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Sesamol Attenuates Meta-Chlorophenyl Piperazine Induced Obsessive-Compulsive Disorder in Mice: Integration of Behavioural, Neurochemical, and Histopathological Metrics

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Abstract:
Background: As we know, the Obsessive Compulsive Disorder (OCD) is a debilitating neuropsychiatric condition characterized by persistent obsessions and repetitive compulsions. Current frontline pharmacotherapies, such as Selective Serotonin Reuptake Inhibitors (SSRIs), exhibit suboptimal efficacy, leaving approximately 40 to 60 percent of patients treatment-resistant. The serotonergic agonist meta-chlorophenylpiperazine (m-CPP) is widely utilized to model compulsive-like states in rodents, which are heavily aggravated by secondary neurochemical disruption and central oxido-nitrosative stress. Sesamol, a natural phenolic lignan isolated from sesame oil (Sesamumindicum L.), exhibits potent blood-brain barrier permeability alongside established antioxidant and neuroprotective activities.
Objective: The present study investigates the therapeutic potential and neuroprotective architecture of sesamol against a stable, progressive obsessive-compulsive-like phenotype chronically induced by m-CPP in Swiss albino mice.
Methods: For the study, thirty-six male Swiss albino mice were randomly assigned to six experimental cohorts (n = 6 per group) over a consecutive 21-day timeline. The disease state was induced via daily administration of m-CPP (0.1 mg/kg, i.p.). Treatment groups received daily oral doses of sesamol (2, 4, and 8 mg/kg, p.o.), while fluoxetine (15 mg/kg, i.p.) served as the standard reference drug. On day 21, compulsive phenotypes and psychomotor scores were indexed via the marble burying test and an actophotometer. On day 22, whole-brain homogenates were isolated to quantify alterations in central neurotransmitters (serotonin, gamma-aminobutyric acid [GABA]), membrane lipid peroxidation (malondialdehyde [MDA]), and endogenous antioxidant defenses (reduced glutathione [GSH], catalase).
Results: Chronic challenge with meta-chlorophenyl piperazine precipitated robust obsessive-compulsive-like phenotypes, as demonstrated by a significant surge in buried marbles and a severe depletion in exploratory motor activity compared to the healthy control group. This behavioral disruption was directly mirrored by a deep exhaustion of central serotonin, gamma-aminobutyric acid, reduced glutathione, and catalase pools alongside a massive accumulation of toxic lipid peroxidation signatures. Qualitative histopathological analysis verified these findings, revealing that m-CPP induced notable vacuolar degeneration and nuclear pyknosis within a substantial subset of resident striatal neurons. Daily oral pre-treatment with sesamol brought about a highly significant, dose-dependent reversal of these deficits. Notably, the highest dose of sesamol showcased optimal anti-compulsive efficacy by reducing buried marbles below the standard drug baseline, completely renormalizing psychomotor activity indices, restoring neurochemical availability, suppressing membrane lipid peroxidation close to baseline physiological dimensions, and exceptionally preserving striatal neuronal morphology against neurodegenerative injury.
Conclusion: These collective findings demonstrate that sesamol attenuates chronically induced obsessive-compulsive-like phenotypes in mice. This therapeutic performance is mediated by its combined capacity to restabilize monoaminergic and inhibitory neurotransmitter pathways while simultaneously preserving striatal cellular integrity against central oxido-nitrosative damage, highlighting it as a promising bioactive candidate for advanced psychiatric drug discovery.

Keywords: Sesamol; Obsessive-Compulsive Disorder; meta-chlorophenyl piperazine; Serotonin; Gamma-aminobutyric acid; Oxidative stress.

1. Introduction

Obsessive-Compulsive Disorder (OCD) is a severe, chronic, and highly disabling neuropsychiatric condition characterized by the manifestation of distressing, intrusive thoughts (obsessions) alongside ritualistic, repetitive behaviors (compulsions). Clinically, these persistent patterns severely compromise an individual's social functionality, occupational capacity, and overall quality of life [1]. Neuroanatomical and functional imaging

studies heavily implicate abnormalities within the cortico-striato-thalamo-cortical (CSTC) circuit as the primary pathway driving these repetitive motor loops [2]. Currently, Selective Serotonin Reuptake Inhibitors (SSRIs), such as fluoxetine, serve as the frontline pharmacotherapy for managing OCD. However, clinical outcomes remain suboptimal, with approximately 40–60% of patients exhibiting inadequate therapeutic responses or total treatment resistance to traditional SSRI regimens [3]. Furthermore, the mandatory therapeutic latency of several weeks and a heavy burden of adverse systemic effects underscore an urgent, unmet medical need to explore novel biomolecules with superior efficacy and safety profiles.

The pathophysiology of OCD is complex and deeply intertwined with severe neurochemical imbalances and cellular stress within the central nervous system. Beyond classic serotonergic (5-HT) dysfunction, growing preclinical evidence highlights a crucial regulatory role for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) within the CSTC loop [4,5]. A breakdown in GABAergic tone leads to a failure in striatal gating mechanisms, resulting in unchecked hyper-reflectivity and compulsive behavior. Crucially, these chronic neurochemical disruptions do not occur in isolation; they are tightly coupled with central oxido-nitrosative stress. Sustained cellular stress drives lipid peroxidation (evidenced by elevated malondialdehyde levels) and drains the brain's endogenous antioxidant defenses—depleting vital defensive components like catalase and reduced glutathione (GSH) [6, 7]. This destructive cascade damages neuronal integrity within core striatal and cortical networks, fixing compulsive behavioral patterns in place.

To investigate these precise pathways in vivo, the administration of meta-chlorophenylpiperazine (m-CPP) has become an established, highly reliable preclinical model. As a non-selective serotonin receptor agonist with prominent high affinity for 5-HT2C and 5-HT1B receptors, m-CPP reliably generates stereotypic and repetitive behaviors in rodents. In this study, a chronic 21-day administration protocol of m-CPP (0.1 mg/kg, i.p.) was selected to induce a stable, progressive obsessive-compulsive-like phenotype in Swiss albino mice. This prolonged structural approach models the persistent and progressive nature of clinical OCD far more effectively than acute drug challenges. It actively alters marble-burying behavioral indexes while triggering profound imbalances in brain 5-HT, GABA, and cellular oxidative status [8].

Natural bioactive compounds have emerged as promising candidates for neuropsychiatric research due to their multi-target mechanisms and low toxicity profiles. Sesamol (3,4-methylenedioxyphenol) is a principal, low-molecular-weight phenolic lignan isolated from sesame oil (Sesamumindicum L.). Sesamol possesses remarkable free-radical scavenging, antioxidant, and anti-inflammatory properties, and its lipophilic nature allows it to readily cross the blood-brain barrier [9]. While previous literature has documented its neuroprotective effects in models of chronic unpredictable stress, cognitive decline, and depression, its therapeutic potential against serotonergic-driven, chronic compulsive states remains entirely unexplored.

Consequently, the objective of the present study was to comprehensively evaluate the therapeutic potential of Sesamol (2, 4, and 8 mg/kg, p.o.) against chronically induced m-CPP obsessive-compulsive-like behavior in experimental mice. By correlating horizontal locomotor scores and marble-burying paradigms with central neurotransmitter fluctuations (Serotonin, GABA), detailed oxido-nitrosative biochemical parameters, and qualitative histopathological assessments of the subcortical striatum, this investigation aims to clarify the precise multi-target neuroprotective architecture of sesamol in the management of structural and functional deficits defining obsessive-compulsive states.

2. Material and Methods

2.1. Drugs and Chemicals

Sesamol, meta-chlorophenylpiperazine (m-CPP), and fluoxetine were procured from Sigma Aldrich, St. Louis MO, USA. Carboxymethyl cellulose (CMC) and other analytical grade chemicals were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). For experimental administration, m-CPP and fluoxetine were dissolved in 0.9% w/v normal saline. Sesamol was freshly dissolved in distilled water prior to treatment.

2.2. Experimental Animals

Thirty-six male Swiss albino mice, weighing between 25 and 35 g and aged 3 to 5 months, were selected for the investigation. The animals were maintained under controlled laboratory conditions characterized by a strict 12-hour light and 12-hour dark cycle. Mice were housed in groups of 10 per standard polypropylene cage (43 × 27 × 15 cm) with free access to standard laboratory chow pellets and water. To eliminate any confounding nutritional effects on gastrointestinal drug absorption, food access was restricted 2 hours prior to and 2 hours following drug administration. All experimental procedures were conducted in strict accordance with standard institutional guidelines for animal care and welfare.All experimental procedures were conducted between 09:00 and 17:00 hours. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) during its meeting held on 13.06.26 at Rodent Research Pvt. Ltd, Jind, Haryana under project proposal no. RRI/IAEC/2026/11. Animal care and handling were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [10].

2.3. Experimental Design

The mice were randomly assigned to six experimental groups (n = 6 per group) to evaluate the therapeutic potential of sesamol over a consecutive 21-day timeline:

- **Group- 1: (Vehicle Control):** Animals received 0.5% w/v carboxymethyl cellulose via oral (p.o.) administration daily for 21 consecutive days.

