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Sesame oil protects against permethrin-induced memory decline and oxidative stress in the hippocampus of Wistar rats

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ABSTRACT

Permethrin is a synthetic pyrethroid commonly used for the control of insect pests. It is neurotoxic at high doses and its exposure has been associated with tremor, salivation, paresthesia, depressed reflexes and nerve damage, in animal studies. Sesame seed oil is derived from the seeds of the flowering sesame plant, known for its beneficial antioxidative and anti-inflammatory properties. This study investigated the possible ameliorative effects of Sesame seed oil on permethrin-induced neurotoxicity. A total of 16 male Wistar rats were divided into four groups: the control group given standard rat feed, permethrin (PER) group treated with 100 mg/kg permethrin, sesame seed oil (SO) group administered with 5 ml/kg SO and SO+PER group co-treated with SO and PER, all for 14 days. The rats were assessed for memory and spatial learning and thereafter anesthetized and sacrificed after 14 days of treatment. The hippocampus was excised from the brain and processed for tissue histology, histochemistry and immunohistochemistry, using haematoxylin & eosin stain, cresyl fast violet stain and antibody against ionised calcium binding adaptor molecule 1 (for microglial expression) respectively. Oxidative status was assessed using glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzymes as biomarkers. Results revealed that permethrin caused memory deficits, varying degrees of disruption of the normal hippocampal microarchitecture, microglial activation and depletion of endogenous antioxidants. On the other hand, these activities were either prevented or minimised in the rats that received sesame seed oil intervention. Our findings revealed that sesame seed oil is both anti-oxidative and anti-inflammatory and capable of mitigating permethrin-induced hippocampal neurotoxicity.

Keywords: *Sesame seed oil, Permethrin, memory deficits, oxidative stress, neuroinflammation*

INTRODUCTION

Permethrin is a synthetic pyrethroid commonly used as insecticide for the control of insect pests and disease vectors [1]. It is a derivative of natural pyrethrins from the plant *Chrysanthemum cinerariaefolium*. Permethrin has been explored in the treatment of lice, scabies and to protect against bites from disease vectors such as mosquitoes and ticks when applied on the surface of clothing and mosquito nets [2-4]. However, exposure to insecticides has been documented to adversely affect various organs and systems of the body including the nervous system. The neurotoxic effects could be a direct or indirect effect that perturbs the normal metabolic processes and disrupt the structural integrity and functional status of the nervous system [5].

Insecticides such as permethrin cause reversible and dose-dependent neurotoxic effects, being able to interfere with the transmission of nervous impulses or ion channels [6, 7]. Clinical neurological features that have been documented in animal studies following permethrin exposure include tremors, paresthesia, splayed gait, depressed reflexes and reversible axonal injury [8]. Permethrin has been implicated in striatal mitochondrial dysfunction and oxidative stress [9]. Oxidative stress is a result of an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products [10].

Microglia are the first line of defense against central nervous system (CNS) injury [11]. They are activated in response to CNS injury, and this is a critical process in the pathogenesis of neuroinflammation in the central nervous system [12, 13]. Microglial activation is a critical process in the pathogenesis of neuroinflammation in the central nervous system [13].

Sesame oil is derived from the seeds of the flowering sesame plant, also known as *Sesamum indicum*; these plants are native to East Africa and India, but they are currently grown in many countries around the world [14, 15]. Sesame oil is an effective natural antioxidant. Natural antioxidants have shown a remarkable reduction in oxidative stress due to excess formation of reactive oxygen species by enhancing anti-oxidative mechanism in the neurodegenerative disorders [16]. Sesame oil has bactericidal and insecticidal activities and also act as an antioxidant; such antioxidant activity appears to be closely related to the prevention of degenerative diseases such as cancer, cardiovascular diseases, atherosclerosis and the process of aging [17].

Sesame seeds contain flavonoids and other phenolic compounds with antioxidative properties [18]. The phytochemicals/phytonutrients of this plant include Sesamin, Sesamol, saponin, alkaloids, flavonoids, glycosides, tannins and others. Many of these constituents have beneficial effects. Sesamin is anti-hypertensive, anti-inflammatory, anti-allergic and protects against oxidative damage [19]. Sesamol (3, 4-methylenedioxyphenol), a phenolic compound found in sesame seed and sesame oil, is the major antioxidant component of the oil. It is a metabolic regulator, inhibits lipid peroxidation,and suppresses carcinogenesis when combined with other antioxidants. Sesamol and its derivatives have been found to be extremely useful in several diseases including neurodegenerative diseases and metabolic disorders [20]. This study aimed to assess the protective effects of sesame oil on permethrin-induced neurotoxicity in the hippocampus of rats.

