

Neuroprotective Effects of Sesame Seed Oil Against Chronic Stress-Induced Prefrontal Cortex Dysfunction in Wistar Rats

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Abstract

Stress disrupts physiological homeostasis and induces neurobehavioural, biochemical, and structural alterations in the brain, including impairment of prefrontal cortical function. Sesame seed oil, rich in lignans, tocopherols, phytosterols, and unsaturated fatty acids, possesses potential antioxidant and neuroprotective properties. This study investigated the effects of sesame seed oil on stress-induced neurobehavioural, biochemical, and histomorphological alterations in the prefrontal cortex of Wistar rats. Sixty adult Wistar rats weighing 150–200 g were randomly assigned to six groups (n = 10): Group A (normal control), Groups B and C (sesame seed oil, 1 and 2 mL/kg, respectively), Group D (stress control), and Groups E and F (stress + sesame seed oil, 1 and 2 mL/kg, respectively). Sesame seed oil was administered for 21 days, while unpredictable stress was applied during days 17–21. On day 22, behavioural assessments were conducted using the open field test, Y-maze, and elevated plus maze. Animals were subsequently euthanised, blood samples collected for biochemical analyses, and the prefrontal cortex processed for histological examination. Stress exposure significantly reduced body weight, feed intake, horizontal locomotion, rearing, Y-maze performance, and open-arm time, while increasing self-grooming and closed-arm time. Stress also significantly increased malondialdehyde, corticosterone, interleukin-6, and tumour necrosis factor- α levels, while reducing total antioxidant capacity and interleukin-10. Histomorphological examination revealed degeneration of pyramidal and glial cells in stressed rats. Sesame seed oil significantly attenuated these behavioural, biochemical, and histological alterations. These findings demonstrate the potential neuroprotective effects of sesame seed oil against stress-induced neural dysfunction and may warrant further investigation in stress-related disorders.

KEYWORDS: Lignans, Tocopherols, Phytosterols, Neurobehavioural, Sesame seed oil

1. Introduction

Stress refers to the physiological and psychological response of an organism to stimuli or conditions that threaten or disrupt homeostasis [1]. Although the stress response is essential for survival and adaptation, prolonged or chronic stress can exert deleterious effects on multiple organ systems, particularly the brain [2]. Stress exposure activates the hypothalamic–pituitary–

adrenal (HPA) axis, resulting in the release of glucocorticoids, including corticosterone in rodents [3]. Persistent elevation of glucocorticoids has been associated with neuronal dysfunction, oxidative stress, neuroinflammation, and structural alterations in several brain regions, particularly stress-sensitive regions such as the prefrontal cortex, hippocampus, and amygdala [4].

Stressors are internal or external factors capable of disrupting physiological or psychological equilibrium [5]. They can be broadly classified as physical, psychological, environmental, metabolic, and social stressors [6]. Physical stressors include pain, restraint, temperature extremes, and food or water deprivation [7]. Psychological stressors encompass fear, anxiety, novelty, and predator exposure, whereas environmental stressors include excessive noise, overcrowding, cage tilting, and alterations in light–dark cycles. Social stressors may involve social isolation, overcrowding, or aggressive interactions [8]. Under normal conditions, the prefrontal cortex plays a central role in regulating cognition, behaviour, working memory, and emotional responses [9]. However, this region is particularly vulnerable to the detrimental effects of chronic stress, partly because of its high density of glucocorticoid receptors and extensive excitatory neuronal networks [10]. Evidence indicates that physiological stressors, including inflammation, head injury, and hypoxia, as well as psychological stress associated with perceived lack of control, can adversely affect prefrontal cortical function [11].

Sesame seed oil, derived from the seeds of the sesame plant (*Sesamum indicum*), is obtained from an oilseed crop widely cultivated in Africa and Asia [12]. Sesame seeds are an important source of nutritional and economically valuable oil because they contain several bioactive phytochemicals, including lignans such as sesamin, sesamol, and sesamolin, as well as tocopherols, phytosterols, and unsaturated fatty acids [13]. These bioactive constituents have demonstrated potential neuroprotective, antioxidant, and anti-inflammatory effects in various experimental models of neurotoxicity [14]. Sesamin and sesamolin, two major lignans present in sesame seed oil, have been associated with several biological activities, including antioxidant, anti-inflammatory, hypocholesterolaemic, neuroprotective, and antihypertensive effects [15]. Despite growing evidence supporting the biological benefits of sesame-derived compounds, relatively limited research has specifically examined the effects of sesame seed oil on prefrontal cortical alterations following stress exposure.

Therefore, the present study investigated the potential of sesame seed oil to mitigate stress-induced behavioural, biochemical, and structural alterations in the prefrontal cortex of Wistar rats. Specifically, the study assessed body weight and feed intake; neurobehavioural performance, including open-field activity, spatial working memory, and anxiety-related behaviour; oxidative stress and inflammatory markers; corticosterone levels; and prefrontal cortical histoarchitecture.

2. Materials and Methods

2.1 Chemicals and Drugs

Sesame seed oil (KTC Food Group Ltd., United Kingdom) was used in the study. Commercial assay kits for malondialdehyde (MDA), total antioxidant capacity (TAC), interleukin-6 (IL-6),

interleukin-10 (IL-10), and tumour necrosis factor-alpha (TNF- α) were obtained from BioVision Inc., Milpitas, CA, USA. Distilled water, normal saline, syringes, and other routine laboratory materials were also used.

2.2 Experimental Animals

Healthy Wistar rats used in this study were obtained from the Neurobehavioral Research Laboratory, Ogbomoso, Oyo State, Nigeria. The animals were housed in hardwood cages measuring 20 × 10 × 12 inches under standard laboratory conditions at a room temperature of 25 ± 2.5°C, with a 12-hour light–dark cycle (lights on at 07:00 h and off at 19:00 h). The animals had unrestricted access to standard laboratory chow and water throughout the experimental period, except during the specified water-deprivation stress procedure.

All experimental procedures were conducted in accordance with the approved protocols of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, and in compliance with the provisions for the care and use of laboratory animals outlined in the European Council Directive 2010/63/EU on the protection of animals used for scientific purposes.

2.3 Experimental Design

Sixty Wistar rats weighing 150–200 g were allocated using a computer-generated randomisation schedule into six groups (n = 10 per group). Group A served as the normal control and received distilled water (1 mL/kg, orally) and standard laboratory chow. Groups B and C received sesame seed oil (SO) at doses of 1 and 2 mL/kg, respectively, administered orally. Group D served as the stress-only group and was exposed to the stress protocol without sesame seed oil administration. Groups E and F were exposed to the stress protocol and received sesame seed oil at 1 and 2 mL/kg, respectively.

All treatments were administered orally using an appropriate cannula for 21 consecutive days. The sesame seed oil doses were selected based on previously published literature [16]. Following completion of the treatment period, animals underwent behavioural assessments using the open-field test [17], Y-maze test [18], and elevated plus maze [17].

Twenty-four hours after completion of the behavioural assessments, the animals were euthanised by cervical dislocation. Blood samples were collected for biochemical analysis of tumour necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-10 (IL-10) [19]. The brains were subsequently excised, fixed in 10% neutral buffered formal saline, and processed for histological examination of the prefrontal cortex [20]. Treatment administration could not be blinded because the investigators responsible for treatment preparation and administration were necessarily aware of group allocation. However, outcome assessment, including behavioural scoring, oxidative-stress and inflammatory-marker analyses, corticosterone assessment, histological evaluation, image

analysis, and statistical analysis, was performed by investigators blinded to treatment allocation.

2.3.1 Stress Procedure

Chronic unpredictable mild stress (CUMS) was employed during the final five days of the 21-day experimental period to induce a combination of psychological, physical, and environmental stress. The stressors were administered once daily in an unpredictable sequence to minimise habituation while avoiding physical injury. On Day 1, cage shaking was employed. The cage containing each rat was gently agitated for 30 minutes to induce mild physical and psychological stress. The intensity of agitation was controlled to minimise the risk of injury while providing sufficient environmental disturbance. On Day 2, water deprivation was employed. Water bottles were removed from the cages for 24 hours, while standard laboratory chow remained available *ad libitum*. On Day 3, rats were exposed to predator sounds. Recorded cat and dog sounds were played through an audio speaker for 30 minutes at an intensity sufficient to evoke a stress response without causing physical harm. On Day 4, animals were exposed to an empty water bottle for 24 hours. This served as a psychological stressor by providing the visual cue of a water source without access to water. On Day 5, the cage-shaking procedure described for Day 1 was repeated for 30 minutes. The sequence was designed to provide repeated exposure to mild and heterogeneous stressors over the final five days of the experimental period. Immediately following completion of the stress protocol, behavioural assessments were conducted according to the study design, followed by collection of blood and tissue samples for biochemical and histological analyses.

