GMR
The Original

COMPARISON OF AN INDUCTION MODELS FOR THE DEVELOPMENT OF PSYCHOSIS CONDITION AND ITS ASSESSMENT TARGETING D2 RECEPTORS BY PRECLINICAL STUDIES

Ms. V.M. Barethiya [1*], Dr. Mrs. S. A. Deshpande [2]

[1] Ph.D. Research Scholar and Assistant Professor, Priyadarshini J. L. College of Pharmacy, Affiliated to Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440016
[2] Professor and Head of Pharmacology department, Priyadarshini J. L. College of Pharmacy, Affiliated to Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440016
*Corresponding Author: Ms. V.M. Barethiya, Email: barethiyavarsha@gmail.com

ABSTRACT
Preclinical animal models are essential for elucidating the neurobiological mechanisms underlying psychosis, stress-related psychopathology and for evaluating potential therapeutic agents. Among the various experimental paradigms, Chronic Unpredictable Mild Stress (CUMS), Olfactory Bulbectomy (OBX) and Dexamethasone-induced glucocorticoid exposure model are widely employed in rats to behavioural, neuroendocrine, and neurochemical alterations relevant to depressive-like states leading to cause depression induced psychosis. The behavioural parameters such as hyperactivity, stereotypy, etc., biochemical estimation such as dopamine, lipid peroxidation and histopathological studies were studied. It was concluded that among all the three models of induction- olfactory bulbectomy induces psychosis model had shown the good result.

KEYWORDS: Psychosis, Chronic Unpredictable Mild Stress (CUMS), Olfactory Bulbectomy (OBX), Hypothalamic–Pituitary–Adrenal (HPA) axis, Glucocorticoid dysregulation

1. INTRODUCTION

Psychosis is broadly described as a state in which a person loses contact with reality, manifesting symptoms such as hallucinations (perceiving things not present) or delusions (fixed false beliefs), and/or disorganized thinking or behaviour. For example, in one review, psychosis is defined as “the presence of delusions, hallucinations without insight, or both.” (1) “The concept of psychosis still lacks

a unified definition, but denotes a clinical construct composed of several symptoms. Delusions, hallucinations, and thought disorders are the core clinical features." (2) In clinical and research practice, psychosis may be considered along a spectrum of severity, and can occur in many different disorders (primary psychotic disorders, mood disorders with psychosis, substance-induced psychosis, medical/neurologic-caused psychosis). (1) The present study deals with the preclinical studies of the induction models of psychosis and their comparison to find out the preferred model for depression induced psychosis with the help of the behavioural, biochemical and histopathological evidences. It is estimated that about **1.5% to 3.5%** of people will meet diagnostic criteria for a primary psychotic disorder in their lifetime; however, many more may experience one or more psychotic symptoms (e.g., hallucinations or delusions) without necessarily meeting full diagnostic criteria.(3)

Typical manifestations include:

- **Hallucinations**: perceptions in the absence of an external stimulus (e.g., hearing voices when no one is speaking).(4)
- **Delusions**: firmly held false beliefs that are resistant to contrary evidence (e.g., believing someone is plotting against you). (5)
- **Disorganised thinking and behaviour**: speech may jump between topics, be derailed or incoherent; behaviour may become bizarre or inappropriate.
- **Negative symptoms / cognitive impairment**: especially in primary psychotic disorders, symptoms such as reduced motivation, social withdrawal, and cognitive deficits contribute heavily to disability.

PATHOPHYSIOLOGY OF PSYCHOSIS

Psychosis results from complex interactions among neurochemical, structural, genetic, and environmental factors that disrupt brain networks involved in perception, cognition, and reality testing.

1. Neurochemical Hypotheses

a. Dopamine Hypothesis

- In Neuropsychopharmacology, the mesolimbic dopamine pathway—originating in the Ventral Tegmental Area and projecting to the Nucleus Accumbens—plays a central role in reward, motivation, and salience attribution. Hyperactivity of dopamine transmission in this pathway, particularly involving excessive stimulation of D_2 receptors, leads to abnormal assignment of importance (aberrant salience) to otherwise irrelevant internal or external stimuli. As a result, neutral thoughts or perceptions may be misinterpreted as highly significant or threatening, giving rise to positive symptoms such as hallucinations and delusions.
- Hypoactivity of dopamine in the mesocortical pathway

In the framework of the Dopamine Hypothesis of Schizophrenia, the mesocortical dopamine pathway—this projects from the ventral tegmental area to the prefrontal cortex—is crucial for regulating cognition, motivation, and executive functions. Hypoactivity of dopamine transmission in this pathway, particularly reduced stimulation of D_1 receptors, leads to impaired cortical processing and diminished neural signaling involved in attention, working memory, and decision-making. This dopaminergic deficit results in negative symptoms such as apathy, anhedonia, and social withdrawal, as well as cognitive symptoms like poor concentration, reduced problem-solving ability, and impaired executive control. Unlike positive symptoms, these features are less responsive to conventional antipsychotics, highlighting the importance of mesocortical dysfunction in the persistent functional impairment seen in schizophrenia.(6)

b. Glutamate Hypothesis

- Hypofunction of NMDA receptor on GABA interneurons reduces inhibitory control, leading to disinhibition of glutamatergic neurons.
- This results in excessive glutamate-driven activation of dopaminergic neurons, contributing to dysregulated dopamine transmission and psychotic symptoms.(7)

c. GABAergic Dysfunction

- Reduced activity of GABAergic interneurons in the Prefrontal Cortex and Hippocampus lowers inhibitory tone, leading to cortical disinhibition.
- This imbalance disrupts neural synchrony and information processing, resulting in cognitive deficits such as impaired attention, memory, and executive function.(8)

3. Neurodevelopmental & Genetic Factors

- Prenatal insults such as maternal infection, malnutrition, or hypoxia can disrupt normal neurodevelopment, leading to abnormal neuronal migration and defective synaptic pruning; these early structural alterations impair the organization and connectivity of cortical and limbic circuits, increasing vulnerability to later psychiatric dysfunction. In addition, genetic susceptibility—through polymorphisms in genes like COMT, DISC1, NRG1, and DTNBP1—affects dopamine metabolism,

synaptic plasticity, and glutamatergic transmission, thereby contributing to the neurochemical imbalances underlying disorders such as schizophrenia..(9)

4. Environmental & Stress Factors

- Exposure to Cannabis, psychosocial stress, and trauma increases dopamine synthesis and receptor sensitivity, heightening the risk of dopaminergic dysregulation.
- Hyperactivity of the Hypothalamic–Pituitary–Adrenal axis (↑ cortisol) further amplifies dopamine transmission and promotes neurotoxic effects in limbic brain regions. (10)

5. Integrative View

Psychosis arises from dopaminergic dysregulation secondary to glutamate and GABA imbalance, influenced by genetic predisposition**,** neurodevelopmental anomalies**,** and environmental stressors**.** These disruptions impair frontal–limbic connectivity, leading to distorted perception and thought.