- **Group- 2: (m-CPP Model):** Animals received meta-chlorophenylpiperazine (0.1 mg/kg, i.p.) daily for 21 consecutive days to induce stable obsessive-compulsive disorder (OCD)-like symptoms.
- **Group- 3: (Standard Drug Control):** Animals were treated with fluoxetine (15 mg/kg, i.p.) daily for 21 successive days. From day 1 to day 21, m-CPP (0.1 mg/kg, i.p.) was injected for 10 minutes following fluoxetine administration.
- **Group- 4: (Sesamol Low Dose):** Animals received sesamol (2 mg/kg, p.o.) for 21 successive days. Chronic m-CPP (0.1 mg/kg, i.p.) was administered 10 minutes after sesamol treatment.
- **Group- 5: (Sesamol Mid Dose):** Animals received sesamol (4 mg/kg, p.o.) for 21 successive days. Chronic m-CPP (0.1 mg/kg, i.p.) was administered 10 minutes after sesamol treatment.
- **Group- 6: (Sesamol High Dose):** Animals received sesamol (8 mg/kg, p.o.) for 21 successive days. Chronic m-CPP (0.1 mg/kg, i.p.) was administered 10 minutes after sesamol treatment.

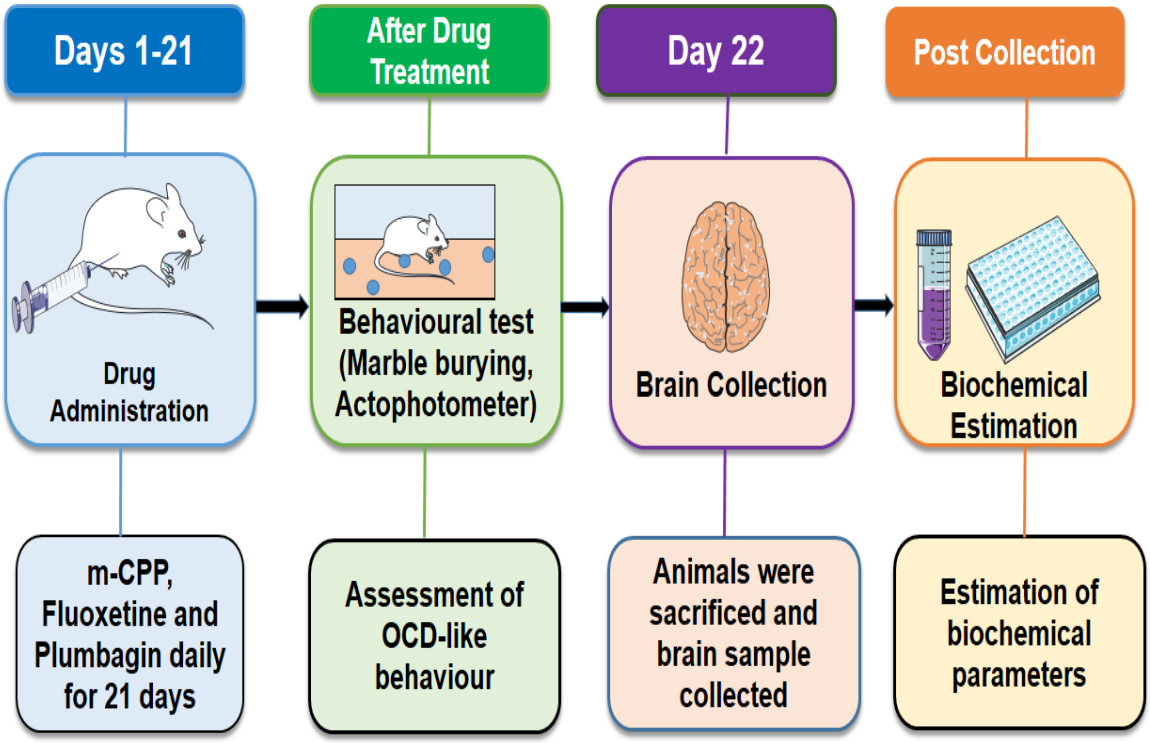


Figure- 1: Assessment timeline of experimental design.

2.4. Behavioral Paradigms

All behavioral indices, including compulsive phenotypes and horizontal locomotor scores, were systematically recorded on the 21st day of the treatment protocol.

2.4.1. Marble Burying Behavior

Compulsive and stereotypic behaviors brought on by chronic m-CPP exposure were quantified using the standard marble-burying paradigm. Individual mice were placed into separate plastic cages (21 × 38 × 14 cm) filled with a 5-cm thick layer of fresh husk bedding. Twenty shiny glass marbles (15 mm in diameter) were distributed evenly across the bedding surface in a grid layout. Each animal was introduced to the cage center and observed undisturbed for 30 minutes. A marble was scored as buried if at least two-thirds of its surface area was covered by the husk bedding. The cumulative number of buried marbles served as the behavioral index for obsessive-compulsive-like behaviour [11].

2.4.2. Assessment of Locomotor Activity

To confirm that changes in marble-burying phenotypes were not artifactual outcomes of drug-induced sedation or general neuromuscular deficits, horizontal locomotor activity was assessed using an actophotometer. Individual animals from control, model, and treatment groups were placed sequentially into the activity chamber. Total locomotor activity scores were automatically recorded across a standard 5-minute experimental window [12].

2.5. Tissue Isolation and Homogenization

On the 22nd day of the study (24 hours following the final behavioral assessment), mice were euthanized by cervical dislocation. Intact brains were rapidly excised, thoroughly rinsed with ice-cold isotonic saline to remove excess surface blood, and weighed. The isolated brain tissues were subsequently transferred to a cold phosphate

buffer (0.1 M, pH 7.4) and completely homogenized using an electrical homogenizer. The resulting crude brain homogenates were stored under appropriate refrigeration and subsequently utilized for quantitative neurochemical and biochemical estimations.

2.6. Biochemical Estimations

2.6.1. Estimation of Brain Serotonin Levels

Endogenous brain serotonin (5-hydroxytryptamine, 5-HT) levels were measured using the standard colorimetric established by Schlumpf et al. (1974). This microanalytical protocol allowed for the highly sensitive, simultaneous extraction and spectrofluorometric quantification of serotonin profiles within small amounts of harvested brain tissue [13].

2.6.2. Estimation of Brain GABA Levels

Central gamma-aminobutyric acid (GABA) concentrations were evaluated via the quantitative fluorometric micromethod developed by Lowe et al. (1958). This assay utilizes specific fluorometric coupling reactions to establish precise alterations in central inhibitory GABAergic neurotransmitter tone [14].

2.6.3. Estimation of Total Protein Content

Total protein concentrations in brain tissue homogenates were measured spectrophotometrically via the standard Biuret method. Absorbance values were translated into absolute protein values using a standard curve constructed from known concentrations of bovine serum albumin (BSA).

2.6.4. Estimation of Brain Reduced Glutathione Levels

Brain reduced glutathione (GSH) levels, serving as a primary internal non-enzymatic antioxidant indicator, were measured according to the methodology outlined by Jollow (1974). The concentration of free sulfhydryl groups was determined colorimetrically by reacting tissue supernatant fractions with Ellman's reagent [15].

2.6.5. Estimation of Brain Catalase Levels

The enzymatic activity of central catalase was determined via the kinetics protocol described by Claiborne (1985). The reduction in absorbance was tracked over time to measure the decomposition rate of hydrogen peroxide (H_2O_2) by tissue catalase at a standard wavelength [16].

2.6.6. Estimation of Brain Lipid Peroxidase Levels

The extent of central oxidative damage and membrane lipid degradation was estimated by measuring tissue lipid peroxide (LPO) profiles using the classic colorimetric method described by Wills (1965). The generation of thiobarbituric acid reactive substances (TBARS), specifically malondialdehyde (MDA), served as the direct quantitative readout for cellular lipid peroxidation [17].

2.7. Histopathological Studies

To evaluate structural alterations and cellular architecture within deep brain tissue, one representative animal from each experimental group was sacrificed under mild anesthesia, and the intact brain was carefully isolated. The harvested brain specimens were immediately fixed in a 10% neutral buffered formalin solution for 48 hours to preserve structural integrity. Fixed tissues were processed, sectioned, stained, and evaluated under light microscopy according to the standardized histological methods described by Slaoui et al. (2011) [18].

2.8. Statistical Analysis

All experimental data obtained from behavioral and biochemical evaluations are expressed as Mean ± Standard Error of the Mean (SEM). Statistical comparisons among the different experimental arms were processed using One-way Analysis of Variance (ANOVA). Post-hoc evaluations to establish specific inter-group differences were performed using Tukey's multiple comparisons test. Statistically significant variances were identified at a confidence ceiling of $p < 0.05$. All statistical graphs and computations were executed utilizing GraphPad Prism software. Analysis was performed using GraphPad Prism Version 6.0, with$p < 0.05$ considered statistically significant.

2.9. Results

2.9.1. Effect of Sesamol on Compulsive Phenotypes in the Marble Burying Test

The chronic administration of m-CPP (0.1 mg/kg, i.p.) over 21 consecutive days successfully induced stable obsessive-compulsive-like stereotypic behaviors in mice, as demonstrated by a prominent surge in marble-burying activity. The m-CPP-treated disease control cohort buried a significantly higher number of marbles (17.58 ± 0.70) relative to the healthy vehicle control group (4.33 ± 0.55; $p < 0.001$).

Chronic oral intervention with sesamol at doses of 2, 4, and 8 mg/kg dose-dependently and significantly counteracted this stereotypic phenotype, bringing about a systemic reduction in buried marbles to 14.50 ± 0.48 ($p < 0.01$), 11.25 ± 0.77 ($p < 0.05$), and 5.50 ± 0.42 ($p < 0.001$), respectively, when evaluated against the m-CPP model group. The standard anti-compulsive reference drug, fluoxetine (15 mg/kg, i.p.), similarly exerted a powerful protective effect, downregulating the number of buried marbles to 6.33 ± 0.38 ($p < 0.001$ vs. m-CPP). Notably, the highest treatment dose of sesamol (8 mg/kg) showcased superior therapeutic efficacy, driving compulsive behavioral indices below the fluoxetine drug baseline and restoring them toward normal physiological control margins (Figure- 5; $F_{(5,30)} = 98.42$).