2.4 Body Weight and Feed Intake

Body weight was measured weekly, whereas food intake was measured daily using an electronic weighing balance, as previously described [21]. For each animal, changes in body weight and food intake were calculated from the recorded measurements. The individual values were subsequently used to obtain group means for statistical analysis.

$$\frac{\text{Final body weight (or feed intake)} - \text{Initial body weight (or feed intake)}}{\text{Initial body weight (or feed intake)}} (g)$$

2.5 Behavioural Tests

2.5.1 Open-Field Novelty-Induced Behaviour

Exposure to the open-field paradigm in rodents is commonly used to assess exploratory and locomotor behaviours. Self-grooming behaviour may also provide an index of stereotypic or repetitive activity. In general, open-field behaviours provide information about the animal's exploratory activity and response to a novel environment.

Each rat was placed individually in the open-field apparatus and observed for 10 minutes. Horizontal locomotion, rearing, and self-grooming behaviours were recorded as previously described [17]. The open-field apparatus consisted of a square enclosure with a rigid floor measuring 36 × 36 × 26 cm. The

wooden apparatus was painted white, and the floor was divided by permanent blue markings into 16 equal squares.

Horizontal locomotion was quantified as the number of floor units traversed by all four paws. Rearing frequency was recorded as the number of instances in which the rat stood on its hind limbs, either with the forelimbs supported against the walls of the apparatus or freely in the air. Grooming frequency was recorded as the number of body-cleaning behaviours involving paw licking, body and perineal licking, and face-washing behaviours during the 10-minute observation period, as previously described [21].

2.5.2 Spatial Working Memory (Y-Maze Test)

The Y-maze was used to assess spatial working memory based on spontaneous alternation behaviour. Each rat was placed in one arm of the maze and allowed to explore the apparatus freely for 5 minutes. An arm entry was recorded when the rat entered an arm such that all four paws were within the arm.

The sequence of arm entries was recorded, and spontaneous alternation behaviour was calculated as previously described [18]. The maximum possible number of spontaneous alternations was determined as the total number of arm entries minus two. The percentage alternation was calculated using the following equation:

$$\text{Percentage alternation} = \frac{\text{Number of actual alternations}}{\text{Maximum alternation} - 2} \times 100$$

The apparatus was thoroughly cleaned with 70% ethanol and allowed to dry between animals to minimise the influence of residual odours on subsequent behavioural performance.

2.5.3 Elevated Plus Maze Test

The elevated plus maze (EPM) was used to assess exploratory and anxiety-related behaviour. The apparatus consisted of two opposing open arms measuring 50 × 10 cm and two opposing enclosed arms measuring 50 × 10 × 40 cm. The maze was elevated 60 cm above the floor. Each rat was placed at the central intersection of the maze facing an open arm [22].

Each animal was observed for 5 minutes. The following behavioural parameters were recorded: total distance travelled, number of entries into the open and closed arms, time spent in the open and closed arms, head-dipping behaviour, and open-arm preference. Open-arm preference was calculated as the ratio of open-arm entries to the total number of arm entries. The apparatus was cleaned with 70% ethanol and allowed to dry between animals to minimise the influence of residual odours on subsequent behavioural responses.

2.6 Biochemical Analyses

2.6.1 Lipid Peroxidation

Lipid peroxidation was assessed by determining the concentration of malondialdehyde (MDA) in plasma samples using the thiobarbituric acid-reactive substances (TBARS) assay according to the method described by [23]. The assay is based on the reaction of MDA and other thiobarbituric acid-reactive substances with thiobarbituric acid, producing a coloured MDA-TBA adduct. The absorbance of the resulting complex was measured spectrophotometrically at 532 nm. MDA concentration was calculated and expressed as µmol/L.

2.6.2 Antioxidant Status

Total antioxidant capacity (TAC) was determined using an assay based on the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical cation, as previously described [24]. Briefly, 10 mg of ABTS was dissolved in 10 mL of an aqueous solution containing 2.5 mmol/L potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 1–4 hours to facilitate oxidation of ABTS. Before use, the oxidised ABTS stock solution was diluted with deionised water to obtain an absorbance of 0.70 at 734 nm. For sample analysis, 1 mL of the diluted ABTS solution was mixed with 10 μ L of serum, and the absorbance was measured 10 minutes after mixing. TAC was determined using Trolox® as the standard and expressed as mEq Trolox®/L.

2.6.3 Pro- and Anti-Inflammatory Markers and Corticosterone

Serum concentrations of tumour necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), interleukin-10 (IL-10), and corticosterone were determined using enzyme-linked immunosorbent assay (ELISA) kits obtained from Enzo Life Sciences Inc., New York, USA, according to the manufacturer's instructions and the method previously described [19]. The assays measured the total concentration of the respective analytes, including both bound and unbound fractions.

2.7 Tissue Histology

Following euthanasia, the brains were carefully dissected and fixed in neutral-buffered formal saline. The prefrontal cortex was processed using standard paraffin-embedding procedures, sectioned at a thickness of 5 μ m, and stained with haematoxylin and eosin (H&E) and cresyl fast violet for general histological evaluation, as previously described [25].

2.8 Photomicrography

Histological sections of the prefrontal cortex were examined using an Olympus binocular light microscope. Representative photomicrographs were captured using a Canon PowerShot 2500 digital camera.

2.9 Histomorphometric and Computational Image Analysis

Histomorphometric and computational image analyses were performed on representative haematoxylin and eosin (H&E)- and cresyl fast violet-stained sections using ImageJ software.

2.10 Statistical Analysis

Data were analysed using Chris Rorden's ezANOVA for Windows (version 0.98). Differences among experimental groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Data are presented as mean $\pm$ standard error of the mean (SEM), and statistical significance was set at $p < 0.05$.

3. Result

3.1 Effect of Sesame Seed Oil on Body Weight

Figure 1 shows the effect of sesame seed oil on the relative change in body weight of stress-induced rats. There was a significant $F(5, 54) = 52.9$, $p = 0.001$ increase in relative body weight in Group C (sesame seed oil, 2 mL/kg), and a significant decrease in Groups D (stress only) and E (sesame seed oil, 1 mL/kg + stress) compared to control. Stress exposure significantly reduced body weight; however, body weight was significantly increased in both Groups E (sesame seed oil, 1 mL/kg + stress) and F (sesame seed oil, 2 mL/kg + stress), suggesting recovery of body weight following sesame seed oil co-treatment.

3.2 Effect of Sesame Seed Oil on Weekly Feed Intake

Figure 2a shows the effect of sesame seed oil on relative changes in feed intake in stress-induced rats. There was a significant $[F(5, 54) = 1834, P=0.0]$ increase in relative feed intake in Groups C (sesame seed oil, 2 mL/kg) and F (sesame seed oil, 2 mL/kg + stress), and a significant decrease in Groups D (stress only) and E (sesame seed oil, 1 mL/kg + stress) compared to control (Group A). Both Groups E and F demonstrated a significant improvement in feed intake ($p < 0.05$) compared with Group D (stress only). Figure 2b shows the effect of sesame seed oil on weekly feed intake. Feed intake was significantly $[F(10, 162) = 1225, p=0.003]$ lower across all treatment groups compared with the control group (Group A), indicating an overall reduction in feed intake associated with stress and/or experimental handling, irrespective of treatment.

3.3 Effect of Sesame Seed Oil on Line Crossing and Rearing

Figure 3a shows the effect of sesame seed oil on line crossing in the open field during the 10-minute observation period. There was a significant $[F(5, 54) = 17.6, p < 0.001]$ increase in horizontal locomotor activity in Group C (sesame seed oil, 2 mL/kg) and a significant decrease in Groups D (stress only) and E (sesame seed oil, 1 mL/kg + stress) compared with the control group (Group A). The overall effect was significant. Compared with Group D (stress only), locomotor activity was significantly increased in both Groups E (sesame seed oil, 1 mL/kg + stress) and F (sesame seed oil, 2 mL/kg + stress), indicating an attenuation of stress-induced reductions in locomotor activity following sesame seed oil co-treatment.