CURRENTLY AVAILABLE INDUCTION MODELS FOR PSYCHOSIS

A. Drug induced psychosis

Dizocilpine induced psychosis- One of the best proposed pharmacological animal models is dizocilpine, as it can mimic the full spectrum of schizophrenic disorder including positive and negative symptoms along with cognitive deficits. Dizoclipine is n-methyl-d-aspartate (NMDA) receptor antagonist known to induce hyperlocomotion and stereotyped behaviour in rodents. To develop an animal model of sz via intraperitoneal administration of dizocilpine in rats (100-150g) at a dose of 0.3 mg/kg for eight days. Dizocilpine injected rats exhibited significant hyperlocomotor behavior, depressive symptoms and cognitive deficits.

Amphetamine induced psychosis: On the acute administration of amphetamine, it shows the result as decreased latent inhibition; increased locomotion while on the chronic administration it shows the result same as acute but with decreased PPI. A molecular alteration was observed as increased mesolimbic dopamine response; decreased acetylcholine in PFC.

Glutamatergic manipulation or phencyclidine/MK-801/ketamine induced psychosis: It shows the result in which the increased locomotion; decreased working memory; decreased reversal learning performance; decreased social interaction and the molecular alteration was observed as decreased PPI decreased pv-immunoreactive neurons in PFC and hippocampus.

B. Induction of psychosis by genetic manipulation

1. Missense mutations models: in this model, shows molecular alteration in decreased brain volume; decreased pde4b activity and binding to disc1; decreased pv-immunoreactive; decreased dendritic density and it was evaluated by decrease in PPI activity; decreased latent inhibition; increased depressive-like phenotype
2. Dominant-negative isoforms of disc1: this model shows increase in increased amphetamine sensibility; decreased working memory because of alteration in reduction of level of dopamine, dopac and decrease in pv-immunoreactive
3. Knockdown model: in this model, there is decreased dopamine; decreased pv-immunoreactive and it was evaluated by increased amphetamine sensibility; decreased ppi; decreased working memory [35]

C. Substance induced psychosis

Substance-induced psychoses are a class of psychotic disorders defined as being triggered by the use of alcohol or other substances (WHO, 1993). The psychosis manifests in the presence of intoxication or withdrawal, and by definition dissipates once the person stops using the substance in question, usually and loosely defined as within a month of such substance-use cessation. The relative risk of developing schizophrenia after incident substance-induced psychosis, compared to people without substance-induced psychosis is extremely high.

Table no. 1 Theoretical comparison of the three induction models of the psychosis

Model	Induction Method	Key Behavioral Outcomes	HPA Axis / Neuroendocrine Findings	Neurobiological Alterations	Relevance to Psychosis
Chronic Unpredictable Mild Stress (CUMS)	Exposure to varied mild stressors (e.g., food/water deprivation, cage tilt, overnight illumination) for 4–8 weeks	Decline Sucrose preference (anhedonia); Incline immobility in forced swim test; poor social interaction; cognitive deficits	↑ Basal corticosterone; exaggerated stress-induced corticosterone response; impaired negative feedback	Reduced BDNF; synaptic plasticity deficits; hippocampal and prefrontal cortical changes	Models negative and cognitive symptom dimensions related to stress-induced psychosis

Model	Induction Method	Key Behavioral Outcomes	HPA Axis / Neuroendocrine Findings	Neurobiological Alterations	Relevance to Psychosis
Olfactory Bulbectomy (OBX)	Bilateral surgical removal of olfactory bulbs	Hyperlocomotion; impulsivity; anhedonia; memory and attention deficits	Elevated basal corticosterone; impaired dexamethasone suppression	Altered serotonergic, noradrenergic, dopaminergic transmission; reduced hippocampal neurogenesis	Strong face and predictive validity for psychosis-like hyperactivity and affective dysregulation
Dexamethasone (Chronic Glucocorticoid Exposure)	Repeated parenteral dexamethasone (i.p./s.c.) for days–weeks	↑ Immobility in forced swim test; anxiety-like behavior; reduced reward sensitivity	HPA axis suppression initially; later GR resistance and impaired feedback	↓ Glucocorticoid receptor (GR) expression; astroglial loss; hippocampal atrophy	Models stress-hormone–induced vulnerability and endocrine aspects of psychosis

2. MATERIAL AND METHODS

Drugs and Chemicals Procurement:

Dexamethasone 5mg/kg, Ketamine hydrochloride and xylazine, Dimethyl Sulfoxide (DMSO) solution was obtained from Loba chemicals. Thiopentone sodium, Formalin 1% Diclofenac sodium and distilled water were used. The surgical method olfactory bulbectomy were used. All the reagents used in this research work are of analytical grades.

Approval of Institutional Animal Ethical Committee: An experimental animal used were healthy Rats (150-200g) obtained from Kusum life science A-3, MIDC, Hingoli, approved by Institutional Animal Ethics Committee (IAEC) having **protocol no.** PJLCP/2023-2024/IAEC/02. Registration no. 648/PO/Ere/S/02/CPCSEA dated 19/07/2002. An instrument and software was used are namely: VJ master software, UV spectrophotometer (model number: SHIMADZU CORP Serial no A 116355, 80698) and digital microscope (model number: MOTIC DM111 6120084).

EXPERIMENTAL WORK:

For the induction of the psychosis, three models were assessed, namely-

A. Chemically (Dexamethasone) induced psychosis model
B. Chronic Unpredictable Mild Stress induced psychosis model
C. Surgery (Olfactory bulbectomy) induced psychosis model

The study was conducted by an experimental on 24 male albino rat, 200—300 grams. For this experiment, the six distinct groups (six rats per group) were used.

Group 1 (Control): Distilled water (10 ml/kg).
Group 2 (Induction): Olfactory bulbectomy surgery
Group 3(Induction): Chronic Unpredictable Mild Stress (CUMS)
Group 4 (Induction): Dexamethasone Induced

Table no. 2 Grouping of an animal:

Sr. No.	Group (n=6)	Treatment
1	Control group (positive control)	0.9% saline solution
2	Induction group (negative control)	Dexamethasone 5mg/kg
		CUMS model
		Olfactory bulbectomy

Inclusion Criteria: Rat with no previous history of psychiatric disorders were included. Rat showing normal baseline behavioral activity.