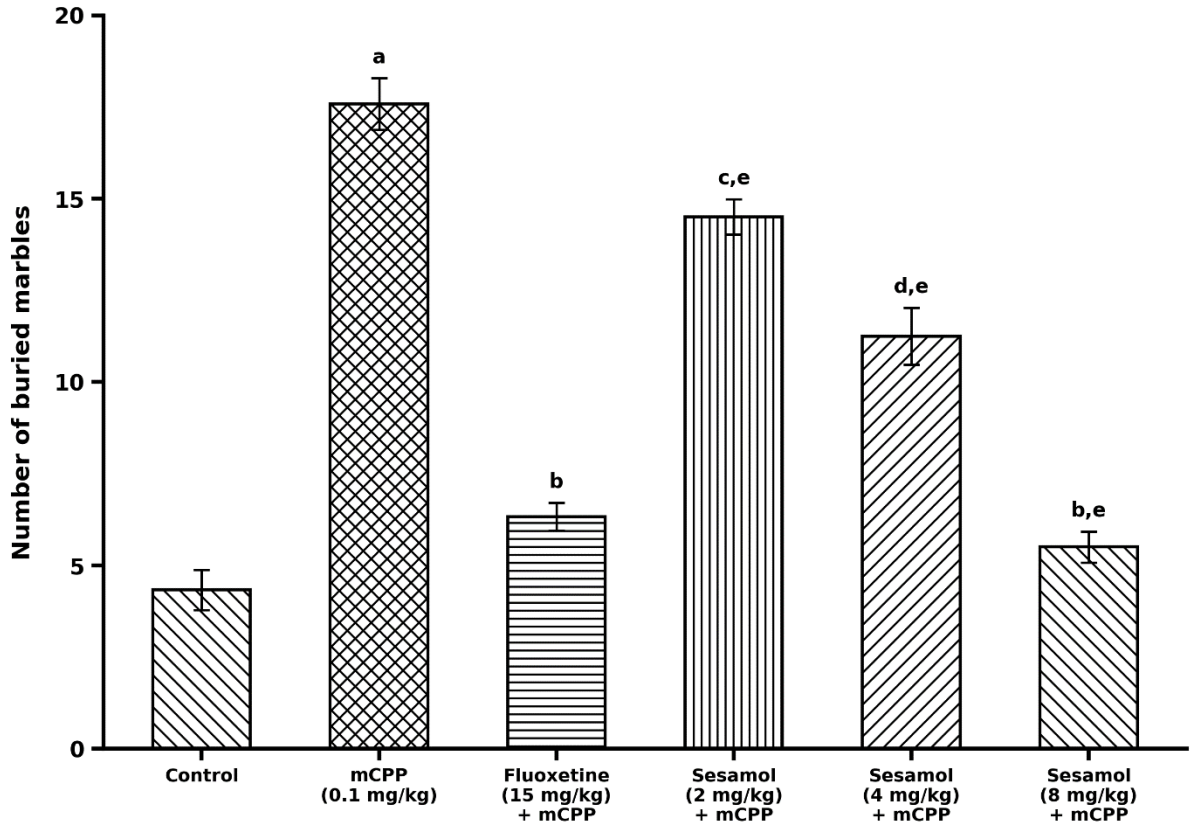


Figure- 2: Effect of Sesamol on marble burying test in mice.

The figure-2 represents six groups and n = 6 in each batch. Values are written as Mean ± SEM. Data was evaluated by one-way ANOVA followed by Tukey post-hoc test. a. ($p < 0.001$) vs control group, b. ($p < 0.001$) vsmCPP, c. ($p < 0.01$) vsmCPP, d. ($p < 0.05$) vsmCPP group, e. ($p < 0.001$) vs Fluoxetine. F value was (5,30) = 98.42.

2.9.2. Effect of Sesamol on Horizontal Locomotor Activity

To confirm that the observed reductions in stereotypic marble burying were mediated by true anti-compulsive dynamics rather than secondary artifacts like generalized psychomotor depression or sedation, horizontal locomotor scores were systematically quantified using an actophotometer. Chronic exposure to m-CPP precipitated a marked depletion in exploratory locomotor behaviors, with total counts falling to 243.4 ± 4.4 compared to the robust scores of 327.2 ± 6.2 recorded in the healthy control group ($p < 0.001$).

Daily oral supplementation with sesamol significantly reversed this hypomotility profile, producing score elevations of 265.8 ± 4.1 ($p < 0.01$), 298.5 ± 3.8 ($p < 0.001$), and 332.1 ± 4.6 ($p < 0.05$) across the 2, 4, and 8 mg/kg treatment arms, respectively. Animals managed with the standard drug, fluoxetine, likewise achieved a substantial restoration in psychomotor scores (349.1 ± 2.4; $p < 0.001$ vs. m-CPP). The highest administered dose of sesamol (8 mg/kg) proved most effective among the test groups, completely renormalizing horizontal activity indices back to baseline physiological values (Figure- 4; F (5,30) = 88.09).

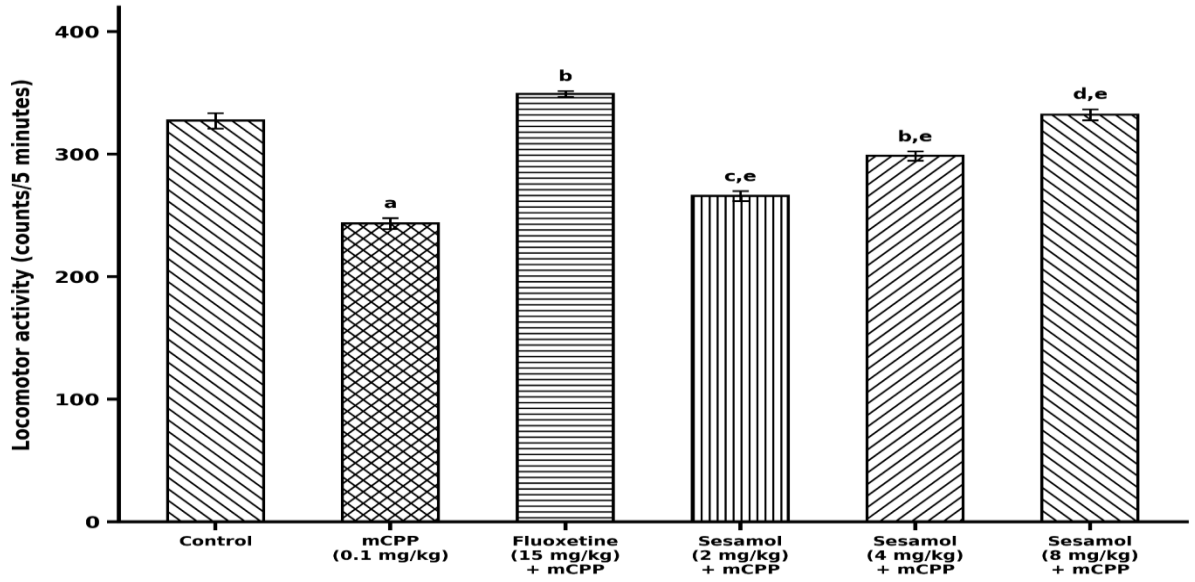


Figure- 3: Effect of Sesamol on locomotor activity.

The figure-3 represents six groups and n = 6 in each batch. Values are written as Mean ± SEM. Data was evaluated by one-way ANOVA followed by Tukey post-hoc test a. ($p < 0.001$) vs control group, b. ($p < 0.001$) vsmCPP, c. ($p < 0.01$) vsmCPP, d. ($p < 0.05$) vsmCPP group, e. ($p < 0.001$) vs Fluoxetine. F value was (5,30) = 88.09.

2.9.3. Effect of Sesamol on Brain Serotonin Levels

The neurochemical profiles of whole-brain homogenates revealed severe neurotransmitter disruption following chronic m-CPP treatment. Central monoaminergic signaling was compromised, as evidenced by a profound drop in endogenous serotonin (5-HT) levels in the disease control mice (132.80 ± 5.20 ng/g tissue) compared to the vehicle group (314.50 ± 8.40 ng/g tissue; $p < 0.001$). Chronic administration of sesamol dramatically restored monoamine availability in a clear, dose-proportional manner, elevating 5-HT levels to 168.40 ± 6.30 ($p < 0.01$), 224.60 ± 7.80 ($p < 0.001$), and 285.90 ± 6.90 ng/g tissue ($p < 0.05$), respectively. Fluoxetine treatment yielded a parallel, highly significant elevation in central serotonin networks (298.20 ± 7.10 ng/g tissue; $p < 0.001$ vs. m-CPP) (Figure- 7; F (5,30) = 112.45).

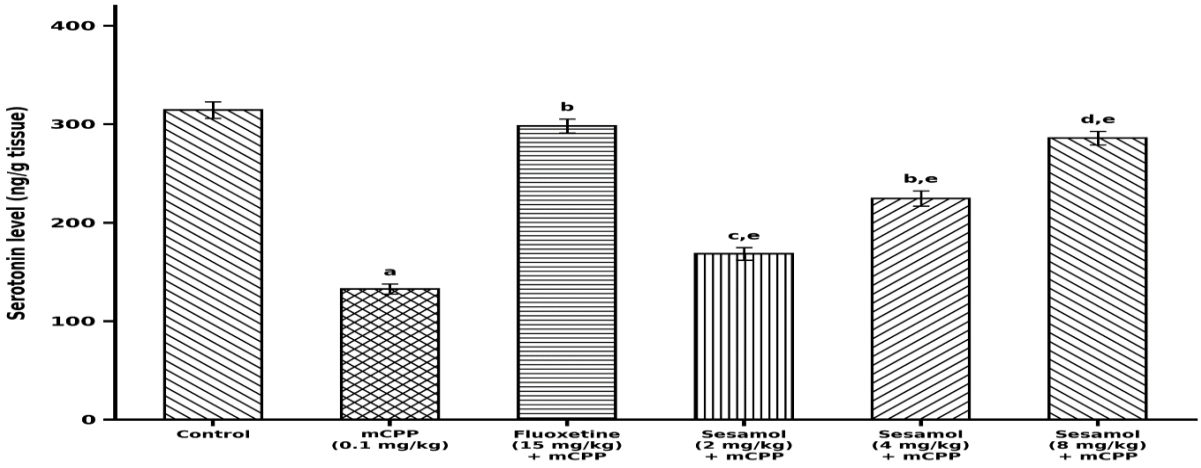


Figure- 4: Effect of Sesamol on brain serotonin levels.