Figure 3b shows the effect of sesame seed oil on rearing frequency during the 10-minute open-field observation period. Rearing activity decreased significantly $[F(5, 54) = 24.1, p < 0.005]$ in Groups D (stress only), E (sesame seed oil, 1 mL/kg + stress), and F (sesame seed oil, 2 mL/kg + stress) compared with the control group (Group A). Compared with Group D (stress only), rearing activity increased significantly in both Groups E and F, indicating attenuation of the stress-associated reduction in exploratory activity following sesame seed oil co-treatment.

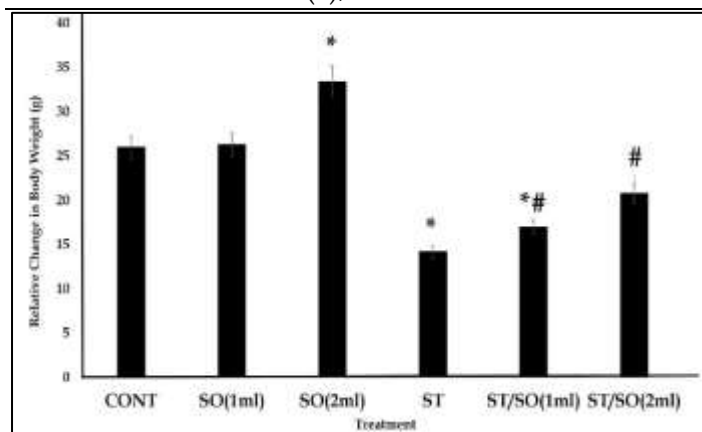


Figure 1: Effect of sesame seed oil on relative change in body weight in stress-induced rats. Each bar represents Mean $\pm$ S.E., * $p < 0.05$ significance difference from CONT. # $p < 0.05$ significance difference from Stress. Number of rats per treatment group = 10. SO: Sesame seed oil, ST: Stress, CONT: Control.

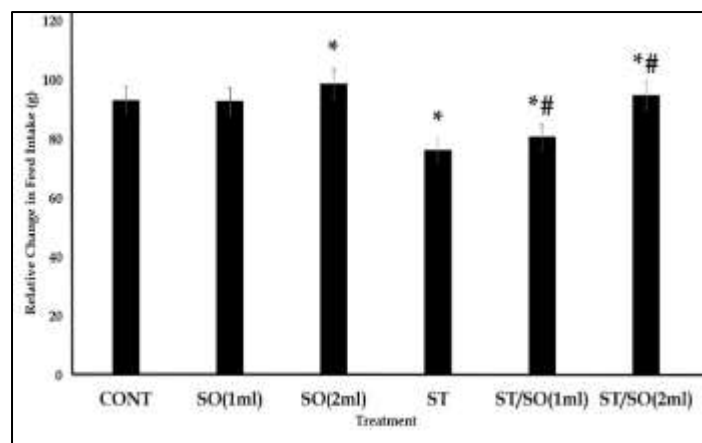


Figure 2a: Effect of Sesame seed oil on relative change in food intake in stress-induced rats. Each bar represents Mean $\pm$ S.E.M., * $p < 0.05$ vs. control, # $p < 0.05$ significant difference from Stress, number of rats per treatment group = 10. SO: Sesame seed oil, ST: Stress, CONT: Control.

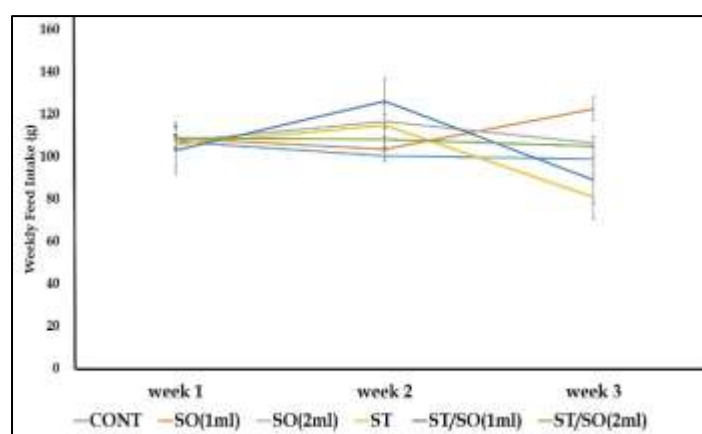


Figure 2b: Effect of Sesame seed oil on weekly food intake in Stress-induced rats. Each bar represents Mean $\pm$ S.E.M., * $p < 0.05$ vs. control, # $p < 0.05$ significant difference from Stress, number of rats per treatment group = 10. SO: Sesame seed oil, ST: Stress, CONT: Control.

3.4 Effect of Sesame Seed Oil on Self-Grooming Behaviour

Figure 4 shows the effect of sesame seed oil on self-grooming behaviour in stress-induced rats during the 10-minute observation period. Grooming increased significantly [$F(5, 54) = 154, p = 0.05$] in Group D (stress only) compared with the control group (Group A). In contrast, grooming decreased significantly in both Group E (sesame seed oil, 1 mL/kg + stress) and Group F (sesame seed oil, 2 mL/kg + stress) compared with Group D (stress only), indicating attenuation of stress-induced alterations in grooming behaviour following sesame seed oil co-treatment.

3.5 Effect of Sesame Seed Oil on Open- and Closed-Arm Time

Figure 5 shows the effect of sesame seed oil on anxiety-related behaviour, assessed by the time spent in the open and closed arms of the elevated plus maze. There was a significant [$F(5, 54) = 512.1, p = 0.001$] decrease in open-arm time in Group B (sesame seed oil, 1 mL/kg), Group D (stress only), and Group E (sesame seed oil, 1 mL/kg + stress) compared with the control group (Group A). Compared with Group D (stress only), open-arm time was increased in both Groups E (sesame seed oil, 1 mL/kg + stress) and F (sesame seed oil, 2 mL/kg + stress). Conversely, closed-arm time was significantly increased in Groups B, D, and E compared with the control group. Compared with Group D (stress only), closed-arm time was reduced in Groups E and F, indicating attenuation of the stress-associated alteration in anxiety-related behaviour following sesame seed oil co-treatment.

3.6 Effect of Sesame Seed Oil on Spatial Working Memory in the Y-Maze

Figure 6 shows the effect of sesame seed oil on Y-maze spontaneous alternation in stress-induced rats. There was a significant [$F(5, 54) = 50.81, p = 0.03$] decrease in percentage alternation in Group D (stress only) and Group E (sesame seed oil, 1 mL/kg + stress) compared with the control group (Group A), $p < 0.05$, suggesting stress-associated impairment of spatial working memory. Compared with Group D (stress only), the alternation index was significantly higher in Group F (sesame seed oil, 2 mL/kg + stress), $p < 0.05$, consistent with attenuation of stress-induced spatial working-memory impairment following sesame seed oil co-treatment at the higher dose.

3.7 Effect of Sesame Seed Oil on Biochemical Parameters

Tables 1 and 2 show the effects of sesame seed oil on oxidative stress markers and inflammatory cytokines in stress-induced rats. Lipid peroxidation measured as malondialdehyde (MDA) levels increased significantly [$F(5, 30) = 338, p = 0.001$] in Group D (stress only) and Group E (sesame seed oil, 1 mL/kg + stress) compared with the control group (Group A). Concentration of MDA was also significantly reduced in Groups E and F (sesame seed oil, 2 mL/kg + stress) compared with Group D (stress only). Total antioxidant capacity (TAC) decreased significantly [$F(5, 30) = 5.04$] in Group D (stress only) compared with the control group (Group A), and increased in Groups E and F compared with

Group D (stress only). Corticosterone concentration increased significantly [$F(5, 30) = 6.64, 0.049$] in Groups D (stress only), E (sesame seed oil, 1 mL/kg + stress), and F (sesame seed oil, 2 mL/kg + stress) compared with the control group (Group A), $p < 0.05$. Compared with Group D, corticosterone concentration was significantly reduced in Group F. Interleukin-6 (IL-6) concentration increased significantly [$F(5, 30) = 53.9, p=0.03$] in Group D (stress only) compared with the control group (Group A), and decreased in Groups E and F compared with Group D.

