Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.100

RESEARCH ARTICLE

Ameliorating Effects of Sesame Seed Oil, a Natural Antioxidant, on Dibenzofuran-Induced Testicular Toxicity and Hormonal Imbalance as an Environmental Pollutant in Adult Albino Rats

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ARTICLE HISTORY (26-440)

Received:	April 22, 2026
Revised:	May 25, 2026
Accepted:	May 27, 2026
Published online:	May 30, 2026

Key words:
Histology
Oxidative markers
Rats
Reproductive Hormones
Sesame Seed Oil
Dibenzofurans
Testes

A B S T R A C T

Herbal plants are considered as a good source of pharmacologically active compounds and used for the treatment of many diseases. Natural products, like sesame oil, are rich in their active ingredients and they have been used in herbal medicine and alternative medicine to treat thousands of diseases. Dibenzofuran (DBF) is a potential environmental toxicant that can cause testicular damage in male animals. The aim of the present study was to investigate the possible role of sesame seed oil in ameliorating DBF-induced testicular toxicity and hormonal imbalance in male rats. Forty adult male albino rats were used in the study (n=10 for each group). T1: Negative control, T2: Sesame oil control (4 mL/kg body weight/day, intraperitoneally). T3: DBF-intoxicated group (oral gavage at 89 mg/kg body weight), and T4: Sesame oil + DBF co-treated. After the study the rats were weighed and blood samples collected. The serum concentrations of testosterone (T), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured and the right and left testes were removed and weighed separately. The right testis was homogenized for measurement of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). The left testis was preserved in Bouin's fixative and used for histological studies. Microscopic observation revealed that DBF produced histological changes in the tubular and interstitial compartments of the testis, which included degenerative changes in the seminiferous tubules, disorganization of the germinal epithelium, degenerative changes in the spermatocytes and arrest of spermatogenesis. Compared to the controls, the DBF-treated group had a significant drop ($P \leq 0.05$) in relative testicular weight, reproductive hormone levels (testosterone, LH, and FSH), and the antioxidant markers SOD and CAT. At the same time, MDA levels raised. That pattern points to serious oxidative damage. The rats administrated sesame seed oil showed reflections of those changes were reversed. The findings concluded that sesame seed oil can help protect against the hormone-disrupting and testicular toxicity effects caused by dibenzofurans in adult male albino rats.

To Cite This Article: Melebary SJ, 2026. Ameliorating effects of sesame seed oil, a natural antioxidant, on dibenzofuran-induced testicular toxicity and hormonal imbalance as an environmental pollutant in adult albino rats. Pak Vet J, 46(5): 1110-1121. http://dx.doi.org/10.29261/pakvetj/2026.100

INTRODUCTION

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are essentially toxic environmental agents that are indegradable. Most POPs are by-products of combustion or heat energy from industrial thermal processes that have become airborne and entered the soil, food chain, and tissues (Oboulbiga *et al.*, 2023). They're lipophilic, so they literally have a "key" to fat cells. They accumulate in organisms rather than being excreted. Because POPs become more concentrated higher up the food chain, wildlife and humans face continued exposure. (Aldeli *et al.*, 2024). Biologically, most toxicities occur after they bind to the aryl hydrocarbon receptor also known as AhR. These pollutants are the key, and AhR is the lock. Together, they move to the nucleus of the cell. From there, the adjustment in gene expression is specifically involved in processing foreign chemicals, managing hormones, and regulating cell growth. (Patrizi *et al.,* 2018; Aldeli *et al.,* 2024). The result has been that dioxins and furans have been associated with cancer, immune system dysfunction, hormone disruption, and developmental disorders – both in laboratory animals and in exposed humans (Kogevinas, 2001; Patrizi *et al.,* 2018). Reproductive function, particularly in males, turns out to be one of the most sensitive targets for these dioxin-like compounds. Specific 2,3,7,8-substituted congeners, when developmentally or peripubertally exposed to males, have the potential to reduce sperm counts and steroid hormone production, alter genes involved in sperm production, and induce testicular damage and testosterone depletion across generations (Johnson *et al.,* 2020; Faiad *et al.,* 2022; 2023).

Dioxins cause a serious disruption of ovarian steroidogenesis, which translates as a disruption in the production of female sex hormones that can lead to hormone-dependent conditions such as endometriosis (Aldeli *et al.*, 2024). The mechanistic basis of this disruption lies in the hijacking of the AhR pathway by dioxins, which triggers a cascade of disruptions in hormone synthesis, oxidative stress, mitochondrial damage, and inflammation in both the ovaries and the brain regions involved in reproductive regulation (Aldeli *et al.,* 2024). Of all the mechanisms involved, oxidative stress is the predominant mechanism behind the gonadotoxicity caused by dioxins, furans and related toxins (Ahmed *et al.*, 2017; Rehman *et al.*, 2019).

Extracted from sesame seeds (*Sesamum indicum* L.), sesame seed oil is rich in bioactive lignans (sesamin, sesamolin, and sesamol), tocopherols, and unsaturated fatty acids. These have strong antioxidant and anti-inflammatory activity (Oboulbiga *et al.,* 2023; Hadipour *et al.,* 2023). Animal studies have demonstrated that sesame oil and lignans significantly enhance endogenous antioxidant capacity. They attenuate lipid peroxidation (fat damage) and maintain key stress-response pathways – such as Nrf2, NF-κB, and MAPK – functioning properly (Shi *et al.*, 2022; Hadipour *et al.*, 2023).

Ultimately, sesamol and its related chemical compounds function by decreasing deleterious stress markers such as TBARS, malondialdehyde, and superoxides, while concurrently enhancing essential protective enzymes including glutathione, catalase, and glutathione peroxidase in critical organs such as the liver, heart, brain, and vasculature (Shi *et al.*, 2022; Hadipour *et al.*, 2023).Sesame essential oils attenuate pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), cyclooxygenase-2 (COX-2), and adhesion molecules (Afroz *et al.*, 2019; Hadipour *et al.,* 2023). These anti-inflammatory actions have been associated with protection in experimental models of atherosclerosis, myocardial ischemia, diabetes, fatty liver disease, and neurodegeneration (Ren *et al.,* 2018; Jayaraj *et al.,* 2020).

Given that DBF/PCDF-induced reproductive toxicity is strongly associated with oxidative stress, endocrine disruption, and inflammatory signaling in reproductive organs (Rehman *et al.,* 2019; Johnson *et al.,* 2020), the antioxidant and anti-inflammatory profile of sesame oil makes it a promising candidate for chemoprotection. However, despite extensive evidence for dioxin-related reproductive damage and the well-documented redox-modulating effects of sesame oil, data directly addressing whether sesame oil can mitigate DBF/ PCDF-induced reproductive dysfunction remain scarce.