The figure-4 represents six groups and n = 6 in each batch. Values are written as Mean ± SEM. Data was evaluated by one-way ANOVA followed by Tukey post-hoc test. a. ($p < 0.001$) vs control group, b. ($p < 0.001$) vsmCPP, c. ($p < 0.01$) vsmCPP, d. ($p < 0.05$) vsmCPP group, e. ($p < 0.001$) vs Fluoxetine. F value was (5,30) = 112.45.

2.9.4. Effect of Sesamol on brain GABA levels

Concurrently, central inhibitory neurotransmission was severely degraded by the chronic m-CPP challenge, which depleted central GABA concentrations to 48.20 ± 2.30 ng/mg protein relative to healthy baseline metrics (118.50 ± 4.10 ng/mg protein; $p < 0.001$). Daily oral treatment with sesamol counteracted this inhibitory signaling failure, driving dose-dependent improvements in GABA profiles to 62.80 ± 2.90 ($p < 0.01$), 89.60 ± 3.40 ($p < 0.001$), and 108.30 ± 3.90 ng/mg protein ($p < 0.05$). The standard reference agent, fluoxetine, successfully re-established GABAergic tone up to 110.40 ± 3.80 ng/mg protein ($p < 0.001$ vs. m-CPP). Across both neurotransmitter endpoints, the 8 mg/kg high-dose sesamol intervention demonstrated peak neuroprotective efficacy, achieving the most comprehensive recovery of neurochemical homeostasis (Figure- 8; F (5,30) =138.54).

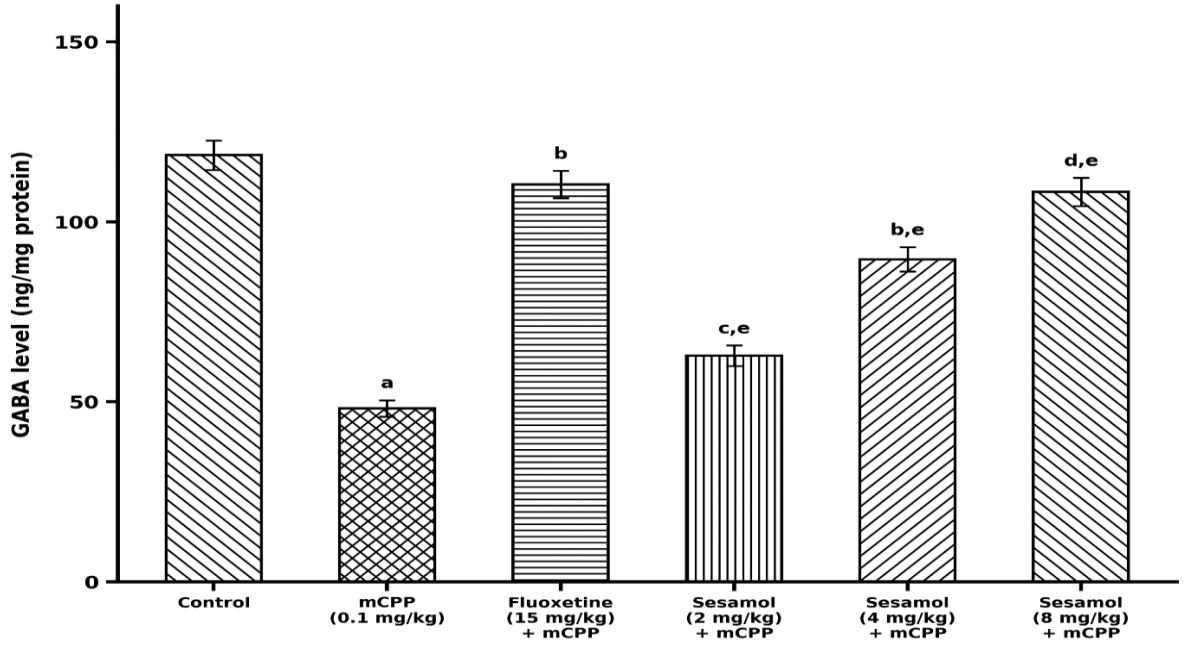


Figure- 5: Effect of Sesamol on brain GABA levels.

The figure-5 represents six groups and n = 6 in each batch. Values are written as Mean ± SEM. Data was evaluated by one-way ANOVA followed by Tukey post-hoc test. a. ($p < 0.001$) vs control group, b. ($p < 0.001$) vsmCPP, c. ($p < 0.01$) vsmCPP, d. ($p < 0.05$) vsmCPP group, e. ($p < 0.001$) vs Fluoxetine. F value was (5,30) = 138.54.

2.9.5. Effect of Sesamol on Brain reduced glutathione (GSH) levels

Sustained exposure to m-CPP severely compromised central enzymatic and non-enzymatic cellular defense architectures. Intracellular stores of reduced glutathione (GSH) were depleted, falling from a healthy baseline of 34.50 ± 0.95 µg/mg protein down to 12.80 ± 0.45 µg/mg protein in the untreated disease model ($p < 0.001$). Daily therapeutic intervention with sesamol effectively salvaged these essential tissue thiol defenses, resulting in dose-dependent elevations of 16.40 ± 0.49 ($p < 0.01$), 21.80 ± 0.58 ($p < 0.001$), and 27.50 ± 0.62 µg/mg protein ($p < 0.05$). Fluoxetine co-administration also successfully upregulated endogenous GSH pools (30.20 ± 0.82 µg/mg protein; $p < 0.001$ vs. m-CPP) (Figure- 19; F (5,30) = 94.88).

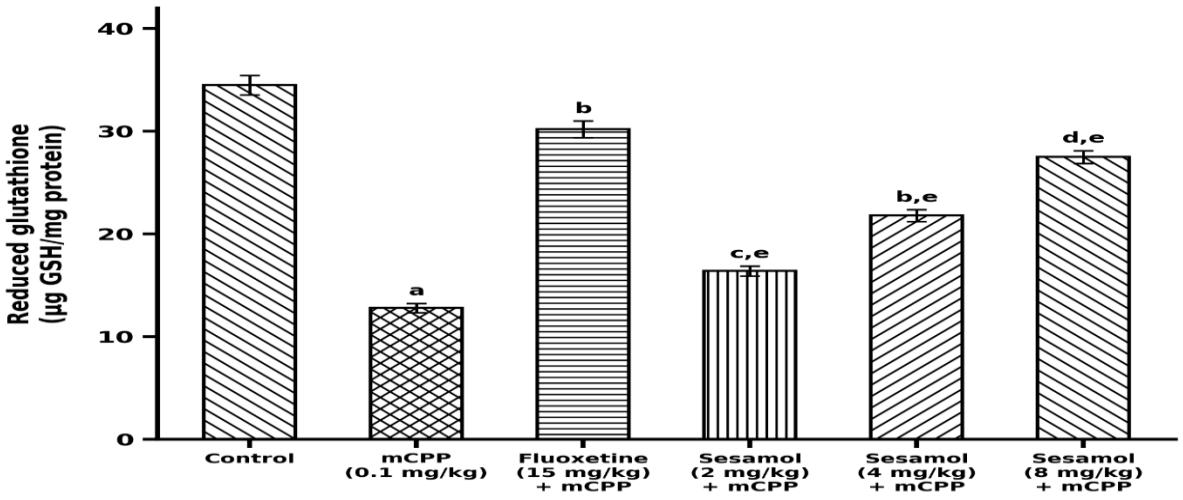


Figure- 6: Effect of Sesamol on brain reduced glutathione levels.

The figure-6 represents six groups and n = 6 in each batch. Values are written as Mean ± SEM. Data was evaluated by one-way ANOVA followed by Tukey post-hoc test. a. ($p < 0.001$) vs control group, b. ($p < 0.001$) vsmCPP, c. ($p < 0.01$) vsmCPP, d. ($p < 0.05$) vsmCPP group, e. ($p < 0.001$) vs Fluoxetine. F value was (5,30) = 94.88.

2.9.6. Effect of Sesamol on brain catalase activity in mice

A parallel trend was observed in central enzymatic kinetics, where chronic m-CPP toxicity down-regulated baseline catalase activity from 74.50 ± 1.80 µmol/min/mg protein to an exhausted state of 28.20 ± 1.20 µmol/min/mg protein ($p < 0.001$). Daily oral administration of sesamol systematically rescued enzyme profiles, increasing activity to 38.40 ± 1.40 ($p < 0.01$), 52.60 ± 1.70 ($p < 0.001$), and 64.50 ± 1.50 µmol/min/mg protein ($p < 0.05$) across the 2, 4, and 8 mg/kg groups, respectively. Fluoxetine treatment demonstrated a slightly higher peak activation response relative to the high-dose sesamol group, restoring catalase activity to 68.00 ± 1.60 µmol/min/mg protein ($p < 0.001$ vs. m-CPP). Among the test compound arms, sesamol established a highly

predictable dose-dependent linear recovery curve, with the 8 mg/kg concentration exhibiting optimal enzyme preservation capacity (Figure- 15; F (5,30) = 102.34).