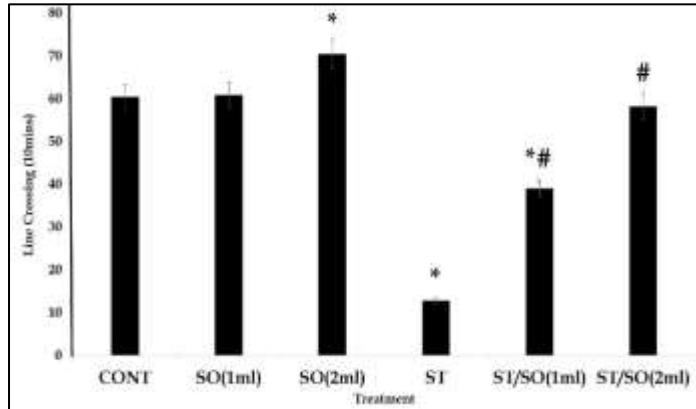


Figure 3a: Effect of sesame seed oil on horizontal locomotion activity in stress-induced rats. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ significance difference from control. # $p < 0.05$ significance difference from Stress. Number of rats per treatment group = 10. SO: Sesame seed oil, ST: Stress, CONT: Control.

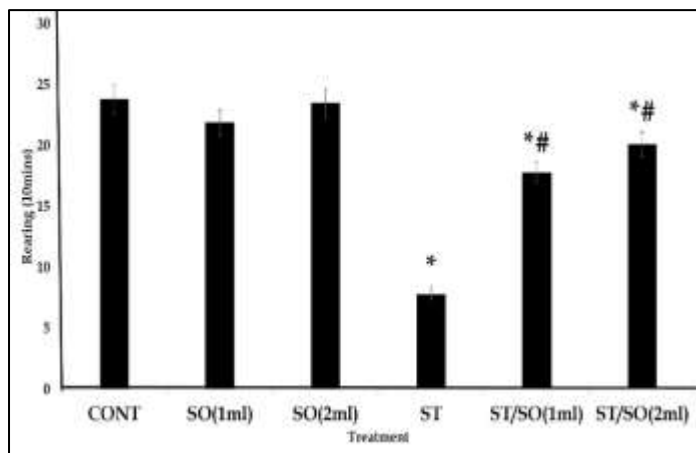


Figure 3b: Effect of sesame seed oil on rearing activity in stress-induced rats. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ significance difference from control. # $p < 0.05$ significance difference from Stress. Number of rats per treatment group = 10. SO: Sesame seed oil, ST: Stress, CONT: Control.

Tumour necrosis factor-alpha (TNF- α) decreased significantly [$F(5, 30) = 15.75, p=0.05$] in Groups C (sesame seed oil, 2 mL/kg) and F (sesame seed oil, 2 mL/kg + stress), and an increase in Group D (stress only), compared with the control group (Group A). Compared with Group D, TNF- α concentration significantly decreased in Groups E and F. Interleukin-10 (IL-10) decreased significantly [$F(5, 30) = 45.1, p=0.003$] in Groups D (stress only) and E (sesame seed oil, 1 mL/kg + stress) compared with control

(Group A). Compared with Group D, IL-10 concentration significantly increased in Groups E and F.

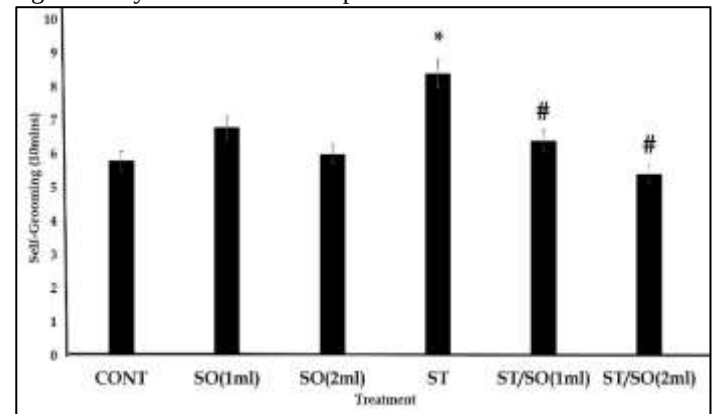


Figure 4: Effect of sesame seed oil on self-grooming behaviour in stress-induced rats. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ significance difference from A. # $p < 0.05$ significance difference from Stress. Number of rats per treatment group = 10. SO: Sesame seed oil, ST: Stress, CONT: Control.

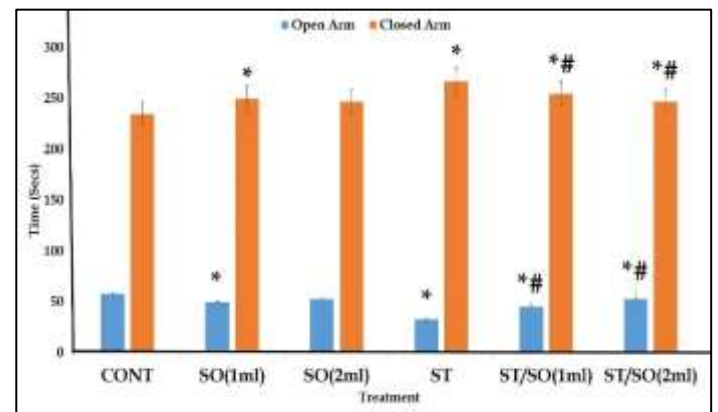


Figure 5: Effect of Sesame seed oil on time spent(sec) Open and Closed arm time in elevated plus maze in stress-induced rats. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs. control, # $p < 0.05$ significant difference from Stress, number of rats per treatment group = 10. SO: Sesame seed oil, ST: Stress, CONT: Control.

3.8 Effect of Sesame Seed Oil on the Histomorphology of the Prefrontal Cortex

Plate 1 (A–F) shows the histomorphological effects of sesame seed oil on the prefrontal cortex of stress-induced Wistar rats following haematoxylin and eosin (H&E) staining. The control group (A) demonstrated normal cortical histoarchitecture, characterised by evenly distributed neuronal cells with intact vesicular nuclei, preserved neuropil, and minimal pyknotic changes. Glial cell distribution appeared physiologic, with no appreciable vacuolation or tissue rarefaction.

The SO1-treated group (B) showed mild alterations in cortical morphology compared with the control group, including slightly reduced neuronal density, occasional pericellular spaces, and mild glial prominence. However, the overall cortical architecture remained preserved. The SO2-treated group (C) showed relatively better neuronal organisation compared with the SO1

group. The cortical layers remained largely intact, with moderate neuronal preservation and minimal vacuolar degeneration. Neuronal somata retained relatively normal morphology, while glial activation appeared mild and localised. The stress-only group (D) exhibited pronounced histopathological alterations, characterised by marked disruption of cortical architecture, extensive neuronal shrinkage, pyknotic nuclei, vacuolar degeneration, and neuropil rarefaction. Tissue pallor and poorly defined cellular boundaries were also observed. The reduction in neuronal profiles and distortion of cortical organisation were consistent with substantial stress-associated neuronal injury. Co-treatment with sesame seed oil attenuated these histological alterations. The ST/SO1 group (E) showed moderate restoration of cortical organisation and increased neuronal preservation compared with the stress-only group, although residual vacuolation, mild cortical disorganisation, and reactive gliosis remained evident. The ST/SO2 group (F) demonstrated the greatest degree of morphological preservation among the stress-exposed groups, with relatively preserved cortical layers, improved neuronal density, reduced vacuolation, and improved neuropil continuity. Neuronal somata appeared more distinct and structurally preserved, while pyknotic changes were reduced compared with the untreated stress group.

