5.68**. Chronic mCPBG (10 mg/kg, i.p.) injections in HFD mice showed no marked (P>0.05) alteration in the body weight as compared to HFD control group. Multiple treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) exhibited no significant effect on the initial [F (5, 30) = 0.02, P>0.05] and final [F (5, 30) = 0.03, P>0.05] body weight of HFD and mCPBG injected HFD mice as compared to HFD and HFD+mCPBG control groups, respectively.

Table 5.68: Effect of 4a on body weight of HFD fed mice injected with mCPBG

Groups	Initial body weight (g)	Final body weight (g)
HFD control	40.50 ± 2.81	41.33 ± 2.17
HFD + mCPBG (10 mg/kg, i.p.)	40.17 ± 3.30	41.17 ± 2.14
HFD + 4a (2 mg/kg, p.o.)	40.00 ± 3.15	40.83 ± 2.82
HFD + mCPBG + 4a (2 mg/kg, p.o.)	41.00 ± 2.73	42.00 ± 2.85
HFD + 4a (4 mg/kg, p.o.)	40.33 ± 2.95	41.50 ± 3.28
HFD + mCPBG + 4a (4 mg/kg, p.o.)	39.67 ± 2.69	40.67 ± 3.25

The values are expressed as mean ± S.E.M., n = 6/group

5.22.1.2. Effect of 4a on SPT in HFD mice injected mCPBG

Repetitive injections of mCPBG (10 mg/kg, i.p.) in HFD mice showed no remarkable (P>0.05) effect on consumption of sucrose solution compared to HFD control group. Chronic administration of **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed significantly [F (5, 30) = 6.72, P<0.05] increased sucrose consumption in HFD mice as compared to HFD control group, whereas showed no significant (P>0.05) effect on sucrose solution consumption in HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group, as shown in **Fig 5.82**.

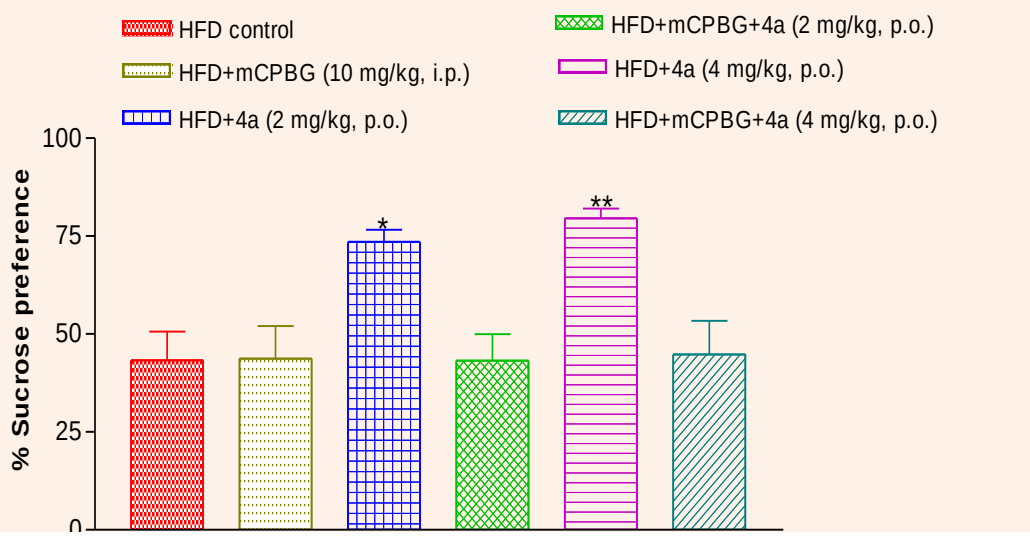


Fig 5.82: Effect of **4a** on SPT in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.22.1.3. Effect of 4a on SLA score in HFD mice pre-treated with mCPBG

The effect of **4a** treatment on locomotor activity score in mCPBG injected HFD is demonstrated in **Fig 5.83**. Chronic injections of mCPBG (10 mg/kg, i.p.) showed no remarkable (P>0.05) change in the basal locomotor activity score of HFD mice as compared to HFD control group. Chronic dosing with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) showed no significant [F (5, 30) = 0.002, P>0.05] alteration in the locomotor activity score in HFD and mCPBG injected HFD mice as compared to HFD and HFD+mCPBG control groups, respectively.

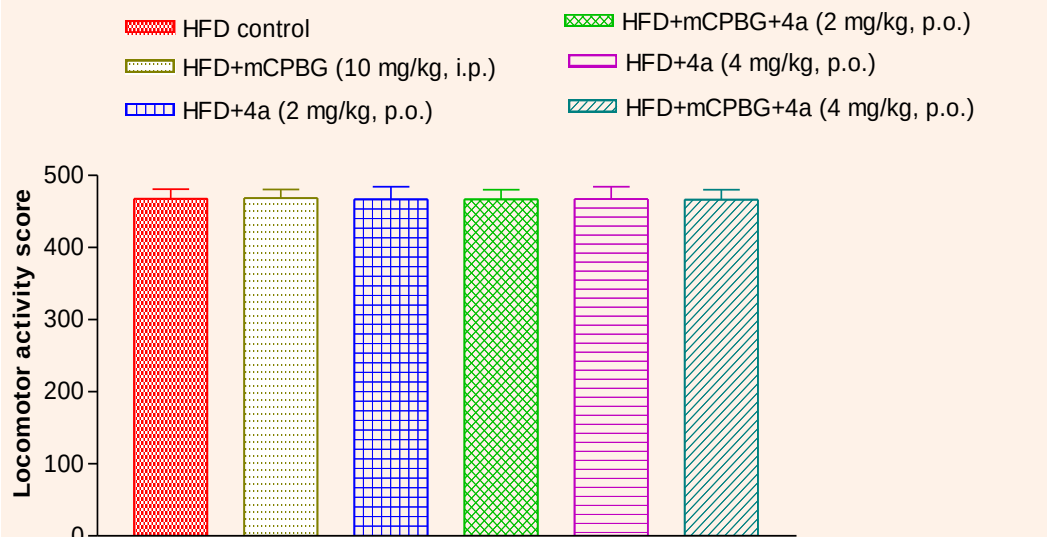


Fig 5.83: Effect of **4a** on SLA score in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., n=6/group.

5.22.1.4. Effect of 4a on FST in HFD mice pre-treated with mCPBG

Fig 5.84 showed the effect of **4a** treatment on immobility time in FST in mCPBG injected HFD mice. Chronic injections of mCPBG (10 mg/kg, i.p.) in HFD mice showed no marked (P>0.05) change in the immobility time in FST as compared to HFD control group. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (5, 30) = 11.25, P<0.05] reduced the immobility time in HFD mice compared HFD control group, whereas showed no significant (P>0.05) change in the immobility time of HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

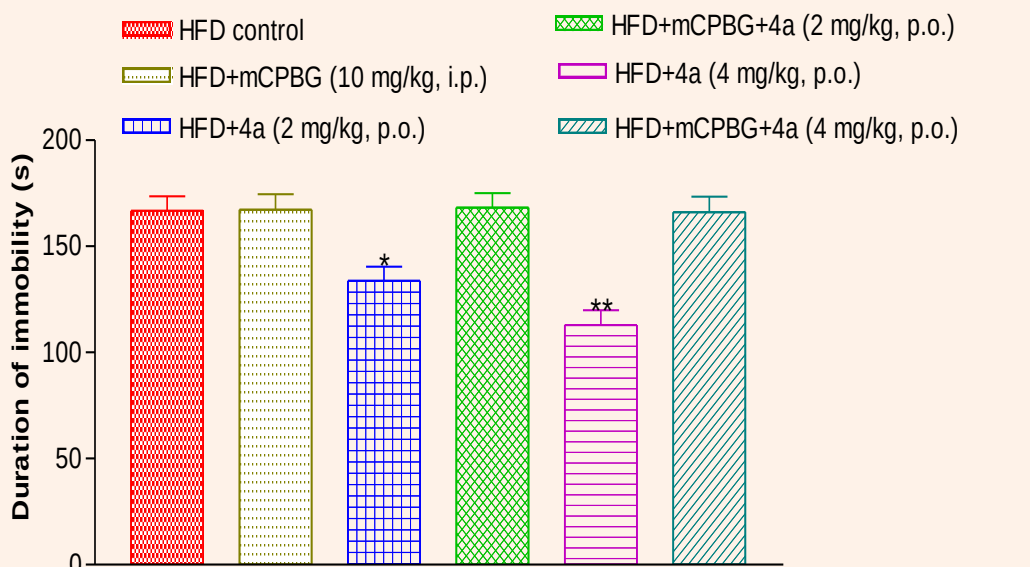


Fig 5.84: Effect of **4a** on duration of immobility in HFD fed mice injected with mCPBG in FST. The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n=6/group.

5.22.1.5. Effect of 4a on TST in HFD mice injected mCPBG

The effect of **4a** on immobility time in TST in mCPBG injected HFD mice is represented in **Fig 5.85**. Chronic injections of mCPBG (10 mg/kg, i.p.) in HFD mice showed no remarkable (P>0.05) change in the immobility time in TST as compared to HFD control group. Chronic treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) significantly [F (5, 30) = 13.76, P<0.01] inhibited the immobility time in HFD mice compared HFD control group, whereas showed no significant (P>0.05) alteration in the immobility time of HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

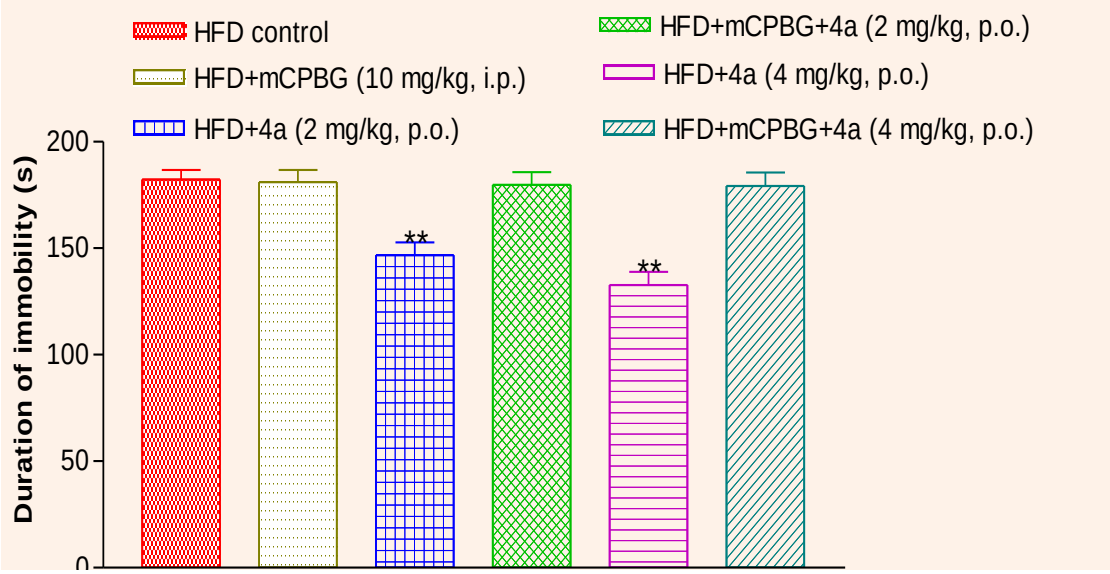


Fig 5.85: Effect of **4a** on duration of immobility in HFD fed mice injected with mCPBG in TST. The values are expressed as mean ± S.E.M., **P<0.01 vs HFD control group, n=6/group.

5.22.1.6. Effect of 4a on EPM in HFD mice pre-treated with mCPBG

Chronic mCPBG (10 mg/kg, i.p.) injections in HFD mice showed no marked (P>0.05) alteration in % OAE and % OAT as compared to HFD control group. Repetitive administration of **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in HFD mice showed significantly higher % OAE [F (5, 30) = 8.71, P<0.05] and % OAT [F (5, 30) = 6.63, P<0.05] as compared to HFD control group, whereas no significant (P>0.05) effect was observed in HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group, as represented in **Table 5.69**.

Table 5.69: Effect of 4a on EPM in HFD fed mice injected with mCPBG

Groups	% OAE	% OAT
HFD control	12.63 ± 3.65	8.17 ± 1.31
HFD + mCPBG (10 mg/kg, i.p.)	13.25 ± 3.38	8.83 ± 1.43
HFD + 4a (2 mg/kg, p.o.)	32.12 ± 5.05*	14.33 ± 1.07*
HFD + mCPBG + 4a (2 mg/kg, p.o.)	14.56 ± 3.84	9.06 ± 1.39
HFD + 4a (4 mg/kg, p.o.)	42.26 ± 5.46**	15.78 ± 1.37**
HFD + mCPBG + 4a (4 mg/kg, p.o.)	15.33 ± 3.47	9.28 ± 0.98

The values are expressed as mean ± S.E.M., *P<0.05; **P<0.01 vs HFD control group, n = 6/group

5.22.1.7. Effect of 4a on L/D in HFD mice pre-treated with mCPBG

The effect of **4a** treatment on the behavior of mCPBG injected HFD fed mice in L/D test is demonstrated in **Table 5.70**. Chronic mCPBG (10 mg/kg, i.p.) injections in HFD mice showed no remarkable (P>0.05) change in the parameters of L/D test as compared to HFD control group. Multiple treatment with **4a** (2 and 4 mg/kg, p.o.) and standard antidepressant ESC (10 mg/kg, p.o.) in HFD mice exhibited significantly higher time in light chamber [F (5, 30) = 7.16, P<0.05] and transition score [F (5, 30) = 8.96, P<0.05] compared to HFD control group, whereas showed no significant (P>0.05) alterations were observed in HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

Table 5.70: Effect of 4a on L/D in HFD fed mice injected with mCPBG

Groups	Time in light chamber (s)	Transition Score
HFD control	14.33 ± 2.30	10.00 ± 2.06
HFD + mCPBG (10 mg/kg, i.p.)	14.00 ± 3.18	10.17 ± 2.14
HFD + 4a (2 mg/kg, p.o.)	30.17 ± 2.90*	25.17 ± 3.55*
HFD + mCPBG + 4a (2 mg/kg, p.o.)	14.50 ± 4.12	10.83 ± 2.39
HFD + 4a (4 mg/kg, p.o.)	34.17 ± 4.51**	29.33 ± 3.66**
HFD + mCPBG + 4a (4 mg/kg, p.o.)	15.00 ± 3.19	11.00 ± 2.92

The values are expressed as mean ± S.E.M.,*P<0.05; **P<0.01 vs HFD control group, n = 6/group.

5.22.2. Effect of 4a on hippocampal 5-HT concentration in HFD mice pre-treated with mCPBG

The effect of **4a** treatment on hippocampal 5-HT level in HFD mice injected with mCPBG is represented in **Fig 5.86**. Chronic mCPBG (10 mg/kg, i.p.) injections in HFD mice showed no marked (P>0.05) change in the hippocampal 5-HT level as compared to HFD control group. Repetitive treatment with **4a** (2 and 4 mg/kg, p.o.) and reference antidepressant ESC (10 mg/kg, p.o.) exhibited significantly [F (5, 30) = 28.77, P<0.01] higher hippocampus 5-HT concentration in HFD mice as compared to HFD control group, whereas showed no significant (P>0.05) change in 5-HT level of HFD fed mice pre-treated with mCPBG as compared to HFD+mCPBG control group.

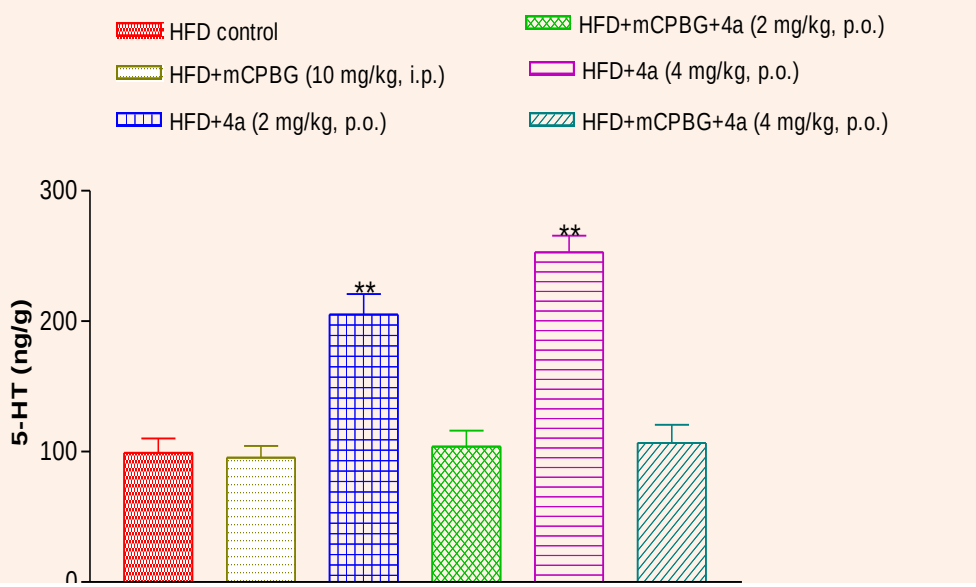


Fig 5.86: Effect of **4a** on hippocampus 5-HT concentration in HFD fed mice injected with mCPBG. The values are expressed as mean ± S.E.M., **P<0.01 vs HFD control group, n=6/group.

