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Therapeutic Potential of *Nigella sativa* Oil against Phthalate-induced Testicular Damage in Adult Wistar Rats

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ABSTRACT

Di-butyl phthalate (DBP) is a widely distributed environmental toxicant known to compromise male reproductive function through oxidative and inflammatory damage to testicular tissue. Nigella sativa oil (NSO), a natural compound with established antioxidant and anti-inflammatory properties, has been proposed as a potential protective agent against such toxicity. This study investigated the protective effects of NSO on DBP-induced testicular injury in adult male Wistar rats. Forty rats were randomly assigned to five groups: control, NSO-only (2 mL/kg), DBP-only (500 mg/kg), NSO plus DBP, and DBP withdrawal. Treatments were administered for 35 days. Testicular histomorphology was assessed using hematoxylin and eosin and periodic acid–Schiff staining, while oxidative stress (malondialdehyde, glutathione peroxidase, reduced glutathione), inflammatory markers (myeloperoxidase, interleukin-10), and reproductive hormones (testosterone, luteinizing hormone, follicle-stimulating hormone) were quantified. DBP exposure resulted in severe testicular degeneration, including seminiferous tubular disruption, germ cell loss, and basement membrane thickening, accompanied by increased malondialdehyde and myeloperoxidase levels, reduced glutathione peroxidase and interleukin-10, and altered antioxidant balance ($p < 0.05$). Co-administration of NSO significantly attenuated these changes by preserving testicular architecture, reducing oxidative and inflammatory markers, and enhancing antioxidant defenses. Testosterone levels were significantly elevated in the NSO-treated group compared with controls, while luteinizing and follicle-stimulating hormones remained unchanged. Although DBP withdrawal led to partial recovery, residual oxidative and inflammatory damage persisted. These findings revealed that NSO demonstrates significant protective potential against DBP-induced testicular toxicity through antioxidant, anti-inflammatory, and hormonal modulatory mechanisms.

Keywords: *Nigella sativa* oil, di-butyl phthalate, oxidative stress, testosterone, histopathology, antioxidants

INTRODUCTION

Phthalates are a group of synthetic chemicals widely used as plasticizers to enhance the flexibility, durability, and longevity of polyvinyl chloride (PVC) and other plastic product[1]. These compounds are present in a vast array of industrial and consumer goods, including medical devices, packaging materials, flooring, wall coverings, toys, food containers, cosmetics, and personal care products[2,3]. Among the most commonly encountered phthalates are diethylhexyl phthalate (DEHP), diisononyl phthalate (DINP), dibutyl phthalate (DBP), and diethyl phthalate (DEP), many of which are not covalently bound to plastics, thereby allowing for environmental leaching and human exposure[4,5].

Dibutyl phthalate (DBP), in particular, is a colorless or pale-yellow ester compound with widespread industrial use. It is found in adhesives, printing inks, paints, personal care products, and plastic consumer goods[6]. Due to its lipid solubility, DBP can easily penetrate biological membranes and accumulate in tissues, posing potential risks to human and animal health[7]. Of notable concern is its effect on the male reproductive system, where it has been shown to disrupt endocrine functions and impair testicular architecture and spermatogenesis[8]. Such disruptions are often mediated by oxidative stress pathways, resulting in elevated levels of reactive oxygen species (ROS) that overwhelm the body's antioxidant

defenses, thereby damaging lipids, proteins, and DNA within testicular tissue.

The use of natural antioxidants to mitigate such chemically induced reproductive toxicity has garnered significant scientific interest. *Nigella sativa* (Family: Ranunculaceae), commonly known as black cumin or black seed, is a medicinal plant traditionally revered across Middle Eastern, Asian, and North African cultures for its broad pharmacological activities[9]. The oil derived from *Nigella sativa* seeds contains potent bioactive constituents such as thymoquinone, which exhibit strong antioxidant, anti-inflammatory, immunomodulatory, and cytoprotective properties[10]. Historically, *Nigella sativa* oil has been used in the management of numerous ailments, including asthma, hypertension, gastrointestinal disturbances, diabetes, and infertility. Emerging evidence also suggests its potential protective role against chemical-induced organ toxicity, including testicular damage.