Exclusion Criteria: Pregnant or lactating female rat were excluded. Rat showing signs of illness or injury. Rats that do not complete the pre-experiment acclimatization excluded.

PROCEDURE OF AN INDUCTION

A. Chemically induced psychosis model

In the chemical induced psychosis model, Dexamethasone 5mg/kg was used by intraperitoneal injection for 21 days of treatment. An observation was noted at every 7th day of each week. After the

completion of week 3, the brain of an animal was isolated, homogenized and subject to the biochemical analysis and histopathological studies.

B. Chronic Unpredictable Mild Stress Model

For inducing the depression induced psychosis, Chronic Unpredictable Model Stress was used. The protocol for said model was set for one month with different stressful condition created for the animals. In this model, an animal was exposed to different stressor on each day for the five weeks. On the 7th day, rest was given to animal. Before exposing to a different stressor, animal was given pre training and acclimatized to the stressors. An observation was noted at every 7th day of each week. After the completion of week 5, the brain of an animal was isolated, homogenized and subject to the biochemical analysis and histopathological studies. Below, in the table no., the protocol of the depression induced psychosis by CUMS model with the different stressor was provided.

Table : 3 Stressors of Chronic unpredictable stress model

	Week 1 stressors	Week 2 stressors	Week 3 stressors	Week 4 stressors	Week 5 stressors
Day 1	Sloped cage (30° angle)	Constant light exposure (24 h)	Humid sawdust (120 ml water will be put on bedding) – 24 hours	Water deprivation (maximum 18 hours)	Constant dark exposure (24 h)
Day 2	Constant light exposure (24 h)	Water deprivation (maximum 18 hours)	Feed deprivation (maximum 18 hours)	Sloped cage (30° angle)	Humid sawdust
Day 3	Water deprivation (maximum 18 hours)	Constant dark exposure (24 h)	Sloped cage (30° angle)	Constant light exposure (24 h)	Constant light exposure (24 h)
Day 4	Constant dark exposure (24 h)	Feed deprivation (maximum 18 hours)	Constant light exposure (24 h)	Constant dark exposure (24 h)	Water deprivation (maximum 18 hours)
Day 5	Feed deprivation (maximum 18 hours)	Humid sawdust	Water deprivation (maximum 18 hours)	Humid sawdust (120 ml water will be put on bedding) – 24 hours	Sloped cage (30° angle)
Day 6	Humid sawdust (120 ml water will be put on bedding) – 24 hours	Sloped cage (30° angle)	Constant dark exposure (24 h)	Feed deprivation (maximum 18 hours)	Feed deprivation (maximum 18 hours)
Day 7	Rest	Rest	Rest	Rest	Rest

C. Olfactory bulbectomy induced psychosis model

Olfactory bulbectomy (OBX) is a surgical removal of the olfactory bulbs in rodents, commonly used as an experimental model for depression and neuropsychiatric disorders.

It causes widespread neurochemical and structural changes, particularly in limbic and cortical regions connected to the bulbs.

These alterations lead to behavioral symptoms resembling human depression, such as anhedonia and hyperactivity.

Additionally, OBX induces dopaminergic and glutamatergic dysregulation, linking it mechanistically to psychosis-like features.

Procedure: After the interpretation of the results, it was observed that an animal was induced with depression but no evidence of psychosis like condition was observed in CUMS model. After it the second for depression induced psychosis was developed and standardized by performing the olfactory bulbectomy surgery in the animals. The procedure was given- bilateral olfactory bulbectomy, rats were anaesthetized with ketamine (80 mg/kg) and Xylazine (5 mg/kg) through intra-peritoneal route. The animal was placed in stereotaxic frame, head was shaven and midline scalp sagittal incision (1 cm) was made. Bilateral burr holes were drilled (2 mm diameter), 8 mm anterior to bregma and 2 mm lateral from midline. The olfactory bulbs from both the burr were aspirated using a blunt hypodermic needle without damaging frontal cortex. The burr holes were then filled with haemostatic sponge to prevent bleeding. The incision was sutured and topical soframycin gel was applied to that region. Intramuscularly, diclofenac sodium (2 mg/kg) was administered as analgesic. The animals were housed in an individual cage and observed for 15 days and further on 16th day, the animals were grouped as per the protocol. A behavioural parameter of an animal was observed on alternative days to know the progression of disease. An animal was kept under observation 42 days.

5. EVALUATION PARAMETERS

1. Behavioural Parameters:

Behavioural assessments such as forced swim test, locomotor activity, % alternation in YMT, sucrose preference test, was done on Zero day 7th, 14th, 21th days, 28th, 35th and 42th days of treatment. On the 42th day, animals were sacrificed using overdose of Thiopentone Sodium (100 mg/ml). The brain was isolated for biochemical estimations and histopathological studies.

Force swim test

The Forced Swim Test (FST) is a common behavioral assay used in rodents to assess antidepressant-like activity. It involves placing animals in a container of water from which they cannot escape, and observing their behavior, specifically the duration they spend immobile (floating passively). This immobility is often interpreted as a measure of behavioral despair.

Locomotor activity

On the days 1, 7, 14 and 21 of the experimental protocol locomotor activity was counted using actophotometer, having a square-shaped sound-free chamber with six pairs of light receivers and transmitters. The animal was put on the floor of the actophotometer for 5 min for familiarization. The locomotor score was calculated for 5 min after 15 min of drug administration.

Stereotypy Behaviour

Rearing is a stereotype vertical locomotor activity involving an animal standing on its hind limbs, while raising up with its forearms in the air or placed on the wall of the cage, indication of an increase in exploratory behaviour, which is a measure of central nervous system excitation. Induced falling describe as number of falls on the floor.These stereotypic behaviors were checked in animals for a total period of 5 minutes in circular cage (20H X 25D). The rat was placed into a plastic cage. Before start of the experiment rat was allowed to explore the plastic cage for 30 minutes. After the previous dose of drug stereotypic behaviours (number of grooming, rearing, sniffing) were counted.

Y-maze test (YMT)

Animals were gently placed individually in the Y-maze apparatus, which consisted of three identical arms (55L X 10W X 15H cm each) in which the arms are symmetrically separated at120o . Specifically, each rat was placed at the end of arm A, and allowed to explore all the three arms (labelled A, B, C) freely for 5 min, taking the following parameters: the number of arm visits and sequence (alternation) of arm visits with video tracker software. An alternation was defined as entries in all three arms on consecutive occasion. The percentage of alternation was calculated as [total of alternations/(total arm entries -2)], as previously described (Ben-Azu, Aderibigbe, et al., 2016). After each rat session, the observation chamber was cleaned with 10% ethanol to remove residual odour

Open-field test (OFT)

Locomotor behaviour was monitored using the open field apparatus. The apparatus consisted of a wooden box measuring (60 X 60 X 60 cm) with visible lines drawn to divide the floor into16 squares(15 cm X 15 cm) with a frontal glass wall and placed in a sound free room (Ben- Azu, Omogbiya, et al., 2016) . The animals were placed in the rear left square and allowed to explore it. The number of square crossed were recorded for 5 min by using a VJ Master software. After each rat session, the observation chamber was cleaned with 10% ethanol to remove residual odour (Hetzler et al.,1985).