6. DISCUSSION

Depression and obesity are among the most prevalent and frequently occurring disorders that affect the quality of life, productivity, job efficiency, socio-economic status and increase the global burden of diseases. Depression and obesity affects around 350 and 500 million people across the globe, respectively. Obesity has been identified as a major risk factor for depression, where more than 50% of the obese individuals are twice susceptible for depression. Despite the enormous research in the area of neuropsychopharmacology, the biological mechanisms involved in depression co-morbid with obesity still remains unclear. The present pharmacotherapy is not efficient to treat depression co-morbid with obesity due to serious side effects such as modest efficacy, suicidality, tolerability, slow onset of response, withdrawal symptoms, weight gain, etc.

The major challenge for the scientists remain to conduct the research based on the biological mechanisms involved in depression co-morbid with obesity and to practice the novel therapeutic alternatives that could potentially combat such serious co-morbid disorders. The present study deals with the biological mechanisms linking depression and obesity such as HPA axis hyperactivity, oxidative stress, insulin and leptin resistance, decreased BDNF level, altered hippocampal DG neuronal morphology and increased p53 protein expression in the DG region of hippocampus, and reduced neurotransmitter 5-HT level. 5-HT_3 receptor antagonists were used as a novel therapeutic approach in the present study.

5-HT_3 receptors present in the hippocampus are involved in the regulation of mood disorders through modulation of the 5-HT neurotransmitter systems (Bétry et al., 2011). Vortioxetine has been reported to have multimodal therapeutic actions such as on mood and anxiety disorders by acting differently at various 5-HT receptors, namely; as a agonist at 5-HT_{1B}, antagonist at 5-HT_3 and 5-HT_7 receptors, respectively (Mørk et al., 2012). The mechanism for antidepressant effect of the 5-HT_3 receptor antagonists suggest that, they bind to the post-synaptic ligand gated ion channel receptors and hence, inhibit fast synaptic transmission of 5-HT. This increases the synaptic availability of 5-HT that acts through other receptors of 5-HT family mainly as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2B}, 5-HT_{2C} (Ramamoorthy et al., 2008, Rajkumar and Mahesh, 2010).

Based on the pA_2 and log P values, **QCM-4** and **4a** were selected from the newly synthesized in-house 5-HT_3 receptor antagonists in the present study. In the preliminary screening, **QCM-4** and **4a** compounds were studied for the antidepressant and anxiolytic activities in acute and chronic rodent models of depression. Once the potency of **QCM-4** and **4a** compounds against depression and anxiety in the preliminary screening was confirmed, they were used for depression co-morbid with obesity in HFD fed mice along with **OND** (as one of the test drug).

One of the major issues in the research of depression co-morbid with obesity at the experimental laboratory level is lack of ideal animal model that mimic the biological mechanisms involved in depression co-morbid with obesity. HFD induced obesity is a very commonly used model for developing obesity in laboratory animals. Initially, mice fed with HFD were screened for depressive behavior and HFD fed animals were treated with **OND**, **QCM-4** and **4a**. CUMS is an important rodent model of depression as it mimics most of the clinical phenotypes of depression. Hence, HFD fed mice were subjected to CUMS procedure and then screened for depressive behavior using **OND**, **QCM-4** and **4a**, as treatment regimen. Both the models, HFD and HFD+CUMS reflected most of the pathological mechanisms involved in depression co-morbid with obesity as observed from the behavioral, biochemical and molecular assays in the present study.

The results obtained in the present neuropsychopharmacological investigation showed the antidepressant potential of 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** on depression co-morbid with obesity. In the preliminary screening, **QCM** and **4a** exhibited antidepressant effect in high predictive validity tests such as FST, TST and anxiolytic effect in behavioral assays including EPM, HBT and L/D. In the chronic models of depression, such as OBX and CUMS, **QCM-4** and **4a**, showed potent antidepressant effect. Furthermore, chronic treatment with **OND**, **QCM-4** and **4a**, showed antidepressant effect in HFD and HFD+CUMS induced depressive behavior in mice. In addition, pre-treatment of 5-HT_3 receptor agonist mCPBG in HFD fed mice inhibited the antidepressant effect of 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** thus, confirming the role of 5-HT_3 receptor antagonism for antidepressant effect in depression co-morbid with obesity.

6.1. Antidepressant potential of novel 5-HT_3 receptor antagonists QCM-4 and 4a in behavioral assays of depression

The psychomotor stimulation or sedation is a very important parameter to be considered while evaluating the antidepressant effect of any novel or standard drug candidates. In the high predictive validity tests of depression such as FST and TST, the observation is duration of immobility. The test compounds/drugs could produce false positive or negative results in the assessment of antidepressant activity if the basal SLA score is altered (Porsolt et al., 1977, Steru et al., 1985). SLA score was measured by using actophotometer apparatus to rule out the chances of non-specific motor effects of **QCM-4** and **4a** with graded dose range. The dose range for **QCM-4** (0.5, 1, 2 and 4 mg/kg) and **4a** (0.5, 1, 2, 4 and 8 mg/kg) was used for SLA. **QCM-4** (0.5, 1 and 2 mg/kg) and **4a** (0.5, 1, 2 and 4 mg/kg) did not influence the basal locomotor activity score, as compared to vehicle treated group. The same doses were used for evaluating the antidepressant effect using FST and TST assays. The most effective two doses of **QCM-4** (1 and 2 mg/kg) and **4a** (2 and 4 mg/kg) in FST and TST were used for further preliminary screening.

FST and TST are very commonly used high predictive validity behavioral assays to evaluate the antidepressant potential of new chemical entities or standard drugs in the experimental laboratories (Porsolt et al., 1977, Steru et al., 1985, Cryan et al., 2002, Cryan and Slattery, 2007). High predictive validity indicates that the treatment/drugs used in clinic, are effective in animal model representing the respective disease. Moreover, FST and TST reflects high sensitivity towards monoamine alterations (Steru et al., 1985, Holmes, 2003) and hence, are ideal behavioral assays for studying neurobiological and genetic mechanisms involved in antidepressant response of drugs (Steru et al., 1985, Porsolt, 2000, Lucki et al., 2001, Nestler et al., 2002). Basically, the animal is allowed to swim in FST and suspended by tail in TST, upon which the animal tries to escape initially, making vigorous movements and later on suffers the inescapable situation, termed as ‘hopelessness’ and acquires immobile position. It is well reported that decrease in duration of immobility and increase in the swimming time in FST and TST, reflect the antidepressant potential of the compound(s)/drug(s) under investigation (Porsolt et al., 1977, Steru et al., 1985).

In preliminary screening, **QCM-4** and **4a** dose dependently inhibited the immobility time in FST and TST and showed antidepressant activity.

6.2. Anxiolytic-like potential of novel 5-HT_3 receptor antagonists QCM-4 and 4a in behavioral assays of anxiety

In the present study, the anxiolytic potential of the in-house synthesized 5-HT_3 receptor antagonists **QCM-4** and **4a** were screened using experimental behavioral assays of anxiety such as EPM, HBT and L/D test. Anxiety reflects the expression of number of complex components and it is hardly possible to mimic all such components in a single experimental behavioral assay. Hence, a battery of behavioral tests was used to evaluate the anxiolytic potential of **QCM-4** (1 and 2 mg/kg) and **4a** (2 and 4 mg/kg) compounds.

The EPM test has high etiological validity that is reflected through the natural stimuli such as fear of novel, brightly-lit open space and fear of balancing on a relatively narrow raised platform. The EPM is a highly practiced behavioral test of unconditioned anxiety, used for screening the anxiolytic or anxiogenic effect of the novel compounds or standard drugs (Dawson and Tricklebank, 1995). The parameters measured in EPM are percent OAE and OAT. Increase in percent OAE and OAT indicates the anxiolytic potential, whereas decrease reflects the anxiogenic potential of the compound/drug under investigation (Weiss et al., 1998). The data obtained from the present study, indicated that **QCM-4** and **4a**, dose dependently increased the percent OAE and OAT in EPM, and showed potential anxiolytic effect.

Hole board test (HBT) is an important behavioral assay for evaluating anxiolytic activity of any known or unknown compounds/drugs, in the experimental laboratory settings. The HBT offers a simple assay for measuring the behavioral response of the test animal, to an unfamiliar or novel environment (Takeda et al., 1998). In HBT, the parameters measured are the head dipping score and frequency of crossings which are highly sensitive to measure the emotionality, anxiety, and/or responses to stress, in the animal under study. The head dip score correlates to the sensitivity towards the changes in emotional state of the animal and reflects the fearless state with the rise in head dipping behavior (Nolan and Parkes, 1973). In the present investigation, novel 5-HT_3 receptor antagonists **QCM-4** and **4a**, were observed to increase the head dip score and frequency of crossings and showed potential anxiolytic activity in mice using HBT paradigm.

Light/dark (L/D) test is another very commonly practiced model for screening anxiolytic or anxiogenic potential of the compound/drug under investigation. In L/D test, animal uses the natural preference for dark spaces and spontaneous exploratory behavior of animal is reflected in response to mild stressors, such as novel environment and brightly-lit area (Imaizumi et al., 1994). The parameters measured in L/D were time spend in light area and transition score. Increased time spent in light chamber and transitions suggest to the anxiolytic effect (Crawley and Goodwin, 1980). In the present study, **QCM-4** and **4a**, dose dependently increased the time spent in light chamber and transition score and showed potential anxiolytic effect in L/D test.

The preliminary antidepressant and anxiolytic potential of **QCM-4** and **4a** compounds, was confirmed using behavioral assays. Further, **QCM-4** and **4a**, were evaluated for antidepressant potential in the chronic models of depression that reflects most of the clinical symptoms and behavior of depression. OBX and CUMS models are among the commonly practiced high validity models in the experimental laboratories which include induction of disease, treatment regimen, and post recovery/treatment, followed by behavioral assays. The novel 5-HT_3 receptor antagonists **QCM-4** and **4a**, were screened in OBX and CUMS models of depression.

6.3. Evaluation of 5-HT_3 receptor antagonists QCM-4 and 4a in OBX model of depression

OBX induced depression is a very popular rodent model in experimental laboratory settings. OBX is a surgical model in which olfactory bulbs are removed and animals are allowed to recover for two weeks from surgery followed by sub-chronic treatment regimen of two weeks and assessment of depressive symptoms using behavioral test battery. Important clinical symptoms of depression-like behavior simulated in OBX model is anhedonia, which indicates loss of interest or pleasure (Song and Leonard, 2005). The possible biological mechanism involved in anhedonia behavior in OBX model, suggest the imbalance in serotonergic neurotransmission in various regions of brain leading to depression-like symptom in terms of inability to experience the pleasure or happiness (Watanabe et al., 2003). In SPT, the OBX rats showed reduced consumption of sucrose solution and treatment with **QCM-4** and **4a** compounds, improved the consumption of sucrose solution.

In modified OFT, the OBX rats exhibited a specific hyperactive behavioral pattern when exposed to the brightly lit, circular, open field arena characterized by increased ambulation, rearing, and fecal pellets (Kelly et al., 1997, Song and Leonard, 2005). In the modified OFT, repetitive treatment with **QCM-4** and **4a** compounds, reversed the hyperactivity of OBX rats, as indicated by reduced ambulatory score, rearing and fecal pellets.

EPM is another behavioral paradigm that has been well reported in OBX rats to be influenced by various variables such as colour of the maze, illumination source and conditions, strains of the animals under experimentation (Song et al., 1996, Kelly et al., 1997, Wieronska et al., 2001). In the present investigation, EPM test was used to study the psychomotor agitation of OBX rats rather than anxiolytic activity, in terms of open arm activity. OBX rats exhibited an increased open arm activity, which suggested the inhibition of defensive behavior upon exposure to novel environment, in concurrence of the earlier studies from our laboratory (Ramamoorthy et al., 2008, Jindal et al., 2015), may be attributed to OBX induced destructions of serotonergic and GABAergic outputs (Jancsár and Leonard, 1984). Chronic treatment with the 5-HT_3 receptor antagonist **QCM-4** and **4a**, reversed these behavioral anomalies and exhibited antidepressant-like behavior. Taking into consideration the results obtained in present study and earlier reports (Van Riezen and Leonard, 1990, Song et al., 1996, Kelly et al., 1997, Mar et al., 2000), it is observed that minor changes in the test conditions do not influence the result significantly and hence, the parameters measured in EPM such as open arm time and open arm entries remains important and reliable indices.

Taken together, in OBX model of depression novel 5-HT_3 receptor antagonists **QCM-4** and **4a**, reversed the behavioral alterations and showed significant antidepressant-like effect.

6.4. Evaluation of 5-HT_3 receptor antagonists QCM-4 and 4a in CUMS model of depression

6.4.1. Mechanism of CUMS induced depression in rodents

The exact aetiology of depression still remains unclear despite several theories as they vary widely in scope and perspective. Researcher have focused on the biological mechanisms of depression induced by chronic stress such as HPA axis hyperactivity and the resultant toxicities from excessive glucocorticoid release in the circulation (Lupien et al., 2009).

Moreover, disrupted hippocampal neurogenesis (Kempermann and Kronenberg, 2003) and genetic or epigenetic factors (Menke et al., 2012) are also involved in depression. The depletion of brain 5-HT in depressed patients, is mostly noted. Hence, SSRIs, remains first line pharmacotherapy for depression (Rodriguez Bambico et al., 2009). However, depletion of 5-HT alone is not a causative factor in depression as SSRIs are not effective, in all patients, suggesting the involvement of other biological factors such as deficits in other neurochemicals, BDNF, alteration in hippocampal neurogenesis, HPA axis hyperactivity and disruption in circadian rhythm (Hasler, 2010, Albert et al., 2012) as shown in **Fig 6.1**.

Furthermore, it is clear that connection between CUMS induced neurogenic alterations result in 5-HT dysfunction through HPA axis hyperactivity and antidepressant acting through 5-HT system, may possibly drive hippocampal neurogenesis mediated antidepressant effects, by regulating stress (Mahar et al., 2014). CUMS induced depressive phenotypes include wide spectrum of alterations such as emotional activity, anhedonia, body weight, cognitive functions, grooming behavior and motivation, most of which are found in depressed patients that are reversed with chronic antidepressant treatment (Willner, 1997, 2005).

i) Alteration in serotonergic activity

CUMS induced depression involves alteration in several important processes such as neurogenesis, morphological and immunological functions. Earlier reports suggest that CUMS inhibits cell proliferation and neurogenesis (Jayatissa et al., 2009), and intracellular signaling in hippocampus (Qi et al., 2006). CUMS directly affects the 5-HT system which results in depressive behavior (Mahar et al., 2014). CUMS leads to reduction in 5-HT release, tissue concentration and synaptic activity in hippocampus in similar way as those observed in depressed patients displaying reduced 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (Luo et al., 2008). Moreover, CUMS induces 35% decline in the neuronal firing activity of 5-HT neurons in the dorsal raphe resulting in behavioral alterations such as anhedonia, as observed from reduced sucrose consumption in SPT. (Bambico et al., 2009) and behavioral despair shown by increased immobility time in FST (Banasr and Duman, 2008). On the other hand, facilitation of 5-HT neurotransmission is very well known to show antidepressant effect (Bell et al., 2001).

