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Full-Length Research Article

Modulatory Effects of Sesame Seed Oil on Some Reproductive Parameters and Testicular Dysfunctions in Subchronic Permethrin-Administered Male Wistar Rats

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Summary: Permethrin (PM) is a synthetic type 1 pyrethroid insecticide, and excessive exposure can cause a wide range of toxic effects. This study evaluated the protective effects of sesame seed oil (SSO) on the testicular histology and male reproductive parameters in permethrin-induced toxicity in adult Wistar rats. Forty (40) adult male Wistar rats were randomly allocated into four (4) groups of 10 animals each. Group A received 0.5 ml of normal saline; Group B was given feed mixed with 1,000 mg/kg of 0.6% permethrin; Group C received 5 ml/kg of sesame seed oil, and Group D received feed mixed with 1,000 mg/kg of 0.6% permethrin and 5 ml/kg of sesame seed oil. All treatments were administered daily for 4 weeks via oral gavage. The animals were anesthetized and transcardially perfused with normal saline and 10% buffered formalin. Testicular tissues were processed for tissue histological analysis and biochemical assays, including superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA). Serum levels of testosterone, luteinizing hormone, and follicle-stimulating hormone were evaluated. Furthermore, sperm count, motility, morphology, and viability were analyzed using the caudal epididymis. The results showed reduced body weight and testicular weight in the permethrin (PM) group. There was a reduction in semen parameters in the PM-exposed rats. The results also showed a reduction in the level of testosterone, with an increased level of LH and FSH in the PM- treated group. Permethrin disrupted the antioxidant capacity of SOD and GSH, induced an elevated level of MDA, and caused degenerative changes in the testicular histoarchitecture. The sesame seed oil protected against oxidative damage in male Wistar rats. It regulated the hormonal profiles and improved caudal epididymal sperm quality, as well as testicular histoarchitectural integrity. This study showed that sesame seed oil improved testicular histoarchitecture and enhanced reproductive parameters in permethrin-induced testicular damage.

Keywords*:* Permethrin, sesame seed oil, glutathione, testosterone, histoarchitecture

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INTRODUCTION

The need for increased crop production and control of disease-causing pathogens both at home and agricultural settings have increased the use of insect control chemicals i.e. insecticides and subsequently increased exposure of humans and other mammals to these chemicals which often caused adverse effects in various body systems (Hamadache *et al*. 2016; Imam *et al*. 2024).

Pyrethroids are synthetic insecticides derived from natural pyrethrins found in Chrysanthemum plants. They are classified into Type I and Type II categories based on their distinct chemical structures and toxicological profiles (Chrustek *et al.,* 2018; Costa, 2015). Although designed to selectively target voltage-gated sodium channels in insects, their lipophilic nature and isomer composition particularly the more potent cis-isomers pose significant risks to non-target organisms, including humans (Hughes and Edwards, 2016; Navarrete-Meneses *et al.,* 2023). Chronic exposure is linked to severe physiological disruptions, such as neurobehavioral deficits, molecular target damage, and reproductive impairment, specifically within the testes (Hamadache *et al.,* 2016; Imam *et al.,* 2024).

Permethrin, a versatile Type I pyrethroid, is widely utilized in public health and agriculture for vector control and crop protection (Ahmad *et al.,* 2024). While valued for its efficacy in combating diseases like malaria, it presents substantial health risks through environmental and occupational exposure (Kalyabina *et al.,* 2021). Research indicates that permethrin induces multisystemic toxicity, including hepatotoxicity, immunosuppression via natural killer cell inhibition, and neurobehavioral abnormalities (Mostafa *et al.,* 2016; Huang *et al.,* 2024). Furthermore, its capacity to induce genotoxic damage and impair male reproductive health underscores the need for rigorous evaluation of its long-term biological impact (Omotoso *et*