Accordingly, the current study aimed to evaluate the protective effect of sesame oil against DBF/PCDF-induced reproductive toxicity. Specifically, the objective was to evaluate whether dietary or therapeutic administration of sesame oil can attenuate oxidative stress, endocrine disruption, and structural damage in the reproductive system following exposure to DBF/PCDFs, thereby providing a mechanistic basis for using this traditional oil as a nutraceutical intervention against dioxin-like reproductive toxicity.

MATERIALS AND METHODS

Drugs and animals: Dibenzofuran (DBF, CAS-No: 132-64-9) was obtained from Sigma-Aldrich Chemical Company, Saint Louis, Missouri, USA. Sesame seed oil was obtained from a local supermarket in Jeddah, Saudi Arabia, 100% pure, in a solution form. Animals: The 40 adult male albino rats were obtained from the Faculty of Pharmacy, King Abdulaziz University, Saudi Arabia, each weighing about 180-200 g. Rats were allowed free access to water and a diet. All groups were maintained with a 12-h:12-h photoperiod cycle at room temperature (25 ± 2°C). All animal procedures were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. The experimental protocol received approval from the Institutional Animal Care and Use Committee (IACUC), ensuring compliance with standard ethical oversight for animal research.

Experimental design: Following two weeks of rat acclimatization, they were randomly allocated into four groups, with eight rats in each group, as detailed below: The rats were categorized into four groups (n=10).

- **Control group**: no treatment was done, and they were administered distilled water.
- **Sesame oil group:** Sesame oil (4 mL/kg body weight/day, intraperitoneally) was administered for 35 consecutive days. This dose and route were selected based on prior studies demonstrating biological activity of 3–5 mL/kg i.p. sesame oil over 7–28 days and the known capacity of chronic i.p. oily vehicles to induce peritoneal and endocrine changes in rats (Ali Jasim, 2024).
- **DBF group:** rats received 89 mg/kg DBF three times/week for 35 days by oral gavage. DBF was first dissolved in [0.5% CMC as vehicle] and administered at a volume of 89 mL/kg. The dose selected in this study was 1/10 of the LD50 concentration of DBF (Brewster *et al.*, 1988).
- **DBF + sesame oil treated group:** 89 mg/kg DBF + 4 ml/kg/day of sesame oil.

Animal euthanasia and biochemical analyses: At the end of the study duration (35 days, comparable to one cycle of spermatogenesis in rats), the animals were weighed, blood samples were extracted from the retro-orbital venous plexus of each rat (under isoflurane anesthesia), and subsequently, the rats were euthanized by decapitation. Subsequently, biochemical analyses were conducted on the serum of the rats (acquired post-clotting and centrifugation of blood samples at 5000 rpm for 10 minutes) and homogenates from the rats' testicular tissues (following the excision and cleansing of these organs, homogenization in 0.1 mM phosphate buffer, and centrifugation at 9000 rpm at 4 °C for 20 minutes). The evaluated biochemical parameters in rat serum—testosterone (T; MBS9424769), luteinizing hormone (LH; MBS161787), and follicle-stimulating hormone (FSH; MBS2021901) were quantified using rat ELISA kits (MyBioSource, Inc., USA). Standard curves were performed according to manufacturer's instructions and only values within the linear range were accepted. Detection limits were in the low pg/mL to sub-ng/mL range, similar to validated testosterone and gonadotropin ELISAs (Pappa *et al.*, 1999; Verma *et al.*, 2022). The assay follows the standard protocol of Gholib *et al.* (2016). Both testes were excised, trimmed of fat and weighed. Relative testis weight was calculated as testis weight divided by body weight (Ali Jasim, 2024). The right testis was frozen in liquid nitrogen and kept at −40°C for homogenization; the left was placed in Bouin's fixative for histology. Right testis homogenates were analyzed for MDA (MBS738685), SOD (MBS036924), and CAT (MBS726781) levels. Right testis homogenate levels were detected using rat-specific ELISA kits (MyBioSource, Inc.).

Light microscopy preparation: The left testicular specimens were procured and promptly fixed in Bouin's fixative, subjected to progressive dehydration, subsequently cleaned in xylol solution, and finally embedded in paraffin. Sections were acquired at a thickness of 5-6 µm using a microtome. Testicular specimens were prepared for staining using standard histological analysis, utilizing hematoxylin and eosin (H&E) staining (Bancroft and Gamble, 2008) and Masson's trichrome stain for the detection of collagen fibers (Kiernan, 2015). All photomicrographs acquired for histological assessments were examined and compiled utilizing an Olympus BX41 research optical microscope (Tokyo, Japan) outfitted with an Olympus DP25 digital camera. The magnification scale bar was recorded on the acquired photomicrographs.

Morphometric study: Using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA), 10 distinct, non-overlapping, randomly selected fields from each slide of every group at 400x magnification were analyzed in H&E-stained sections to determine the mean diameter of the seminiferous tubules and the mean height of the seminiferous epithelium. The diameter of seminiferous tubules was measured along both the minor and major axes, and the mean value was calculated. In addition, the mean area percentage of collagen fibers was quantified in Masson's trichrome–stained sections (×400) (Ibrahim and Sadek, 2022). The appearance of germ cells in the seminiferous epithelium was classified based on the Johnsen score (Johnsen, 1970). Each tubule in the segment was evaluated and assigned a score ranging from one to ten (Table 1). The average Johnsen score was determined by randomly selecting 10 seminiferous tubules. (Akhtar *et al.*, 2020).

Table 1: Johnsen score and the degree of germ cell maturity

Johnsen Score	Criteria Description
10	Complete spermatogenesis with perfect tubular structure
9	Many spermatozoa present, but architecture disorganized
8	Only a few spermatozoa seen
7	No spermatozoa, but many spermatids
6	Only a few spermatids present
5	Many spermatocytes present; no spermatids or spermatozoa
4	Only a few spermatocytes
3	Only spermatogonia present
2	Only Sertoli cells (no germ cells)
1	No germ cells, no Sertoli cells – total absence

Statistical analysis: The data were analyzed using GraphPad software (GraphPad Inc., La Jolla, CA, USA), which was also used to generate the graphs. Data are presented as mean ± standard error of the mean (SEM). Before applying one-way ANOVA, model assumptions of normality and homogeneity of variances were assessed (Shapiro–Wilk test for normality, and Bartlett's test for normal data or Levene's test for non-normal or skewed data) (Kim, 2017). When these assumptions were satisfied, group differences were evaluated using one-way ANOVA followed by Tukey's multiple comparisons test. For multiple comparisons, significance levels are reported as $P<0.05$, $P<0.01$, and $P<0.0001$.