Sucrose preference test (SPT)

SPT is used as an indicator of anhedonia which is described as lack of interest in presence of any reward or pleasurable state. It exists in few forms of affective disorder including depression. In psychosis, anhedonia is one of the parameters to assess negative symptoms of the psychosis. This test was performed in order to assess rat's interest in seeking out a sweetened drink over plain drinking water. Since a bias towards the sweetened drink is normal and failure to do so is indicative of anhedonia or depressive symptoms (Ahmad et al., 2017; Shahzad et al., 2017). Sucrose preference testing is carried out in home cage and cages were randomly allocated with two identical graduated water bottles for consecutive 3 days. One bottle contained plain drinking water while the second contained 100ml of 1-2% w/v sucrose solution (Kandratavicius et al., 2015). Sucrose solution and water intake was measured on daily basis and the position of the two bottles was switched after every 24hours to reduce any confound produced by a side biasness. After testing, sucrose consumption was calculated as volume of sucrose intake over total volume of fluid intake and converted into percentage (Serchov et al.,2016).

Biochemical Estimation

Isolation and preparation of brain homogenate

Immediately after the behavioural tests, animals in groups 1–11 were sacrificed using overdose of thiopentone sodium (100 mg/ml). After perfusion with ice cold saline, brain was carefully dissected out, washed, weighed, divided into two equal parts and again weighed. Thereafter, the whole brains were homogenized with 5 mL of 10% w/v phosphate buffer (0.1 M, PH 7.4) (Yadav et al., 2017). The homogenates were centrifuged at 10,000 g for 10 min at 4°C, the pellet was discarded and the

supernatant was immediately separated into three portions for the biochemical assays such as CAT, GSH and Dopamine.

Estimation of catalase activity

Catalase causes rapid decomposition of hydrogen peroxide to water and oxygen. The assay mixture consisted of 1.95 ml of phosphate buffer (0.05 M, pH 7.0), reaction started by the addition of 1.0 ml of hydrogen peroxide (0.019 M), and 0.05 ml of brain tissue homogenate in a final volume of 3.0 ml. After cooling absorbance was taken at 240 nm (Ben-Azu, Omogbiya, et al., 2016) using UV spectrophotometer.

Estimation of brain dopamine levels

To 0.02 ml of aqueous, 0.05ml 0.4M EDTA and 0.01ml Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stopped after two minutes by addition of 0.01ml Na_2SO_3 in 5M NaOH (0.5 g Na_2SO_3 in 2 ml H_2O + 18 ml 5 M NaOH). Acetic acid (0.01 ml, 10 M) was added 1.5 minutes later. Then the solution was heated to 100°C for 6 minutes. When the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter at 330/375nm. Internal standard was prepared by adding 500 µg/ml of dopamine in distilled water: HCl-butanol in 1:2 ratios and following the whole above-mentioned procedure (K. Sharma et al., 2016). Tissue blanks and internal reagent blank were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine).

Data Analysis

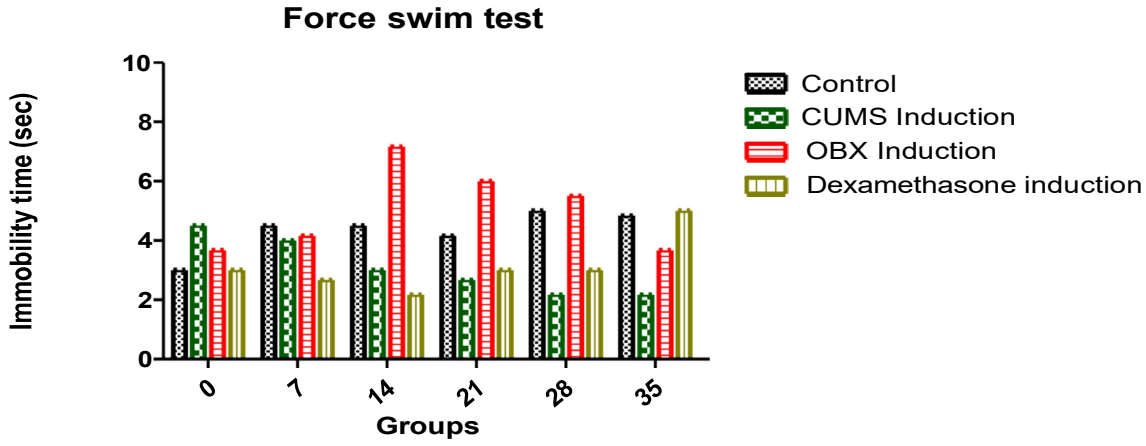
The study employed GraphPad Prism (software, V.5.0.) for data analysis, including "one and two-way ANOVA followed by post hoc Bonferroni's multiple comparison tests" for the respective parameters. The study data were represented as the mean of respective parametric values and $P<0.05$ was considered as significant.

Data Analysis The study employed GraphPad Prism (software, V.5.0.) for data analysis, including "one and two-way ANOVA followed by post hoc Bonferroni's multiple comparison tests" for the respective parameters. The study data were represented as the mean of respective parametric values and $P<0.05$ was considered as significant.

6. RESULT

The comparative evaluation of the three induction models—Dexamethasone, Chronic Unpredictable Mild Stress (CUMS), and Olfactory Bulbectomy (OBX)—was performed using behavioral, biochemical, and histopathological parameters. Statistical analysis was carried out using GraphPad Prism (Version 5.0), and data were expressed as mean ± SD.