al., 2022). Prolonged human exposure to permethrin is associated with neurotoxic manifestations such as muscular asthenia, dyspnea, and cephalalgia. In severe instances, it may trigger autonomic disruptions, including hypersalivation and acute seizure activity. Specifically, permethrin adversely impacts male reproductive function by inhibiting the motility of mature spermatozoa and interfering with spermatogenesis (Jin *et al.,* 2012; Mostafa *et al.,* 2016). The testes are uniquely vulnerable to oxidative stress due to their high concentration of polyunsaturated fatty acids (PUFAs), which renders the testicular membrane susceptible to lipid peroxidation (Okpalanduka and Agu, 2018). Permethrin-induced reactive oxygen species (ROS) production subsequently depletes essential antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), while causing significant mitochondrial disruption (Mozhdeganloo *et al.,* 2016). The use of natural antioxidants is increasingly recognized as a vital intervention for mitigating oxidative damage to the reproductive system. Sesame seed oil (SSO), derived from *Sesamum indicum*, is a potent bio-active agent characterized by high concentrations of PUFAs, Vitamin E, and unique lignans such as sesamin and sesaminol (Gharby *et al.,* 2017; Arab *et al.,* 2022). These components confer antioxidant, anti-inflammatory, and antimutagenic properties by scavenging hydroxyl and peroxy radicals to inhibit lipid peroxidation (Imran *et al.,* 2020). Beyond systemic benefits like improved lipid profiles and glycemic control, SSO mitigates DNA oxidative damage and suppresses ROS (Gharby *et al.,* 2017). Previous studies have established that sesame supplementation enhances the epididymal sperm reserve and increases the cross-sectional diameter of spermatocytes (Abbasi *et al.,* 2013; Fawzia *et al.,* 2021). These findings suggest that the bioactive constituents of sesame exert a positive trophic effect on the germinal epithelium, thereby improving sperm storage capacity and overall reproductive health in animal models.

MATERIALS AND METHODS

Laboratory Animals and Care: A total of forty (40) adult male Wistar rats (130 - 150) g were purchased from the Department of Vetrinary Medicine University of Ilorin and housed in the Animal House facility of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria. The animals were allowed to acclimatized for two weeks to allow them adapt to the new environment. The rats were treated under standard environmental condition exposed to normal day/night cycle between 23ºC to 26ºC and allowed access to feed (commercial pelletised chow) and water ad libitum.

Ethical Approval: Ethical approval for the study was obtained from university of Ilorin Ethical Review Committee (UERC/ASN/2024/2977). All institutional and national guidelines for the care and use of Animals were followed.

Animal Treatment: Rambo insect powder (Rambo®; Gongoni Co. Ltd, Kano, Nigeria) containing 0.6% Permethrin and 99.4% inert carriers was purchased and used for the study. The animals were divided into four groups of 10 animals each. Group A received 0.5 ml of normal saline; Group B was given feed mixed with 1,000 mg/kg of 0.6% permethrin; Group C was administered 5 ml/kg of sesame seed oil, and Group D received feed mixed with 1,000 mg/kg of 0.6% permethrin and 5 ml/kg of sesame seed oil. Treatment was for 28 consecutive days.

At the end of the experiment, 4 rats were anesthetized intramuscularly with 0.5 ml/kg ketamine. They were subjected to transcardial perfusion with 50 ml of normal saline, followed by 10% buffered formalin. After the perfusion, the testes were excised for histological analysis. Six rats were sacrificed by cervical dislocation after which blood was taking for serum analysis of reproductive hormone, caudal epididymis was used for semen parameter, and testis was remove and homogenate was used for oxidative markers analysis.

Assay for Biochemical Parameters: superoxide dismutase, glutathione peroxidase, and malondialdehyde were assays. Assay kits for superoxide dismutase (SOD; KT-60703), glutathione peroxidase (GPx; MBS744364), and malondialdehyde (MDA; MBS9389391) were used to assess the testicular oxidative state of rats using spectrophotometric technique (Vadhana *et al*., 2011). All reagents and samples were placed at room temperature before starting the procedures. Equal testis was homogenized in ice-cold 30% sucrose solution with an automated homogenizer at 4°C. The homogenate was scooped and poured into a 5 mL plain specimen bottle and placed in a centrifuging tube containing ice. The homogenate was centrifuged for 15 minutes at 3,000 rpm. The supernatants were aspirated into plain labeled glass cuvettes placed in ice. The assay was carried out according to the manufacturer's instructions on the assay pack.

The serum level of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were determined using Accu-bind ELISA Microwells from Calbiotech, with CAT Nos: FS046F, LH231F, and TE187S and the procedure was performed according to the manufacturer's instruction.