RESULTS

Rats' testicular relative weight results: In comparison to the control group, the DBF-treated group exhibited a statistically significant ($P=0.0300$) reduction in the relative weight of the rats' testes. The co-administration of sesame oil and DBF resulted in a statistically significant increase ($P=0.0327$) in the relative testicular weight of rats compared to those treated with DBF alone.

Oxidative stress and antioxidant markers results: Fig. 1 illustrates the concentrations of MDA and the activity of antioxidant enzymes (CAT and SOD) in the testes of both the control and experimental groups. The DBF-treated group exhibited elevated MDA levels, but CAT and SOD activity were diminished ($P<0.001$). Compared with the sesame seed oil group, the DBF group showed a greater increase in MDA levels and a marked decrease in CAT and SOD activities. In contrast, in the DBF + sesame seed oil–treated group, MDA levels and CAT and SOD activities were restored to values comparable to the control group ($P<0.001$). No statistically significant changes were seen between the sesame oil-treated group and the DBF + sesame oil-treated groups.

Hormonal profile results: The mean serum testosterone levels in the DBF-treated group were significantly lower ($P<0.0001$) than in both the control and sesame oil-treated groups. The DBF + sesame oil-treated group demonstrated a significant increase compared to the DBF-treated group ($P<0.0001$) but showed no significant difference from the control group ($P=0.9936$) (Fig. 2).

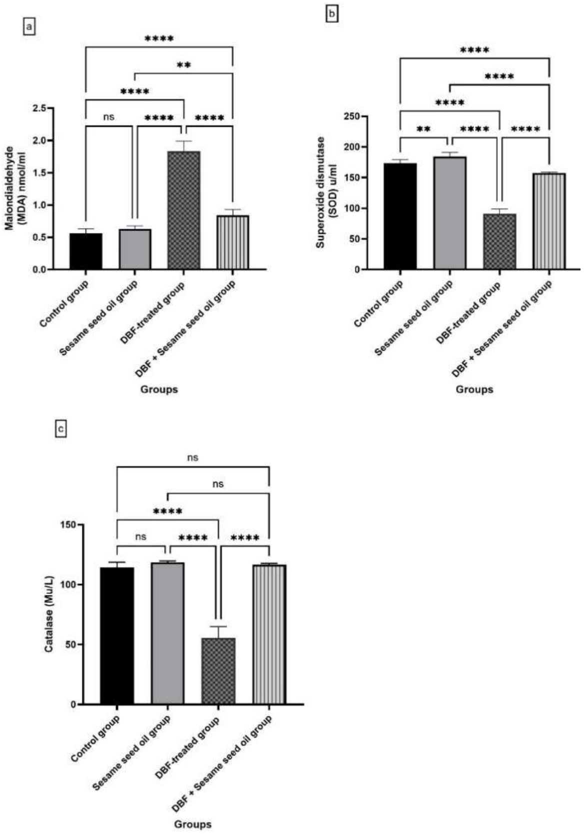

Fig. 1: The impact of sesame seed oil consumption on oxidative stress and antioxidant indicators in adult male rats administered dibenzofuran (DBF). The indicators measured include (a) Malondialdehyde (MDA), (b) Superoxide dismutase (SOD), and (c) Catalase (CAT). The data is presented as mean ± SEM. "ns" denotes non-significant, "**" indicates significance at P<0.01, and "****" signifies significance at P<0.0001.

The mean serum FSH and LH levels were markedly reduced in DBF-treated rats relative to the control groups, with a significant (P<0.0001) decline compared with the DBF + sesame oil-treated group. No significant differences (P=0.0519, P=0.9978, respectively) were seen in blood FSH and LH levels between the DBF + sesame oil-treated group and the control groups (Fig. 2).

Histological results

Hematoxylin & eosin-stained section: The testicular tissue of the sesame oil–treated group appeared similar to that of the control group (Fig. 3 A–D). Seminiferous tubules and interstitial tissue showed normal architecture. Control sections revealed closely packed seminiferous tubules with narrow interstitial spaces containing Leydig cells and blood vessels. The testis was surrounded by a tunica albuginea of connective tissue and fibroblasts, underlain by a vascular tunica vasculosa. Seminiferous tubules were oval to round, lined by germinal epithelium composed of Sertoli and spermatogenic cells arranged from spermatogonia to spermatozoa. The DBF group told a different story. Under H&E, everything looked beat up. The seminiferous tubules had thin germinal linings, hardly any sperm, and loose cells floating in the lumen. Lots of spermatogenic and Sertoli cells showed empty vacuoles and shrunken, dark nuclei. Tubules showed serious damage with gaps, widened spaces, sparse or absent sperm and often degraded or absent lining with dead cells. Leydig cells were unhealthy with shrunken nuclei and vacuoles. Interstitium had pink hyaline material and dilated, bleeding vessels. The tunica albuginea was thick and fragmented. The addition of sesame oil improved the conditions greatly, the tunica albuginea was thinned to resemble more normal tissue, and most tubules were healthy with germinal epithelium and mature sperm, although some tubules still had wide spaces and vacuoles. Sertoli cells appeared nearly normal. The interstitium had normal vessels and less hyaline, and most Leydig cells appeared healthy, although some had vacuoles.