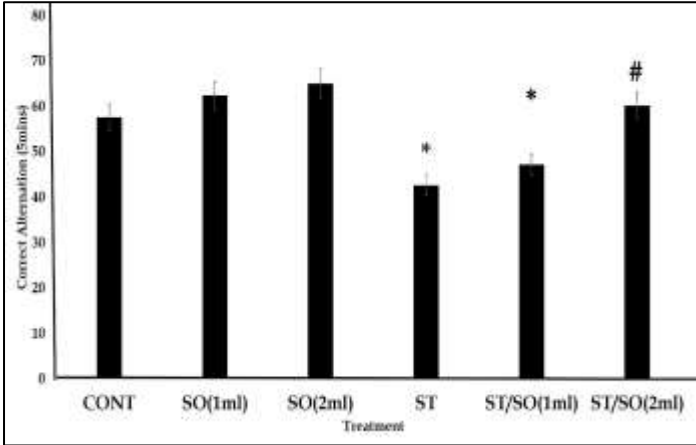


Figure 6: Effect of sesame seed oil on percentage alternation in stress-induced rats. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs. control, # $p < 0.05$ significant difference from Stress, number of rats per treatment group = 10. SO: Sesame seed oil, ST: Stress, CONT: Control.

3.9 Quantitative Histomorphometric and Colour Histogram Analysis of H&E Sections

Quantitative histomorphometric analysis are presented in Table 3. Cellular profile density varied across the experimental groups. The control group showed a relatively high cellular profile count (267 ± 13 cells/mm³), while the SO1 group showed a mild reduction (241 ± 11 cells/mm³). In contrast, the SO2 group demonstrated a higher cellular profile count (289 ± 16 cells/mm³). The stress-only group exhibited the lowest cellular profile density (149 ± 12 cells/mm³), accompanied by a marked reduction in

stained area percentage ($18.43 \pm 5.28\%$) and mean cellular area ($10,872 \pm 42$ μ m²). These findings were consistent with the histological alterations observed in the stress-exposed animals. Sesame seed oil co-treatment was associated with improvement in these parameters. The ST/SO1 group showed increased cellular profile density (214 ± 14 cells/mm³), whereas the ST/SO2 group demonstrated the highest cellular profile count among the stress-exposed groups (318 ± 18 cells/mm³). Mean cellular area and stained area percentage were also improved in the ST/SO2 group.

Table 1. Effects of sesame seed oil on oxidative stress markers and corticosterone in stress-induced rats

Group	MDA	TAC	Corticosterone
Control	0.68 $\pm$ 0.01	13.89 $\pm$ 0.56	9.73 $\pm$ 0.51
SO-1	0.71 $\pm$ 0.03	12.41 $\pm$ 0.61	10.20 $\pm$ 0.85
SO-2	0.69 $\pm$ 0.01	13.68 $\pm$ 1.55	9.86 $\pm$ 0.85
ST	4.74 $\pm$ 0.01*	5.41 $\pm$ 0.61*	16.73 $\pm$ 0.67*
ST/SO-1	1.91 $\pm$ 0.03*#	11.25 $\pm$ 0.17#	14.38 $\pm$ 1.32*
ST/SO-2	0.74 $\pm$ 0.01#	11.98 $\pm$ 0.55#	11.04 $\pm$ 1.01*#

Values are presented as mean $\pm$ SEM (n = 10 per group). SO = sesame seed oil; ST = stress; MDA = malondialdehyde; TAC = total antioxidant capacity. Values are mean $\pm$ SEM. $p < 0.05$ vs control; # $p < 0.05$ vs ST.

Table 2. Effects of sesame seed oil on inflammatory cytokines in stress-induced rats

Group	IL-6 (pg/mL)	TNF- α (pg/mL)	IL-10 (pg/mL)
Control	23.40 $\pm$ 2.42	27.11 $\pm$ 0.01	30.48 $\pm$ 2.03
SO-1	23.12 $\pm$ 0.45	25.04 $\pm$ 0.58	30.68 $\pm$ 2.83
SO-2	22.81 $\pm$ 0.59	25.12 $\pm$ 1.50*	28.23 $\pm$ 4.62
ST	28.13 $\pm$ 0.08*	41.95 $\pm$ 0.58*	10.79 $\pm$ 0.91*
ST/SO-1	25.94 $\pm$ 0.83#	26.94 $\pm$ 2.14#	27.11 $\pm$ 0.01*#
ST/SO-2	23.62 $\pm$ 0.45#	24.95 $\pm$ 3.18*#	27.79 $\pm$ 0.91#

SO = sesame seed oil; ST = stress; SO-1 = sesame seed oil, 1 mL/kg; SO-2 = sesame seed oil, 2 mL/kg; IL-6 = interleukin-6; TNF- α = tumour necrosis factor-alpha; IL-10 = interleukin-10. Values are presented as mean $\pm$ SEM. *Significantly different from control; #significantly different from stress-only group ($p < 0.05$).

Colour histogram analysis (Figure 7) demonstrated differences in staining characteristics among the experimental groups. The control sections showed relatively balanced red, green, and blue channel intensities, consistent with the observed H&E staining pattern. The stress group exhibited reduced red-channel intensity with relative increases in the green and blue channels, corresponding to tissue pallor and altered staining characteristics. In the ST/SO2 group, the colour intensity pattern was shifted towards that observed in the control group. Further quantitative histomorphometric analysis demonstrated marked alterations following stress exposure. The ST group had

the lowest neuronal count (132 ± 9 cells/mm³), highest vacuolation percentage ($16.82 \pm 2.4\%$), lowest optical density (0.49 ± 0.04), and lowest cortical layer thickness (932 ± 30 μm). Texture entropy was highest in this group (6.91 ± 0.21), indicating increased tissue heterogeneity. Sesame seed oil co-treatment improved these histomorphometric parameters, with greater changes observed in the ST/SO2 group. This group exhibited an increased neuronal count (301 ± 15 cells/mm³), reduced vacuolation ($6.02 \pm 1.0\%$), increased optical density (0.79 ± 0.02), and greater cortical thickness (1218 ± 25 μm). Texture entropy was also reduced relative to the stress-only group.

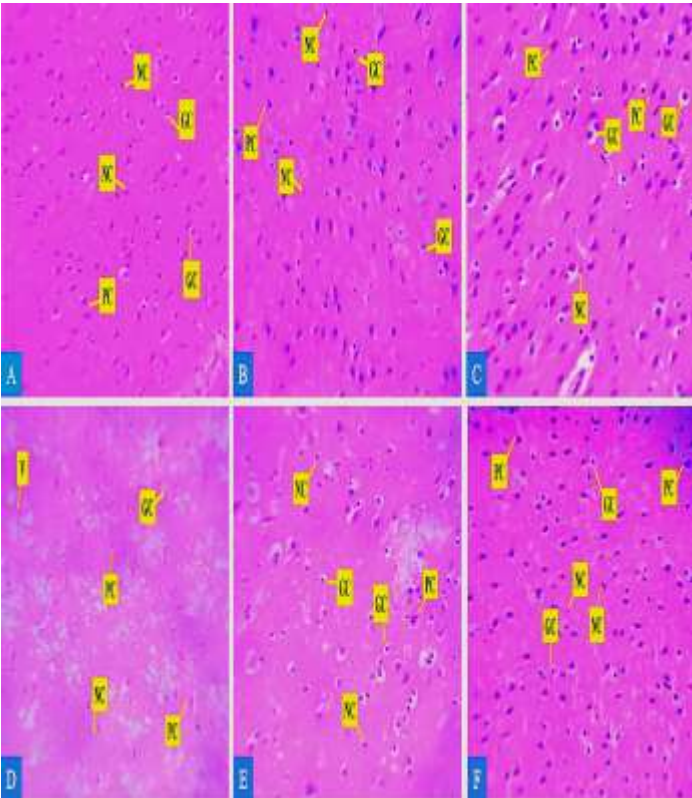


Plate 1: Photomicrograph of H&E-stained prefrontal cortex sections (X400). (A) Control: normal layered architecture with pyramidal cells (PC) showing large, rounded, vesicular nuclei, granule cells (GC) with prominent nucleoli, and physiologically distributed glial/neuroglial cells (NC); no pyknosis or vacuolation. (B) SO1 and (C) SO2: preserved cortical architecture with minimal glial reactivity. (D) Stress only: numerous pyknotic pyramidal neurons (arrows) with shrunken, hyperchromatic nuclei, prominent vacuolation (V), and neuropil rarefaction. (E) ST/SO1: partial preservation of pyramidal neuron morphology with residual vacuolation and glial activation. (F) ST/SO2: near-normal neuronal density with markedly reduced pyknosis and vacuolation. Scale bar: 9 μm/pixel.