Given the increasing global concern over environmental endocrine disruptors and reproductive health, the present study investigates the therapeutic efficacy of *Nigella sativa* oil in ameliorating testicular damage induced by dibutyl phthalate (DBP) in adult Wistar rats. By evaluating oxidative stress parameters, histological changes, and reproductive markers, this study aims to provide insight into the protective mechanisms of *Nigella sativa* and its potential application as a natural remedy against phthalate-induced reproductive toxicity.

MATERIALS AND METHODS

Ethical approval

All experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Al-Hikmah University, Ilorin, Nigeria, with ethical approval number HUI-FHS-ERC-25-0039, in accordance with international guidelines for the care and use of laboratory animals.

DBP and NSO

The two primary experimental substances utilized in this study were *Nigella sativa* oil (NSO) and dibutyl phthalate (DBP). *Nigella sativa* oil was manufactured by Hermani International KPZ, Karachi, Pakistan, while DBP was sourced from Central Drug House (P) Ltd., New Delhi, India (Batch No: 171019). Both substances were procured from certified distributors in Lagos, Nigeria.

Animals and experimental design

This experimental study was conducted in 2024 at the Animal Research facility, Department of Human Anatomy, Faculty of Health Sciences, Al-Hikmah University, Ilorin, Nigeria. All animal handling procedures were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC), ensuring humane treatment and ethical use of laboratory animals throughout the study. A total of forty (40) healthy male Wistar rats, weighing between 160 g and 244 g, were obtained from the animal breeding facility of the University of Ilorin. The animals were housed in well-ventilated plastic cages within the animal holding facility of Al-Hikmah University under standard laboratory conditions. They were maintained on a 12-hour light/dark cycle, fed commercial pelleted feed ad libitum, and provided unrestricted access to clean drinking water. All rats were acclimatized for one week before the commencement of the experiment. During acclimatization, cage cleaning was performed regularly, sawdust was replaced, and any notable physical changes were recorded. Following acclimatization, the animals were randomly assigned into five groups (n = 8 per group) as follows:

Group I (Control); Received normal saline, Group II (NSO only); Administered *Nigella sativa* oil at 2 mL/kg body weight, Group III (DBP only); Administered dibutyl phthalate at 500 mg/kg body weight, Group IV (NSO + DBP); Co-administered *Nigella sativa* oil (2 mL/kg) and DBP (500 mg/kg), Group V (DBP Withdrawal); Administered DBP (500 mg/kg) and left untreated for a withdrawal period. The DBP withdrawal group was included to distinguish between spontaneous recovery following toxicant cessation and the therapeutic effects of *Nigella sativa* oil. All substances were administered orally using an appropriately sized oral cannula. Each rat was carefully restrained by positioning the neck between the index and middle fingers while supporting the lower body with the palm. This positioning allowed accurate delivery of the treatment without choking or damaging the trachea. Special care was taken to minimize stress and avoid injury, particularly in agitated or vulnerable animals. The experimental period lasted for 35 days (5 weeks) for all groups except for the DBP withdrawal group, which lasted for 21 days.

Body and organ weight measurements

Body weights of the animals were recorded biweekly using a non-digital mechanical scale with a pre-tared weighing basin to ensure accuracy. Upon sacrifice, the testes and associated tissues were excised and weighed using a digital precision scale (Mini Electronic Balance, capacity: 1000 g; accuracy: 0.1 g) to evaluate any changes attributable to treatment.

Euthanasia and tissue collection

At the end of the experimental period, all animals were humanely euthanized via cervical dislocation, in accordance with institutional ethical guidelines for the humane treatment of laboratory animals. Post-euthanasia, dissection was performed under aseptic conditions using sterile surgical instruments to harvest relevant tissues and organs for subsequent histological and biochemical analyses. Whole blood samples were collected immediately following euthanasia for the

evaluation of serum hormone levels. The carcasses were securely placed in sealed biohazard bags and disposed of by burial in a designated pit located at a safe distance from the animal housing facility. The euthanasia and dissection areas were thoroughly cleaned and disinfected, and all disposable materials, including gloves, sawdust, and surgical waste, were discarded following standard protocols for biohazard waste management.