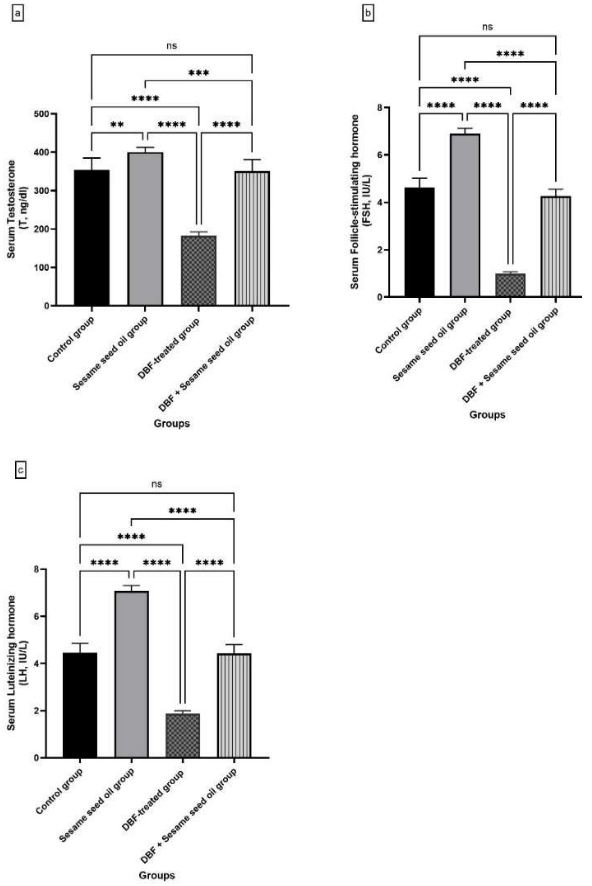

Fig. 2: The impact of sesame seed oil consumption on the hormonal profile of mature male rats that were fed dibenzofuran. The hormones measured include (a) Testosterone, (b) Follicle-stimulating hormone (FSH), and (c) Luteinizing hormone (LH). The data is presented as mean ± SEM. "ns" denotes non-significant, "**" indicates significance at P<0.01, and "****" signifies significance at P<0.0001.

Masson staining: Masson's trichrome staining showed that the control and sesame oil groups had very little blue-green collagen, which was limited to small deposits in the basement membranes of seminiferous tubules and blood vessels (Fig. 6A–B). In contrast, the DBF group had a large amount of collagen in the interstitium, tubule basement membranes and perivascular areas (Fig. 6C). When the DBF and sesame oil were given together, collagen deposition was reduced, with only mild interstitial and focal staining of the basement membrane and perivascular areas (Fig. 6D). Histomorphometric measurements were consistent with these observations.

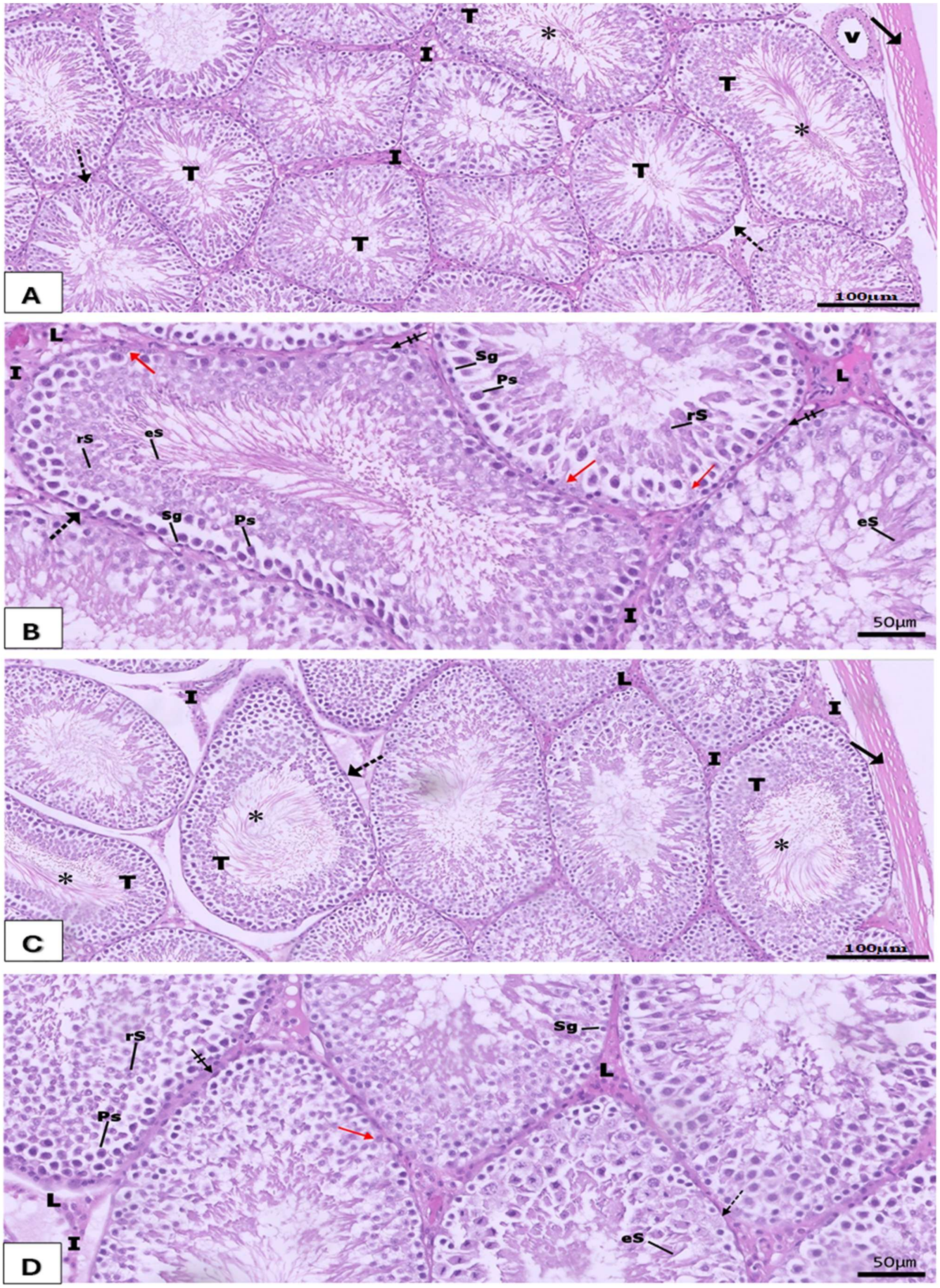

Fig. 3: Testicular section from (A&B) the control group and (C&D) the sesame seed oil group shows closely packed seminiferous tubules (T) with sperm (*) detected in the lumina and little interstitium in between (I). The testis was surrounded by the tunica albuginea (black arrow) and the tunica vasculosa, which contained blood vessels (v). The seminiferous tubules (T) are surrounded by a thin, regular basal lamina (dot arrow) and flattened peritubular myoid cells (double cross arrow). The tubules are lined with stratified germinal epithelium composed of spermatogonia (Sg), primary spermatocytes (Ps), rounded spermatids (rS), and elongated sperms (eS) towards the lumina. Sertoli cells (red arrow) are observed among the spermatogenic cells. Groups of Leydig cells (L) are detected in the interstitium (I). (H&E A &Cx10, B&D x20).