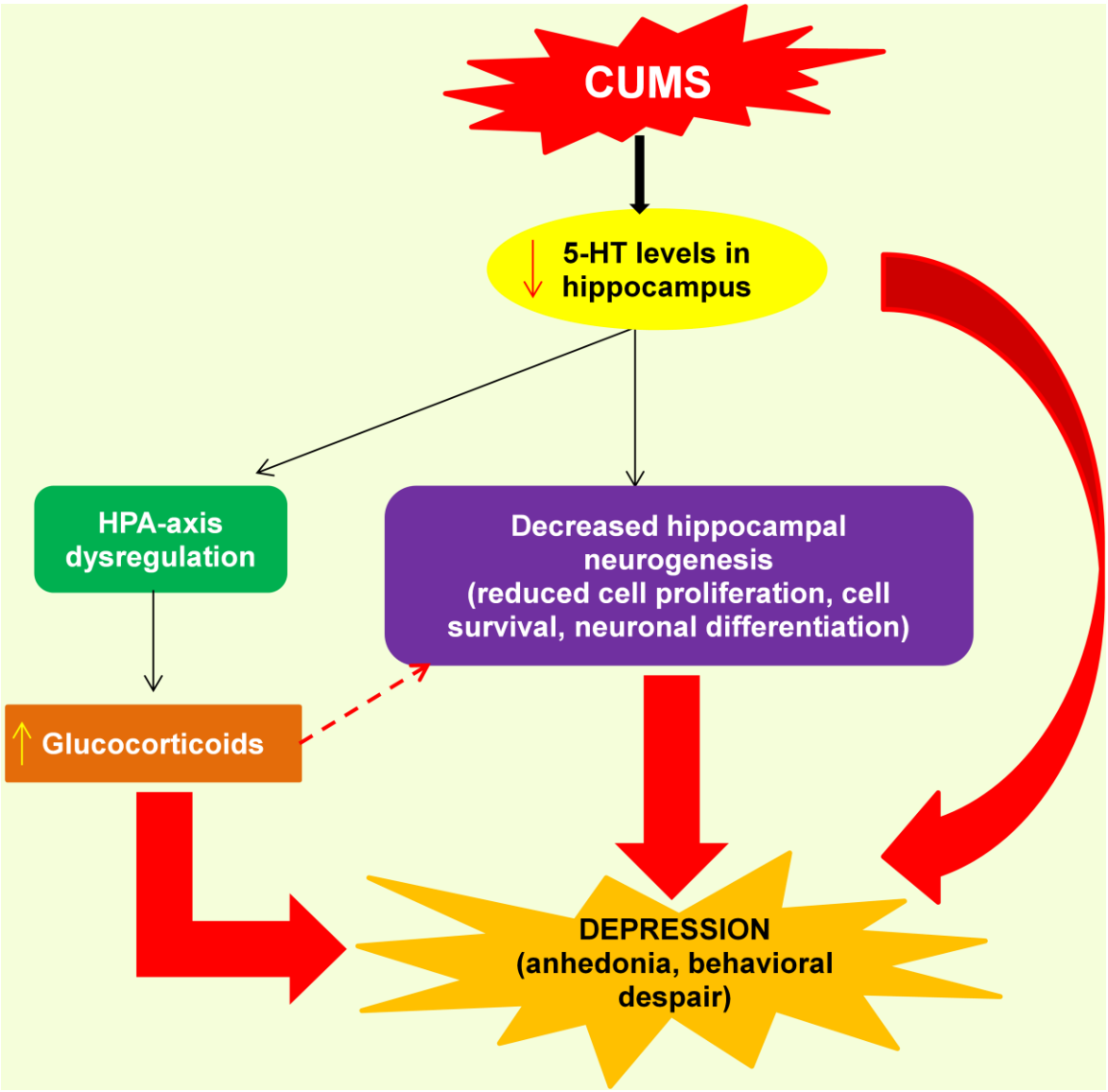


Fig 6.1: Schematic representation of CUMS induced alterations in HPA axis, hippocampal neurogenesis and 5-HT resulting in depression

ii) Neurogenesis in depression: It was hypothesized that deficits in hippocampal neurogenesis could play an important role in the pathology of depression. This was supported by reduced hippocampal volume as observed in decreased granule cells and granule cell layer volume in DG region of depressed patients and improved hippocampal neurogenesis as observed from increased granule cell layer volume in depressed patients those have taken antidepressant treatment (Kempermann, 2002, Kempermann and Kronenberg, 2003).

Moreover, delayed efficacy with antidepressant treatment in humans and animal models appears due to the time taken by the newly formed neurons to become functional and hyperplasic (Ge et al., 2007). Antidepressants have been effective in adult hippocampal neurogenesis and supporting the relevance of late clinical benefit in terms of the developmental latency of newly formed neurons and antidepressants mediated behavioral activity may require hippocampal DG neurogenesis (Hanson et al., 2011, Perera et al., 2011).

iii) 5-HT system and neurotrophic factors in regulation of neurogenesis

Hippocampal neurogenesis is regulated by various neurotrophic factors and monoamines. Hippocampus is densely innervated with 5-HT fibers. Removal of 5-HT neurons from dorsal raphe and median raphe, results in reduced neurogenesis, which is reversed with 5-HT re-innervation (Brezun and Daszuta, 2000). Effect of various 5-HT receptors was studied on neurogenesis, suggesting that 5-HT_{1A} stimulation is pro-neurogenic, no effect with 5-HT_{1B} stimulation, 5-$HT_{2A/C}$ blockers reduces the neuronal cell proliferation, 5-HT_{2B} stimulation facilitate neurogenesis and shows antidepressant effect, with 5-HT_{2C} stimulation results in no effect on neurogenesis process (Diaz et al., 2012) (**Table 6.1**). Moreover, inhibition of 5-HT transporters promotes hippocampal neurogenesis and leads to antidepressant effect (Ferrés-Coy et al., 2013).

Table 6.1: Influence of 5-HT system and BDNF on hippocampal neurogenesis

Type of receptor	Agonism	Antagonism
5-HT_{1A}	↑	↓
5-HT_{1B}	No effect	No effect
5-HT_{2A}	↑	↓
5-HT_{2B}	↑	↓
5-HT_{2C}	No effect	↓
5-HT	↑	↓
5-HT transporters	↓	↑
BDNF	↑	↓

Neurotrophic factor BDNF, has been known to promote adult hippocampal neurogenesis and modulate antidepressant-related behaviors. Central and peripheral BDNF infusion promotes neurogenesis in hippocampus, neuronal survival and also exhibits antidepressant activity (Schmidt and Duman, 2010).

BDNF knock out animals and reduced BDNF in hippocampal DG region, showed reduced hippocampal neurogenesis, reduced survival rate, and neuronal differentiation, showed depressive behavior and resistance to antidepressant treatment (Adachi et al., 2008, Taliaz et al., 2010). Importantly, hippocampal BDNF levels are depleted in response to CUMS and improved in response to antidepressant treatments (Hanson et al., 2011).

iv) CUMS and neurogenic function on hippocampus: CUMS has been reported to show depressive behavior in rodents, decrease hippocampal neurogenesis, proliferation and survival (Dagytė et al., 2010, Schmidt and Duman, 2010, Mahar et al., 2014). The dorsal hippocampus in rodents (analogous to posterior hippocampus in humans) is mainly involved in memory and learning functions and the ventral hippocampus (analogous to anterior hippocampus in humans) are concerned with emotional modulation (Fanselow and Dong, 2010). Ventral hippocampus has higher neurotransmitters such as 5-HT, NE and mesolimbic DA innervations, than dorsal hippocampus (Gage and Thompson, 1980, Gasbarri et al., 1994).

The differences in function, are also observed at the level of DG, where dorsal DG, are mainly involved in learning and memory functions and ventral DG is implicated mainly in anxiety and depression-like phenotypes or antidepressant-like effects (Kheirbek and Hen, 2011). Furthermore, CUMS has been found to reduce the ventral DG-specific neurogenesis (Tanti et al., 2013) and antidepressant effect results from improvement in ventral DG-specific neurogenesis (Païzanis et al., 2010). Besides, recently it was shown that fluoxetine treatment reversed the CUMS induced reduction in neuronal progenitor, in ventral DG and reduction in early immature neurons in ventral and dorsal DG (Tanti et al., 2013).

The information regarding CUMS model and pharmacotherapy for depression suggests that;

(i) antidepressants show late clinical benefit as they take several weeks to show clinical efficacy (Jacobs et al., 2000)

(ii) CUMS induction in rodents leads to depressive effect (Hanson et al., 2011)

(iii) certain antidepressant related effect requires neurogenesis (Perera et al., 2011)

Neurogenesis in hippocampus begins soon after a pro/anti-proliferative stimulus that leads to differentiation of new cells into progenitors, that elongates dendrites into molecular layer around DG further forming spines. The newly formed neurons undergo apoptosis or survive and further experiences a hyperplastic period of around 4-6 weeks and becomes functional **(Fig 6.2**). This implicates the importance of maturity of newly formed neurons and there functional state along with proliferation and survival of neurons (Ge et al., 2007). Hence, the affective behavior is observed as a result of modulation of hippocampal neurogenesis when the immature hyperplastic neurons in the ventral DG in rodents and anterior DG in humans are affected at the time of behavioral assays or clinical assessments, respectively (Jayatissa et al., 2009, Mahar et al., 2011).

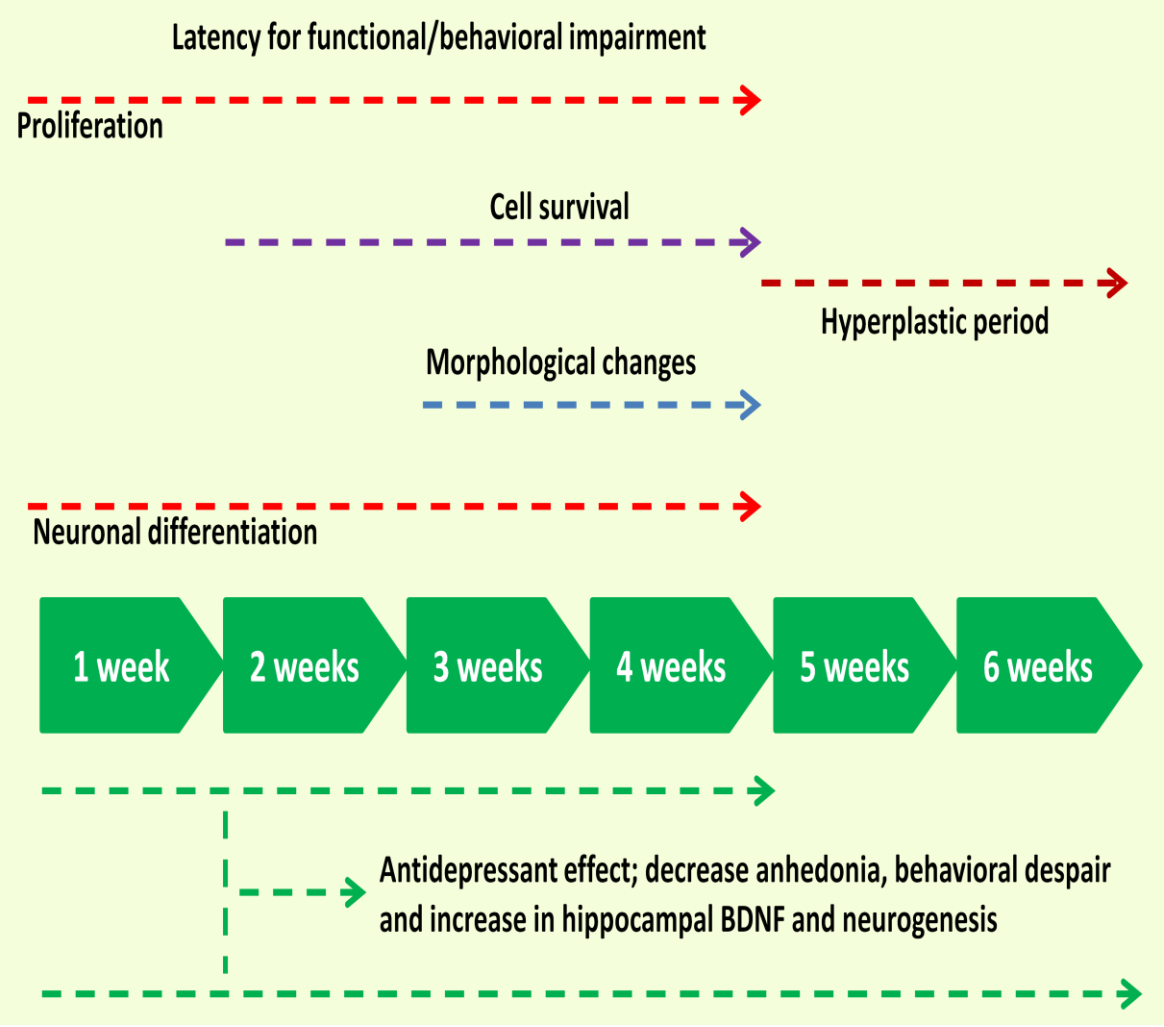


Fig 6.2: Schematic representation of timeline of neurogenic effect in CUMS model

Once the antidepressant potential of **QCM-4** and **4a** compounds, was proved in OBX surgical model of depression in rats, in our next plan of work, it was decided to screen the compounds **QCM-4** and **4a** in CUMS model of depression that reflects most of the clinical symptoms of the disease condition and possess high predictive, face and construct validities. Stress has been one of the most important pathogenic factors in several neuropsychiatric disorders such as depression and anxiety (García-Bueno et al., 2008). CUMS leads to various long-term behavioral, neurochemical, neuroimmune and neuroendocrine alterations, that resemble to those observed in patients suffering from depression (Willner, 2005). CUMS induced depressive symptoms are reversed only by chronic, but not acute, treatment with antidepressants (Cryan and Holmes, 2005). There are various neuropsychiatric complications, such as anxiety, depression, and memory impairment that are generally associated with stress. Many of these effects are thought to be mediated by stress induced neurochemical and hormonal abnormalities that are often associated with oxidative stress (Fontella et al., 2005). CUMS-induced oxidative damage has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases, ranging from psychiatric disorders such as depression, anxiety and cognitive dysfunctions (Bilici et al., 2001).

ROS may play a role in some neuropsychiatric disorders such as MDD. Evidence based study suggests that activation of immune-inflammatory process causes increased monoamine catabolism, abnormalities in lipids that may cause overproduction of ROS, lipid peroxidation as well as reduced antioxidant enzyme activity, and these processes may be related to pathophysiology of depression (Liu et al., 1994, Fontella et al., 2005, Schiepers et al., 2005). Moreover, several studies have reported the antioxidant potential of existing antidepressants, substantially demonstrating the correlation between oxidative stress and depression (Zafir and Banu, 2007, Behr et al., 2012).

In the present study, different types of stress situations were used to investigate the consequences of chronic stress in depression-like behavior in mice. Earlier report have evidenced the CUMS induced depressive phenotypes (Mineur et al., 2006), that can be used for evaluating the antidepressant activity of potential antidepressants using various behavioral assessment studies like SPT, FST, TST and EPM.

Sucrose preference mainly reflects anhedonia, a key symptom of human depression, indicated by loss of interest or pleasure (Strekalova et al., 2006). Preclinical studies have reported the CUMS induced nerve cells damage, in the neural reward system. This damage is thought to be related to the serotonergic and dopaminergic systems leading to the loss of the ability to experience happiness or pleasure (Bekris et al., 2005, Strekalova et al., 2006). In the present study, CUMS animals exhibited reduced preference for sucrose solution as compared with non-stressed mice. Chronic treatment with **QCM-4** and **4a** compounds significantly inhibited this alteration and improved consumption of sucrose solution, in CUMS exposed mice. Chronic stress has been shown to dramatically increase the immobility time of mice in FST and TST, a manifestation of 'behavioral despair' (Mineur et al., 2006). Repetitive administration with 5-HT_3 receptor antagonists **QCM-4** and **4a**, counteracted the CUMS-induced increased immobility time in mice and showed antidepressant-like effect.

EPM remains an important behavioral assay commonly practiced in research laboratories to screen anxiolytic potential of known or novel drugs/compounds (Hogg, 1996). In the CUMS model, stressed animals showed reduced percentage of OAE and OAT, respectively, which was reversed with chronic treatment of **QCM-4** and **4a** compounds.

In the preliminary investigations, the novel 5-HT_3 receptor antagonists **QCM-4** and **4a**, showed potential antidepressant and anxiolytic-like effect in acute and chronic models of depression.

6.5. HFD induced obesity and HFD fed mice subjected to CUMS as models to study depressive-phenotypes associated with obesity

Obesity is well known metabolic disorder characterized by excessive increase in the adiposity and body fat which adversely affect the individual health leading to several metabolic and cardiovascular complications such as coronary artery diseases, hyperlipidemia, hypertension, hyperglycemia, insulin resistance, and T2DM (Goyal, 2012).

Obesity is also referred as a direct consequence of, increased intake of high calorie/fat food and sedentary life style with increased energy intake and decreased energy expenditure, which stores extra calories in the form of lipids, especially triglycerides in the adipocytes (Goyal, 2012, Rosini et al., 2012).