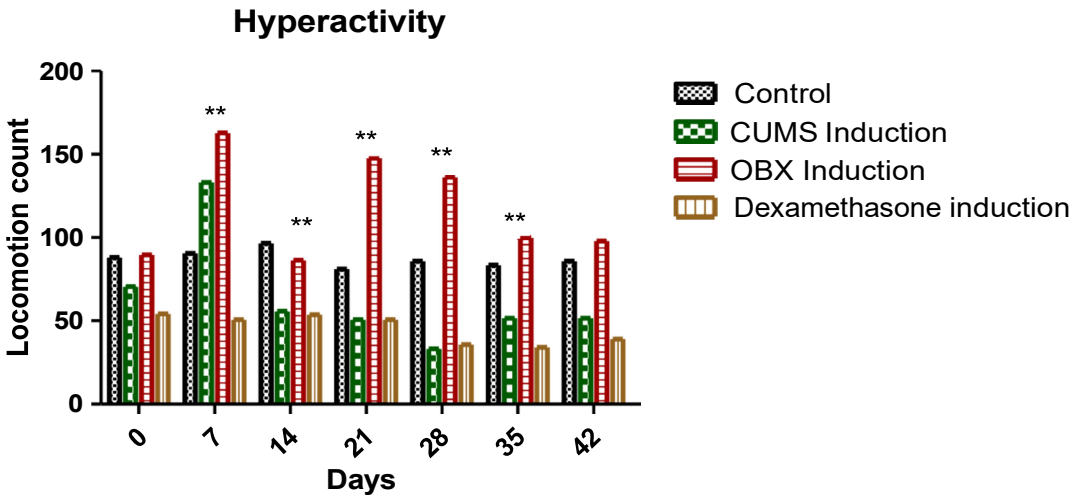
1. Force Swim Test



Graph no.01 Graph of force swim test where the immobility time of rats in seconds was counted.

All induction groups showed a significant increase in immobility time compared to control, indicating depression-like behavior. The CUMS and OBX groups demonstrated a more pronounced increase relative to the dexamethasone group, suggesting stronger construct validity for depressive phenotypes in these models. Force swim test was used to know the induction of depression.

1. **Hyperactivity**

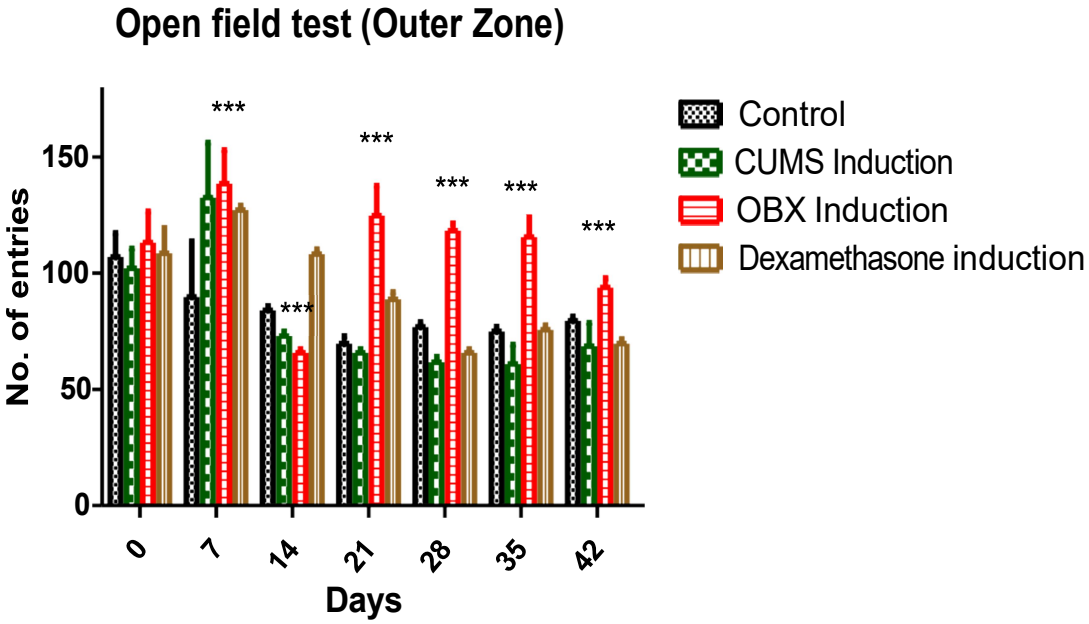


Graph no.02 Result of hyperactivity by using actophotometer apparatus. "The values on the graph for the respective groups (n=6) are represented as mean ± SD and data was analysed by "two-way ANOVA followed by Bonferroni's multiple comparison test". The level of significance is expressed as #P<0.0001 versus control, *, $P<0.001, and **, $$, ẟ P<0.0001 versus the control group.

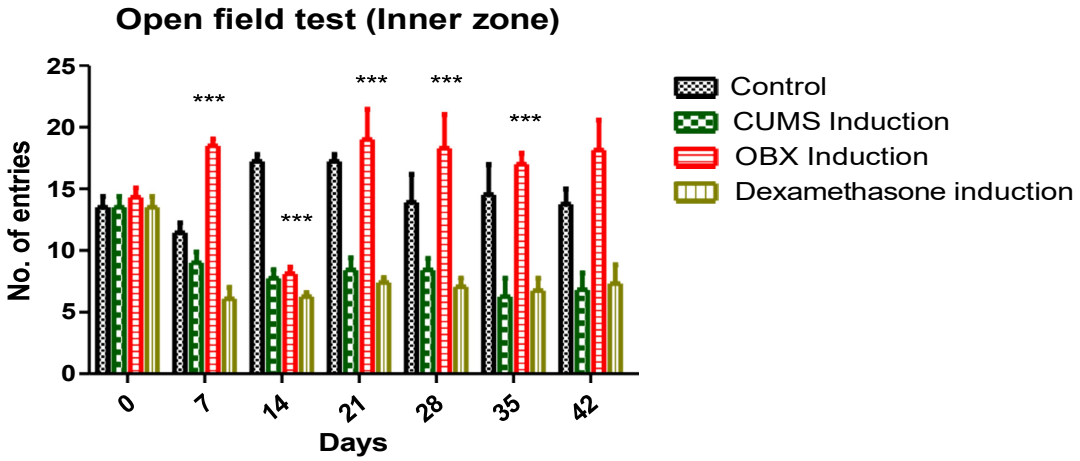
The OBX group exhibited a significant increase in locomotor counts compared to control and other induction groups, indicating hyperactivity. The dexamethasone group showed moderate elevation in activity, whereas the CUMS group did not show significant hyperlocomotion. Increased locomotor activity in OBX supports dopaminergic hyper-responsivity.

2. **Open Field Test**

In the open field test, activity of the rats was evaluated in the outer zone as well as the inner zone by using the VJ Master software. The number of entries in each zone was counted.