MATERIALS AND METHODS

The study was approved by the Ethical Review Committee and the protocols and treatment procedures were carried out according to the Institutional Animal Care and Use Committee (IACUC) guidelines.

Animal Care

Sixteen (16) male Wistar rats with weight range of 81.50±6.06 to 99.50±3.97 g were used for the study. The rats were kept in the Animal House of the Faculty of Basic Medical Sciences. Proper ventilation was maintained by the use of well-spaced and gauzed cages and a hygienic environment was ensured. The rats were fed with standard rat diets and had access to water *ad libitum* and were allowed to acclimatise for 7 days before the commencement of the experiment.

Animal Grouping and Treatment

The rats were randomly divided into four groups, with four rats in each group. Group A (control group) rats were fed with standard rat feeds; group B (permethrin group) received 0.6% permethrin (Rambo®; Gongoni Co. Ltd, Kano, Nigeria) mixed with the standard feed at a dose of 1000 mg/kg of feeds per day; group C (sesame oil group) received 5 ml/kg/day of sesame oil; and group D received both sesame oil and Permethrin at doses stated above. Sesame oil was administered orally via a cannula and all the experiment lasted for 14 days.

Behavioural Studies

After 14 days of treatment, behavioural tests were carried out to assess neural mechanisms of spatial learning and memory using the Morris water maze [21]. Animals acquire information about spatial location

and reach hidden platform in circular pool filled with water. Following an initial period of training, a decrease in the time to reach the hidden platform suggests learning. The Morris water maze apparatus consists of a circular water tank (100 cm diameter and 60 cm height) filled with water (26±2°C) to a depth of 30 cm. The rats were trained 24 hours prior to the main test was conducted. The pool was divided into four hypothetical quadrants, designated as N (North), E (East), W (West), S (South). An escape platform was placed 2.5 cm below the surface of the water in a constant position in the middle of the south-west quadrant in all trials. During the training period, each rat was placed in each of the four quadrants for a maximum of 60 seconds to find the escape platform at an interval of 15 minutes between quadrants until the escape latency period reduced to less than 15 seconds. For each rat, the test was carried out for 15 minutes. The time taken to find the escape platform was noted as the escape latency period.

The Y-maze spontaneous alternation test is used to assess learning and memory in rodents [22]. Alternation behavior is based on the spontaneous tendency in rodents to walk around the maze systematically entering each arm in turn. In the spontaneous alternation task, each rat was placed in a Y maze and allowed to move freely in the maze. Alternation behavior was defined as successive entries into each of the three arms with no repetition. Therefore, spontaneous alternation was assessed using a Y-maze composed of three equally spaced arms (41 cm long and 15 cm high). The floor of each arm was made of a 5 cm wide plywood. Each rat was placed in one of the arm compartments and allowed to move freely until it completely enters another arm. The sequence of arm entries was recorded. For each animal in a group the Y-maze testing was done thrice for 5 minutes each.

Animal Sacrifice and Sample Collection

The rats were euthanised on day 15. The hippocampal tissue of the right cerebral hemisphere was fixed in formal saline while that of the left hemisphere was placed in 0.25 M sucrose solution. The latter was homogenised and the homogenates were centrifuged at 3,000 rpm for 15 minutes to get the supernatant containing tissue lysates. The supernatant was used for the quantitative assay of superoxide dismutase and glutathione peroxidase enzymes using appropriate ELISA kits. The tissues fixed in formal saline was processed for histological preparations using haematoxylin & eosin and cresyl fast violet stains, and viewed under the light microscope.

Data Analysis

Data were analysed using GraphPad software and presented as mean (standard error of mean) at 95% confidence limit.

RESULTS

Sesame oil mitigates memory deficits associated with permethrin neurotoxicity

The escape latency period (ELP) of rats in the permethrin-treated group was highest compared to other groups, while the least was in the Sesame oil group (Fig. 1A). The rats in the Control had slightly higher ELP compared with the Sesame oil group, while the ELP of rats treated with both Permethrin and Sesame oil was higher than those of the Control and Sesame oil group and slightly lower than permethrin-treated group ($p>0.05$).