Recent studies have confirmed that obesity facilitates the development of neuropsychiatric disorder such as cognitive impairments (Nguyen et al., 2015), Parkinson's disease (Chen et al., 2014), Alzheimer's disease (Letra et al., 2014), and depression (Sharma and Fulton, 2013), as briefly represented in **Fig 6.3.**

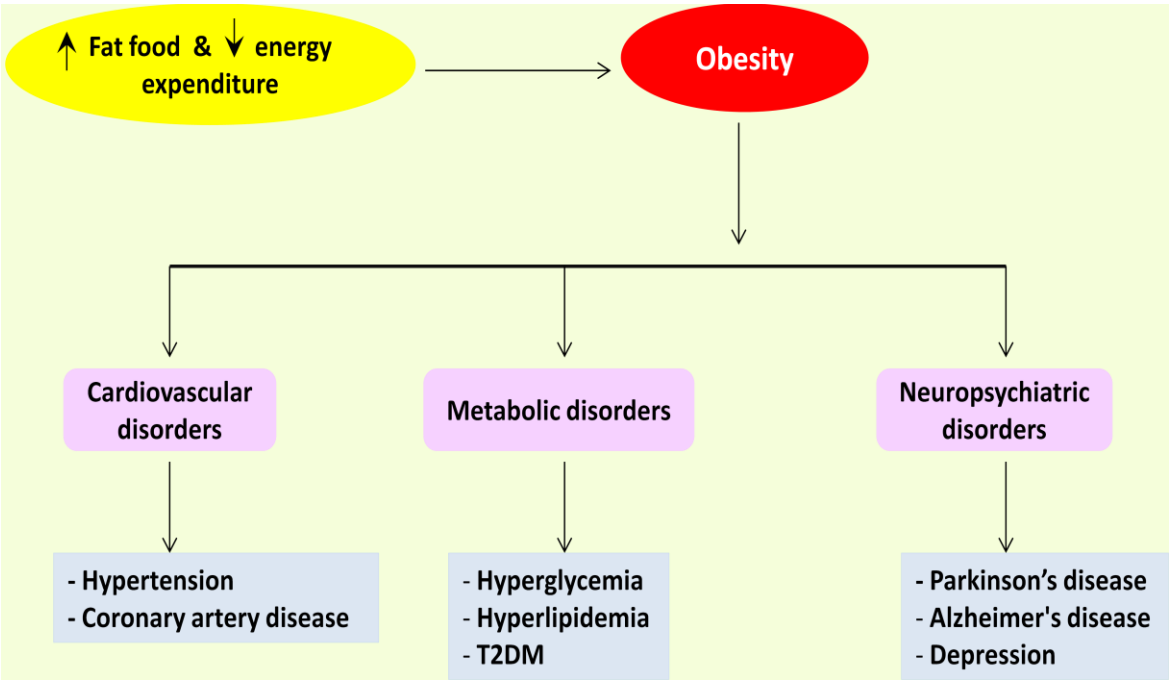


Fig 6.3: Various consequences associated with obesity

Fat intake in diet often has been claimed as a major factor for weight gain and obesity. Clinical studies have confirmed that dietary fat intake can easily induce obesity (French and Robinson, 2003). Meta analysis studies, conducted in different nations, such as United States, Canada and China have demonstrated that, when the average fat content in the regular diet increases, the incidence of obesity also increases (George et al., 1990, Saris et al., 2000). Hence, it is very clear to maintain the dietary fat content of human diet.

Dietary fats not only induces obesity in humans but also makes the experimental laboratory animals mice and rats obese (Buettner et al., 2007). Several studies have reported positive relationship between high dietary fat content and body weight or fat gain faster than, consuming the diet low in fat content, in both mice as well as rats (Judge et al., 2008, Warden and Fisler, 2008).

DIO is commonly used model for induction of obesity in laboratory animals as it resembles most of the features of human obesity (Goyal, 2012). Diet containing high percentage of fat is mostly preferred. Saturated fatty acids (SFA) get deposited in the adipose tissues than mono unsaturated fatty acids (MUFA) and polyunsatured fatty acids (PUFA) respectively, rather than getting used as fuel. SFA are acylated into triglycerides and stored in adipose tissues. Hence, the energy content of the diet is high when the diet is rich in SFA content.

Moreover, the oxidation rate of SFA is reduced with longer carbon chain length (Hariri and Thibault, 2010). Assessment of the HFD induced obesity is generally done in terms of body weight where, 10-25% higher body weight is considered as moderate obesity and 40% or higher body weight is considered as severe obesity, respectively (Levin and Dunn-Meynell, 2002, Woods et al., 2003).

Obesity is one of the most serious health concerns in the human society, across the world as it exacerbates metabolic alterations and has been found as a major risk factor for neuropsychiatric disorder, depression (Aslani et al., 2015). Depression is well characterized with alterations in appetite, anhedonia or loss of interest, body weight etc, (Bekris et al., 2005). Several individuals experience mild environmental stressors in their daily life which may trigger depression and feeding behaviors or appetite pattern (Torres and Nowson, 2007). Hence, it would be interesting to study the CUMS induced depressive phenotypes in HFD fed animals.

The CUMS model is highly sensitive, valid, and reliable to study depression in rodents and evaluate the antidepressant effect of novel or known drugs as it has high construct, face and predictive validities. HFD induced obesity model reflects most of the biological mechanisms involved in depression associated with obesity including HPA axis hyperactivity, oxidative stress, leptin resistance, insulin resistance or altered glucose level, reduced neurotrophic factor BDNF and hippocampal neurogenesis. CUMS model has not been studied much in animals fed with HFD for depressive phenotypes. CUMS model of depression was used in present study, to worsen the depressive phenotypes in HFD fed mice. In the present study to evaluate the effect of chronic stress on the depressive phenotypes, mice were subjected to CUMS procedure, post HFD feeding.

6.5.1. OND, QCM-4 and 4a reverse HFD and HFD+CUMS induced depressive-like behavior

In the present study, HFD induced obesity model was used to induce obesity in mice, according to Srinivasan et al, (Srinivasan et al., 2005). The HFD fed animals were administered with 5-HT_3 receptor antagonists **OND** (0.5 and 1 mg/kg, p.o.), **QCM-4** (1 and 2 mg/kg, p.o.) and **4a** (2 and 4 mg/kg, p.o.) for 28 days. In another model, HFD fed mice were subjected to CUMS for 28 days and treatment with **OND** (0.5 and 1 mg/kg, p.o.), **QCM-4** (1 and 2 mg/kg, p.o.) and **4a** (2 and 4 mg/kg, p.o.), were started on day 8 to 28. Post treatment schedule, HFD fed mice and HFD+CUMS mice were screened for depressive and anxiety-like behavior using SPT, FST, TST, EPM, L/D and HBT assays, biochemical estimations including plasma glucose, lipids, oxidative stress parameters MDA and GSH, plasma CORT, leptin, insulin, followed by molecular assays such as hippocampal 5-HT, cAMP and BDNF concentrations, histological assays of hippocampal DG region using H and E staining and IHC assay of p53 in hippocampal DG region.

HFD feeding leads to the development of depression and anxiety-like behavior, increases the HPA axis response to stress and result in several biochemical modifications (Sharma and Fulton, 2013). Chronic stress plays an important role in the onset and relapse of depression (Lee et al., 2002). One of the very important symptoms of clinical depression is the inability to experience pleasure called as anhedonia and in pre-clinical studies to access the loss of interest or loss of ability to experience pleasure, a high predictive validity model SPT, is preferred, as it indicates the anhedonic behavior (Willner et al., 1992). Obesity is a stressful condition and in the present study, severe anhedonia was observed in HFD fed mice. Chronic treatment with 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a**, improved the sucrose solution consumption in HFD fed mice, and showed antidepressant effect.

CUMS induced depression is probably the most popular and suitable model to study depressive behavior in rodents as it possesses high face, construct and predictive validities, reflecting the similarities in the pathogenic and behavioral alteration in human and animal depression. CUMS model aims to simulate severe depressive-like condition that is developed gradually as those are generally observed in depressed patients (Luo et al., 2008). In addition, CUMS leads to important changes in metabolic disorders with respect to feeding behavior and metabolism.

Stress releases hormone cortisol, which further leads to storage of fat through brain signaling mechanisms (Björntorp, 2001). Considering the raising ratios of co-morbidity of psychiatric and metabolic disorders, HFD fed mice were subjected to CUMS in the present study, to evaluate whether the depressive phenotypes are aggravated in HFD fed mice or not. In SPT, reduced preference for sucrose solution was observed in HFD fed mice exposed to CUMS. Chronic treatment with **OND**, **QCM-4** and **4a** reversed the anhedonia response in HFD fed mice subjected to CUMS and hence, showed anti-anhedonic effect.

SLA was measured to avoid the chances of false positive results, as the behavioral assays such as FST and TST involves immobility time as the main observation. Chronic treatment with **OND**, **QCM-4** and **4a**, did not alter the basal locomotor score in HFD fed and HFD fed mice subjected to CUMS. FST shows high predictive validity and is a good reliability model used widely at laboratory level to screen antidepressant effect of any know or unknown compound(s). Moreover, FST and TST are highly sensitive for the neurochemical modifications and represents one of the very important preliminary behavioral models of depression that is mostly used to evaluate neurobiological mechanisms involved in antidepressant effect of any known or unknown compounds/drugs (Steru et al., 1985, Porsolt, 2000). In the present study, HFD fed mice and HFD fed mice subjected to CUMS showed dramatically increased immobility time in FST and TST, a manifestation of 'behavioral despair'. Chronic treatment with **OND**, **QCM-4** and **4a**, inhibited the immobility time in FST and TST and showed antidepressant effect in HFD fed and HFD fed mice subjected to CUMS.

6.5.2. OND, QCM-4 and 4a reverse anxiety-like behavior in HFD and HFD+CUMS induced anxiety-like behavior

The co-morbid existence of depression and anxiety have been studied for a long time due to the very narrow margin of symptoms between them, consistent appearance and positive association of both disorders (Dobson, 1985). Obesity is a stressful condition and stress induces anxiety-like behavior that worsens the depression related symptoms in rodents (André et al., 2014). Furthermore, it is well evident from literature that CUMS may induce anxiety-like symptoms (Bondi et al., 2008). EPM has been widely used as a behavioral testing paradigm for screening of anti-anxiety activity.

EPM reflects the psychomotor and emotional aspects in rodents which correlate with unconditioned anxiety. Moreover, anti-anxiety agents elevate the frequency of entries and time spent in open arms in EPM, and vice-versa is true with anxiogenic agents (Hogg, 1996). In EPM test, HFD fed and HFD fed mice exposed to CUMS, exhibited severe anxiety-like behavior. Chronic treatment with **OND**, **QCM-4** and **4a**, increased the percent OAE and OAT in HFD fed and HFD fed mice subjected to CUMS and showed anxiolytic effect.

HBT is another behavioral test displaying anxiety-like, emotional or stress response upon exposure to an unusual environment generally expressing the exploratory behavior along with the emotional disturbances in rodents (Takeda et al., 1998). Head dip score is major parameter that shows the exploratory behavior of animal and displays the sensitivity for changes in the emotional state of the test animal (Nolan and Parkes, 1973). In the present study, HFD fed mice showed reduced head dips and crossing scores and exhibited severe anxiety-like behavior in HBT. Chronic treatment with **OND**, **QCM-4** and **4a**, increased the number of head dips and crossings and showed anxiolytic effect in HFD fed mice.

L/D test represents one of the highly practiced laboratory animal models for anxiety. The new environment and light are known as mild stressors and the innate aversion response to brightly lit chamber represents the exploratory behavior of rodents (Imaizumi et al., 1994). In L/D test, time spent in light chamber and transition score are mainly observed. In the present study, the time spent in light chamber and transition scores were decreased in HFD fed and HFD fed mice subjected to CUMS, thus exhibiting anxiety-like phenotypes, which were reversed by chronic treatment with 5-HT_3 receptor antagonists, namely **OND**, **QCM-4** and **4a**.

Taken together, the behavioral assays showed severe depressive and anxiety-like behavior in HFD and HFD+CUMS induced depression models that were inhibited by chronic treatment with 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a**.

6.6. Mechanism of action

6.6.1. OND, QCM-4 and 4a prevent HFD and HFD+CUMS induced alteration in plasma glucose, total cholesterol, triglycerides and OGTT

Overconsumption of fatty diet and physical inactivity leads to overweight/obesity. The increased body weight with HFD feeding in mice, probably is the result of consumption of diet rich in energy in the form saturated fats over the period of time and deposition of fat pads in various regions of the body (Eisinger et al., 2014). With the HFD feeding for long period, the lipid profile tends to be higher and in particular, total cholesterol and triglycerides resulting as a risk factor for cardiovascular diseases (Katcher et al., 2009). In general, high intake of fatty diet, increases the level of triglycerides, which in turn constitutes a source of fatty acid availability and oxidation. These increased fatty acid availability for oxidation, inhibits the insulin mediated decrease in hepatic glucose output and attenuates the glucose uptake or utilization in skeletal muscles, resulting in compensatory hyperinsulinemia, which is often known as a prominent feature of insulin resistance (Srinivasan et al., 2005). Moreover, increased body weight, plasma glucose and lipid profile have been observed to cause depression in humans suggesting the critical role of insulin resistance (Martinac et al., 2007, Tyrovolas et al., 2009), as mentioned in **Fig 6.4**.

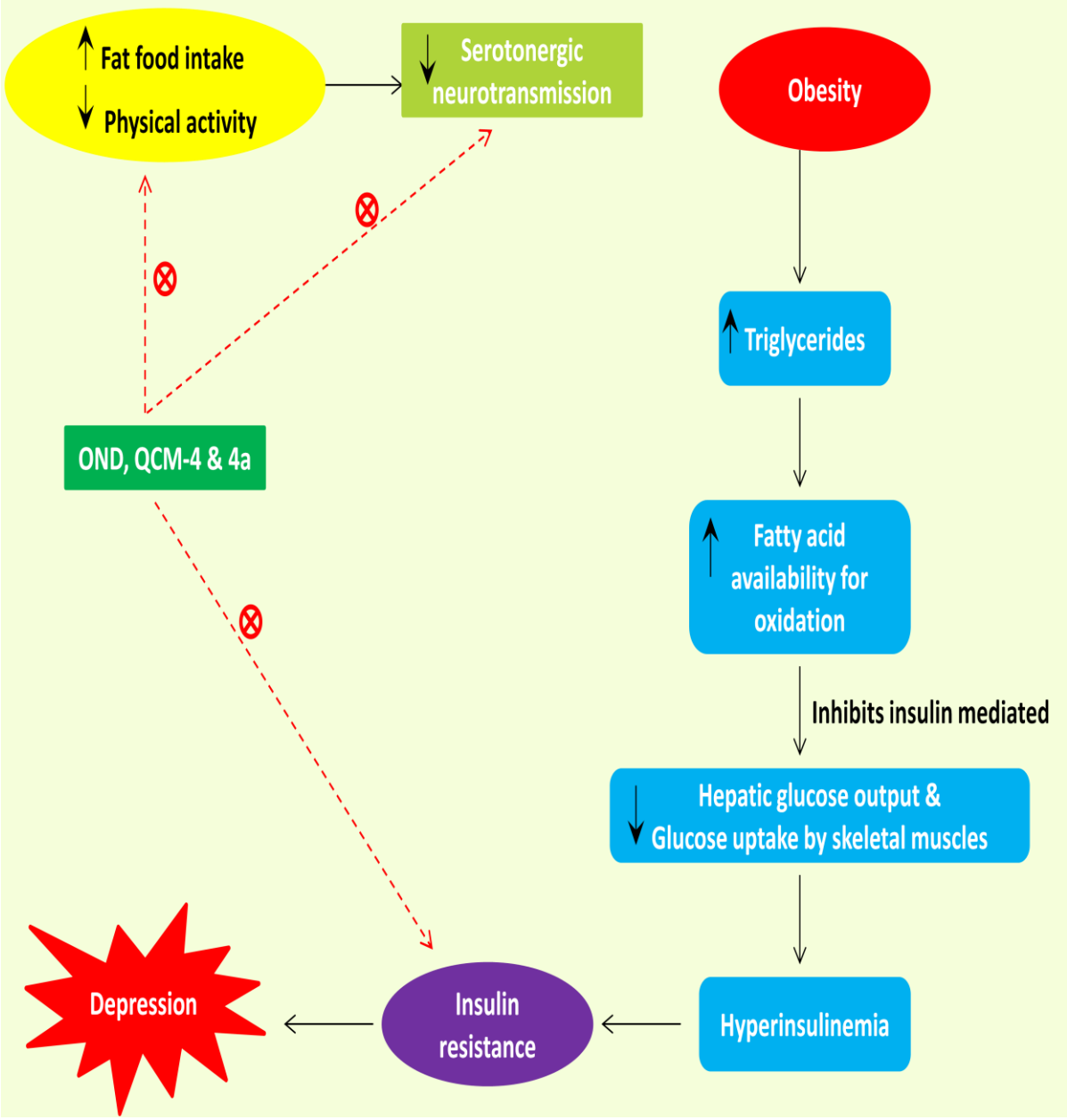


Fig 6.4: Inhibition of HFD induced dyslipidemia by serotonergic modulators **OND**, **QCM-4** and **4a** in depression co-morbid with obesity

HFD feeding inhibits the sensitivity for glucose in OGTT, an early indication for insulin resistance. The 5-HT plays a crucial role in regulation of insulin secretion and hence, in insulin resistance by modifying plasma glucose and lipid profile. The 5-HT is a very important neurotransmitter that regulates mood, sleep-wake cycle and appetite. 5-HT synthesis inhibitor p-chlorophenylalanine, has been reported to induce hyperphagia and weight gain in contrast to 5-HT reuptake inhibitors such as fluoxetine and sibutramine, which reduced the food intake and blood glucose level in animals (Namkung et al., 2015).