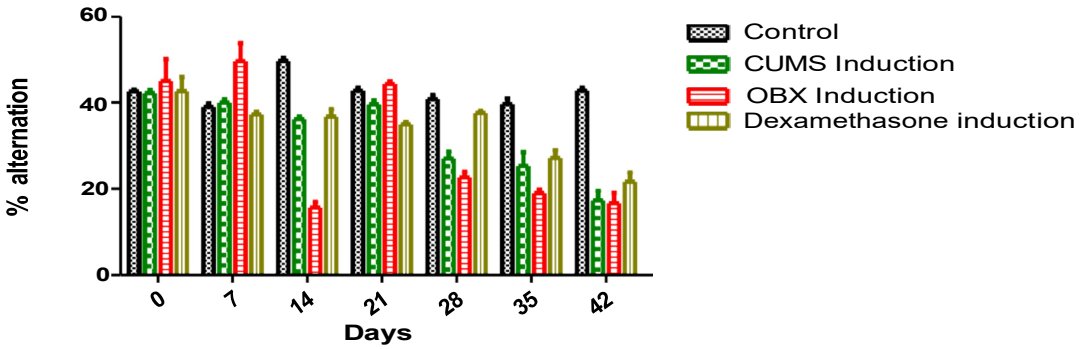
Graph no. 03 a Result of outer zone in the open field test apparatus. "The values on the graph for the respective groups (n=6) are represented as mean ± SD and data was analysed by "two-way ANOVA followed by Bonferroni's multiple comparison test". The level of significance is expressed as #P<0.0001 versus control, *, $P<0.001, and **, $$, ẟ P<0.0001 versus the control group.



Graph no. 03 b Result of inner zone in the open field test apparatus. "The values on the graph for the respective groups (n=6) are represented as mean ± SD and data was analysed by "two-way ANOVA followed by Bonferroni's multiple comparison test". The level of significance is expressed as #P<0.0001 versus control, *, $P<0.001, and **, $$, ð P<0.0001 versus the control group.

OBX rats demonstrated increased entries in both outer and inner zones, indicating heightened exploratory behavior and central excitation. CUMS animals showed reduced exploratory activity, consistent with anxiety- and depression-like behavior. The dexamethasone group showed variable but moderate alterations.

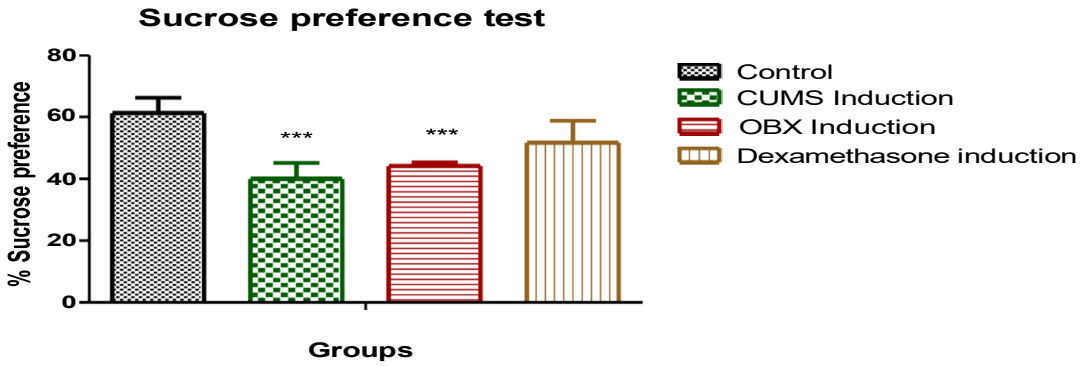
Y-Maze test apparatus



Graph no. 04 Result of inner zone in the open field test apparatus. "The values on the graph for the respective groups (n=6) are represented as mean ± SD and data was analysed by "two-way ANOVA followed by Bonferroni's multiple comparison test". The level of significance is expressed as #P<0.0001 versus control, *, $P<0.001, and **, $$, ð P<0.0001 versus the control group.

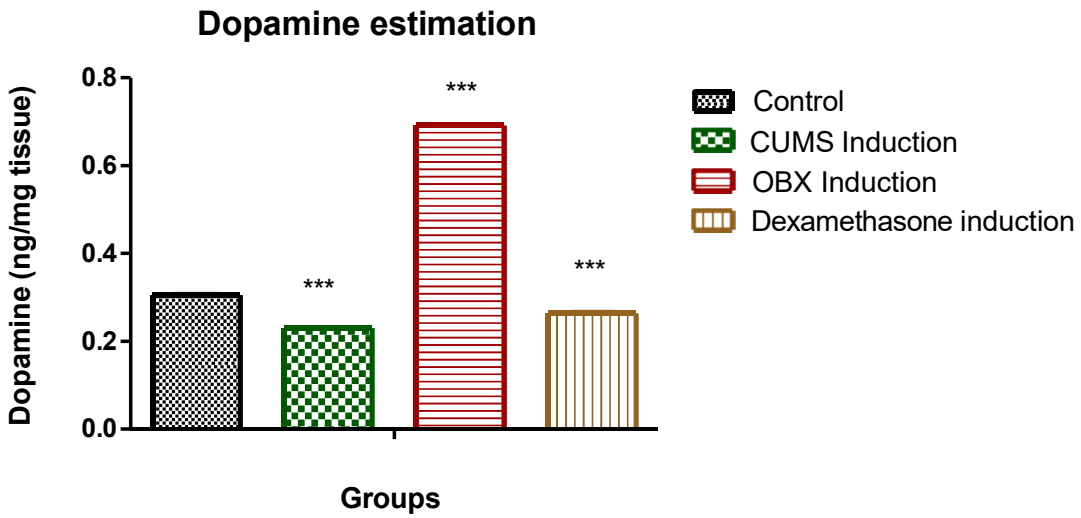
A significant reduction in percentage alternation was observed in OBX and dexamethasone groups, indicating spatial memory impairment and prefrontal-hippocampal dysfunction. The CUMS group showed mild impairment.

3. Sucrose preference test



Graph no. 05 Result of the sucrose preference test. "The values on the graph for the respective groups (n=6) are represented as mean ± SD and data was analysed by "One-way ANOVA followed by Bonferroni's multiple comparison test". The level of significance is expressed as #P<0.0001 versus control, *, $P<0.001, and **, $$, ð P<0.0001 versus the control group.
CUMS and OBX groups showed a significant decrease in sucrose preference percentage, reflecting anhedonia. The dexamethasone group showed moderate reduction.