Permethrin treatment led to reduction in percentage correct alternations (PCA) compared with the Control ($p>0.05$) (Fig. 1B). Rats that received Sesame oil had a higher PCA compared with permethrin-treated rats, while rats co-administered with permethrin and Sesame oil had a slightly lower PCA compared with rats that received only Sesame oil, but higher than the permethrin-treated rats. These differences were not statistically significant ($p>0.05$).

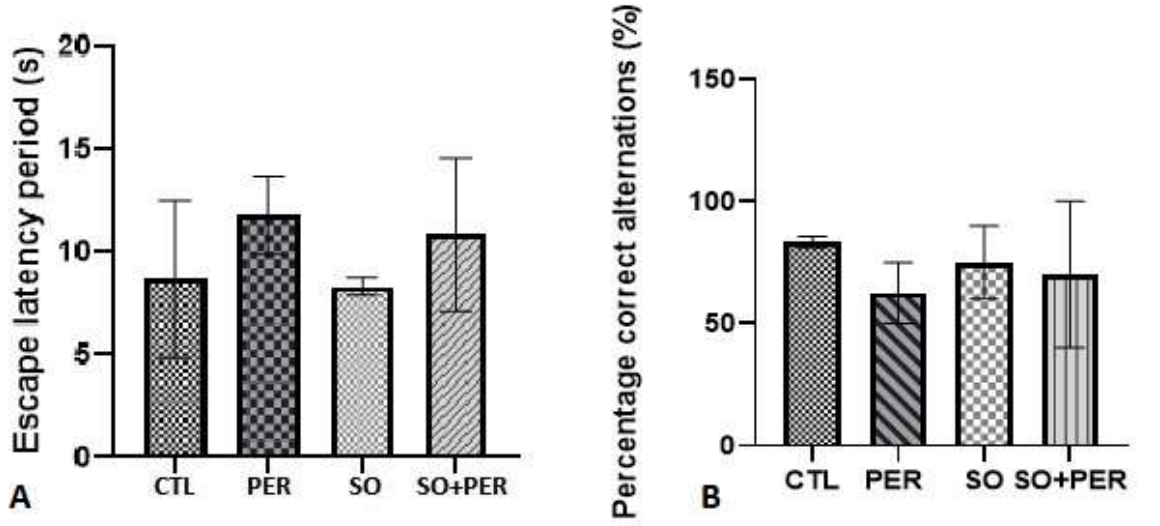

Figure 1: Escape latency period (ELP) (A) for rats in the Morris water maze test, showing increased ELP in permethrin-treated group (PER), but decreased in Sesame oil (SO) group and Sesame oil + permethrin-treated (SO+PER) group ($p>0.05$). (CTL- Control). In Figure 1B, the percentage correct alternations by animals in the Y-maze test, highest percentage in the Control (CTL), lowest in permethrin treatment (PER), while Sesame oil (SO) caused an increase in PCA. Changes were not statistically significant $p>0.05$).

Activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes in the hippocampus

Permethrin induced a remarkable reduction in SOD (Fig. 2A) and GPx (Fig. 2B) activity in rats' hippocampus compared with the Control, while rats treated with Sesame oil had a very high level of SOD and GPx. Rats that received Permethrin and Sesame oil concurrently had higher level of SOD and GPx compared with rats treated with Permethrin only, but lower activity when compared with the Control and Sesame oil-treated rats, though not statistically significant ($p > 0.05$).