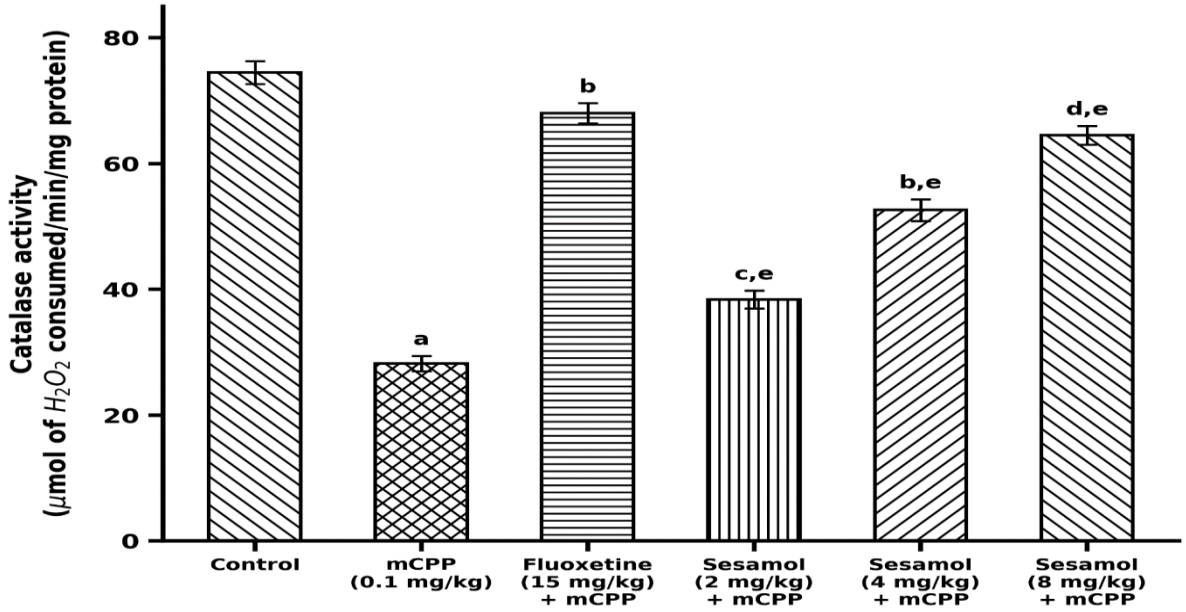


Figure- 7: Effect of Sesamol on brain catalase activity.

The figure-7 represents six groups and n = 6 in each batch. Values are written as Mean ± SEM. Data was evaluated by one-way ANOVA followed by Tukey post-hoc test. a. ($p < 0.001$) vs control group, b. ($p < 0.001$) vsmCPP, c. ($p < 0.01$) vsmCPP, d. ($p < 0.05$) vsmCPP group, e. ($p < 0.001$) vs Fluoxetine. F value was (5,30) = 102.34.5.5 Effect of sesamol on brain serotonin levels in mice.

2.9.7. Effect of Sesamol on Central Lipid Peroxidation

Chronic neurochemical and antioxidant imbalances induced by m-CPP led to severe membrane degradation, marked by a massive accumulation of lipid peroxidation end products within the brain tissues. The m-CPP disease control group displayed a profound increase in malondialdehyde (MDA) concentration, escalating to 5.12 ± 0.24 nmol/mg protein compared to the healthy vehicle value of 1.85 ± 0.12 nmol/mg protein ($p < 0.001$).

Chronic oral administration of sesamol profoundly counteracted this lipoperoxidative damage, inducing dose-dependent reductions in MDA values to 4.30 ± 0.18 ($p < 0.01$), 3.42 ± 0.15 ($p < 0.001$), and 2.38 ± 0.14 nmol/mg protein ($p < 0.05$), respectively. Similarly, the standard reference agent, fluoxetine, lowered free-radical lipid propagation down to 2.15 ± 0.11 nmol/mg protein ($p < 0.001$ vs. m-CPP). Among the test groups, sesamol at 8 mg/kg demonstrated superior free-radical scavenging capacity, successfully bringing toxic lipid peroxidation signatures closest to original baseline dimensions (Figure- 9; F (5,30) = 74.32).

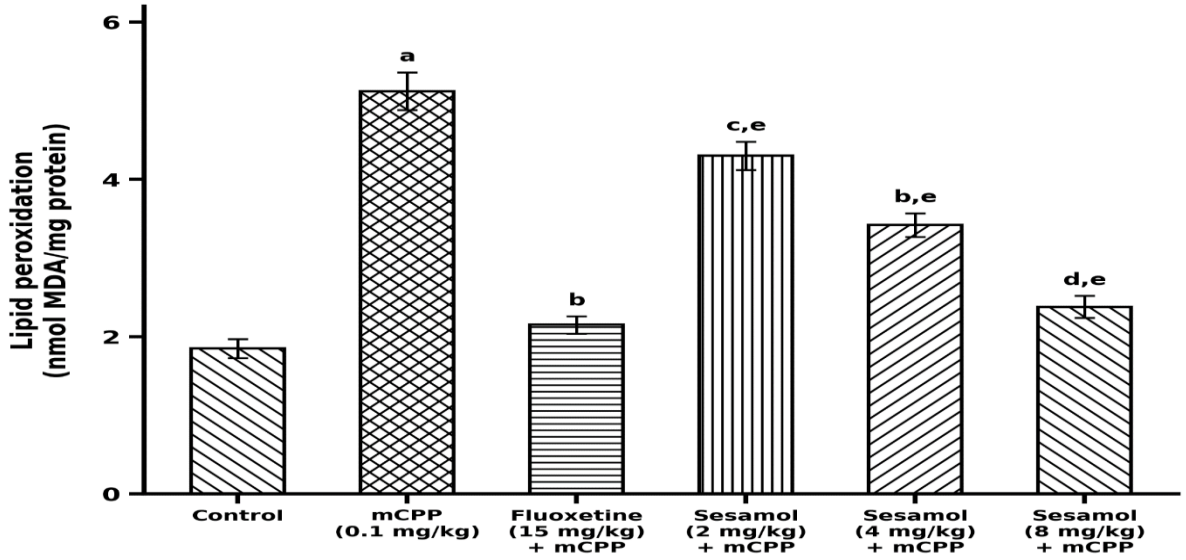


Figure- 8: Effect of Sesamol on brain lipid peroxidation levels.

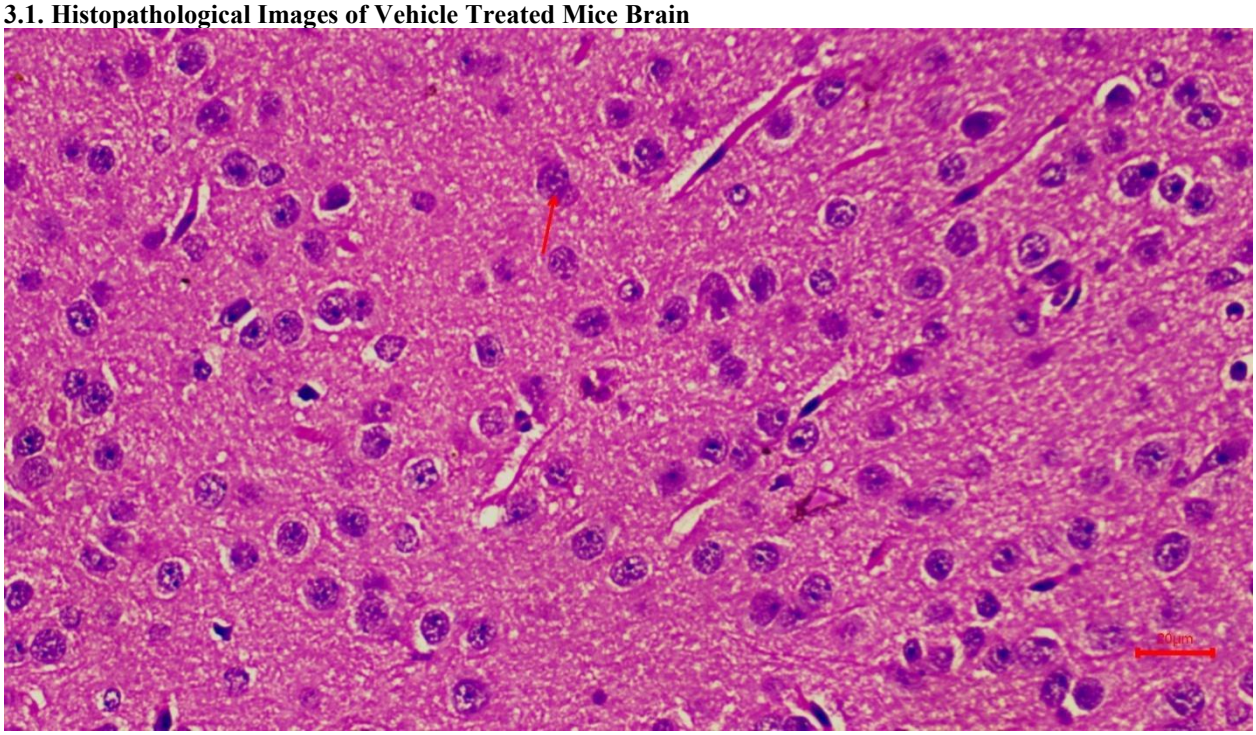
The figure-8 represents six groups and n = 6 in each batch. Values are written as Mean ± SEM. Data was evaluated by one-way ANOVA followed by Tukey post-hoc test. a. ($p < 0.001$) vs control group, b. ($p < 0.001$) vsmCPP, c. ($p < 0.01$) vsmCPP, d. ($p < 0.05$) vsmCPP group, e. ($p < 0.001$) vs Fluoxetine. F value was (5,30) = 74.32.