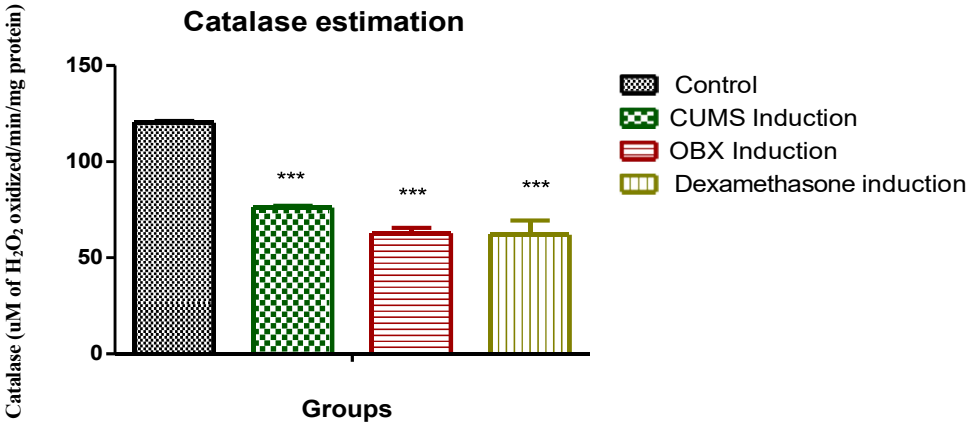
2. BIOCHEMICAL ESTIMATION



Graph no.06 Result of dopamine estimation. "The values on the graph for the respective groups (n=6) are represented as mean ± SD and data was analysed by "One-way ANOVA followed by Bonferroni's multiple comparison test". The level of significance is expressed as #P<0.0001 versus control, *, $P<0.001, and **, $$, ð P<0.0001 versus the control group.

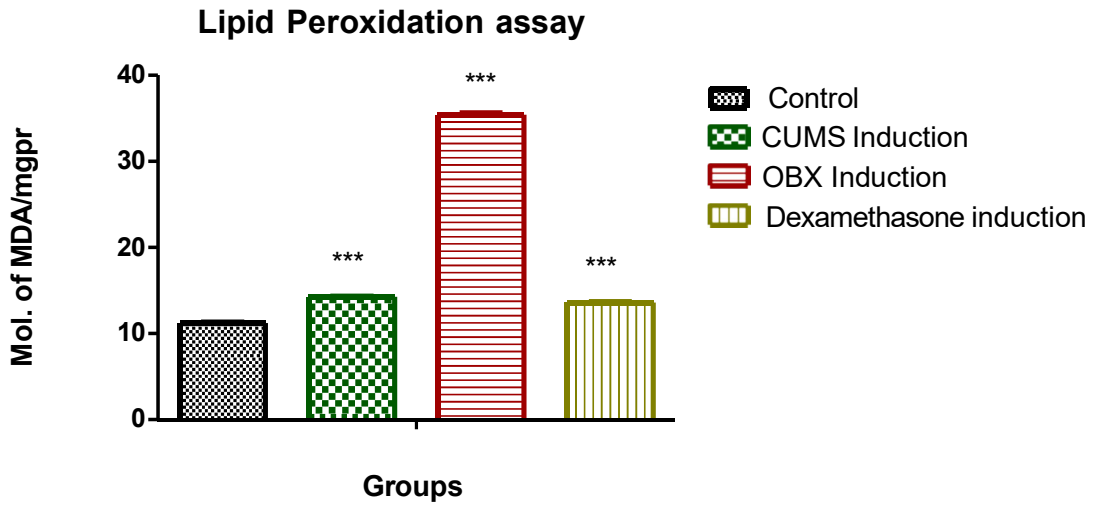
A significant elevation in brain dopamine concentration was observed in the OBX group compared to control and CUMS groups. The dexamethasone group showed moderate dopamine elevation. Increased dopamine in OBX animals suggests mesolimbic dopaminergic hyperactivity, a hallmark neurochemical mechanism associated with psychosis.

CATALASE ESTIMATION



Graph no.07 Result of catalase estimation. "The values on the graph for the respective groups (n=6) are represented as mean ± SD and data was analysed by "One -way ANOVA followed by Bonferroni's multiple comparison test". The level of significance is expressed as #P<0.0001 versus control, *, $P<0.001, and **, $$, ð P<0.0001 versus the control group.
Catalase levels were significantly reduced in all induction groups compared to control, indicating oxidative stress. The reduction was more pronounced in CUMS and OBX models.

LIPID PEROXIDATION ESTIMATION



Graph no.08 Result of lipid peroxidation estimation. "The values on the graph for the respective groups (n=6) are represented as mean ± SD and data was analysed by "One-way ANOVA followed by Bonferroni's multiple comparison test". The level of significance is expressed as #P<0.0001 versus control, *, $P<0.001, and **, $$, ð P<0.0001 versus the control group.
LPO levels were significantly increased in induction groups, with OBX and CUMS showing higher oxidative damage relative to dexamethasone.

7. DISCUSSION

The present study aimed to compare three induction models for psychosis-like conditions using behavioral, biochemical, and histopathological evidence. Psychosis, particularly positive symptoms, is strongly associated with hyperactivity of dopamine signaling in the Mesolimbic pathway. Olfactory Bulbectomy (OBX) induces dopaminergic hypersensitivity, characterized by enhanced dopamine release to novel stimuli and psychostimulants along with D_2 receptor supersensitivity, thereby modeling psychosis-relevant behaviours. Additionally, OBX disrupts NMDA receptor and GABA balance and reduces integrity of cortical and hippocampal regions, reflecting mechanisms implicated in schizophrenia such as NMDA hypofunction and interneuron dysfunction. Consequently, OBX generates a combined pattern of excitation–inhibition imbalance and dopaminergic overactivity that closely aligns with established neurobiological theories of psychosis. The CUMS model involves prolonged exposure to mild, unpredictable stressors and reliably induces anhedonia, behavioral despair, social withdrawal, and cognitive deficits, accompanied by hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis and impaired stress hormone feedback. The CUMS model primarily produced depression-like features characterized by anhedonia, increased immobility, oxidative stress, and mild cognitive deficits. However, hyperactivity and dopaminergic elevation were not prominent. Since psychosis—particularly positive symptoms are mechanistically linked to mesolimbic dopamine hyperactivity, the CUMS model lacks strong neurochemical validity for psychosis induction. Chronic administration of dexamethasone directly perturbs glucocorticoid signalling leading to behavioral despair, anxiety-like behaviour, glucocorticoid receptor dysfunction, and altered neuroplasticity, modeling endocrine abnormalities associated with stress-related psychiatric disorders. The dexamethasone-induced model produced moderate behavioral and biochemical alterations. Corticosteroid exposure disrupts the hypothalamic–pituitary–adrenal (HPA) axis, potentially increasing dopamine transmission and reducing GABAergic inhibition. Although some psychosis-like features were observed, the model predominantly reflected stress-hormone–mediated mood disturbances rather than robust dopaminergic sensitization. In contrast, the OBX model demonstrated a combination of depression-like behavior (increased immobility, anhedonia), psychosis-relevant hyperactivity, significant dopaminergic elevation, oxidative stress, and structural cortical damage. Mechanistically, OBX produces sensory deafferentation leading to limbic disinhibition, excitatory/inhibitory imbalance, NMDA–GABA dysfunction, and increased mesolimbic dopamine responsivity. This cascade closely aligns with established neurobiological theories of psychosis, particularly dopamine hyperfunction and cortical dysregulation. OBX produces robust hyperlocomotion, affective dysregulation, and memory impairments, along with marked disturbances in monoaminergic neurotransmission and HPA axis function, thereby demonstrating strong face and predictive validity for psychosis-like phenotypes. Furthermore, OBX animals exhibited cognitive

impairment in the Y-maze, supporting prefrontal-hippocampal dysfunction—another hallmark observed in psychotic disorders. Histopathological evidence of neurodegeneration strengthens the construct validity of OBX as a dual depression–psychosis model.