The deficiency of 5-HT is very well known to be involved in depression. Chronic HFD feeding has, showed reduced 5-HT level in obesity. 5-HT has been known as anorexigenic neurotransmitter as it inhibits food intake (Kim et al., 2013). Hence, depression co-morbid with obesity is often associated with decreased level of 5-HT in brain.

In the present study, HFD feeding for 14 weeks increased the body weight, caused hyperglycemia, hyperlipidemia in mice and produced insulin resistance syndrome. Chronic treatment with 5-HT_3 receptor antagonist **OND**, **QCM-4** and **4a**, ameliorated the elevated plasma glucose, lipid level, and improved the glucose sensitivity irrespective of any change in the body weight, thus showed antidepressant effect in depression co-morbid with obesity in HFD and HFD+CUMS induced depression.

6.6.2. OND, QCM-4 and 4a inhibit HFD and HFD+CUMS induced oxidative stress

Another important pathological marker observed in HFD induced obesity model is increased brain oxidative stress, that leads to development of co-morbid depression (Carey et al., 2015). HFD feeding for long time causes neuronal degeneration due to increased oxidative stress markers and reduced anti-oxidants (Kang et al., 2015). Obesity is a pro-inflammatory state that leads to increased oxidative stress markers such as lipid peroxidation or MDA and diminished antioxidant enzymes like GSH in the brain (Esposito et al., 2006). Oxidative stress in obesity, alters the glucose utilization in fat and skeletal muscles, further altering the synthesis of insulin (Festa et al., 2001).

The role of oxidative stress induced damage in the pathogenesis of depression is reported earlier (Chung et al., 2013). Literature survey revealed that exposure to chronic stress can stimulate numerous pathways that leads to the increased production of free radicals (Matsumoto et al., 1999). The free radicals results in a cascade of events such as lipid peroxidation, protein oxidation, DNA damage and cell death, and contribute to the occurrence of pathological conditions. Stress alters the balance between oxidant and antioxidant factors/parameters, and leads to oxidative stress induced neuronal damage (Eren et al., 2007). Moreover, HPA axis hyperactivity is being reported to induce oxidative stress through activation of cytokines and inflammatory pathways. The abnormally increased glucocorticoids in response to stress induced hyperactivation of HPA axis disturbs the cellular redox system (Spiers et al., 2014).

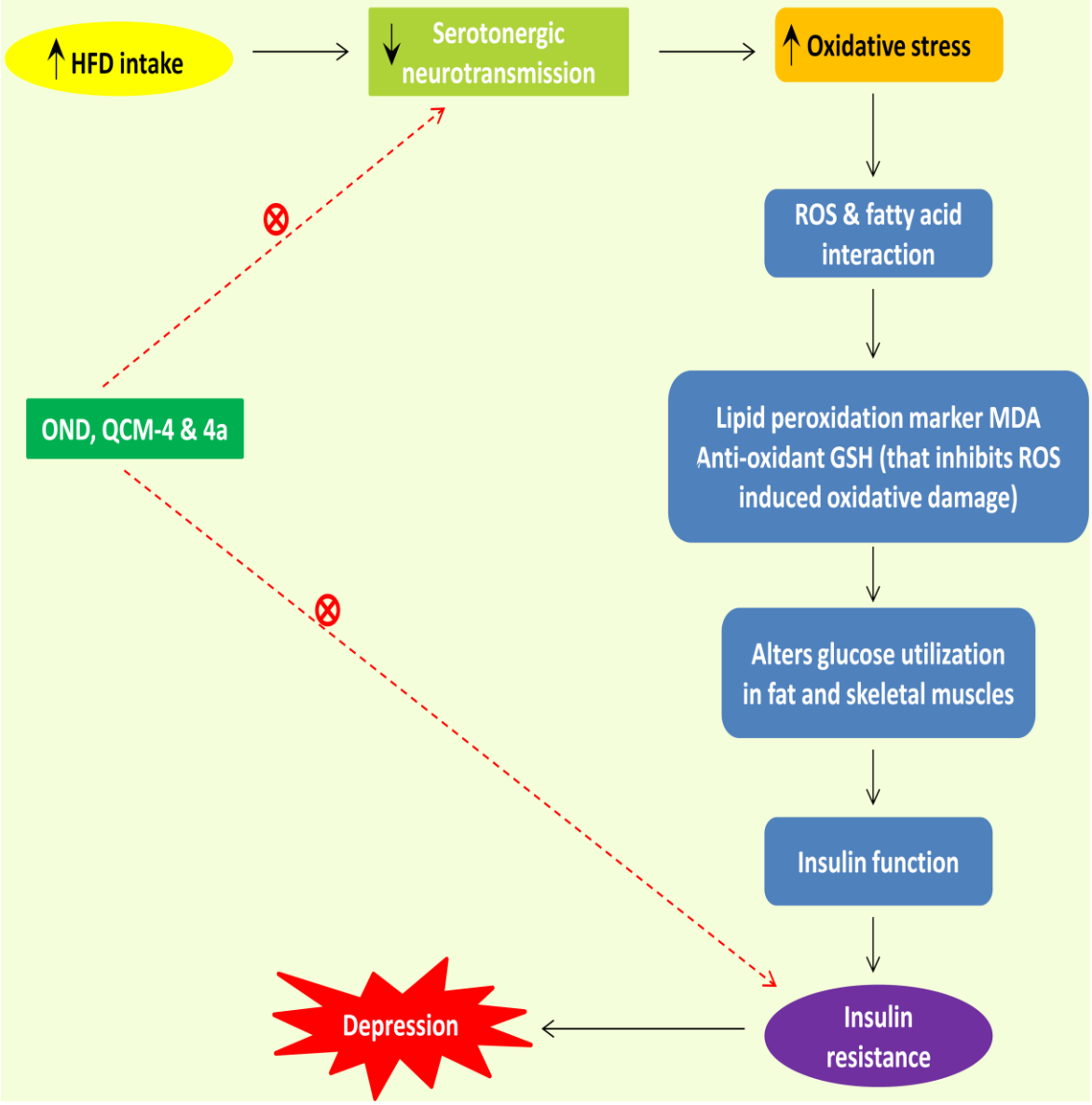


Fig 6.5: Inhibition of HFD induced oxidative stress by serotonergic modulators **OND**, **QCM-4** and **4a** in depression co-morbid with obesity

The imbalance between oxygen free radicals and antioxidant defense system increases the oxidative stress (Balaban et al., 2005) that generates ROS and causes neurodegeneration (Bruce-Keller et al., 2010). An interesting study has demonstrated, abnormally raised brain oxidative stress marker lipid peroxidation, in animals fed with HFD (Charradi et al., 2012). In obesity, the production of lipid peroxidation is often high due to the interaction of PUFA and ROS in brain. Results obtained in the present study, were in compliance with the earlier report, where remarkably raised MDA was measured in the mouse brain (Charradi et al., 2012).

The concentration of antioxidant GSH, an important thiol, present in the brain cells, that exhibit protective action against ROS induced oxidative damage (Wang and Ballatori, 1998) was decreased with HFD feeding, as shown in **Fig 6.5**. Elevated ROS, results in the oxidation of GSH to GSSG, which leads to decrease in GSH concentration. Nicotinamide adenine dinucleotide phosphate (NADPH), released from glucose-6-phosphate dehydrogenase (G6PDH), makes another enzyme glutathione reductase, to form GSH again from GSSG. The level of these antioxidants is reduced in both HFD fed and T2DM mice (Dincer et al., 2002, Baydas et al., 2003).

The increased chronic stress, leads to the imbalance of MDA and GSH that results in severe depressive and anxiety-like behaviors in HFD fed mice. Some of the reports concluded that oxidative stress, leads to degeneration of 5-HT neurons in specific regions of the brain, that further causes neurodegenerative diseases (Van Luijtelaar et al., 1989, Ueda et al., 2008). These observations were consistent with the earlier reports, suggesting degeneration of monoaminergic neurons, due to alteration of serotonergic neurotransmission (Kosofsky and Molliver, 1987, O'Hearn et al., 1988).

In the present study, chronic treatment with 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a**, showed neuroprotective effect against oxidative stress in HFD and HFD+CUMS induced depression and hence, showed beneficial action in depression co-morbid with obesity.

6.6.3. OND, QCM-4 and 4a reverse HFD and HFD+CUMS induced HPA axis hyperactivity and excess glucocorticoids (GCs)

HPA axis hyperactivity and raised GCs are observed in the pathophysiology of both depression and obesity. In normal physiological state of acute stress after activation of HPA axis, GCs are released, that have some adaptive value to restore the energy balance by promoting the release of insulin, moving palatable food, mobilizing the stored energy and directing it to central stores. HPA axis hyperactivity in individuals with abdominal obesity is noticed through raised response to CRH stimulation, elevated basal and stimulated response to stress. Elevated circulatory cortisol, increases visceral adipose tissue by 2 to 5 fold and thus higher levels of total cholesterol and triglycerides (Kyrou and Tsigos, 2009).

In mammals, a clear correlation exists between the nutritional status and stress hormone regulation in different situations. Hyperactivity of HPA axis is well characterized by abnormal production of cortisol and the inflammatory response has great impact in depressed patients (Lamers et al., 2013). The activity of the HPA axis, is modulated by factors involved in weight regulation, despite the controversy on whether weight gain results from early activation of the HPA axis or from comfort eating as a way to control stress (Tissue, 1990). The raised levels of GCs in pre-clinical as well as clinical studies of depression and anxiety is reported earlier (Plotsky et al., 1998). Increased GCs, disturb the blood glucose in chronic stress. Obesity itself is a stressful condition with high blood GCs that further causes diabetogenic action by altering the action of insulin and causing insulin resistance (Bruder-Nascimento et al., 2013). Several earlier studies have reported the association of depression, anxiety and insulin resistance (Everson-Rose et al., 2004, Murray et al., 2008, Pan et al., 2008), as represented in **Fig 6.6**. Elevated cortisol in obese patients, indicates the hyperactivity of HPA axis, which is related to the higher body mass and altered cortisol binding globulin (Dallman et al., 2004).

Clinical studies, have reported elevated GCs, in depressed patients (Stetler and Miller, 2011). GCs, maintain energy homeostasis by acting peripherally and exert feedback inhibition on CNS to modulate HPA axis activity, along with emotional and behavioral effects of stress (Herman et al., 2003). Elevated GCs, lead to hippocampal atrophy and hippocampal neurodegeneration in depressed individuals (Schoenfeld and Gould, 2012). Association of HPA axis hyperactivity, depression and abdominal fat have been reported earlier (Weber-Hamann et al., 2002). Elevated GCs, in depression are associated with inhibition of GC-mediated negative feedback, accompanied with raised CRH released from PVN (Holsboer, 2000). Beside this, anxiety-like behavior was noticed in rats, fed with HFD accompanied with elevated GC level and decreased GC-mediated negative feedback control. However, HFD elicited direct emotional disturbances, have not been studied and established. Hence, it can be concluded that diet rich in saturated fat and fat accumulation are crucial mediators of HPA axis disturbances that reflect depressive symptomlogy associated with obesity (Hryhorczuk et al., 2015).

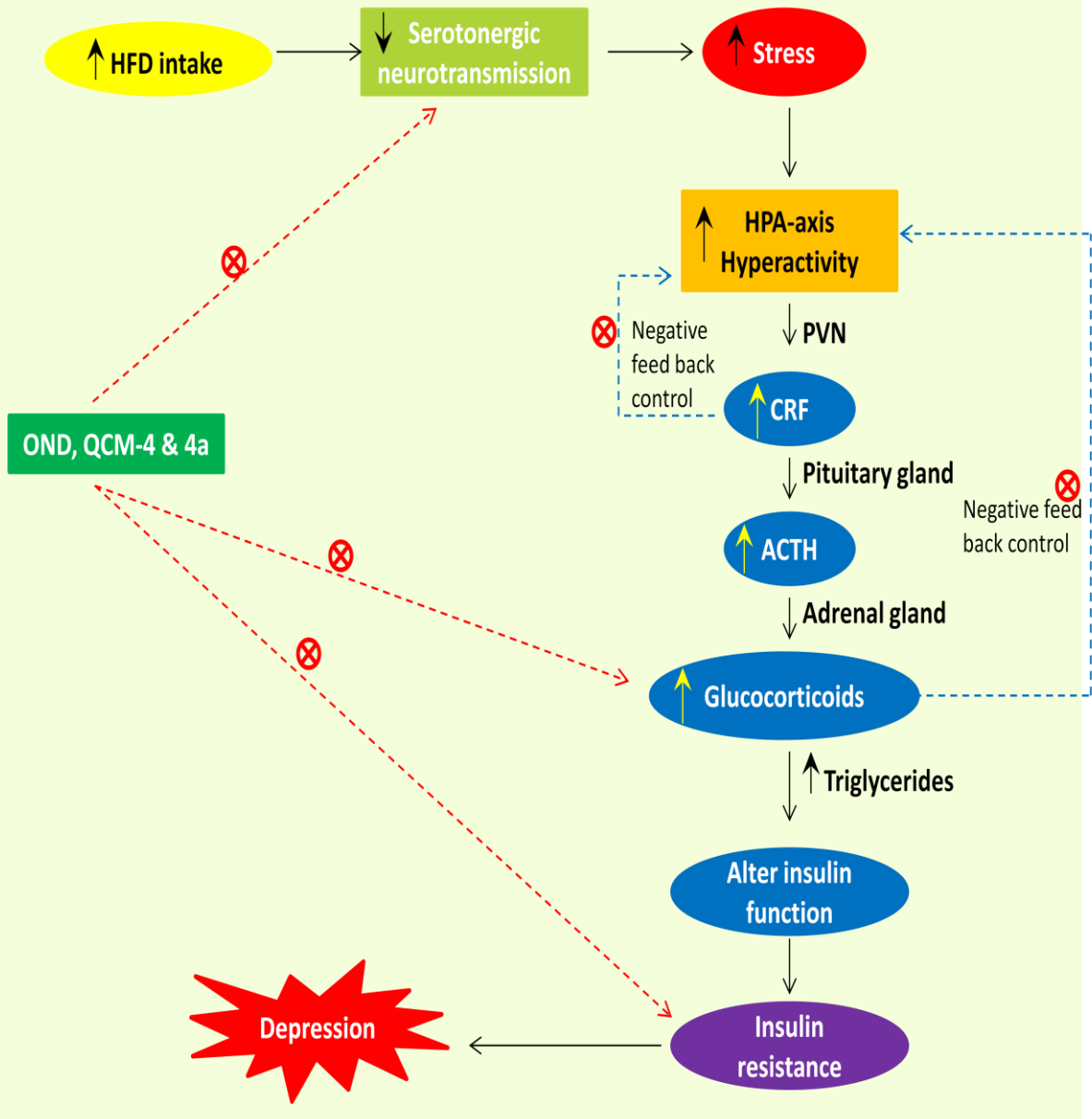


Fig 6.6: Inhibition of HFD induced HPA axis hyperactivity by serotonergic modulators **OND**, **QCM-4** and **4a** in depression co-morbid with obesity

HPA axis and 5-HT system are involved in stress related disorders such as depression and anxiety. Hippocampus which plays an important role in regulation of mood is abundantly innervated by serotonergic projections and hippocampus is also very sensitive to GCs (Hügin-Flores et al., 2004). Under normal physiological conditions, 5-HT system and HPA axis, are cross regulated in mammals, thus showing reciprocal relationship. Moreover, interactions of HPA axis and 5-HT system are of particular relevance with concern to pathological conditions including depressive disorder.

Depression involves dysfunctioning of both HPA axis and 5-HT system where, deficiency in brain 5-HT activity and raised tonic activity of HPA axis are observed (Porter et al., 2004). In support to this hypothesis, stress induced increase in plasma CORT has been reported in animals with lesions in the 5-HT nerve terminals (Lanfumey et al., 2008). These observations suggest that reduced 5-HT tone, resulting in raised tonic activity of HPA axis, are atleast in part involved in stress related disorder such as depression.

In the present study, HFD fed and HFD fed mice subjected to CUMS, resulted in severe depressive phenotypes with reduced 5-HT level and increased HPA axis hyperactivity. Chronic treatment with 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a**, inhibited the HPA axis hyperactivity by reducing the plasma CORT and improving 5-HT neurotransmission in HFD and HFD+CUMS induced depression in mice and prevented depressive behavior associated with obesity.