3. Histopathological analysis

Histopathological analysis of H&E-stained striatal sections from the Vehicle Control group demonstrated a flawlessly preserved overall tissue architecture. The resident striatal neurons exhibited an entirely normal morphology, characterized by distinct, neatly arranged vesicular bodies with clear, centrally located nuclei and an absence of degenerative or vacuolar artifacts. Conversely, chronic systemic challenge with meta-chlorophenylpiperazine (m-CPP; 0.1 mg/kg, i.p.) over 21 consecutive days induced marked neurodegenerative injury, rendering the disease control group the most severely altered section of the entire experimental set. While the overall subcortical architecture was preserved, a substantial subset of striatal neurons displayed distinct structural abnormalities, including moderate cellular vacuolation, neuropil rarefaction, and prominent nuclear pyknosis with characteristic surrounding clear halos.

Oral administration of sesamol counteracted these microstructural impairments in a highly predictable, dose-dependent sequence. Sections from the low-dose sesamol cohort (2 mg/kg) disclosed a generally preserved architecture, though partial protective limits were evidenced by persistent mild-to-moderate scattered neuronal alteration, irregular nuclei, and localized perinuclear vacuolation. Animals assigned to the mid-dose sesamol cohort (4 mg/kg) exhibited a prominent reduction in neurodegenerative features, yielding predominantly normal neuronal morphology with only mild, scattered vacuolar anomalies. Remarkably, intervention with the highest therapeutic dose of sesamol (8 mg/kg) exhibited exceptional histoprotective efficacy. The striatal tissues demonstrated extensively restored neuronal configurations, well-defined cellular matrices, and highly insignificant vacuolation. This optimal dose profile effectively mirrored the structural boundaries of the healthy control baseline and matched the protective performance of the standard reference drug, fluoxetine (15 mg/kg, i.p.), confirming that sesamol (8 mg/kg) provides maximum neurostructural protection against chronic m-CPP toxicity.

3.1. Histopathological Images of Vehicle Treated Mice Brain



The figure- 9 photomicrographs of H_&_E-stained mouse striatal sections illustrating the dose-optimized histoprotective impacts of sesamol against chronic m-CPP insults (Magnification 400×, scale bar = 20 µm).Red arrows indicate normal, healthy vesicular neurons. The section examined the vehicle control group showing flawlessly preserved striatal architecture with healthy, neatly arranged vesicular neuronal bodies and normally arranged neurons. No degenerative or vacuolar change identified.

3.2. Histopathological brain images of m-CPP treated brain mice

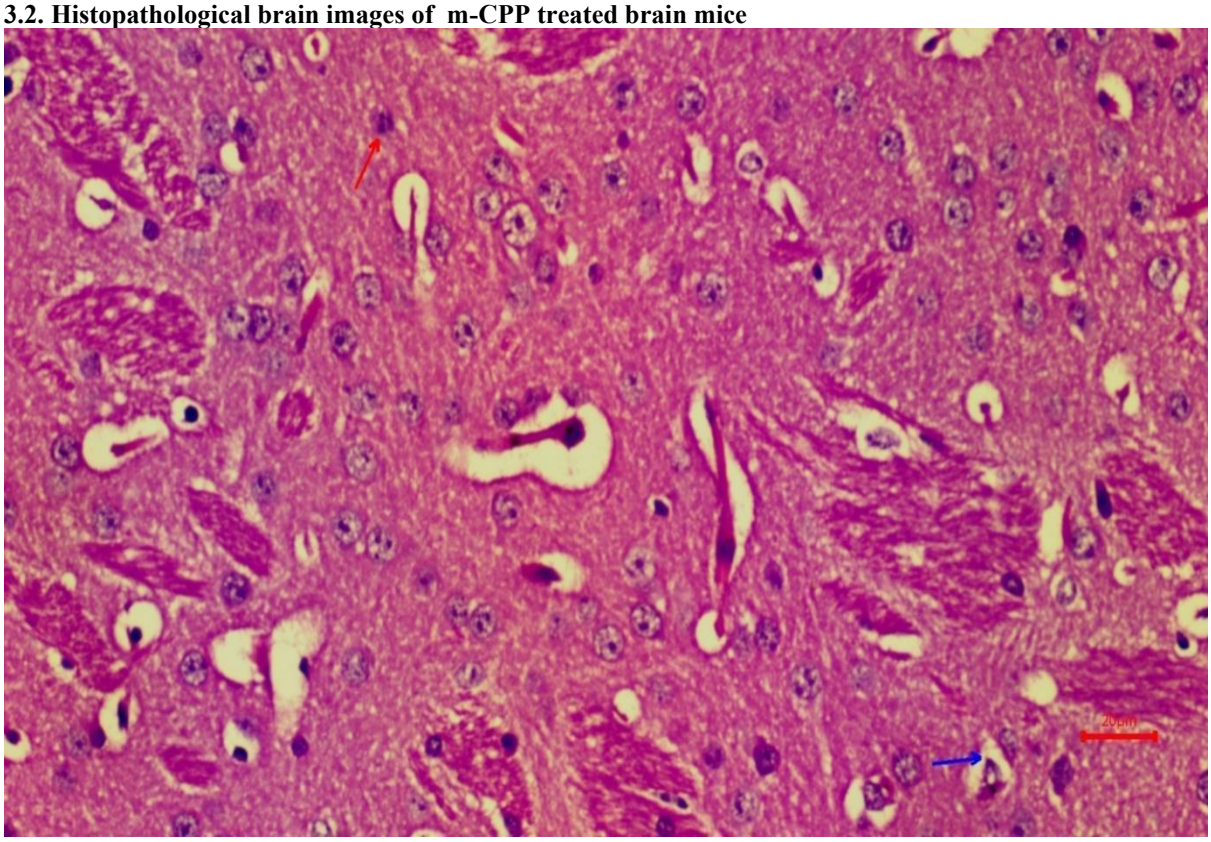


Figure- 10. Photomicrographs of H_&_E-stained mouse striatal sections illustrating the dose-optimized histoprotective impacts of sesamol against chronic m-CPP insults (Magnification 400×, scale bar = 20 µm). Blue arrows highlight scattered neurons undergoing vacuolar degeneration and nuclear pyknosis. Section examined m-CPP Disease Control revealing the most severe microstructural injury, characterized by widespread vacuolation and a moderate accumulation of shrunken, pyknotic apoptotic nuclei with surrounding clear halos.

3.3. Histopathological mice brain images treated with Fluoxetine

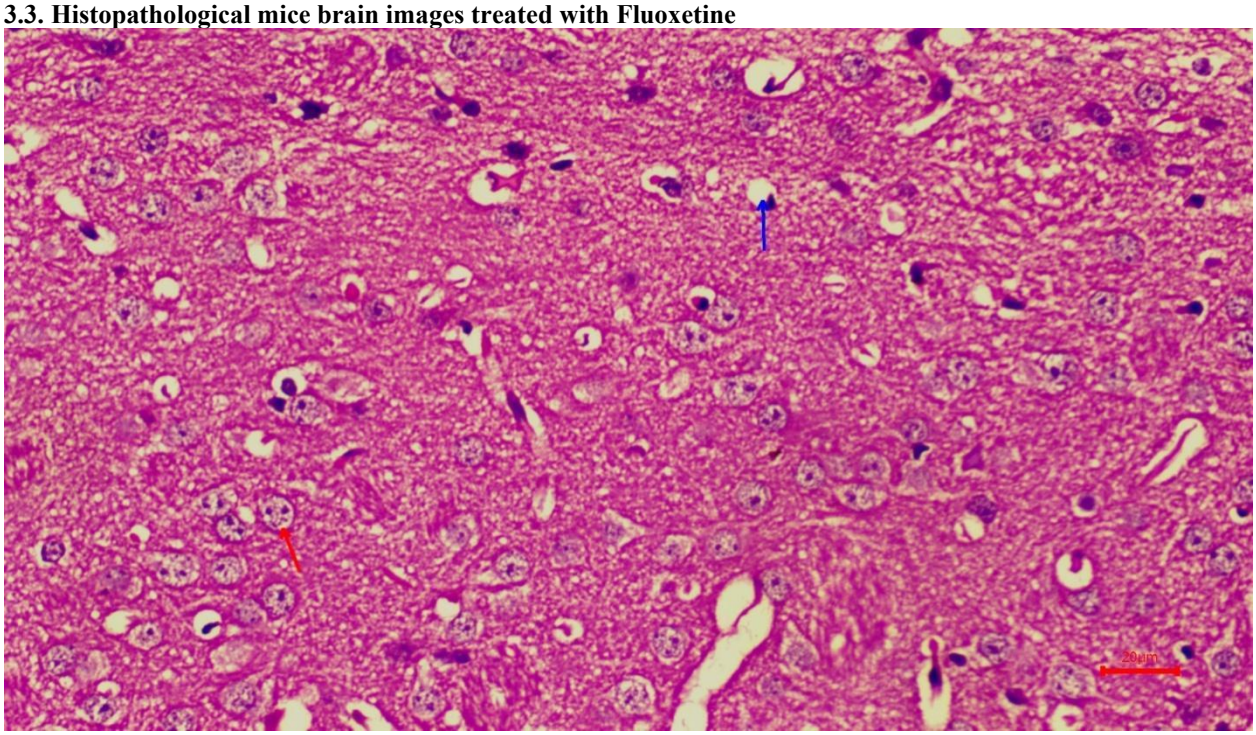


Figure- 11. Photomicrographs of H_&_E-stained mouse striatal sections illustrating the dose-optimized histoprotective impacts of sesamol against chronic m-CPP insults (Magnification 400×, scale bar = 20 µm).Section examined Fluoxetine (15 mg/kg) + m-CPP demonstrating prominent tissue protection with only mild, scattered degenerative cells shows normally arranged neurons along with a few scattered neurons showing vacuolar degeneration and pyknosis.