Estimation of Epididymal Sperm Characteristics : The epididymis of each testis was dissected and transferred into the Petri dish containing 1 ml of normal saline. Epididymal Sperm Concentration: The sperm were counted according to the method of (Robb *et al*., 1978) by using a Neubauer hemocytometer chamber.

Sperm Motility: The percentage of epididymal sperm motility was measured depending upon the graduation basis that was suggested by Chemineau *et al*., 1991).

Sperm abnormality and Sperm Viability: The percentage of abnormal spermatozoa based on either short tail, cut tail, long head. The sperm viability was express as ratio of live spermatozoa to dead sperm (life/death ratio). these were by counting 200 sperms under a light microscope (X200) according to (Chemineau *et al*., 1991).

Histological Analysis: Testes tissues were fixed in Bouin's solution, dehydrated in ascending-graded alcohol, cleared in xylene, and
embedded in paraffin. Then they were cut into 5µm sections using a microtome and stained with hematoxylin and eosin (H&E) according to the method of (Sheehan and Hrapchak, 1987). Images were viewed under a light microscope and images were taken with an amscope digital camera.

Data Analysis
All quantitative data from this study were analyzed using one way analysis of variance (one-way ANOVA) and Tukey post hoc test for multiple comparison with the GraphPad Prism version 10.0.2 and expressed as mean ± standard error of the mean (M±SEM), and $p<0.05$ is considered statistically significant.

RESULTS

Effects of Sesame Seed Oil on Body and Testicular Weight in Permethrin Toxicity: Permethrin exposure showed a reduction in the body weights of experimental animals progressively sesame seed oil intervention preserved the body weight. The testis weight was reduced in PM-exposed rats compared to the control, the sesame seed oil administered with permethrin showed further reduction in testicular weight. Table 1.

Effects of Sesame Seed Oil on Permethrin-induced Reproductive Hormones Disruption: The result of this study showed a non-significant increase in the serum level of LH of PM compared to the NS and SSO groups, but significant when compared with the PM + SSO group. This study also revealed a statistically non-significant increase in the concentration of the FSH of the PM-exposed group compared to the NS, SSO, and PM + SSO groups. The study also found a reduction in the testosterone level of the PM group compared to the NS, and SSO groups with a statistically non-significant increase compared to the PM + SSO group at $p<0.05$. Table 2.

Effects of Sesame Seed Oil on Lipid Peroxidation following Permethrin Exposure
The testicular glutathione peroxidase (GPx) was reduced than that of the normal saline (NS) figure 1, but not statistically significant. The SOD level of the PM group was higher than that of the NS and other experimental groups but not statistically significant at $p<0.05$. The testicular MDA concentration of the PM-treated rats was significantly higher at $p<0.05$ compared to the NS, SSO, and PM + SSO groups (Figure 1).

Effects of Sesame Seed Oil on Seminal Fluid Parameters Rats exposed to Permethrin: The findings of this study revealed a significant reduction in the percentage of sperm count (SC) of the PM group compared to all other experimental groups (Figure 2) at $p<0.05$. The sperm morphology was significantly reduction in the PM group compared to NS, SSO, and PM + SSO groups. The life/death ratio (viability) of the NS, SSO, and PM + SSO groups were significantly higher than that of the PM group at $p<0.05$. Figure 2. The percentage of sperm motility was statistically significant lower in the PM group compared to the NS, SSO, and PM + SSO groups at $p<0.05$. Figure 2.

Table 1:
Effects of Permethrin and Sesame Seed oil on Body and Organ Weight

Groups	Initial weight (g)	Final weight (g)	weight Difference (g)	Testis Weight (g)
NS	170 ± 11.20	222.3 ± 13.76	52.3 ± 2.56	2.527
PM	167.3 ± 14.66	166.7 ± 16.95	-0.6 ± 2.29	2.070
SSO	176.7 ± 14.08	218.7 ± 10.97	42.0 ± 3.11	2.143
PM + SSO	162.3 ± 12.41	164.7 ± 11.85	2.4 ± 0.56	1.853

NS (Normal saline); PM (Permethrin); SSO (Sesame seed oil); PM + SSO (Permethrin with Sesame seed oil)