6.6.4. OND, QCM-4 and 4a reverse HFD and HFD+CUMS induced leptin resistance

Leptin is an anti-obesity hormone, secreted in proportion to the fat mass regulating metabolism and behavior (Maffei et al., 1995). Along with the regulation of physiological process such as neuroendocrine function, energy expenditure and appetite, leptin has been studied for antidepressant and anxiolytic effects (Liu et al., 2010, Yamada et al., 2011). Clinical studies have presented conflicting results with concern to leptin, where some studies suggested MDD patients have reduced leptin levels (Atmaca et al., 2008) whereas, other suggested that leptin level were increased in depressed women (Zeman et al., 2009), and with antidepressants treatment, either increases leptin or shows no change in leptin level (Schilling et al., 2013).

Preclinical studies have displayed more precise and convincing results as observed with lack of leptin (ob/ob mice) or its receptor (db/db mice) showing raised behavioral despairs in FST (Yamada et al., 2011), systemic and central administration of leptin produced antidepressant effect in FST, TST, and anxiolytic effect in EPM and social interaction test (Liu et al., 2010, Yamada et al., 2011).

Leptin shows significant effect on emotions, through its influence on HPA axis activity and reciprocal relationship exists between leptin and CORT level (Komorowski et al., 2000). CUMS model of depression in rodents activates HPA axis, which in turn inhibits leptin level (Ge et al., 2013).

Apart from modulating HPA axis activity, leptin also acts on leptin receptors (LepRb), present in the forebrain and midbrain loci, concerned with emotions. Genetic deletion of LepRb in the hippocampus, results in depressive phenotypes (Lu et al., 2006). In addition, it is reported that antidepressant effect of leptin, are mediated through LepRb signaling in limbic and prefrontal nuclei (Guo et al., 2012) and anti-anxiety action of leptin, is mediated by dopaminergic neurons that innervate the amygdala (Liu et al., 2011).

In individuals with higher visceral fat mass, leptin levels are found to be at risk for onset of depression (Milaneschi et al., 2012). In central obesity, insulin resistance, T2DM and leptin resistance have been observed. Leptin resistance, a characteristic of obesity, which is a result of lower CNS entry through BBB and problem with LepRb signalling, that could possibly turn as risk for mood disorders (Myers et al., 2012). Mice fed with HFD, showed decreased sensitivity of leptin for its antidepressant effect and improvement in the hippocampal BDNF levels, further resulting in depression (Yamada et al., 2011).

Leptin resistance could affect several neuronal hippocampal, mesolimbic dopaminergic and endocrine pathways mainly HPA axis activity that are concerned with regulation of mood and emotions and hence, remains an important factor in biological mechanisms underlying depression co-morbid with obesity, as depicted in **Fig 6.7**. HFD induced obesity shows leptin resistance in rodents.

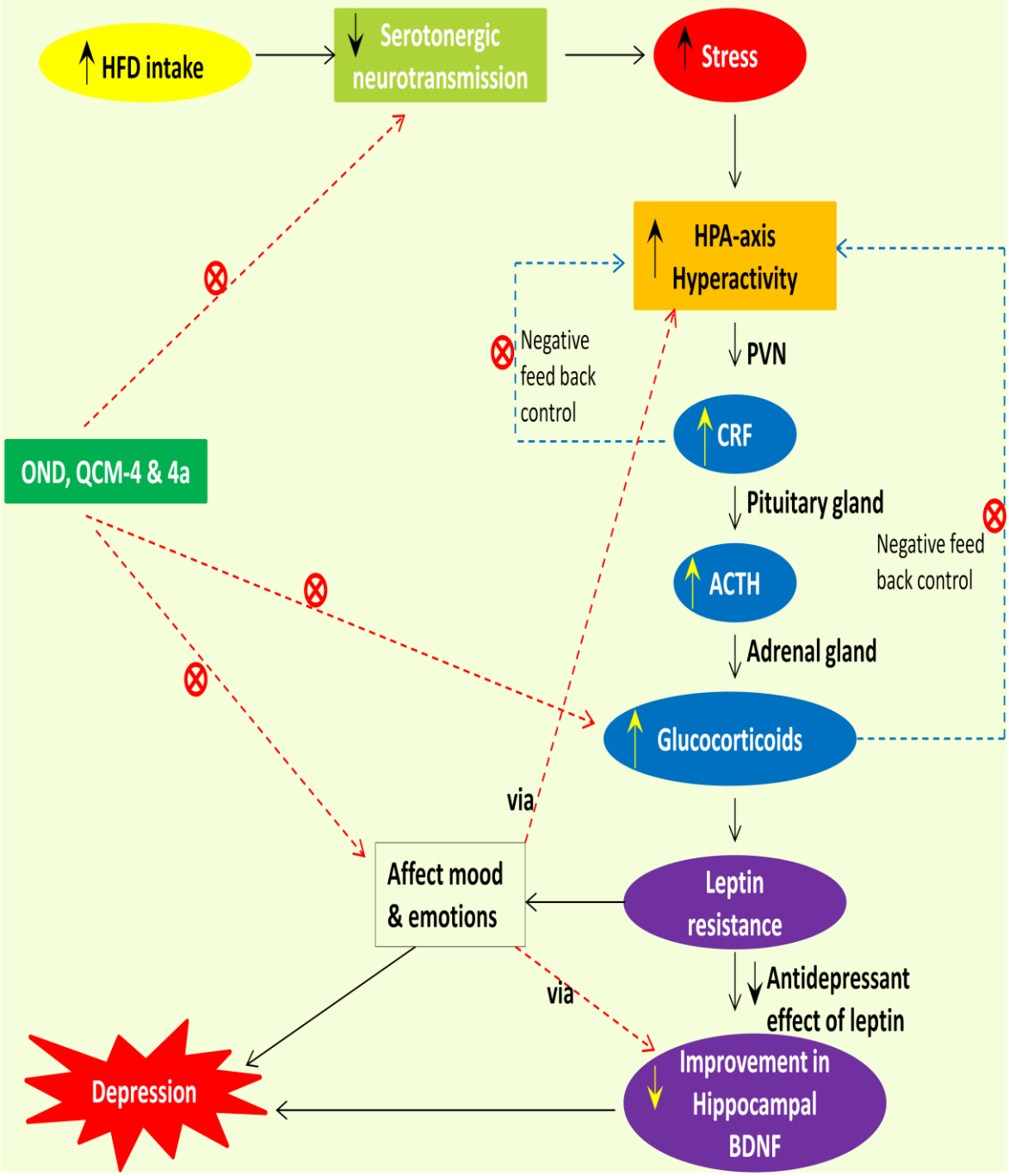


Fig 6.7: Inhibition of HFD induced leptin resistance by serotonergic modulators **OND**, **QCM-4** and **4a** in depression co-morbid with obesity

The results obtained in our studies, were in line of earlier report where HFD fed mice showed leptin resistance. Chronic treatment of 5-HT_3 receptor antagonists **OND, QCM-4 and 4a** reversed the abnormally elevated leptin levels and improved leptin sensitivity in HFD fed and HFD fed mice subjected to CUMS and exhibited antidepressant effect.

6.6.5. OND, QCM-4 and 4a inhibit HFD and HFD+CUMS induced insulin resistance

Several cross-sectional studies have shown the association of depression and insulin resistance suggesting that treatment of depression improves insulin sensitivity (Pearson et al., 2010, Silva et al., 2012). Insulin sensitizer pioglitazone reduced the signs of depression and anxiety in MDD patients (Kemp et al., 2012, Kan et al., 2013). In rodents, rosiglitazone treatment inhibited the depressive behavior (Ahmed et al., 2009). One of the theories, explains that depressive patients are associated with HPA axis hyperactivity causing abnormal elevation in plasma cortisol concentration. Furthermore, excess cortisol disturbs the glucose regulation and causes hyperinsulinemia and insulin resistance (Ryan et al., 2012). It is also evident from literature that the abnormal cortisol due to HPA axis dysregulation in clinical depression and anxiety, leads to the accumulation of excessive fat in the abdominal region, further disturbing the glucose metabolism and increasing the plasma glucose and insulin concentrations, which support the association of depression and insulin resistance (Weber-Hamann et al., 2002, Timonen et al., 2004, Adriaanse et al., 2006).

The association of obesity and insulin resistance is well documented earlier (Gallagher et al., 2010). In obesity, several mechanisms are involved in the development of insulin resistance. One such mechanism suggests that the elevated FFA in the liver produced by fatty tissues in obesity, leads to insulin resistance that further elevates the glucose level abnormally (Tissue, 1990). In addition, in obesity HPA axis dysregulation results in the excess secretion of cortisol that further interferes with the metabolism of adipocytes and promotes insulin resistance (Lee et al., 2014) as shown in **Fig 6.8**. Obese or overweight individuals, affected with insulin resistance, are prone to develop depression (Hamer et al., 2012, Platt et al., 2013).

Insulin administration through intranasal route, bypasses the BBB and has been found to produce antidepressant effect by reducing CORT level and visceral adiposity (Benedict et al., 2004, Chapman et al., 2013). In rodents fed with HFD, intranasal treatment of insulin exhibited anxiolytic effect in EPM and marble burying test (Marks et al., 2009). HFD induced obesity leads to anxiety behavior, due to impairments in central insulin signaling especially hypothalamic insulin signaling (De Souza et al., 2005). Insulin improves the mood, which indicates relationship between insulin resistance and depression.

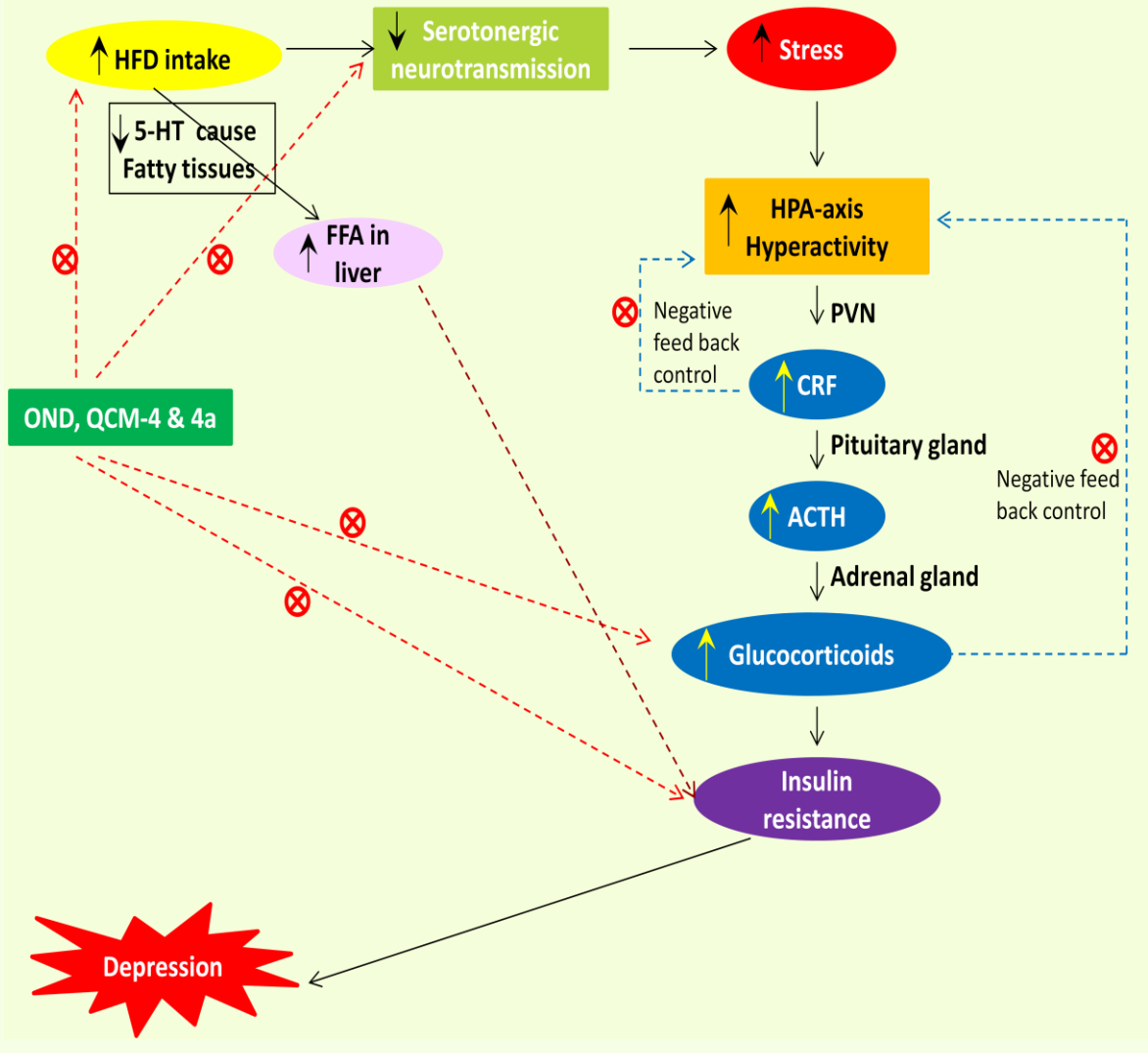


Fig 6.8: Inhibition of HFD induced insulin resistance by serotonergic modulators **OND**, **QCM-4** and **4a** in depression co-morbid with obesity

Fatty acid content of the diet, plays significant role in development of insulin resistance, as diet rich in SFA and MUFA have positive correlation with insulin resistance (Lovejoy et al., 2001). In rodents, HFD feeding, results in insulin resistance, due to reduced insulin receptors, glucose transport and glucose metabolism, decreased glycogen synthase activity in liver and muscles, and reduced storage of glucose as glycogen (Lichtenstein and Schwab, 2000, Riccardi et al., 2004). HFD feeding, leads to hypertrophy and hyperplasia, that exerts stress on endoplasmic reticulum and stimulates the secretion of pro-inflammatory cytokines, which reduces the cellular responsiveness to insulin (de Ferranti and Mozaffarian, 2008).

Hence, insulin resistance may result in depressed mood in obese population and improving insulin sensitivity may show antidepressant effect.

5-HT, maintains the balance between energy and glucose in the brain which improves insulin sensitivity (Horáček et al., 1999) and enhances the hepatic glucose uptake, facilitating the glycogen storage in liver especially in hyperglycemia and hyperinsulinemia conditions (Moore et al., 2005). Literature, has evidenced the involvement of central 5-HT, in regulating the glucose level through improving the insulin sensitivity. Thus, the agents acting by modulating the level of central 5-HT, could be useful for improving the insulin sensitivity and hence, glucose homeostasis (Pestell et al., 1989, Scheen et al., 1991). 5-HT and insulin are co-localized associated in beta-pancreatic cells (Paulmann et al., 2009). The glucose stimulation activates the transglutaminase (TGase) through elevated intracellular calcium (Guilluy et al., 2007). TGase further uses intracellular 5-HT for covalent coupling with Rab3a and Rab27a resulting in receptor independent process called as 'serotonylation' in beta cells, thus modulating the insulin secretion (Paulmann et al., 2009).

In the present investigation, chronic treatment with 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a**, inhibited the insulin resistance in HFD fed and HFD fed mice subjected to CUMS by increasing the 5-HT level, thus making an indication that **OND**, **QCM-4** and **4a**, correlates positively towards improving the insulin sensitivity in depression co-morbid with obesity.

6.6.6. OND, QCM-4 and 4a reverse HFD and HFD+CUMS induced alteration in hippocampal 5-HT level

Inverse relationship exists between central 5-HT and food intake. 5-HT modulates several motor, sensory and behavioral processes and involved in regulating feeding behavior. 5-HT exerts inhibitory effect on food intake and tends to regulate body weight by acting at several 5-HT receptor system (Watanabe et al., 2016). Anorexigenic α-MSH, released by POMC acts on MC4R and inhibits food intake and appetite. Orexigenic NPY/AgRP, improves food intake and appetite by releasing MC4R antagonist AgRP and inhibits POMC neurons, through the release of the inhibitory neurotransmitter GABA (Namkung et al., 2015). 5-HT leads to the hyperpolarization and inhibition of AgRP and decreases an inhibitory drive on POMC cells, through activation of 5-HT1BRs.

Furthermore, 5-HT is found to activate POMC neurons via activation of 5-HT2CRs. Thus, the reciprocal increase in α-MSH release and decrease in AgRP release at MC4R in target sites are observed (Heisler et al., 2006, Heisler and Lam, 2010), as represented in **Fig 6.9**.

The role of 5-HT, in the pathophysiology of depression is well known since the existence of monoamine hypothesis of depression. Marked reduction in the brain 5-HT levels have been reported in clinical studies (Owens and Nemeroff, 1994). Hence, 5-HT, plays a significant role in the pathogenesis of depression and obesity and regulates the mood, appetite and sleep (Owens and Nemeroff, 1994, Watanabe et al., 2016).