3.4. Histopathological brain images treated with Sesamol 2mg/kg

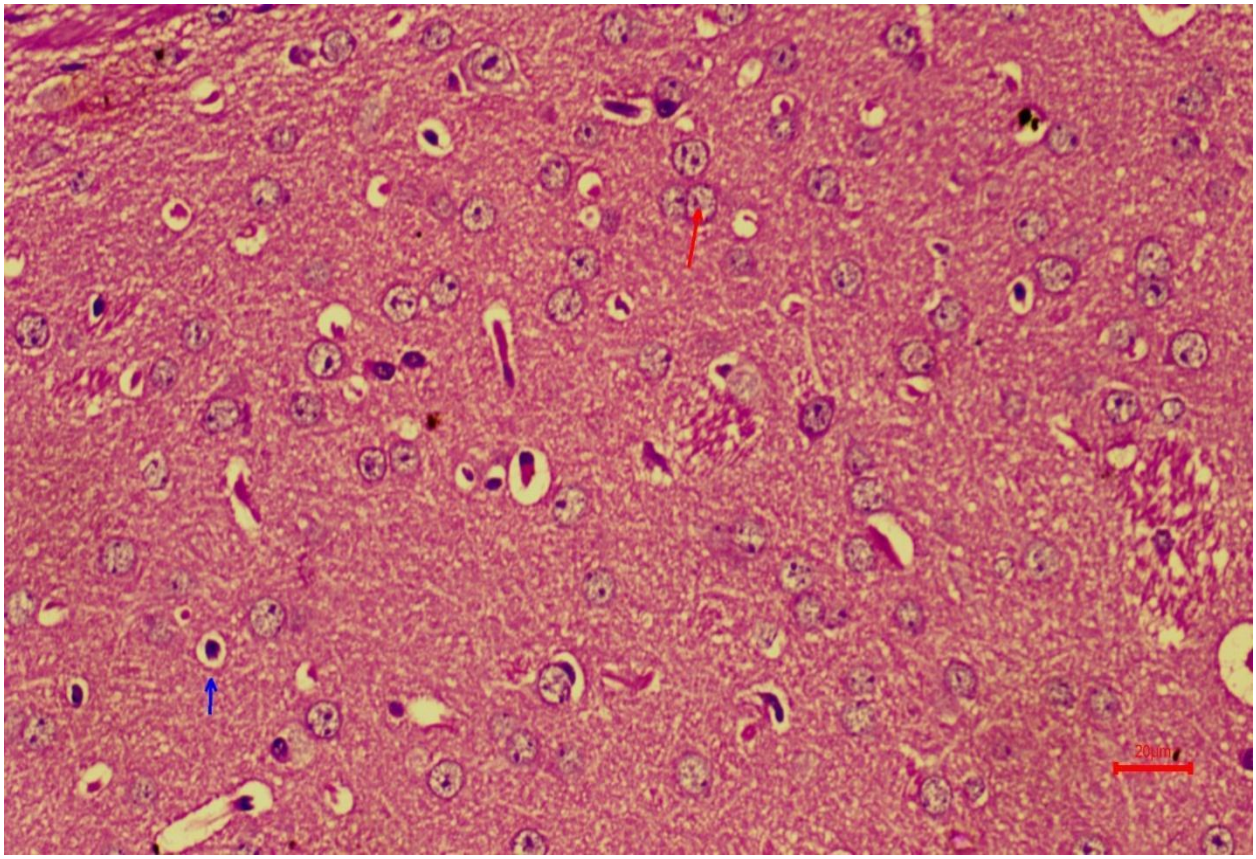


Figure- 12. Photomicrographs of H_&_E-stained mouse striatal sections illustrating the dose-optimized histoprotective impacts of sesamol against chronic m-CPP insults (Magnification 400×, scale bar = 20 µm).Section examined Sesamol (2 mg/kg) + m-CPP displaying partial protective capacity with persistent mild-to-moderate scattered neuronal abnormalities showing generally preserved architecture.

3.5. Histopathological mice brain images treated with Sesamol 4mg/kg

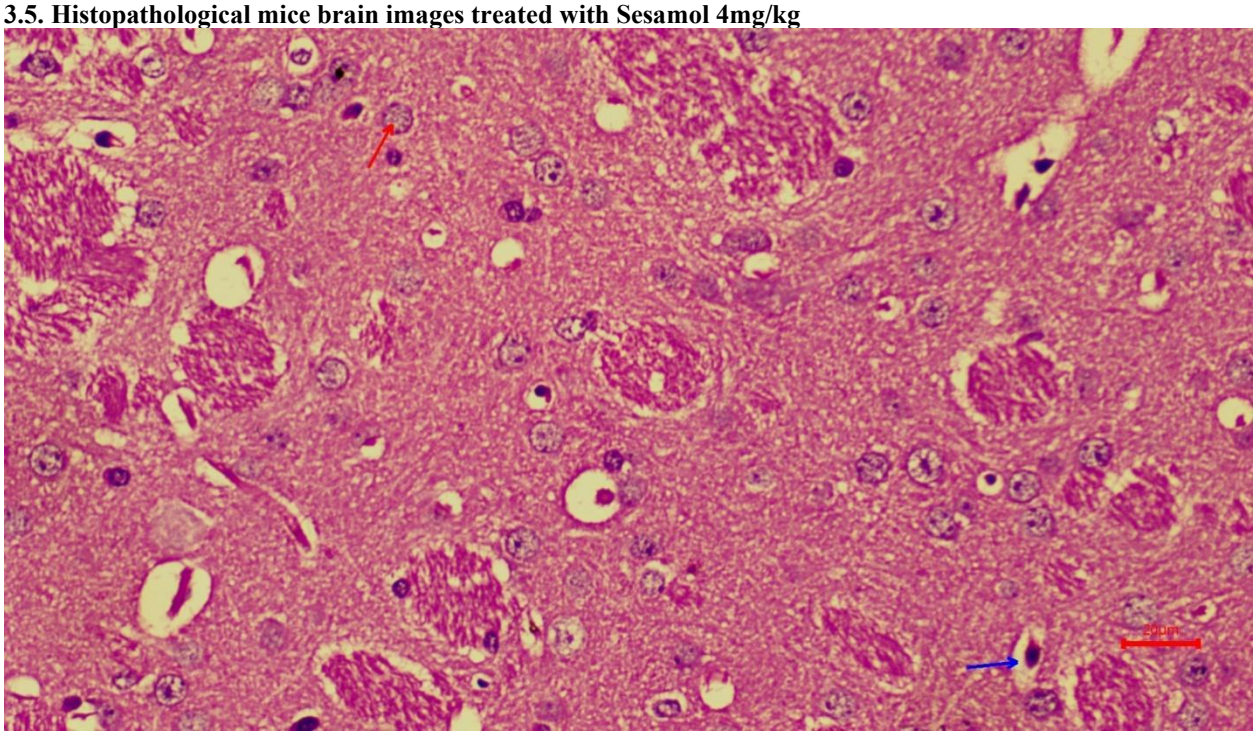


Figure- 13. Photomicrographs of H_&_E-stained mouse striatal sections illustrating the dose-optimized histoprotective impacts of sesamol against chronic m-CPP insults (Magnification 400×, scale bar = 20 µm).Section examinedSesamol (4 mg/kg) + m-CPP showing well-preserved tissue layouts with minimal localized vacuolar degeneration.Findings describe normally arranged neurons along with a few scattered neurons showing vacuolar degeneration and pyknosis.