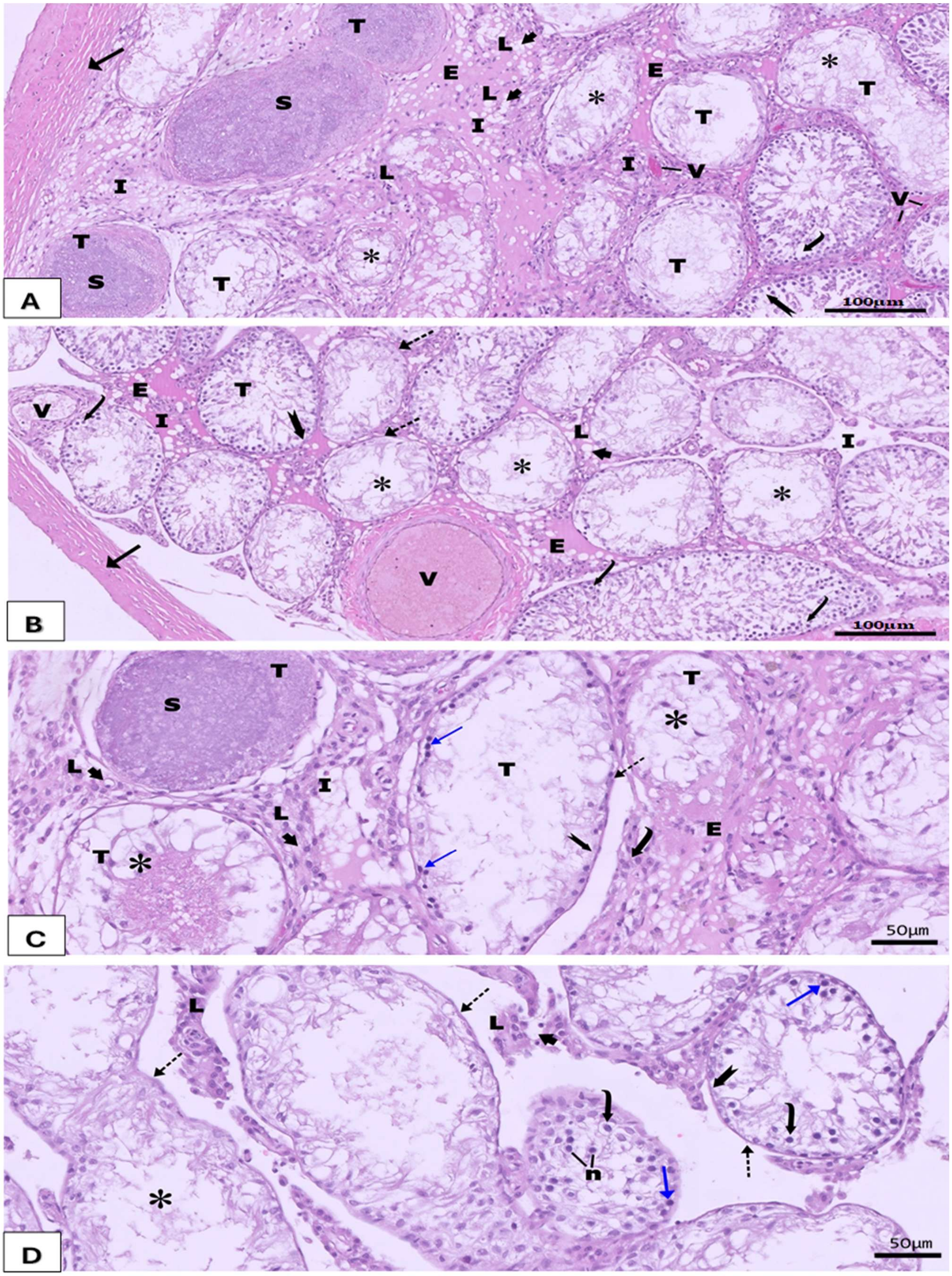

Fig. 4: Testicular section from the DBF-treated group shows massive destructive alterations of the seminiferous tubules (T) with disappearance of almost all germinal epithelium (*) with thickened basal lamina (dotted arrow). Some seminiferous tubules (T) reveal distorted spermatogenic and Sertoli cells (blue arrow) with cytoplasmic vacuolation and pyknotic nuclei. Some seminiferous tubules (T) depict intercellular vacuoles (curved arrow). Some areas are completely depleted of germinal epithelium (bifid arrow). Nuclear pyknosis and cytoplasmic vacuolation (arrowhead) in Leydig cells (L) are noticed. Other seminiferous tubules (T) are replaced by necrotic cells (S). Eosinophilic hyaline material (E) and dilated, congested blood vessels (V) with hemorrhage are observed in the widened interstitium (I). Notice that the testicular sections show a thick, separated tunica albuginea (black arrow). (H&E A&B x10, C&D x20).