Table 2:
Regulatory effects Sesame seed oil on the Perethrin-induced Reproductive Hormonal disruption in adult Rats

NS	1.52 ± 0.40	4.26 ± 1.76	2.065 ± 0.1505
PM	0.60 ± 0.12	9.05 ± 2.57	4.832 ± 1.082
SSO	1.02 ± 0.25	4.66 ± 0.489	1.182 ± 0.3003
PM + SSO	0.54 ± 0.08	1.03 ± 0.19[b]	1.293 ± 0.3578

NS (Normal saline); PM (Permethrin); SSO (Sesame seed oil); PM + SSO (Permethrin with Sesame seed oil). b signifies significant difference when compared to Permethrin at ($p<0.05$). TT (Testosterone), LH (Luteinizing Hormone), FSH (Follicle Stiulating Hormone). Data presented as Mean ± standard error of the mean (M±SEM). (N = 6)

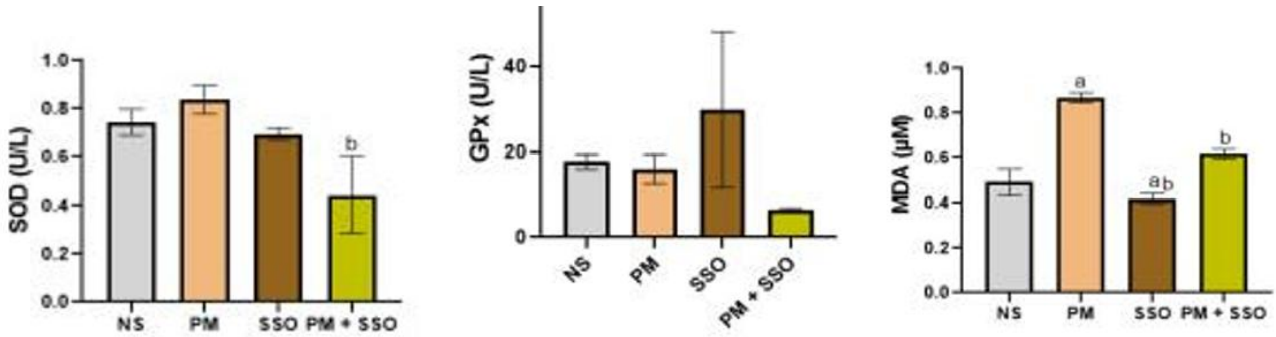

Figure 1:
Effects of Sesame Seed Oil on Testicular oxidative Stress biomarkers. (a) shows statistically significant difference compared to NS; (b) shows statistically significant difference compared to PM at $p<0.05$. Data presented as Mean ± standard error of the mean (M±SEM). (N =

6). SOD (Superoxide Dismutase); GSH (Glutathione); MDA (Malondialdehyde). NS (Normal saline); PM (Permethrin); SSO (Sesae seed oil); PM + SSO (Permethrin with Sesame seed oil).

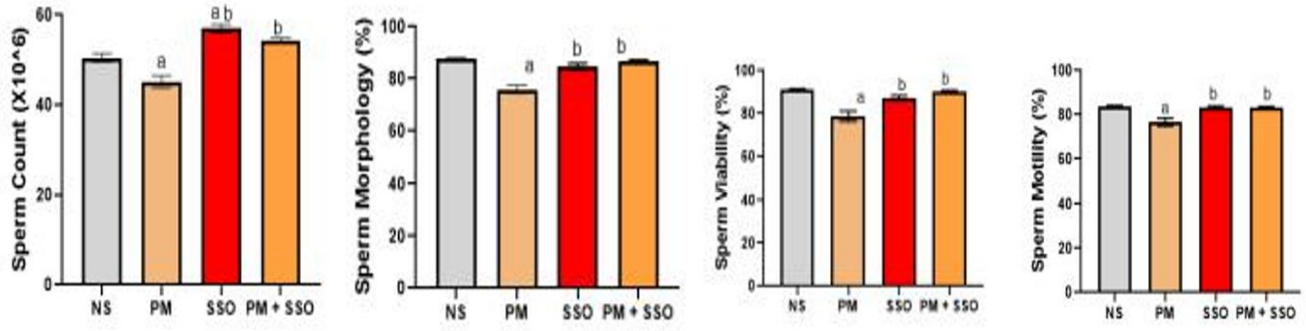

Figure 2:
Effects of Sesame Seed Oil on Epididymal sperm qualities (a) shows statistically significant difference compared to NS; (b) shows statistically significant difference compared to PM at $p<0.05$. Data presented as Mean ± standard error of the mean (M±SEM). (N = 6)