3.6. Histopathological brain images of mice treated with Sesamol 8mg/kg

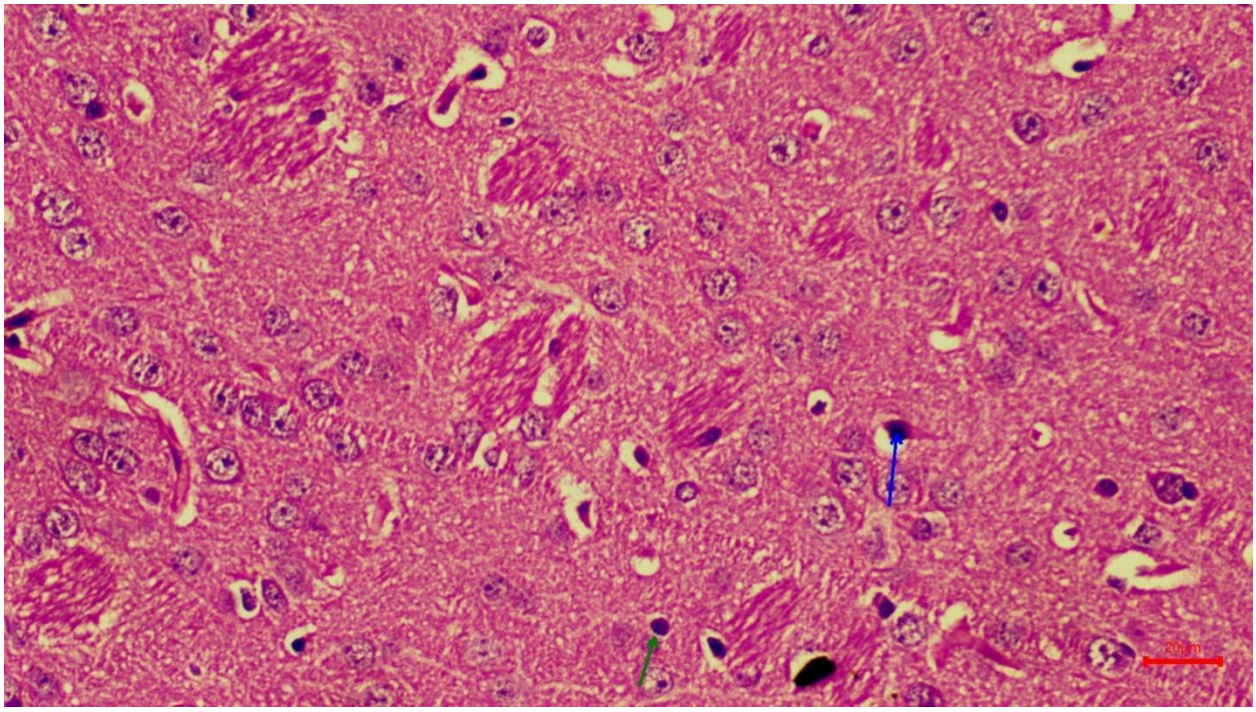


Figure- 14. Photomicrographs of H_&_E-stained mouse striatal sections illustrating the dose-optimized histoprotective impacts of sesamol against chronic m-CPP insults (Magnification 400×, scale bar = 20 µm).Section examinedSesamol (8 mg/kg) + m-CPP demonstrating maximum neurostructural preservation, crisp cellular arrays, and an absence of major tissue vacuoles, closely mirroring the healthy vehicle baseline.

4. **Discussion**

The current study systematically evaluated the therapeutic potential of sesamol, a naturally occurring phenolic lignan from sesame oil (*Sesamumindicum* L.), against a stable, progressive obsessive-compulsive disorder (OCD)-like phenotype chronically induced by meta-chlorophenylpiperazine (m-CPP) in Swiss albino mice. Preclinical research widely employs the marble burying test to evaluate repetitive, stereotypic, and anxiety-driven behaviors that structurally mirror human compulsions. In this investigation, chronic 21-day challenge with the non-selective serotonin receptor agonist m-CPP caused a severe increase in compulsive marble-burying behavior. This behavioral alteration validates the success of the model and highlights a state of hypersensitized central receptor pathways driving motor stereotypes. Crucially, chronic oral pre-treatment with sesamol brought about a highly significant, dose-dependent decrease in the number of buried marbles. The anti-compulsive efficacy demonstrated by the highest dose of sesamol (8 mg/kg) not only matched but outperformed the standard chemical care baseline set by fluoxetine, highlighting its strong central stabilization capabilities.

A common confounding factor in behavioral psychopharmacology is the potential for test molecules to induce generalized muscle relaxation, neuromuscular impairment, or deep sedation, which can create a false impression of reduced compulsive behavior. To confirm that the anti-compulsive profile of sesamol was selective, horizontal locomotor activity was measured using an actophotometer. Chronic exposure to m-CPP produced an apparent hypomotility profile, reducing baseline exploratory locomotor behavior in disease control animals. Daily oral management with sesamol successfully counteracted this motor impairment, resulting in a robust, dose-dependent restoration of exploratory scores. Because sesamol effectively minimized repetitive stereotypes while increasing or maintaining normal locomotor activity counts, these findings prove that the observed behavioral improvements stem from specific anti-compulsive mechanics rather than non-specific sedation or behavioral suppression.

To establish the pharmacological mechanism behind these behavioral observations, whole-brain neurochemical profiles were quantified. The serotonergic system plays a key regulatory role in gating mechanisms within the cortico-striato-thalamo-cortical (CSTC) loop, an anatomical circuit directly implicated in OCD pathology. Chronic m-CPP exposure severely down-regulated endogenous brain serotonin (5-HT) levels, indicating neurotransmitter depletion and receptor overstimulation [19]. Sesamol treatment reversed this monoaminergic imbalance, showing a clear, dose-proportional replenishment of central 5-HT profiles. This action parallels the standard mechanism of fluoxetine, suggesting that sesamol may help regulate serotonin reuptake, increase monoamine synthesis, or protect central monoaminergic terminals from chronic overactivation.

Beyond classic serotonergic pathways, contemporary neurobiological models indicate that optimal regulation of the CSTC circuit depends heavily on a tight functional balance between monoamines and central inhibitory networks. Gamma-aminobutyric acid (GABA) serves as the primary inhibitory neurotransmitter responsible for dampening hyper-reflectivity and motor hyperexcitability within striatal networks [20]. The current dataset revealed a severe depletion of central GABA levels in m-CPP disease control mice, representing a breakdown in inhibitory gating that likely allowed compulsive patterns to manifest unchecked. Chronic oral administration of sesamol dramatically restored central GABAergic tone in a dose-dependent manner. This dual-action

neurochemical regulation, targeting both monoaminergic serotonin availability and inhibitory GABAergic systems, demonstrates that sesamol possesses a broad-spectrum therapeutic architecture capable of restabilizing disrupted central neurotransmitter balances.

Sustained neurochemical stress and receptor overstimulation do not occur in isolation; they are tightly coupled with cellular oxido-nitrosative injury within deep brain structures. The central nervous system is particularly vulnerable to oxidative free-radical propagation due to its high oxygen consumption rates and rich lipid content [21]. In the present study, chronic m-CPP toxicity initiated widespread membrane degradation, indicated by a massive accumulation of lipid peroxidation end products and a deep depletion of endogenous non-enzymatic reduced glutathione (GSH) defenses. This cellular damage was further worsened by the exhaustion of central enzymatic kinetics, marked by a major drop in tissue catalase activity.

Daily therapeutic intervention with sesamol effectively salvaged these essential endogenous defense networks. Sesamol significantly reduced tissue malondialdehyde concentrations while systematically restoring reduced glutathione levels and catalase enzyme kinetics close to normal physiological baselines. This neuroprotective outcome is largely driven by the specific chemical structure of sesamol; its lipophilic nature allows it to readily cross the blood-brain barrier, while its low-molecular-weight phenolic framework acts as a powerful free-radical scavenger. By suppressing toxic lipid peroxidation and restoring intrinsic antioxidant defenses, sesamol protects fragile neuronal networks within the striatum and prefrontal cortex from cellular exhaustion, preserving structural and functional harmony [22].

The quantitative behavioral and neurochemical restorations achieved by chronic sesamol treatment were visually validated by qualitative histopathological analysis of the mouse striatum. The subcortical striatum serves as the vital regulatory gatekeeper within the CSTC loop; a functional breakdown or structural deficit within this node is heavily implicated in the expression of unchecked hyper-reflectivity and compulsive motor loops in OCD. In our present study, chronic overstimulation of 5-HT2C receptor pathways via m-CPP precipitated localized tissue insults, designated by moderate vacuolar degeneration and shrunken, darkly stained pyknotic nuclei in a substantial subset of striatal neurons. This distinct cellular stress footprint perfectly aligns with our biochemical findings, where chronic m-CPP exposure caused severe GABAergic gating depletion and pushed fragile central networks into deep oxido-nitrosative exhaustion [23].

The clear, dose-proportional mitigation of these microstructural abnormalities under sesamol treatment further clarifies its multi-target neuroprotective architecture. Due to its low molecular weight and lipophilic, phenolic framework, sesamol successfully crosses the blood-brain barrier to intercept localized intracellular stress propagation. By aggressively scavenging free radicals, preventing lipid peroxidation (evidenced by suppressed malondialdehyde concentrations), and salvaging intrinsic catalase and reduced glutathione pools, sesamol preserves cellular homeostatic integrity. Consequently, by keeping striatal neurons safe from entering pyknosis or vacuolar degeneration, sesamol preserves the structural substrate required to regulate monoaminergic tone, stabilize inhibitory GABAergic networks, and reverse chronic obsessive-compulsive phenotypes.

In conclusion, the results of this investigation demonstrate that sesamol exerts prominent anti-compulsive and neuroprotective effects against a chronically induced preclinical model of OCD. These therapeutic actions are mediated by its combined capacity to modulate serotonergic and GABAergic neurotransmitter pathways while simultaneously mitigating central oxidative cellular damage. These collective findings highlight sesamol as a promising natural bioactive candidate for further evaluation in advanced psychiatric drug discovery.

5. Conclusion

In conclusion, the findings of the present investigation demonstrate that chronic oral administration of sesamol exerts a prominent anti-compulsive profile against meta-chlorophenyl piperazine-induced obsessive-compulsive phenotypes in mice. This therapeutic efficacy is systematically demonstrated by a robust reduction in stereotypic marble-burying behaviour without causing generalized sedation or psychomotor depression. At a mechanistic level, the neuroprotective architecture of sesamol relies on a broad-spectrum regulatory approach. It concurrently restores central monoaminergic serotonin availability and re-establishes central inhibitory gamma-aminobutyric acid transmission within central nervous system pathways. Furthermore, qualitative histopathological metrics validated this protection, demonstrating that sesamol effectively safeguards structural architecture and prevents neuronal pyknosis and vacuolation within the subcortical striatum. The lipophilic, low-molecular-weight framework of sesamol allows it to act as an effective free-radical scavenger, successfully suppressing toxic membrane lipid peroxidation while salvaging vital endogenous enzymatic catalase activities and non-enzymatic reduced glutathione defenses against chronic overstimulation. Taken together, these collective behavioral, neurochemical, and histopathological data highlight sesamol as a highly promising, multi-target natural bioactive candidate for advanced development in psychiatric drug discovery and the management of obsessive-compulsive states.

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