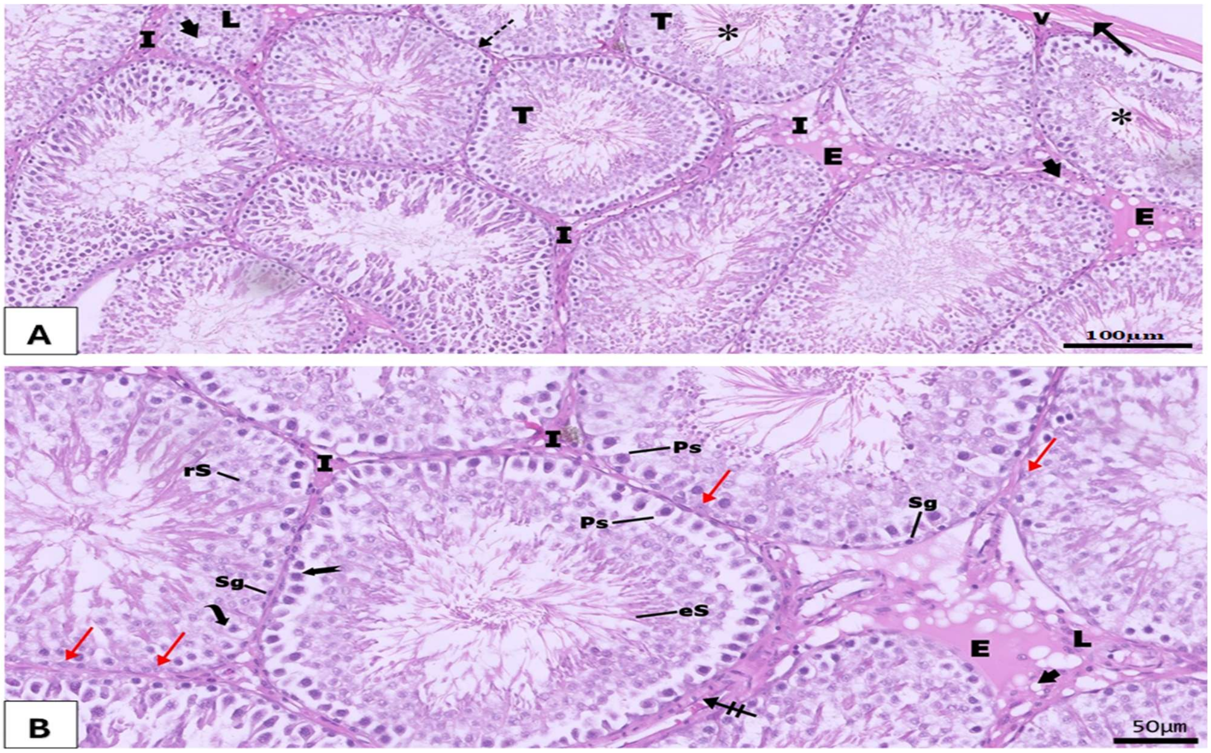

Fig. 5: A photomicrograph of a section in the testis of the DBF + sesame oil-treated group showing apparently normal oval to rounded seminiferous tubules (T) with regular intact basal lamina (dotted arrow) and normal different stages of spermatogenesis: spermatogonium (Sg), primary spermatocytes (Ps), rounded spermatids (rS), and elongated sperms (eS) towards the lumina. The testis was surrounded by apparently normal tunica albuginea (black arrow) with small blood vessels (V). Abundant sperm are detected in the lumina (*). Sertoli cells (red arrow) are observed among the spermatogenic cells. Notice some widened intercellular spaces (bifid arrow), and some vacuolated cells (curved arrow) are seen. Notice the little interstitium (I) in between the tubules with some eosinophilic hyaline material (E). Clusters of Leydig cells (L) can be seen in between the seminiferous tubules in the interstitium (I), while some of them appear with vacuolated cytoplasm (arrowhead), and some hyaline material (E) is detected within the interstitium (H&E Ax10, Bx20).

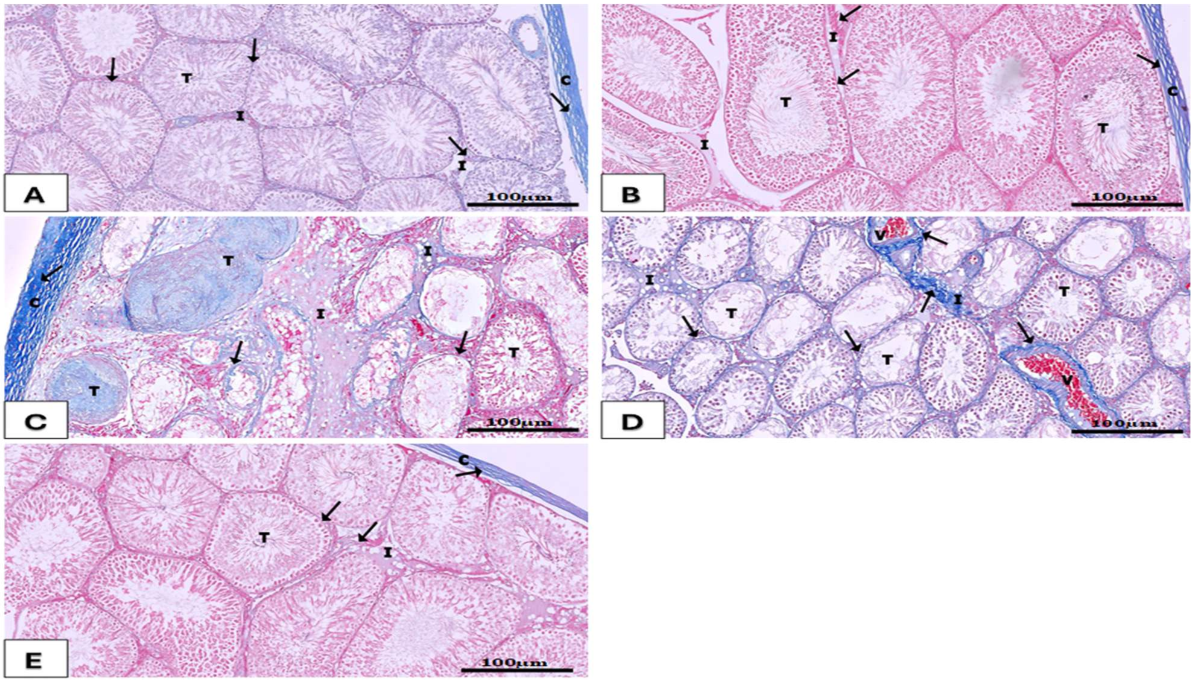

Fig. 6: Masson trichrome-stained Testicular section from:(A): control group and (B) the sesame seed oil group show a minimal amount of bluish-green stained collagen fibers (↑) in the connective tissue capsule (C) and around the blood vessels (V), in the basal lamina of seminiferous tubules (T), and normal interstitial tissue (I) with absent collagen fibers. (C) & (D): The dibenzofuran-treated group shows a massive increase in collagen fiber (↑) deposition in the connective tissue capsule (C) and around dilated congested interstitial blood vessels (v), in the basal lamina of seminiferous tubules (T), and the interstitial tissue (I). (E): The dibenzofuran + sesame oil-treated group shows a moderate amount of collagen fibers (↑) in the basal lamina of seminiferous tubules (T), interstitium (I), and around the blood vessels (V). Notice minimal collagen fibers in the connective tissue capsule (C). (Masson's trichrome stain A-Ex20).

Johnsen index: Besides the Johnsen index, the study assessed some quantitative parameters. The mean diameter of seminiferous tubules and the height of germinal epithelium were significantly reduced in DBF treated rats compared to controls and sesame oil group (P<0.0001). Co-administration of sesame oil + DBF significantly restored these measures (P<0.0001), returning them close to control values without significant differences (diameter: P=0.3658; height: P=0.1295; Figs. 7a,b). The spermatogenic index showed a similar pattern, declining sharply in the DBF group (P<0.0001), with many tubules scoring 1 or 2, indicating absence or near absence of germ cells. In contrast, the DBF-plus-sesame-oil group showed scores in the 8–9 range, comparable to controls and sesame oil alone (P=0.0919 and P=0.2509; Fig. 7c).