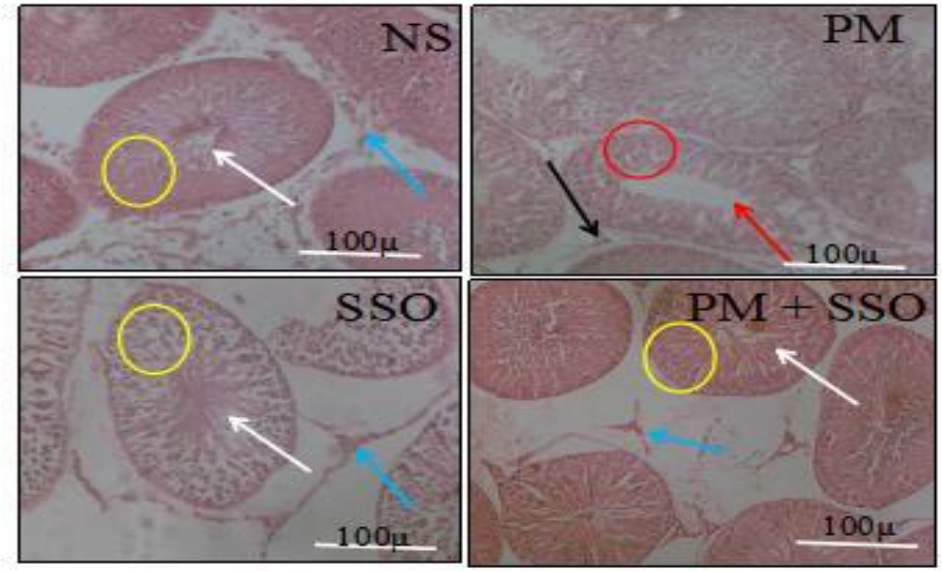

Plate 1:
Photomicrograph of Seminiferous tubule of rat stained with H & E. NS (Normal saline); PM (Permethrin); SSO (Sesame seed oil); PM + SSO (Permethrin with Sesame seed oil). Scale bar 100 μ (Mag. X100)

Histological Observation: Representative photomicrograph of testes showing normal histoarchitecture of the seminiferous tubule for the control (NS), The PM group shows deformed seminiferous tubule with mildly damaged germ cells (red ring), lengthened lumen (red arrow) and scanty Leydig cells (black arrow) and the SSO group shows fairly normal seminiferous tubule (white arrow), with scanty but well organized (yellow ring), the PM + SSO group shows normal histological presentation similar to that of the control

DISCUSSION

In this study, permethrin (PM) exposure resulted in a slight reduction in both body and testicular weight in rats. This aligns with previous findings by Khaki *et al.,* (2017), who reported decreased weights of male reproductive organs, including the testes and epididymis, following PM exposure. Similarly, Ayomide *et al.,* (2025) observed PM-induced body weight reduction in female rats, further strengthening our results. However, our findings contrast with the report by Omotoso *et al.,* (2021), who observed an increase in these parameters following 14 days of exposure. The sesame seed oil (SSO) intervention increased body weight but was associated with a decrease in testicular weight compared to the PM-only group. This may be attributed to SSO's antioxidant capacity, which prevents reactive oxygen species (ROS) generation and subsequent lipid peroxidation of cell membranes. This is consistent with Khaneshi *et al.,* (2013), who showed that SSO increases body weight in streptozotocin (STZ)-induced diabetic rat models. Furthermore, SSO has been shown to enhance testicular diameter, lumen, and volume (Mohammadzadeh *et al.,* , 2021).

Proper male reproductive function relies on the integrity of the hypothalamic-pituitary-testicular (HPT) axis to maintain spermatogenesis and fertility. Under physiological conditions, Luteinizing Hormone (LH) stimulates Leydig cells to synthesize testosterone, while Follicle-Stimulating Hormone (FSH) acts on Sertoli cells to produce androgen-binding protein.