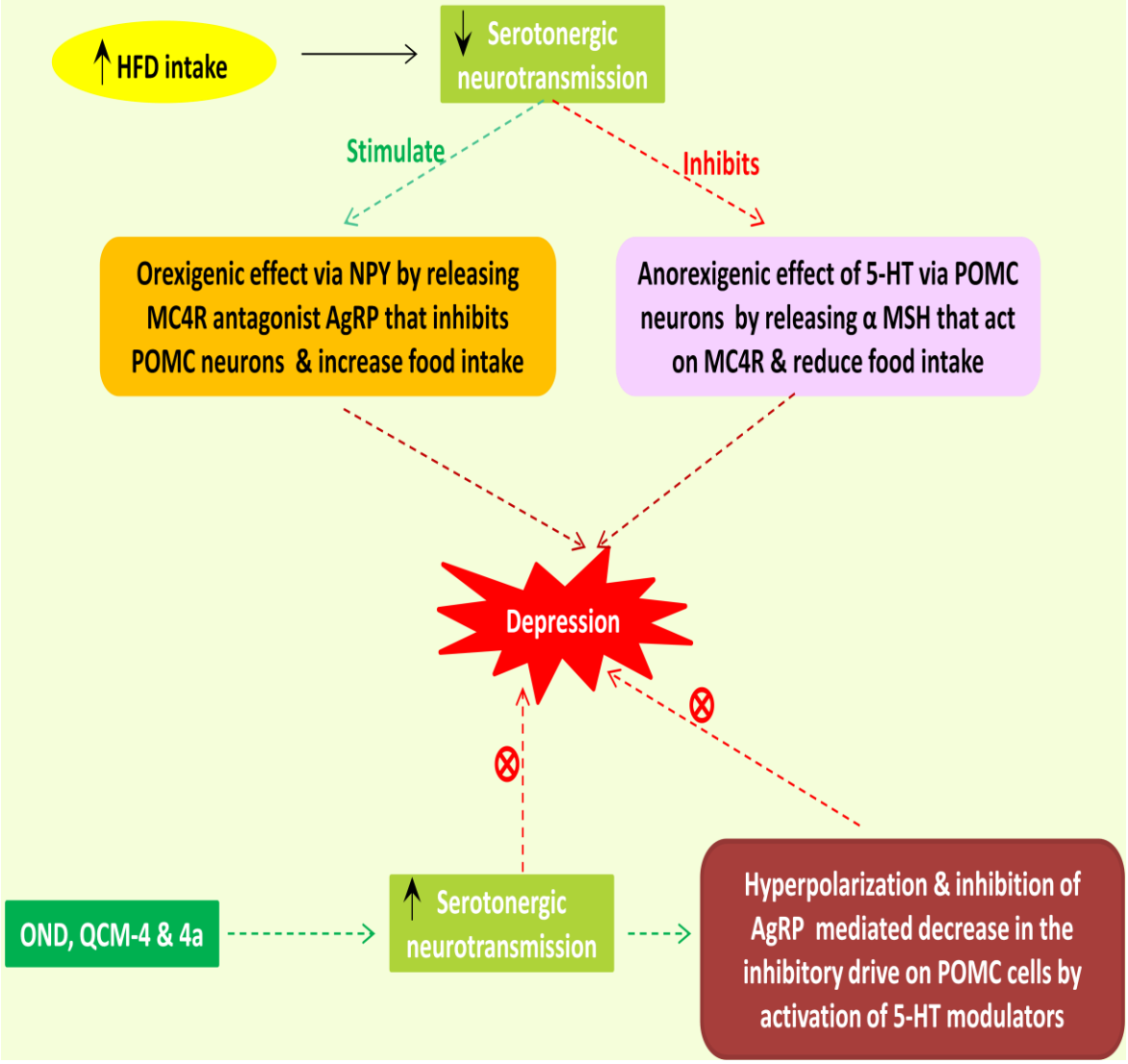


Fig 6.9: Inhibition of HFD induced diminished serotonergic neurotransmission by serotonergic modulators **OND**, **QCM-4** and **4a** in depression co-morbid with obesity

In recent studies, it was well reported that 5-HT levels are decreased with experimental HFD induced obesity in laboratory animals (Namkung et al., 2015, Watanabe et al., 2016). Hence, low levels of 5-HT could be an important risk factor for obesity due to increased food intake. 5-HT inhibits the appetite in the CNS and the peripheral 5-HT concentrations may correlate to the central 5-HT levels, as it cannot cross the BBB (Park et al., 2014).

Taken together, 5-HT is a crucial marker in the disease pathogenesis of depression and obesity. HFD induced obesity and HFD fed mice subjected to CUMS model showed reduced hippocampal 5-HT levels and depressive phenotypes and hence, these models could be used to study depression co-morbid with obesity in the experimental laboratories. Chronic treatment with 5-HT_3 receptor antagonists **OND, QCM-4 and 4a**, improved the 5-HT levels in the hippocampus of the HFD fed and HFD fed mice subjected to CUMS and showed antidepressant-like effect.

6.6.7. OND, QCM-4 and 4a prevent HFD and HFD+CUMS induced impairment in hippocampal neurotrophic factor BDNF and morphological perturbations

BDNF is a member of neurotrophin family of growth factors, which acts on TrkB receptors and stimulates the development and differentiation of new neurons and facilitates long-term potentiation (Noble et al., 2011). BDNF is a very important marker in neuropsychiatric disorders such as cognition, memory, and depression. Animal models of depression, have shown that chronic stress, decreases the hippocampus expression of BDNF mainly in the DG region (Lee and Kim, 2010). Stressors, aggravate the HPA axis activity which elevate GCs and ultimately affect the activity of BDNF, in the hippocampus. Neurotrophic hypothesis suggests that, reduced hippocampal BDNF levels are involved in the pathogenesis of depression. Chronic treatment with antidepressants improves BDNF expression in the DG region of the hippocampus and hence, inhibits depressive phenotypes (Warner-Schmidt and Duman, 2006).

Reduced levels of BDNF were associated with altered glucose metabolism and hence, as pathogenetic factor for T2DM (Krabbe et al., 2007). BDNF has been found to play an important role in regulation of blood glucose levels, lipid metabolism and inhibits food intake in mice (Ono et al., 2000). Moreover, the BDNF mutant mice developed obesity, characterized by increased body weight, hyperleptenemia, hyperinsulinemia, hyperglycemia, and hyperlipidemia. BDNF was studied as a risk factor for development and pathophysiology of childhood obesity (Araki et al., 2014).

Moreover, low blood BDNF levels were reported in obese children and adults. A recent meta-analysis study correlated decreased BDNF levels with obesity and T2DM in Chinese population (Li et al., 2015).

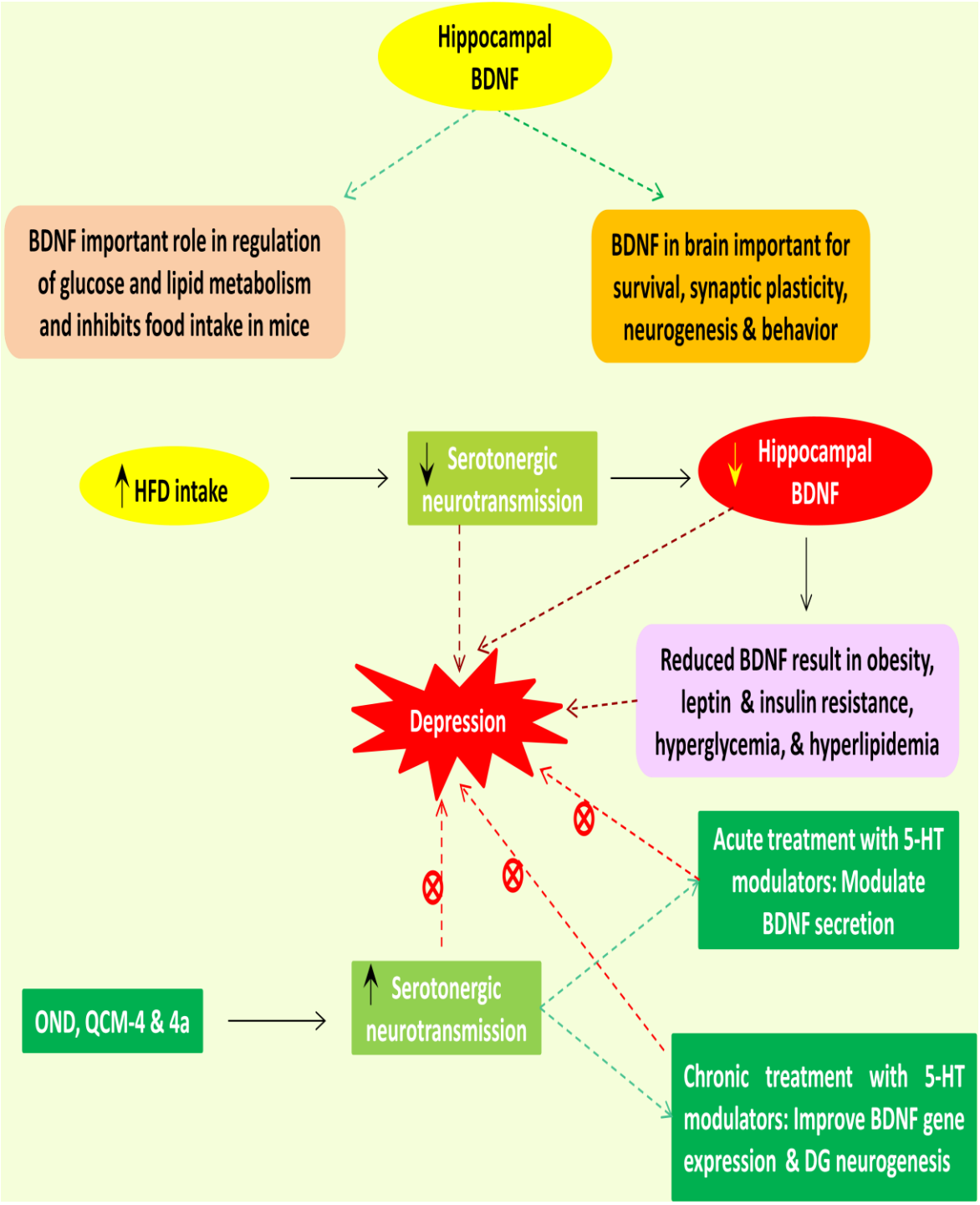


Fig 6.10: Inhibition of HFD induced diminished hippocampal BDNF by serotonergic modulators **OND**, **QCM-4** and **4a** in depression co-morbid with obesity

HFD induced model of obesity and T2DM in rodents, have shown reduced levels of BDNF in the hippocampus, suggesting BDNF as an important biological marker in the disease pathogenesis. Moreover, animals fed with HFD, showed reduced antidepressant activity of leptin by improving the BDNF concentrations, in the hippocampus (Yamada et al., 2011). HFD induced alterations, in energy homeostasis and lipid metabolism including hyperglycemia, hyperleptinemia in animals was similar to that observed in humans. HFD fed animals showed poor cognitive performance which was associated with reduced hippocampal volume and BDNF levels (Freeman et al., 2014) as described in **Fig 6.10**. Moreover, HFD induced reduction in hippocampal BDNF levels have been observed to be associated with depressive and anxiety-like phenotypes in rodents (Kaczmarczyk et al., 2013, Numakawa et al., 2014). Hence, altered BDNF signaling in hippocampus of adult brain, may be involved in the pathophysiology of depression. In rodents, HFD induced reduced hippocampal BDNF, was in correlation with decreased activation of cAMP response element binding protein (CREB), in the hippocampus, which has positive association with the depressive and anxiety-like behavior (Sharma et al., 2012).

Obesity and depression are associated with chronic stress that showed elevated GCs, decreased BDNF, 5-HT and neurogenesis process in the hippocampus region of the brain (Mahar et al., 2014). cAMP acts as a second messenger system, involved in the signal transduction mechanisms that stimulate CREB and finally increases BDNF, the neurotrophic factor responsible for neurogenesis (Nakagawa et al., 2002, Mällo et al., 2008). Delayed clinical antidepressant efficacy, allowed the researchers to study the downstream mechanisms involved in antidepressant effect. BDNF is an important neurotrophin, that is widely distributed throughout the brain and involved in the regulation of neuronal survival, synapse/neurite formation, migration, axonal and dentrite growth along with the key role of synaptic plasticity and behavior (Martinowich and Lu, 2008). It was confirmed through experimental research that BDNF mediated neurogenesis process is involved in the antidepressant activity (Malberg and Blendy, 2005).

Alteration in the serotonergic signaling in the brain, significantly affects the synaptic plasticity and hippocampal neurogenesis (Ferrés-Coy et al., 2013). 5-HT, a neurotransmitter plays a crucial role in the hippocampal neurogenesis through improving the survival, neurite outgrowth and synaptogenesis by reversing the HPA axis hyperactivity (Ferrés-Coy et al., 2013).

5-HT is involved in regulation of BDNF in rodents (Mällo et al., 2008). 5-HT improves the expression of neurogenesis factors, such as CREB and BDNF in the brain (Nibuya et al., 1996). CREB and BDNF are involved in the hippocampal neurogenesis and hence, serve as important markers in stress related disorders such as depression and anxiety (Martinowich and Lu, 2008). 5-HT regulates BDNF in two different ways, one suggesting the fast acting 5-HT modulation of BDNF secretion which leads to changes in synaptic plasticity and the other suggest the repetitive treatment with serotonergic modulators, resulting in improving BDNF gene expression that improves the hippocampal DG region neurogenesis and stability of synapse (Martinowich and Lu, 2008).

Results obtained in the present study, showed depleted hippocampal cAMP and BDNF levels in HFD fed and HFD fed mice subjected to CUMS. Hence, hippocampal neuronal degeneration as observed by H and E staining in HFD fed and HFD fed mice subjected to CUMS. Chronic treatment with 5-HT_3 receptor antagonists **OND, QCM-4 and 4a**, improved the hippocampal cAMP and BDNF levels, and neuronal morphology through modulation of serotonergic neurons, thus showing antidepressant effect, in HFD fed and HFD fed mice subjected to CUMS.

6.6.8. OND, QCM-4 and 4a inhibit HFD and HFD+CUMS induced p53 mediated neuronal damage

Based on the biological mechanisms involved in the pathogenesis of depression co-morbid with obesity, mainly HPA axis hyperactivity and brain oxidative stress, IHC study of p53 protein in the DG region of hippocampus was performed. The p53 protein mediated apoptosis, is strongly recommended in the neurodegenerative disorders (Ghavami et al., 2014), as briefly represented in **Fig 6.11**. The p53 protein is also involved in altering the function of HPA axis and leading to increase oxidative stress mediated neuronal damage (Scrable et al., 2009).

In the IHC study, HFD fed mice, showed higher neuronal damage in the DG region, as confirmed by high percent area of p53. Chronic treatment with 5-HT_3 receptor antagonists **OND, QCM-4 and 4a**, inhibited the p53 mediated hippocampal neuronal degeneration, in HFD fed mice. From the mechanistic point of view, for role of **OND, QCM-4 and 4a**, in inhibition of p53 mediated neuronal damage, further studies are urged in depression associated with obesity.