3.10 Effect of Sesame Seed Oil on the Histopathology of the Rat Prefrontal Cortex (Cresyl Fast Violet)

Plate 2 shows the histopathological effects of sesame seed oil on the prefrontal cortex of rats following Cresyl Fast Violet staining. The control group demonstrated intense violet staining with

abundant Nissl substance, intact neuronal somata, and preserved cortical continuity. Neurons exhibited prominent basophilic cytoplasmic staining with minimal chromatolytic changes. The SO1 group showed a mild reduction in Cresyl Fast Violet staining intensity and slight neuronal sparsity, although the overall cortical architecture remained preserved. The SO2 group demonstrated relatively improved neuronal preservation, characterised by moderate-to-strong Nissl staining and relatively intact neuronal morphology. The stress-only group exhibited marked histopathological alterations, characterised by extensive chromatolysis, neuronal pyknosis, vacuolar degeneration, and disruption of cortical organisation. Nissl substance appeared markedly reduced, accompanied by increased degenerative spaces and reactive gliosis. In the ST/SO1 group, partial preservation of neuronal morphology and Nissl staining was observed, although residual chromatolysis and cortical disorganisation persisted. The ST/SO2 group demonstrated greater preservation of neuronal architecture, with improved Nissl substance retention, reduced pyknosis, and better preservation of cortical continuity compared with the stress-only group.

Table 3: Quantitative histomorphometric and image-analysis parameters of the prefrontal cortex

Group	Cellular profile density (cells/mm ³)	Stained area (%)	Neuron count (cells/mm ³)	Vacuolation (%)	Optical density	Layer thickness (μm)	Texture entropy	Nissl-positive (%)
Control	267 ± 13	31.49 ± 8.32	252.0 ± 11.0	4.38 ± 0.81	0.71 ± 0.03	1185 ± 24.0	5.11 ± 0.12	82.4 ± 3.10
SO-1	241 ± 11	29.84 ± 6.15	228.0 ± 10.0	4.10 ± 0.61	0.68 ± 0.02	1164 ± 22.0	5.28 ± 0.11	79.6 ± 2.80
SO-2	289 ± 16	33.77 ± 7.10	276.0 ± 13.0	7.70 ± 1.10	0.74 ± 0.03	1191 ± 21.0	5.43 ± 0.13	84.8 ± 3.51
ST	149 ± 12	18.43 ± 5.28	132.0 ± 9.0	16.82 ± 2.41	0.49 ± 0.04	932 ± 30.0	6.91 ± 0.21	51.3 ± 4.00
ST/SO-1	214 ± 14	24.86 ± 6.41	201.0 ± 12.0	11.35 ± 1.81	0.61 ± 0.03	1038 ± 28.0	6.18 ± 0.17	67.9 ± 3.31
ST/SO-2	318 ± 18	36.52 ± 7.92	301.0 ± 15.0	6.02 ± 1.02	0.79 ± 0.02	1218 ± 25.0	5.36 ± 0.14	88.6 ± 3.70

Note: SO-1 = sesame seed oil, 1 mL/kg; SO-2 = sesame seed oil, 2 mL/kg; ST = stress; ST/SO-1 = stress + sesame seed oil, 1 mL/kg; ST/SO-2 = stress + sesame seed oil, 2 mL/kg. Values are presented as mean ± SEM

3.11 Quantitative Histomorphometric and Colour Histogram Analysis of Cresyl Fast Violet-Stained Sections of the Rat Prefrontal Cortex

Quantitative analysis of Cresyl Fast Violet-stained sections demonstrated differences in neuronal preservation across the experimental groups. The control group exhibited a high cellular profile density (311 ± 15 cells/mm³), large mean cellular area

($14,218 \pm 28 \mu\text{m}^2$), and extensive stained area ($39.61 \pm 7.42\%$), consistent with the observed preservation of neuronal morphology and Nissl substance. The stress-only group demonstrated the lowest cellular profile density ($156 \pm 11 \text{ cells/mm}^3$), reduced mean cellular area ($10,931 \pm 41 \mu\text{m}^2$), and markedly reduced stained area ($19.25 \pm 4.83\%$). The ST/SO1 group showed partial preservation of Nissl staining, although residual neuronal degeneration and gliosis were observed. Greater preservation of neuronal morphology and Nissl substance was observed in the ST/SO2 group, together with improved cortical continuity and fewer degenerative features.

Colour histogram analysis (**Figure 8**) demonstrated marked reductions in red and green channel intensities in the stress group, whereas the ST/SO2 group showed a restoration of staining intensity. The ST/SO2 group also exhibited the highest stained area percentage ($42.18 \pm 8.10\%$).

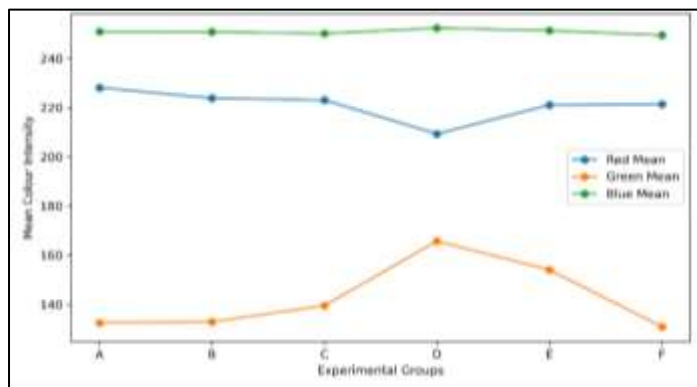


Figure 7: Colour histogram analysis of H&E-stained sections of the rat prefrontal cortex across experimental groups. The graph illustrates the mean red, green, and blue (RGB) channel intensities obtained from digital image analysis of H&E-stained prefrontal cortical sections. The control group demonstrated balanced RGB distribution consistent with preserved cortical architecture and normal staining affinity. Stress induction (ST) produced altered colour intensity patterns characterized by reduced red-channel intensity and relative increases in green and blue distributions, reflecting tissue pallor, vacuolation, and cortical disorganization. Treatment with sesame seed oil, particularly at 2 ml/kg (ST/SO2), restored colour intensity patterns toward control values, suggesting improved tissue preservation and attenuation of stress-induced neurodegeneration.

Further morphometric analysis demonstrated marked alterations in the stress group, including reduced neuronal count ($141 \pm 8 \text{ cells/mm}^3$), increased vacuolation ($18.61 \pm 2.8\%$), reduced optical density (0.46 ± 0.04), and reduced cortical layer thickness ($901 \pm 34 \mu\text{m}$). Texture entropy was increased (7.02 ± 0.24), while the Nissl-positive percentage was reduced ($44.6 \pm 3.8\%$). These findings were accompanied by the histological changes observed in the stress group.

Sesame seed oil administration was associated with improvement in these parameters. The ST/SO2 group exhibited increased neuronal density ($317 \pm 15 \text{ cells/mm}^3$), reduced vacuolation ($5.22 \pm 0.9\%$), increased optical density (0.88 ± 0.03), and increased

cortical thickness ($1236 \pm 24 \mu\text{m}$). Texture entropy was reduced, while the Nissl-positive percentage increased to $93.8 \pm 3.4\%$.

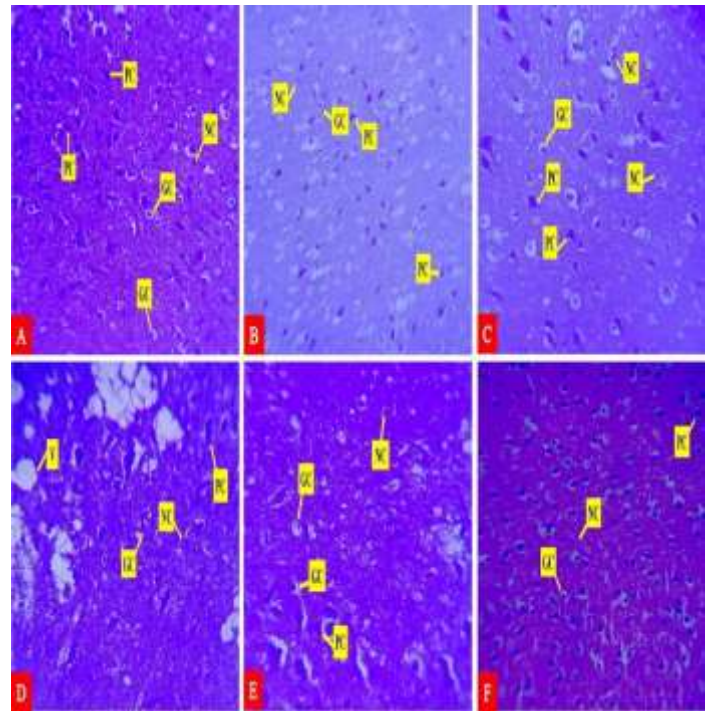


Plate 2: Photomicrograph of prefrontal cortex-stained section by cresyl fast violet X400 Mag. The staining revealed the characteristic layered arrangement of the prefrontal cortex with well-delineated pyramidal cells (PC), deeply stained granule cells (GC), neuroglia cell (NC), and prominent Nissl bodies in (Group A). However, degenerating pyramidal cells with pale-staining nuclei and reduced Nissl substance were observed in group (group D), while protection against neuronal injury, with better preservation of Nissl bodies, was evident in group (E) and more pronounced in group (F). Mag. X400. Scale Bar -9um/pixel.