In this study, PM disrupted this sequence, increasing LH and FSH concentrations while reducing testosterone levels. The elevation of LH and FSH may be attributed to PM's potential anti-melatonin effects; since melatonin acts as a

natural anti-gonadotropin that inhibits gonadotropin synthesis (Luke, 2001), its suppression by PM leads to unregulated LH and FSH secretion. Despite these high gonadotropin levels, testosterone remained low, indicating direct testicular toxicity. This reduction in testosterone is likely due to PM blocking the transport of cholesterol into the mitochondria for steroid synthesis (Hauet *et al., ,* 2005) and decreasing the activity of enzymes involved in testosterone biosynthesis (Okpalanduka and Agu, 2018). SSO was found to regulate the hormonal sequence. SSO intervention helped normalize these pathways and enhanced testosterone levels, though it was unable to fully restore testosterone to control levels when co-administered with PM.

Permethrin markedly disrupted the testicular antioxidant system by reducing antioxidant enzyme levels and increasing malondialdehyde (MDA), a marker of lipid peroxidation (figure 1). Both Type I and Type II pyrethroids generate superoxide ions, which inhibit antioxidant enzyme activity, leading to oxidative and inflammatory damage in various organs, including the testes and brain (Omotoso *et al., ,* 2021; Imam *et al., ,* 2024). The observed decline in glutathione peroxidase (GPx) activity led to increased lipid peroxidation of testicular membranes. This aligns with findings by Mozhdeganloo *et al.,* (2015), which indicated that PM acts as an oxidant agent that lowers glutathione (GSH) and total antioxidant capacity in the liver of rainbow trout. Also supporting this findings previous studies of Omotoso *et al., ,* (2022) and Imam *et al., ,* (2024) which show that pyrethroids-induced decline level of antioxidant enzymes and elevated lipid peroxidation I.e MDA levels in testes and brain tissues. In contrast, SSO protected testicular tissue against oxidative damage by enhancing antioxidant capacity and reducing lipid peroxidation, consistent with its known ROS-scavenging properties.

Normal seminal fluid qualities are vital for male fertility; any structural or functional abnormality in spermatozoa hinders reproductive efficiency (Vasan, 2011). In this study, PM-mixed feed reduced sperm concentration, motility, morphology, and viability (figure 2). The sperm membrane is rich in polyunsaturated fatty acids, making it a primary target for free radical attack and lipid peroxidation. The reduction in sperm quality may stem from energy depletion as pyrethroids like cypermethrin have been reported to cause mitochondrial damage and induced cytochrome c release, impairing the energy production which vital for required for sperm motility (Mohammadzadeh *et al., ,* 2021). according to the findings of previous studies PM was shown to induced reduction in the sperm concentration and morphological alteration (Khaki *et al., ,* 2016; Mohammadzadeh *et al., ,* 2021). this reinforce our findings and reiterate pro-oxidant characteristics of the PM. Sesame seed oil (SSO) improved seminal fluid qualities by enhancing the sperm volume, morphology, motility and viability. This finding is enhanced by the improved antioxidant system as seeing in the low level of lipid peroxidation and restored hormonal sequence.

Histological examination revealed degeneration in the rat testes, characterized by germ cell loss, vacuolation, and detachment. The increased interstitial space and scanty Leydig cells likely contributed to the low expression of LH receptors, further impairing testosterone synthesis. These findings are supported by Omotoso *et al.,* (2022), who observed similar seminiferous tubule degeneration at high doses of PM. SSO treatment successfully restored seminiferous tubule integrity and improved germ cell organization. the earlier studies of Mohammadzadeh *et al., ,* (2021) and Khaneshi *et al., ,* (2013) revealed similar findings which show that SSO improved thickness of the testicular epithelium, seminiferous tubules diameter in laydig cell integrity in rats.

In conclusion, sesame seed oil offers protective potential against the detrimental effects of permethrin on the architecture of the testis, regulate hormonal coordination and enhanced seminal fluid parameters by enhancing antioxidant activities and lowers MDA concentration. Therefore, this study concludes that sesame seed oil has therapeutic advantages and can be used to management of male infertility.

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