8.. CONCLUSION

The relative study demonstrates that while all three induction models successfully produced depressive- such like differences, only the Olfactory Bulbectomy model showed harmonious psychosis-applicable features supported by behavioral hyperactivity, elevated dopamine situations, oxidative stress, cognitive poverties, and histopathological damage. Thus, the OBX model provides superior construct and neurochemical validity for studying depression- convinced psychosis in preclinical settings. It represents a mechanistically presumptive model linking limbic disinhibition, excitatory/ inhibitory imbalance, and mesolimbic dopaminergic hyperactive- responsivity. Further studies should further validate this model through molecular levels similar as D2 receptor expression, NMDA receptor subunit analysis, GABAergic interneuron levels, and seditious cytokine profiling to strengthen its translational applicability.

ACKNOWLEDGEMENTS

The authors are thankful to the LTJSS Management and Shakuntala Pathology Nagpur for providing the necessary facilities and assistance.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

Acknowledgements

The authors are thankful to the LTJSS Management and Shakuntala Pathology Nagpur for providing the necessary facilities and assistance.

Conflict of Interest

No conflict of interest associated with this work.

10. REFERENCES:

1. Arciniegas DB. Psychosis. Continuum (Minneap Minn). 2015 Jun;21(3 Behavioral Neurology and Neuropsychiatry):715-36. doi: 10.1212/01.CON.0000466662.89908.e7. PMID: 26039850; PMCID: PMC4455840.
2. Calabrese J, Al Khalili Y. Psychosis. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK546579/?utm_s)
3. Vilalta-Franch J, Lopez-Pousa S, Calvo-Perxas L, Garre-Olmo J. Psychosis of Alzheimer disease: prevalence, incidence, persistence, risk factors, and mortality. Am J Geriatr Psychiatry 2013; 21 (11): 1135– 1143. doi:10.1016/j.jagp.2013.01.051. - PubMed
4. Rabinowitz J, Berardo CG, Bugarski-Kirola D, Marder S. Association of prominent positive and prominent negative symptoms and functional health, well-being, healthcare-related quality of life and family burden: a CATIE analysis. Schizophr Res 2013; 150 (2–3): 339– 342. doi:10.1016/j.schres.2013.07.014. - PubMed
5. Gayane Kirakosyan * and Alina Frolova. Understanding psychosis: diagnosis and clinical presentation (updates for clinicians). World Journal of Advanced Research and Reviews, 2022, 13(01), 065–071
6. Howes & Kapur, 2009, *Schizophrenia Bulletin*, 35(3):549–562. Howes, O.D., & Kapur, S. (2009). *The dopamine hypothesis of schizophrenia: version III—the final common pathway.* Schizophrenia Bulletin, 35(3), 549–562.
7. Coyle, J.T. (2012). *NMDA receptor and schizophrenia: a brief history.* Dialogues in Clinical Neuroscience**,** 14(3), 293–300.
8. Lewis, D.A., & Sweet, R.A. (2012). *GABAergic dysfunction in schizophrenia: insights from postmortem studies.* Biological Psychiatry, 71(5), 350–356.
9. Owen, M.J., Sawa, A., & Mortensen, P.B. (2016). *Schizophrenia.* The Lancet Psychiatry, 3(3), 221–234.
10. Walker, E.F., et al. (2008). *Stress and the hypothalamic–pituitary–adrenal axis in the developmental course of schizophrenia.* Annual Review of Clinical Psychology, 4, 409–438.
11. Coppola, D. (2025). The Olfactory Bulbectomy Model of Depression: Brief History, Current Status and Critique. *Brain Sciences*, *15*(8), 775. https://doi.org/10.3390/brainsci15080775

12. Morales-Medina JC, Iannitti T, Freeman A, Caldwell HK. The olfactory bulbectomized rat as a model of depression: The hippocampal pathway. Behav Brain Res. 2017 Jan 15;317:562-575. doi: 10.1016/j.bbr.2016.09.029. Epub 2016 Sep 12. PMID: 27633561.
13. Ying-Jui Ho, Tzuo-Mei Liu, Mei-Yun Tai, Zhi-Hong Wen, Richard Shih-Shien Chow, Yuan-Feen Tsai, Chih-Shung Wong, Effects of olfactory bulbectomy on NMDA receptor density in the rat brain: [3H] MK-801 binding assay, Brain Research, Volume 900, Issue 2, 2001, Pages 214-218, ISSN 0006-8993, https://doi.org/10.1016/S0006-8993(01)02297-1.
14. Takahashi K, Nakagawasai O, Nemoto W, Nakajima T, Arai Y, Hisamitsu T, Tan-No K. Alterations in behavioral responses to dopamine agonists in olfactory bulbectomized mice: relationship to changes in the striatal dopaminergic system. Psychopharmacology (Berl). 2016 Apr;233(7):1311-22. doi: 10.1007/s00213-016-4224-y. Epub 2016 Feb 9. PMID: 26856855.
15. Eisenstein SA, Holmes PV, Hohmann AG. Endocannabinoid modulation of amphetamine sensitization is disrupted in a rodent model of lesion-induced dopamine dysregulation. Synapse. 2009 Nov;63(11):941-50. doi: 10.1002/syn.20679. PMID: 19593824; PMCID: PMC2739243.
16. Marcilhac A, Anglade G, Hery F, Siaud P. Olfactory bulbectomy increases vasopressin, but not corticotropin-releasing hormone, content in the external layer of the median eminence of male rats. Neurosci Lett. 1999 Mar 5;262(2):89-92. doi: 10.1016/s0304-3940(98)00981-1. PMID: 10203238.
17. Barethiya, Varsha & Deshpande, Shilpa. (2025). Assessment of the antipsychotic effects of hydroalcoholic extract of Tinospora cordifolia in Animal model.J appl. Bioanalysis.509-514.