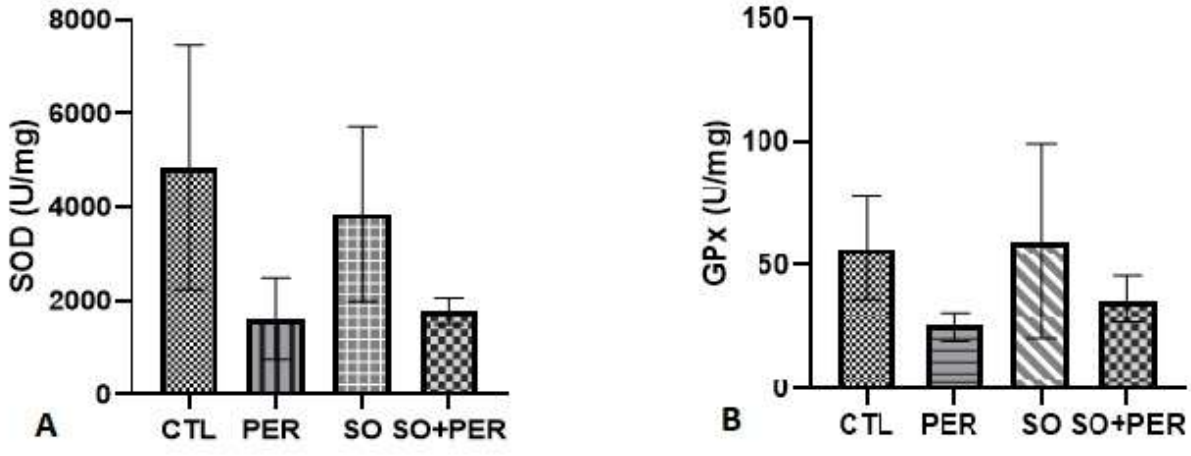

Figure 2: Permethrin-induced neurotoxicity resulted in reduced SOD activity in the hippocampus of rats (A), which slightly improved in rats co-treated with Permethrin and Sesame oil ($p>0.05$). Activity of glutathione peroxidase (GPx) (B) in the hippocampus of rats showing marked reduction in permethrin-treated (PER) group compared with Control (CTL); Sesame oil (SO) group had a higher GPx level compared with Control and rats co-treated with permethrin and Sesame oil (SO+PER), with the latter higher than in PER ($p>0.05$).

Histological Observation

The CA3 region of hippocampus of rats in the control group (Fig. 3; CTL) showed sparsely dispersed pyramidal cells with well-rounded nucleus that were properly stained, while in rats treated with Permethrin it showed sparsely dispersed pyramidal cells but poorly stained, with the presence of vacuolations in the cells (PER). The rats treated with Sesame oil (SO) presented with cells similar in structure to the control and also showed more pyramidal cells compared to the control and the rats treated with Permethrin. The animals co-treated with Sesame oil and permethrin (SO+PER) showed a combination of normal pyramidal cells and distorted cells, with the distorted cells exceeding the normal cells. Vacuolations were also evident in the cells, though the cells were adequately stained.

The dentate gyrus (DG) of the control group showed finely packed granule cells containing well-rounded nuclei with good staining intensity (CTL) while the rats treated with Permethrin showed the presence of poorly stained fragmented cells with the presence of vacuolations and chromatolytic changes in the granule cells (PER). There were also hyperchromatic cells. The rats treated with sesame oil showed well delineated nuclei with cellular structures similar to those of the control, though pale-stained (SO). The dentate gyrus of rats co-treated with sesame oil and permethrin presented with properly stained granule cells but with a combination of normal and fragmented cells (SO+PER).