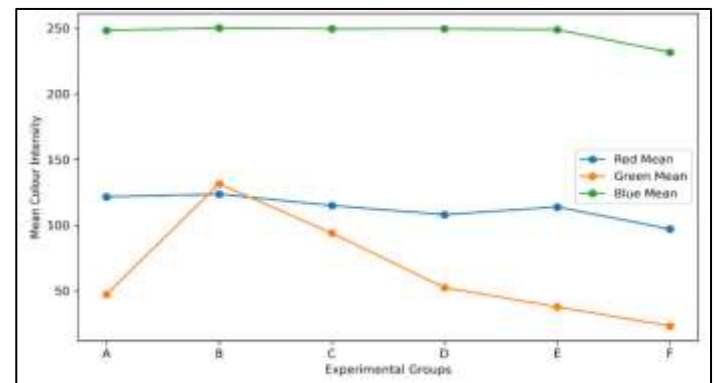


Figure 8: Colour histogram analysis of Cresyl Fast Violet-stained sections of the rat prefrontal cortex across experimental groups. The graph shows the mean red, green, and blue (RGB) channel intensities derived from image analysis of Cresyl Fast Violet-stained prefrontal cortical sections. The control group exhibited strong violet staining intensity indicative of preserved Nissl substance and intact neuronal metabolic activity. Stress induction markedly altered colour distribution patterns with reduced staining intensity, reflecting chromatolysis, neuronal degeneration, and depletion of Nissl-positive material. Sesame seed oil treatment, particularly at the higher dose (ST/SO2), enhanced staining preservation and restored colour intensity profiles, suggesting improved neuronal integrity and metabolic preservation in the prefrontal cortex.

Table 4. Quantitative Histomorphometric and Colour Histogram Analysis of Cresyl Fast Violet-Stained Prefrontal Cortex

Group	Cellular profile density (cells/mm ³)	Stained area (%)	Neuron count (cells/mm ³)	Vacuolation (%)	Optical density	Layer thickness (µm)	Texture entropy	Nissl-positive (%)
Control	311 ± 15.01	39.61 ± 7.42	296 ± 13.10	3.34 ± 0.70	0.83 ± 0.03	1214 ± 26.12	4.92 ± 0.11	91.2 ± 3.21
SO-1	224 ± 12.02	27.82 ± 5.34	211 ± 10.02	3.36 ± 0.82	0.62 ± 0.02	1146 ± 23.21	5.37 ± 0.12	73.8 ± 2.70
SO-2	273 ± 14.10	34.74 ± 6.52	258 ± 12.21	3.55 ± 0.72	0.74 ± 0.03	1181 ± 22.11	5.11 ± 0.10	84.7 ± 3.02
ST	156 ± 11.10	19.25 ± 4.83	141 ± 8.12	18.61 ± 2.80	0.46 ± 0.04	901 ± 34.15	7.02 ± 0.24	44.6 ± 3.82
ST/SO-1	238 ± 13.03	28.44 ± 5.93	223 ± 11.03	11.44 ± 1.91	0.65 ± 0.03	1018 ± 29.22	6.12 ± 0.18	69.3 ± 3.10
ST/SO-2	329 ± 17.04	42.18 ± 8.10	317 ± 15.14	5.22 ± 0.90	0.88 ± 0.03	1236 ± 24.20	5.04 ± 0.12	93.8 ± 3.41

Values are presented as mean ± SEM for histomorphometric and RGB histogram-derived parameters obtained from Cresyl Fast Violet-stained prefrontal cortex sections. Mean profile area was calibrated using a scale conversion factor of 9 µm/pixel. SO-1 = sesame seed oil, 1 mL/kg; SO-2 = sesame seed oil, 2 mL/kg; ST = stress; ST/SO-1 = stress + sesame seed oil, 1 mL/kg; ST/SO-2 = stress + sesame seed oil, 2 mL/kg.

Discussion

The present study investigated the effects of sesame seed oil on behavioural, biochemical, and histomorphological alterations in the prefrontal cortex of stress-induced Wistar rats. Overall, stress exposure was associated with reductions in body weight and feed intake, alterations in exploratory and anxiety-related behaviours, impairment of spatial working memory, increased MDA and corticosterone concentrations, reduced TAC and IL-10, and marked histomorphological alterations in the prefrontal cortex. Sesame seed oil co-treatment attenuated several of these stress-associated changes, with the 2 mL/kg dose generally producing greater improvement than the 1 mL/kg dose. Stress exposure significantly reduced body weight and feed intake compared with the control group. Similar reductions in body weight and food intake have been reported in experimental models of stress [26,27]. The reduction in food intake may reflect alterations in hypothalamic regulation of appetite associated with activation of the hypothalamic–pituitary–adrenal (HPA) axis. Stress-induced activation of corticotropin-releasing hormone (CRH) can influence hypothalamic feeding circuits and reduce food intake, while sustained glucocorticoid elevation may alter energy metabolism and promote a catabolic state [28,29]. Pro-inflammatory mediators such as TNF-α and IL-6 may further contribute to stress-associated anorexia through effects on central feeding pathways [30].

In the present study, sesame seed oil co-treatment was associated with improved body weight and feed intake in stressed animals, particularly at the higher dose. This finding may reflect the ability of bioactive constituents of sesame seed oil to modulate oxidative and inflammatory processes associated with stress; however, the present study did not directly examine hypothalamic signalling or energy metabolism, and therefore these mechanisms remain speculative. The behavioural findings further demonstrated the effects of stress exposure on locomotor, exploratory, anxiety-related, and cognitive functions. Stress significantly reduced horizontal locomotor activity and rearing frequency, while increasing self-grooming behaviour. These findings are consistent with previous reports of stress-induced alterations in exploratory and behavioural responses [32]. The behavioural changes may be related to the effects of sustained glucocorticoid exposure on stress-sensitive neural circuits involving the prefrontal cortex, hippocampus, and amygdala. Chronic stress has been associated with alterations in dendritic architecture, synaptic plasticity, and neurotransmission within these regions [33]. Oxidative and inflammatory disturbances may further compromise neuronal function by affecting cellular metabolism, membrane integrity, and synaptic signalling. In the present study, sesame seed oil co-treatment increased locomotor activity and rearing relative to the stress-only group and reduced the stress-associated increase in self-grooming. These findings indicate an attenuation of the behavioural alterations induced by stress. The elevated plus maze findings provided additional evidence of stress-associated alterations in anxiety-related behaviour. Stress exposure was associated with reduced open-arm time and increased closed-arm time, consistent with an increased avoidance of the open arms. Sesame seed oil co-treatment increased open-arm time and reduced closed-arm time relative to the stress-only group. Although these findings are consistent with an attenuation of stress-associated anxiety-like behaviour, interpretation of the elevated plus maze should consider that locomotor activity can influence arm-entry and time-based measures. Nevertheless, the concurrent improvement in open-field locomotor and exploratory measures provides additional support for an overall improvement in behavioural performance following sesame seed oil treatment. Spatial working memory was also affected by stress exposure. The stress-only group showed a significant reduction in Y-maze spontaneous alternation, indicating impaired spatial working-memory performance. This finding is consistent with evidence that stress can adversely affect cognitive processes mediated by the prefrontal cortex and hippocampus [33]. Glucocorticoid excess, oxidative stress, and alterations in synaptic plasticity have all been implicated in stress-related cognitive dysfunction. In the present study, the 2 mL/kg sesame seed oil co-treatment significantly increased the alternation index compared with the

stress-only group, suggesting attenuation of stress-associated impairment in spatial working memory. The stronger effect observed at the higher dose may indicate a dose-related response, although formal dose–response modelling would be required to establish this relationship.