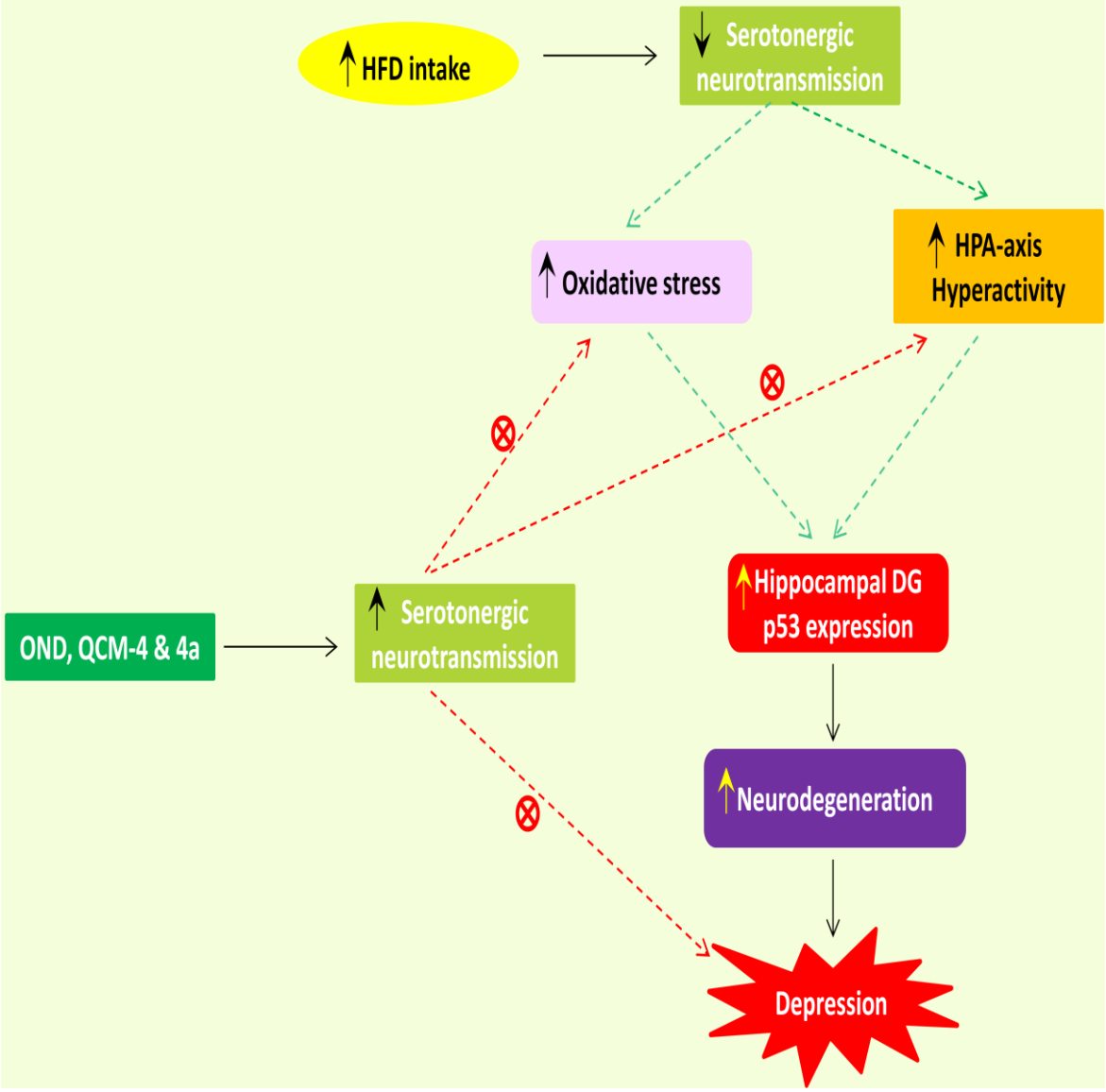


Fig 6.11: Inhibition of HFD induced p53 mediated neuronal damage by serotonergic modulators **OND**, **QCM-4** and **4a** in depression co-morbid with obesity

6.7. Role of 5-HT$_3$ receptors for antidepressant activity of OND, QCM-4 and 4a in depression co-morbid with obesity

Clinically used SSRI class of antidepressants exhibit their effect by facilitating 5-HT neurotransmission and functional antagonism of 5-HT$_3$ receptors (Eisensamer et al., 2003). Hence, there exist a correlation of 5-HT$_3$ receptor antagonism and modulation of 5-HT, in antidepressant effect.

In the present study, in order to confirm that **OND, QCM-4 and 4a**, showed antidepressant effects, through antagonism of 5-HT_3 receptors and modulation of 5-HT neurotransmission, the potency of **OND, QCM-4 and 4a**, was evaluated in HFD fed mice pre-treated with 5-HT_3 receptor agonist mCPBG. 5-HT_3 receptor agonist mCPBG, seems of no clinical importance.

In an in-vitro assay, incorporation of 5-HT_3 receptor agonist mCPBG, initially activated the cells, expressing 5-HT_3 receptors, in the plasma membrane followed by desensitization through internalization of 5-HT_3 receptors thereby decreasing there density (Ilegems et al., 2004). Such internalization of 5-HT_3 receptors was prevented by its antagonist **OND**, thus inhibiting the reduction in the density and functional activity of receptors (Freeman et al., 2006).

In the present study, mice were fed with HFD for 14 weeks and pre-treated with mCPBG followed by administration of **OND, QCM-4 and 4a** for 28 days. Then behavioral assays of depression such as SPT, FST, TST and, anxiety including EPM, L/D test were performed followed by estimation of hippocampus 5-HT level.

The basal locomotor scores were not altered with mCPBG alone and along with **OND, QCM-4 and 4a**, in HFD fed mice. Pre-treatment with mCPBG, attenuated the antidepressant effect of **OND, QCM-4and 4a**, by reducing the sucrose consumption in SPT, increasing immobility time in FST and TST, decreasing the percent OAE and OAT in EPM, inhibiting light chamber time and transition scores in L/D test and inhibiting hippocampal 5-HT levels, in HFD fed mice. This study, experimentally confirmed that, the antidepressant effect of **OND, QCM-4 and 4a**, were produced by antagonism of 5-HT_3 receptors. Our results were similar to earlier report, were pre-treatment of mCPBG, inhibited the antidepressant effect of agents, acting through 5-HT_3 receptor antagonism (Nakagawa et al., 1998).

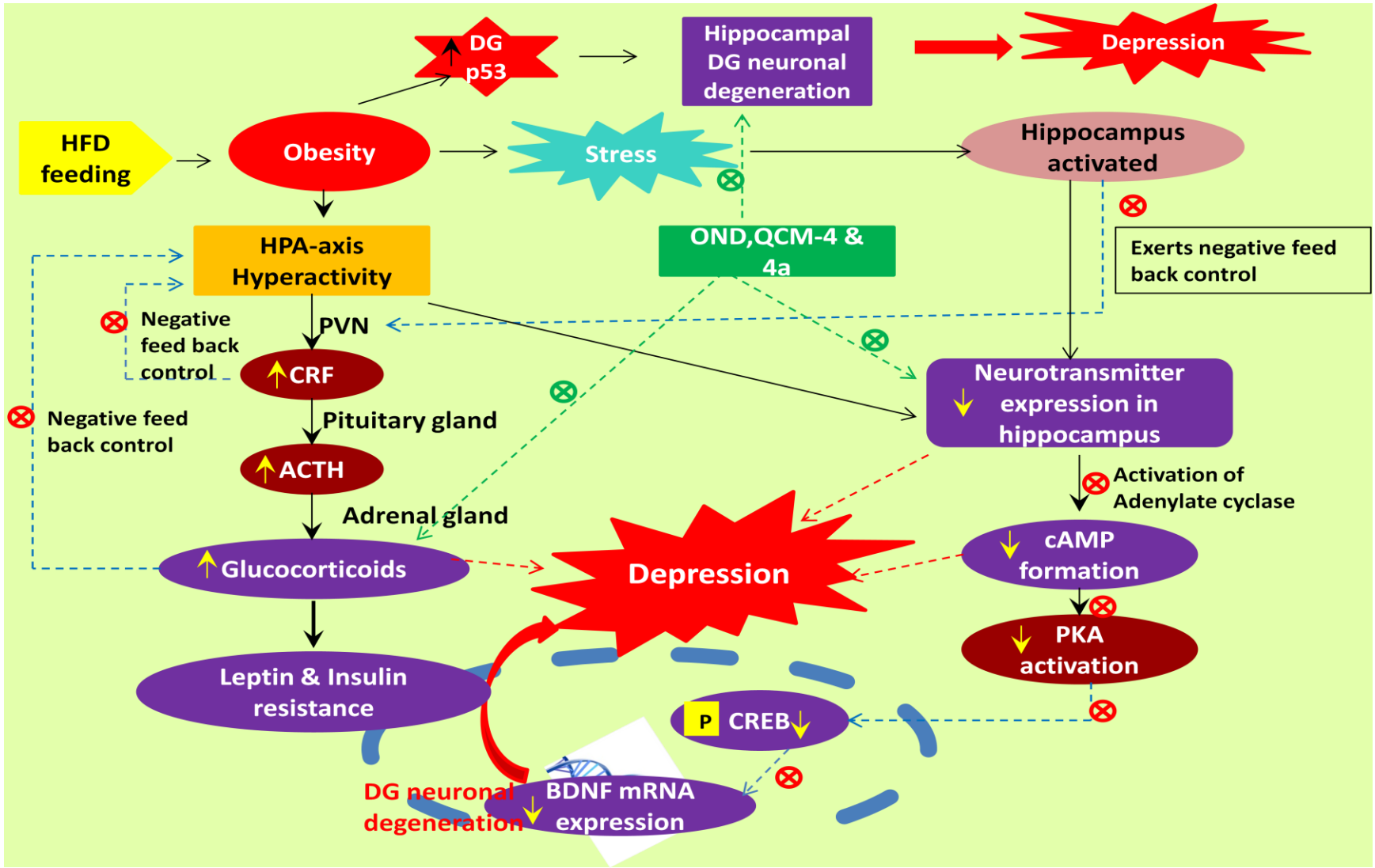


Fig 6.12: Conclusive hypothesis

CONCLUSIONS

From the present study following can be concluded;

- Obesity leads to the risk of depressive behavior

- The biological mechanisms linking depression and obesity are HPA axis hyperactivity, increased oxidative stress, leptin and insulin resistance, impaired BDNF and 5-HT level, neuroanatomical alterations in the hippocampal DG

- HFD and HFD+CUMS induced depression models mimic behavioral, biochemical and molecular alterations of depression co-morbid with obesity

- 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** showed antidepressant effect through serotonergic neuromodulation by reversing behavioral, biochemical and molecular alterations in depression co-morbid with obesity

- Novel drug candidates with potential 5-HT_3 receptor antagonism may be the therapeutic approach for management of depression co-morbid with obesity

SUMMARY

Depression is a neuropsychiatric disorder that is associated with depressed mood, loss of interest or inability to experience pleasure, low self esteem or guilt feeling, low energy, altered sleep and appetite, and poor concentration. In addition, depression has often observed along with the symptoms of anxiety as they share very narrow margin of symptoms. Depression affects around 350 million world population and is a significant contributor to the global burden of diseases. According to WHO, by 2020 depression is expected to stand second from its current 4^{th} position as a leading cause of disability worldwide. Despite of several therapies, no single drug has shown a promising result for treatment of depression in clinic.

Obesity is often associated with increased risk factor for several chronic diseases such as T2DM, hypertension, heart disease and stroke. The prevalence rate of obesity is very high, where 1.9 billion adults of age 18 years and older are overweight, of which around 600 million (13% of world population) of adults are obese. Over a period of time, researchers have come across the neuropsychiatric consequences associated with obesity mainly depression, Parkinson's disease and cognitive impairments. Obesity has been studied as a major risk factor for depression as more than 50% of the obese population are twice susceptible for the development of depression in comparison to non-obese population.

Despite of advances in the neuropsychopharmacological research, biological mechanisms linking depression and obesity are still not studied in details. Hence, a proper pharmacotherapy for treatment of depression co-morbid with obesity has not yet developed.

In the present study, several biological mechanisms involved in the pathogenesis of depression co-morbid with obesity such as HPA axis hyperactivity, oxidative stress, leptin and insulin resistance, reduced BDNF, 5-HT level in hippocampus, altered neuronal morphology in DG region of hippocampus and p53 mediated hippocampal DG region neuronal damage was studied. The high predictive validity models such as HFD induced obesity and HFD fed mice subjected to CUMS were used to mimic the depressive phenotypes in order to study depression co-morbid with obesity. Mice were fed with HFD for 14 weeks to induce obesity and screened for depressive behavior. In another model, HFD fed mice were subjected to CUMS and than screened for depressive phenotypes.

Central 5-HT is involved in regulation of energy homeostasis by controlling food intake, mood and sleep. 5-HT turnover is severely affected in both depression and obesity. 5-HT neurotransmission is involved in regulation of various physiological processes. The drugs that acts by improving the level of 5-HT in brain are basically aimed for the treatment of depression and obesity. Serotonergic system has inverse relationship with HPA axis hyperactivity and hence, is effective in regulation of negative feedback control by inhibiting the excess plasma glucocorticoids. Importantly, 5-HT neurotransmission inhibits oxidative stress induced neuronal damage. 5-HT is also involved in regulation of leptin and insulin resistance. Serotonergic system is involved in controlling the secretion of insulin and hence, improves insulin sensitivity and plasma glucose. BDNF is the neurotrophic factor that is present on the serotonergic neurons in the hippocampus region. In depression co-morbid with obesity, BDNF levels were severely decreased due to degeneration of serotonergic neurons in the hippocampus. Obesity is a stressful condition and stress induces p53 mediated hippocampal DG neuronal damage.

Serotonergic neurotransmission have effective control over the biological mechanisms linking depression and obesity such as HPA axis hyperactivity, oxidative stress, leptin and insulin resistance, BDNF, hippocampal neuronal morphology and p53 mediated neuronal damage.

Hence, the novel therapeutic targets that act by improving the serotonergic neurotransmission could be a sensible and effective therapeutic approach to treat depression co-morbid with obesity. The currently most commonly prescribed SSRI class of antidepressants has showed functional antagonism of 5-HT_3 receptors while producing antidepressant action. Moreover, the recent drug vortioxetine has reported for the management of mood and anxiety-like disorders through its multimodal effect on serotonergic system including agonist at 5-HT_{1B} receptor, and antagonist at 5-HT_3 and 5-HT_7 receptors, respectively. Hence, there exists a correlation between antagonism of 5-HT_3 receptors and neuromodulation of 5-HT in the antidepressant-like effect.

Since long time, 5-HT_3 receptor antagonists are being studied as potential antidepressants through modulation of brain serotonergic neurotransmission in the pre-clinical settings. Hence, in the present study standard **OND** and novel 5-HT_3 receptor antagonists **QCM-4** and **4a** were used as a treatment strategy for depressive phenotypes in HFD and HFD+CUMS models.

Chronic treatment with **OND**, **QCM-4** and **4a** inhibited the depressive-like behavior by improving the sucrose consumption in SPT, inhibiting behavioral despair in FST and TST by reducing the immobility time in HFD and HFD+CUMS induced depression models. In the behavioral assays of anxiety, chronic treatment with **OND**, **QCM-4** and **4a** improved the percent OAE and OAT in EPM, head dip and crossing scores in HBT, time in light chamber and transition scores in L/D test in HFD and HFD+CUMS induced depression models.

In the biochemical estimations, chronic treatment with **OND**, **QCM-4** and **4a** inhibited elevated plasma glucose and lipids, HPA axis hyperactivity by reducing the excess plasma corticosterone, oxidative stress by reducing MDA and increasing GSH level in the hippocampus, improved leptin and insulin sensitivity by reducing their plasma levels in HFD and HFD+CUMS induced depression models.

In the molecular assays, chronic treatment with **OND**, **QCM-4** and **4a** improved the hippocampal 5-HT, cAMP and BDNF levels, inhibited alteration of hippocampal DG region neuronal morphology using H & E staining in HFD and HFD+CUMS models, and ameliorated p53 mediated neuronal damage in hippocampal DG region of HFD fed mice.

The confirmatory assay was performed to evaluate whether **OND**, **QCM-4** and **4a** exhibited antidepressant effect through 5-HT_3 receptor antagonism or not. The potency of **OND**, **QCM-4** and **4a** was investigated in presence of 5-HT_3 receptor agonist mCPBG, which seem to have no clinical effect. HFD fed mice were co-treated with 5-HT_3 receptor agonist mCPBG along with 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** for 28 days. After treatment schedule, in the behavior assays concomitant administration of mCPBG inhibited the antidepressant efficacy of **OND**, **QCM-4** and **4a** by decreasing sucrose consumption in SPT, increasing immobility time in FST and TST, reducing percent OAE and OAT in EPM and decreasing time in light chamber and transition score in L/D test in HFD fed mice. In addition, mCPBG severely inhibited the hippocampal 5-HT level in **OND**, **QCM-4** and **4a** treated HFD fed mice. This study indicated that **OND**, **QCM-4** and **4a** showed the antidepressant effect in depression co-morbid with obesity by antagonism of 5-HT_3 receptors.

Taken together, chronic administration of 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** inhibited behavioral, biochemical and molecular alterations and showed antidepressant effect by modulation of serotonergic neurotransmission on depression co-morbid with obesity in HFD and HFD+CUMS induced depression models.

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Behavioral and Neuro-pharmacological Screening of Potential Serotonergic Modulators for Depression Co-morbid with Obesity

THESIS

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DOCTOR OF PHILOSOPHY

by

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CONCLUSIONS

From the present study following can be concluded;

- Obesity leads to the risk of depressive behavior

- The biological mechanisms linking depression and obesity are HPA axis hyperactivity, increased oxidative stress, leptin and insulin resistance, impaired BDNF and 5-HT level, neuroanatomical alterations in the hippocampal DG

- HFD and HFD+CUMS induced depression models mimic behavioral, biochemical and molecular alterations of depression co-morbid with obesity

- 5-HT_3 receptor antagonists **OND**, **QCM-4** and **4a** showed antidepressant effect through serotonergic neuromodulation by reversing behavioral, biochemical and molecular alterations in depression co-morbid with obesity

- Novel drug candidates with potential 5-HT_3 receptor antagonism may be the therapeutic approach for management of depression co-morbid with obesity