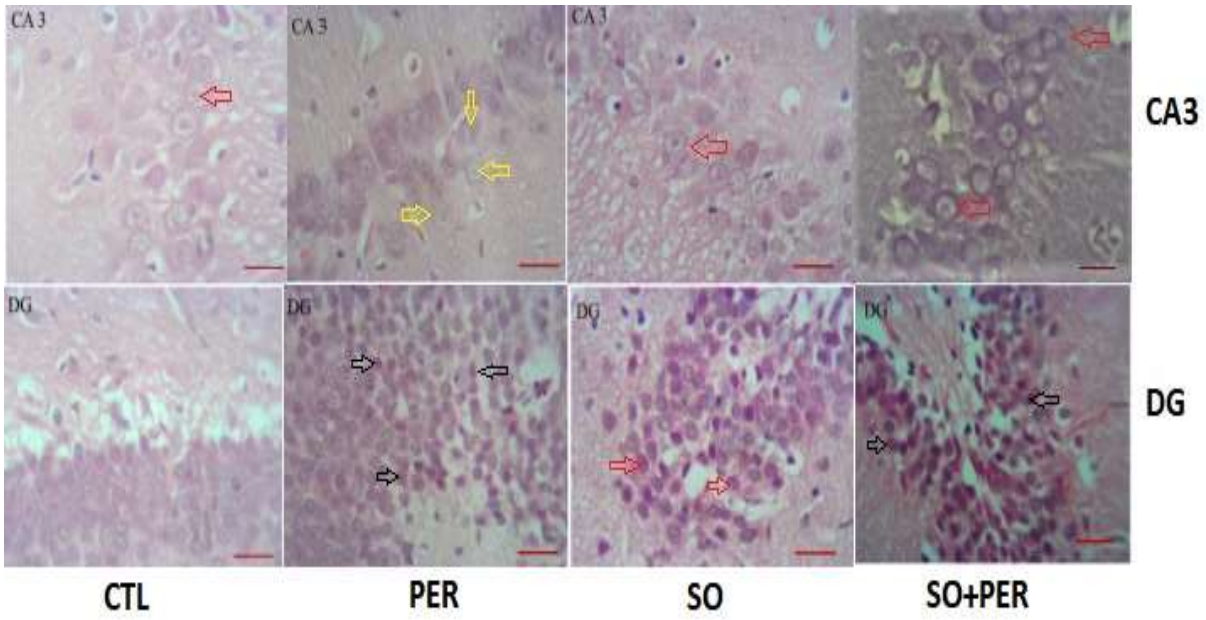

Figure 3: Representative photomicrographs showing the cytoarchitecture of CA3 and dentate gyrus (DG) of the hippocampus of rats: CTL=Control, PER=Permethrin-treated, SO=Sesame oil, and SO+PER=Sesame oil+Permethrin. CTL sections showed fairly stained sparsely dispersed pyramidal cells (red arrows) with well-rounded nucleus. Permethrin-treated groups showed poorly stained sparsely dispersed pyramidal cells, presence of vacuolations in the cells and fragmented cells (yellow arrows), and hyperchromatic cells (black arrows). The SO groups had fairly stained cells (red arrows) and smaller in size. showed that the animals treated with Sesame oil presented with cells similar in structure to the control group, while SO+PER group showed a combination of normal pyramidal cells and hyprchromatic cells (black arrows), vacuolations were also evident in the cells and the cells were adequately stained.

Sesame seed oil protects against permethrin-induced microglial activation

In Figure 4, the expression of microglial cells in the hippocampus of permethrin-treated rats (PER) was increased, whereas there was no evidence of such degree of activation in the control (CTL) group and other groups that received Sesame oil (SO, SO+PER).