Stress exposure also produced a marked disturbance in redox status. MDA, an index of lipid peroxidation, was substantially increased in the stress-only group, whereas TAC was significantly reduced. This pattern indicates an imbalance between oxidative processes and antioxidant capacity following stress exposure. Previous studies have similarly associated stress with increased oxidative burden [36,37]. Increased metabolic activity and mitochondrial dysfunction during prolonged stress may contribute to reactive oxygen species generation, while persistent oxidative challenge can compromise endogenous antioxidant defenses [38]. In the present study, sesame seed oil co-treatment reduced MDA and increased TAC relative to the stress-only group. The effect was particularly evident with the 2 mL/kg treatment. Sesame seed oil contains bioactive constituents, including sesamin, sesamol, and sesamol, which have been reported to possess antioxidant properties [39]. These properties may contribute to the improvement in redox status observed in the present study. However, because specific antioxidant enzymes and Nrf2 signalling were not measured, the precise molecular mechanism cannot be established from the present findings.

Corticosterone concentration was significantly elevated in the stress-only group, consistent with activation of the neuroendocrine stress response [40,41]. Glucocorticoids normally participate in negative feedback regulation of the HPA axis through glucocorticoid receptors [42]. Persistent stress exposure can disrupt this regulatory system and contribute to prolonged glucocorticoid signalling. In the present study, corticosterone remained elevated in the stress + sesame seed oil groups relative to the control group, although the 2 mL/kg sesame seed oil treatment significantly reduced corticosterone relative to the stress-only group. This partial reduction suggests that sesame seed oil may attenuate, rather than completely normalise, the neuroendocrine response to stress. The present findings do not permit direct conclusions regarding glucocorticoid receptor sensitivity or HPA-axis feedback mechanisms because these parameters were not directly assessed.

The inflammatory profile was also altered following stress exposure. IL-6 and TNF- α were increased in the stress-only group, whereas IL-10 was reduced. This pattern is consistent with an inflammatory shift associated with stress exposure and agrees with previous experimental observations [44]. Oxidative stress and inflammatory signalling are closely interconnected, and activation of pathways such as nuclear factor-kappa B (NF- κ B) has been implicated in the regulation of pro-inflammatory cytokine expression [45,46]. Chronic stress may therefore create

a biological environment in which oxidative and inflammatory processes reinforce one another. In the present study, sesame seed oil co-treatment reduced IL-6 and TNF- α concentrations and increased IL-10 relative to the stress-only group. These findings are consistent with previous reports describing anti-inflammatory effects of sesame-derived bioactive compounds [48]. Nevertheless, because NF- κ B activity, microglial phenotype, and related signalling pathways were not directly measured, the proposed molecular mechanisms should be regarded as possible explanations rather than established mechanisms in the present study.

The histological findings provided morphological evidence of stress-associated prefrontal cortical injury. H&E-stained sections from the stress-only group showed neuronal shrinkage, pyknotic nuclei, vacuolar degeneration, neuropil rarefaction, and disruption of cortical organisation. These findings were accompanied by reductions in cellular profile density, neuronal count, stained area, optical density, and cortical layer thickness, together with increased vacuolation and texture entropy. Collectively, these findings indicate substantial alteration of cortical tissue organisation following stress exposure. The increased texture entropy further suggests greater heterogeneity within the analysed tissue, consistent with the observed disruption of normal cortical architecture.

Cresyl Fast Violet staining provided additional evidence of neuronal alterations. The stress-only group showed marked chromatolytic changes and reduced Nissl staining, accompanied by reductions in Nissl-positive percentage and optical density. Nissl substance is associated with the rough endoplasmic reticulum and ribosomal machinery of neurons; therefore, reduced staining is consistent with alterations in neuronal structural and metabolic integrity. However, the present histological findings should not be interpreted as definitive evidence of apoptosis because apoptotic markers were not assessed. Similarly, although neuronal shrinkage and pyknosis may occur during degenerative processes, confirmation of specific cell-death pathways would require additional molecular or immunohistochemical analyses.

Sesame seed oil co-treatment attenuated many of the observed histomorphological alterations. The ST/SO1 group demonstrated partial improvement, whereas the ST/SO2 group generally showed greater preservation of cortical architecture. Quantitative analysis of the ST/SO2 group demonstrated higher cellular profile density and neuronal count, greater stained area, increased optical density and cortical layer thickness, and lower vacuolation and texture entropy compared with the stress-only group. Cresyl Fast Violet analysis similarly showed improved Nissl staining and a higher Nissl-positive percentage following the higher sesame seed oil dose. These morphological findings are broadly consistent with the observed improvements in oxidative, inflammatory, and behavioural parameters.

The colour histogram analysis further demonstrated differences in staining characteristics between the experimental groups. H&E sections from the stress group showed altered RGB intensity distributions compared with the control group, while the ST/SO2 group demonstrated a pattern that was closer to that of the control group. Similarly, Cresyl Fast Violet sections showed altered colour-channel intensity in the stress group and improved staining characteristics following sesame seed oil treatment. Although colour histogram analysis provides an objective quantitative approach to characterising staining patterns, changes in colour intensity may reflect both tissue composition and staining characteristics. Consequently, these measurements should be interpreted as complementary to, rather than replacements for, conventional histological assessment.

Together, the findings indicate that stress exposure produced coordinated behavioural, biochemical, and structural alterations in the prefrontal cortex. The reduction in exploratory and cognitive performance occurred alongside increased oxidative and inflammatory markers, altered corticosterone concentrations, and marked cortical histomorphological changes. Sesame seed oil co-treatment attenuated several of these abnormalities, with the 2 mL/kg dose generally showing greater effects than the 1 mL/kg dose. The convergence of behavioural, biochemical, and histological findings strengthens the evidence for a protective effect of sesame seed oil against the effects of experimental stress. The protective effects observed may be related to the biological activities of sesame seed oil constituents, particularly its lignans and other antioxidant compounds. However, the present study did not directly investigate specific molecular pathways such as Nrf2, NF- κ B, mitochondrial signalling, apoptosis, or glucocorticoid-receptor activity. Therefore, these pathways should be considered plausible mechanisms requiring further investigation rather than definitive explanations for the observed effects. Future studies incorporating molecular markers of oxidative stress, neuroinflammation, synaptic plasticity, mitochondrial function, and neuronal cell death would help clarify the mechanisms underlying the neuroprotective effects of sesame seed oil.

Overall, the present findings demonstrate that experimental stress was associated with significant neurobehavioural, biochemical, and prefrontal cortical histomorphological alterations in Wistar rats. Sesame seed oil, particularly at 2 mL/kg, attenuated several of these changes and was associated with improved behavioural performance, redox balance, inflammatory profile, and cortical structural preservation. These findings support further investigation of sesame seed oil and its bioactive constituents as potential nutritional or adjunctive interventions for stress-related neural dysfunction.

Conclusion

Sesame seed oil administration attenuated several behavioural, biochemical, and histomorphological alterations associated with stress-induced neurotoxicity in Wistar rats. Stress exposure was

associated with impaired spatial working memory, increased pro-inflammatory cytokines (IL-6 and TNF- α), oxidative imbalance, and disruption of prefrontal cortical architecture. Sesame seed oil co-treatment, particularly at 2 mL/kg, was associated with improved Y-maze performance, reduced inflammatory markers, improved antioxidant status, and preservation of prefrontal cortical morphology. These findings suggest that the observed effects may be related to the antioxidant and anti-inflammatory properties of sesame seed oil and its bioactive constituents. However, the specific molecular mechanisms underlying these effects were not directly investigated in the present study. Further studies incorporating molecular and mechanistic assessments are warranted to validate these findings and determine their potential relevance to stress-related neurological disorders.

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Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

Competing interests

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Declarations Ethics approval

Ethical approval for this study was granted by the Ethical Committee of the Faculty of Basic Medical Sciences (ERC/FBMS/099/2025).

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