Fibrosis, quantified as the percentage of collagen area on Masson's trichrome stained sections, was significantly increased in DBF treated animals compared to controls (P<0.0001). Co-administration of sesame oil with DBF resulted in a significant reduction in collagen accumulation compared to DBF alone (P<0.0001), with no significant difference from controls (P=0.1364; Fig. 7d).

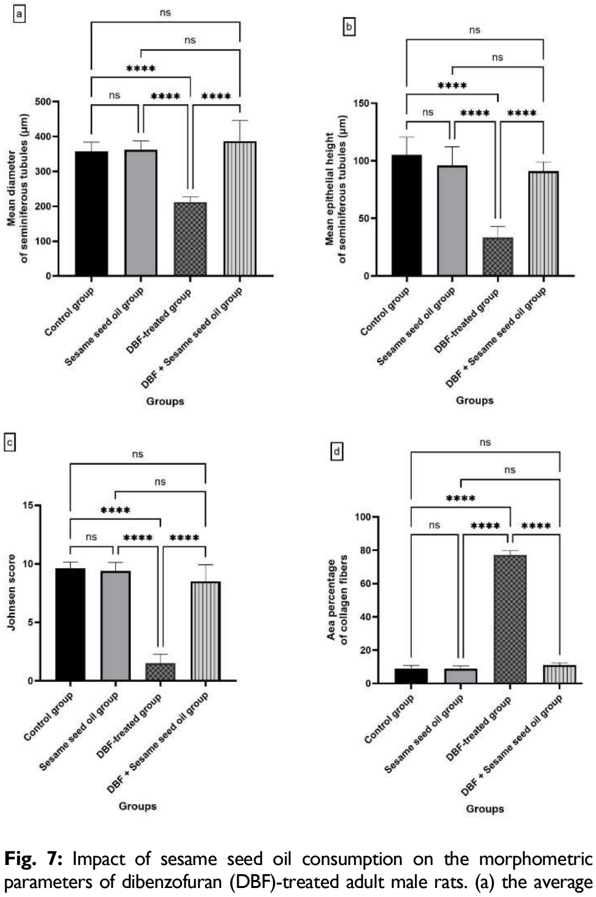

Fig. 7: Impact of sesame seed oil consumption on the morphometric parameters of dibenzofuran (DBF)-treated adult male rats. (a) the average diameter of seminiferous tubules, (b) the average height of germinal epithelium, (c) the Johnsen score (spermatogenic index), and (d) the average area % of collagen fibers. The data is presented as mean ± SEM. "ns" denotes non-significant, while "****" indicates significance at P<0.0001.

DISCUSSION

Male infertility is increasingly acknowledged as an important global health issue and declining semen quality has been reported in recent studies. Decreases in sperm concentration and total sperm count have been linked to increasing exposure to environmental pollutants and an increase in testicular cancer incidence (Gao *et al.*, 2015; Ali *et al.*, 2020). This study is concerned with dibenzofuran (DBF), and its derivatives; polychlorinated dibenzofurans (PCDFs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). These compounds differ in chemical structure, chlorine substitution patterns, potency and metabolism. Of the 210 PCDD/F congeners, those chlorinated in 2,3,7,8 positions – especially TCDD – have the strongest binding to the aryl hydrocarbon receptor (AhR) and the greatest toxicity. Altering or removing these chlorines markedly reduces toxicity (Patrizi and Cumis, 2018; Tuomisto, 2019).

In contrast, unsubstituted DBF does not conform to the classical profile of dioxin-like congeners, and it is inappropriate to assign it the same toxic equivalency factor (TEF) or to assume comparable dose-response effects to those of chlorinated PCDD/Fs (Szymańska *et al.*, 2020).

Therefore, comparisons between DBF and TCDD results should be interpreted as mechanistic parallels via the AhR pathway, indicating similar reproductive and endocrine disruptions without implying equivalent exposure levels or toxic potency (Zubair and Adrees, 2019).

TCDD or PCDF are a distinct congeners that their effects are normalized to TCDD via TEFs and TEQs (Zubair and Adrees, 2019; Szymańska *et al.*, 2020), and clarify that any extrapolation to DBF is inferential and constrained by differences in structure, potency, and kinetics. This aligns with current findings from established risk assessment frameworks for PCDD/Fs and assists in preventing the overgeneralization of high-potency TCDD models to the specific DBF exposure evaluated in the stuy of Zubair and Adrees (2019) and Szymańska *et al.*, (2020).

The existence of SOD and CAT is essential for the defence system in the male reproductive tract (Dias *et al.*, 2020). In this study, DBF treated group downregulated the activity of antioxidant enzymes, while increasing MDA levels. Excessive reactive oxygen species damage sperm by attacking the polyunsaturated fatty acids in the sperm membrane, leading to lipid peroxidation (Ijaz *et al.*, 2020; Nowicka-Bauer and Nixon, 2020; Ortega-Ferrusola *et al.*, 2019). The combined treatments of sesame EOs +DBF improved the antioxidant activity of the testis. This may be due to the presence of bioactive lignans (sesamin, sesamolinol) and other compounds in sesame oil that scavenge free radicals and inhibit lipid peroxidation (Borai *et al.,* 2017; Al-Attar *et al.,* 2018; Borai *et al.,* 2019; Altyar *et al.,* 2024). Pretreatment with sesame oil was found to protect against oxidative stress by acting as an effective free-radical scavenger, chelating metals, and preventing lipid peroxidation (Al-Attar *et al.,* 2018).

The exposure to lufenuron or copper sulfate ramps up lipid peroxidation (TBARS/MDA) and ROS, while tanking SOD, CAT, and glutathione levels in blood or gills exactly the same kind of MDA rise and SOD/CAT drop you see here with DBF (Naz *et al.*, 2023; Al-Saeed *et al.*, 2023). Those researchers specifically call TBARS/MDA a byproduct of PUFA peroxidation and use the drop in SOD/CAT to define oxidative stress as the main driver of toxicity (Al-Saeed *et al.*, 2023).