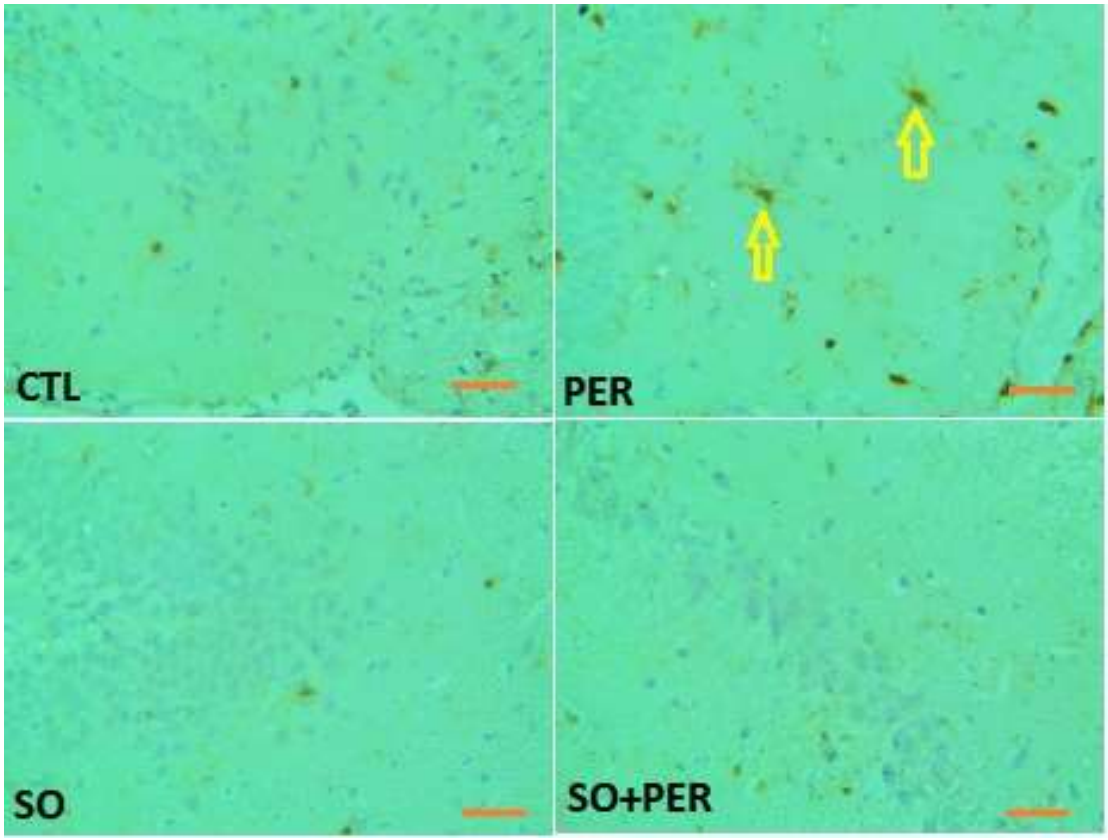

Figure 4: Representative photomicrographs of hippocampus of Wistar rats showing the expression of microglia using antibody against ionised calcium binding adaptor molecule 1 (iba1). There was marked expression in permethrin (PER) treated group (yellow arrows) compared with the control (CTL), Sesame oil (SO) and Sesame oil plus permethrin (SO+PER) groups.

DISCUSSION

As earlier reported, exposure to permethrin has adverse implications on normal working memory and spatial memory in a dose-dependent fashion [7]. In the current study, a higher dose was selected while exploring the beneficial health effects of Sesame oil on the hippocampus of rats exposed to permethrin. The higher escape latency period observed in permethrin-treated animals was an indication that the pyrethroid impairs spatial memory, while the lower percentage correct alternation revealed an impairment in working memory. Other studies have identified the implication of permethrin on cognitive functions. According to Nasuti *et al*. [23], permethrin causes progressive, time-dependent neurodegeneration in animal models with associated depletion in dopamine levels, dopaminergic neurons and cognitive impairments. The neuroprotective roles of Sesame seed oil (SO) have been studied by other researchers too [16]. Like other natural antioxidants, SO counteracts the formation of excessive reactive oxygen radicals, thereby enhancing antioxidant mechanism in the pathogenesis of neurodegenerative disorders [16]. This mechanism contributed to the improvement in cognitive function of rats treated with SO in the present study.

In assessing hippocampal oxidative stress associated with permethrin neurotoxicity, glutathione peroxidase (GPx) and superoxide dismutase (SOD) were employed as enzymatic markers. The activity of GPx and SOD as markers of oxidative stress was quantified in the hippocampus. Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of reactive oxygen species (ROS) in cells and tissues. This is one of the mechanisms by which permethrin exacts its effects on body cells [24]. Sesame oil administration reduced the oxidative stress induced by permethrin toxicity as evident by the increased level of GPx and SOD in the hippocampus of rats co-treated with permethrin and sesame oil. This lends credence to the anti-oxidative activity of sesame oil, hence its neuroprotective role in the hippocampus and ability to ameliorate any dysfunction occasioned by permethrin neurotoxicity. Shasmitha [25] also reported a reduction in oxidative stress and rise in oxidative enzyme markers following sesame oil consumption.

As earlier reported, permethrin causes microarchitectural alterations in various tissues of the body [26]. While the hippocampus of rats administered with SO and the Control were apparently normal with well-rounded nucleus and no fragmented cells, permethrin-treated rats presented with various degrees of alterations, including cytoplasmic vacuolations and fragmentation of cells which is as a result of direct insult of permethrin. The animals co-treated with sesame oil and permethrin showed fewer fragmented cells and vacuolation, which is an evidence of the effect of sesame oil in ameliorating the neurotoxic effects of permethrin. The compromised structural integrity of the hippocampus in the animals treated with permethrin is capable of affecting vital neurologic functions of different hippocampal regions leading to various forms of behavioral deficits, especially as seen in the memory indices.

Microglia are the cellular mediators of inflammation in the central nervous system, and their activation suggests the presence of neuroinflammation. As revealed in this study, permethrin neurotoxicity resulted in the activation of microglia as a response to injury to the hippocampus. Once activated, the initially resting microglia undergo a series of morphological and neurochemical and phenotypic alterations in gene expression and activation of signaling molecules [27]. Activation of microglia adversely affects cognitive functions [28], as we reported impairment in both spatial and working memory in rats exposed to permethrin diet. Sesame oil also played a significant role as an antioxidative and anti-inflammatory agent in ameliorating this deficit in memory function.

Conclusion: Exposure to permethrin is neurotoxic, affecting hippocampal neurochemistry and cognitive functions. Sesame seed oil has both antioxidative and anti-inflammatory properties, which confer some neuroprotection on the hippocampus following permethrin-induced neurotoxicity.

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