The above studies provide a mechanistic basis for sperm membrane damage and redox imbalance. It was observed that combined arsenic trioxide and antimony exposure causes abnormal autophagy and apoptosis of the testis with increased levels of Beclin 1, Atg 5, LC3B/LC3A, p53, Cyt c, caspase 8/3, and Bax and decreased Bcl 2, resulting in compromised sperm quality and testosterone levels (Wu *et al.*, 2021). Similarly, butachlor exposure to the liver increases the expression of Beclin 1, ATG 5, LC3, Apaf 1, Bax, caspase 3/9, p53, and Cyt c and decreases the expression of Bcl 2 and p62. This associates oxidative stress with mitochondria- and death receptor–mediated apoptosis, as well as disrupted autophagy (Yang *et al.*, 2021). These pathways help explain observations such as germ cell loss, pyknotic nuclei, and collagen deposition as outcomes of reactive oxygen species driving mitochondrial and endoplasmic reticulum stress.

Lufenuron and CuSO4 cause dose- and time-dependent damages such as degeneration, necrosis, oedema, congestion of blood vessels and fibrosis in the liver, kidney, gill, brain and heart (Naz *et al.,* 2023; Al-Saeed *et al.,* 2023). These pathological changes have been found to have a strong relationship with biochemical and oxidative markers. The involvement of multiple organs suggests that long-lasting redox imbalance and inflammatory stress cause testicular atrophy, interstitial fibrosis and altered seminiferous architecture.

Some copper exposures can increase antioxidant activities in a dose-, duration- and water chemistry-dependent manner (Naz *et al.,* 2023; Al-Saeed *et al.,* 2023). Fish and rodent sensitivity and target organs differ, making extrapolation to humans problematic (Wu *et al.,* 2021). There is potential for significant oxidative and reproductive toxicity even at low environmental doses, complicating the establishment of safe regulatory limits and the rationale for antioxidant treatments (Yang *et al.,* 2021).

Spermatogenesis is regulated by the hypothalamic–pituitary–gonadal (HPG) axis (Ni *et al.*, 2019; Ijaz *et al.*, 2022a; Ijaz *et al.*, 2022b; Hamza *et al.*, 2023). GnRH stimulates the release of FSH and LH, which are essential for Sertoli cell function and Leydig cell testosterone production, respectively. In our experiment, DBF exposure was associated with reduced gonadotropin and androgen levels, suggesting disruption of the HPG axis. Inadequate FSH compromises Sertoli cell support of germ cells, whereas reduced LH limits Leydig cell–mediated testosterone synthesis, thereby impairing spermatid development and sperm production (Ramaswamy and Weinbauer, 2014; Ijaz *et al.*, 2023). Sesame seed oil co-treatment partially normalized FSH, LH, and testosterone levels, consistent with previous studies suggesting that sesame oil may modulate neuroendocrine pathways involved in GnRH and gonadotropin secretion (AL-Sallami, 2017).

The observed increase in LH and testosterone in the sesame oil–treated groups may be related to the high content of unsaturated fatty acids in sesame oil, which have been implicated in supporting steroidogenesis and testicular function (Zirkin and Papadopoulos, 2018; Lei *et al.*, 2025). However, the precise molecular mechanisms remain unclear, and direct effects on specific protein receptors or ligands cannot be concluded from the present data and therefore are not claimed here (Shittu Lukeman *et al.*, 2008).

Under the microscope, DBF showed effects on the germinal epithelium thinned out, spaces between cells widened, dead cells peeled off into the tubule lumen, and plenty of cells had those shrunken, dark pyknotic nuclei classic signs of apoptosis in spermatogonia and early spermatocytes (Johnson, 2014; Sharma *et al.*, 2023; Zhou *et al.*, 2025). DBF also increased collagen deposits and altered tubule size, which points to both failed sperm production and remodeling of the surrounding tissue. All of that fits with what's known about how spermatogenesis depends on androgens, and what happens when testosterone drops and oxidative stress kicks in (Ramaswamy and Weinbauer, 2014; Celik *et al.*, 2017; Anan *et al.*, 2017).

The addition of sesame oil altered the overall outcome. It mitigated most of the damage, resulting in an improved tubule structure, reduced collagen accumulation, and an enhanced spermatogenic index. Previous studies have correlated such protective effects with two primary factors: sesame oil's capacity to combat fibrosis, partly through the inhibition of fibroblast proliferation and TGF-β1 signaling, and its capacity to support androgen production, attributed to its unsaturated fatty acids. (Ali *et al.*, 2020; Ibrahim and Sadek, 2022).

DBF exposure caused serious damage to the testes. The germinal epithelium got thinner, gaps opened up between cells, dead cells sloughed off into the tubule lumen, and lots of cells had those small, dark pyknotic nuclei textbook signs that spermatogonia and early spermatocytes were undergoing apoptosis (Johnson, 2014; Sharma *et al.,* 2023; Zhou *et al.,* 2025). DBF also enhanced collagen deposition and changed the size and shape of the seminiferous tubules, which fits with failed sperm production and remodeling of the tissue around the tubules. None of that is surprising spermatogenesis depends heavily on androgens, and when testosterone drops and oxidative stress hits, this is exactly the kind of structural fallout you'd expect (Ramaswamy and Weinbauer, 2014; Anan *et al.,* 2017; Celik *et al.,* 2017).

Adding sesame oil attenuates the tissue damage, kept the tubule architecture in better shape, reduced the collagen buildup, and improved the spermatogenic index. Earlier studies have attributed similar protective effects to two factors: sesame oil's ability to fight fibrosis (partly by putting the brakes on fibroblast growth and TGF-β1 signaling) and its support for androgen production, thanks to the unsaturated fatty acids it's packed with Ali *et al.,* (2020) and Ibrahim and Sadek, (2022).

Conclusions: Dibenzofuran exposure caused significant testicular damage in adult male albino rats. Hormone levels, testosterone, LH, and FSH were distributed, oxidative stress markers surged, and testicular tissue showed clear structural deterioration. TDBF acts as both an endocrine disruptor and a pro-oxidant in the male reproductive system. On the other hand, supplementing with sesame seed oil made a striking difference. It helped restore hormonal equilibrium, boosted antioxidant defenses (SOD and CAT), brought down lipid peroxidation (MDA), and markedly improved testicular architecture. Sesame seed EOs appear as a promising natural therapeutic agent for ameliorating DBF-induced reproductive toxicity, mainly via its antioxidant and tissue protective properties.

However, more studies are required to elucidate the underlying molecular mechanisms and to explore the possibility of clinical applications.

Authors contribution: Sahar Jameel Melebary: Conceptualization, visualization, methodology, writing the original draft, writing-review, and editing.

Declaration of interests: The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgments: This work was funded by the University of Jeddah, Jeddah, Saudi Arabia, under grant No. (UJ-25-DR-2225). Therefore, the author thanks the University of Jeddah for its technical and financial support.

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