Histopathological evaluation

Testicular samples from all experimental groups were harvested and immediately fixed in 10% neutral buffered formalin for a minimum of 48 hours to preserve tissue architecture. Following fixation, the tissues were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections of 5 µm thickness were obtained using a rotary microtome and mounted on clean glass slides. The sections were stained with p for routine histological assessment of testicular architecture, including seminiferous tubule organization, spermatogenic cell integrity, and interstitial tissue condition. Histological slides were examined under a compound light microscope (magnification 4× and 10× objectives) to identify morphological alterations such as seminiferous epithelial disruption, tubular atrophy, degeneration of spermatogenic lineage, and Leydig cell density. Photomicrographs were captured using a calibrated digital camera (AmScope imaging system) mounted on the microscope. All evaluations were performed in a blinded fashion by independent observers to ensure unbiased interpretation. This analysis was aimed at assessing the degree of testicular damage induced by dibutyl phthalate (DBP) and the potential protective or restorative effects *conferred by Nigella sativa oil.*

Hormonal assays

Serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were measured using commercially available sandwich ELISA kits following standard protocols. Blood was collected via cardiac puncture, centrifuged at 3000 rpm for 10 minutes, and the serum was stored at –20°C until analysis. Hormone concentrations were determined based on standard curves generated from known concentrations and read at 450 nm using a microplate reader. All assays were conducted in duplicates, and results were expressed as mean ± SEM, with statistical significance set at $p < 0.05$.

Assessment of oxidative stress and inflammation

Following sacrifice, testicular tissues were excised, rinsed, and homogenized in ice-cold 0.25 M sucrose solution. The homogenates were centrifuged to obtain supernatants for biochemical assays. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the TBARS assay, where absorbance at 532 nm indicated oxidative damage. Glutathione peroxidase (GPx) activity was determined by monitoring NADPH oxidation at 340 nm, reflecting enzymatic antioxidant capacity.

Additional markers, including reduced glutathione (GSH), interleukin-10 (IL-10), and myeloperoxidase (MPO), were quantified. GSH levels were estimated colorimetrically using DTNB at 412 nm. IL-10, an anti-inflammatory cytokine, was measured using ELISA, while MPO activity, indicating inflammation, was evaluated by the oxidation of o-dianisidine in the presence of H_2O_2 and measured at 460 nm. These assays collectively assessed oxidative stress, antioxidant defense, and inflammation in the testicular tissue following phthalate exposure and *Nigella sativa* oil treatment.

Statistical analysis

Quantitative data obtained from biochemical, histological, and morphometric evaluations were statistically analyzed using ezANOVA software. One-way analysis of variance (ANOVA) was employed to determine significant differences among the experimental groups, and where significant differences were detected, Tukey's post-hoc multiple comparison test was applied to identify specific group differences. All data were expressed as mean ± standard error of the mean (SEM), and statistical significance was established at a threshold of $p< 0.05$. Results were graphically represented using bar charts, with error bars indicating variability (SEM) within each group, to visually support the statistical outcomes.

RESULTS

Physical observations

Throughout the study, clear physical and behavioral differences were noted among groups. Control and *Nigella sativa* oil (NSO)-treated rats remained active, alert, and healthy, showing normal grooming behavior and consistent weight gain. In contrast, DBP-exposed rats exhibited signs of lethargy, weakness, weight loss, and social withdrawal, indicating systemic toxicity. Rats co-treated with DBP and NSO showed moderate improvements in behavior and activity compared to the DBP-only group, suggesting a partial protective effect of NSO. Similarly, rats in the DBP withdrawal group initially showed DBP-related symptoms but gradually regained weight and activity post-exposure.

Body and organ weights

The study showed that DBP exposure significantly reduced body and testicular weights in male Wistar rats. Rats in the DBP group experienced notable weight loss and weakness. In contrast, control and NSO-only groups maintained consistent weight gain. Interestingly, the DBP withdrawal group demonstrated a marked rebound in weight gain after treatment ceased, suggesting partial recovery (Figure 1).

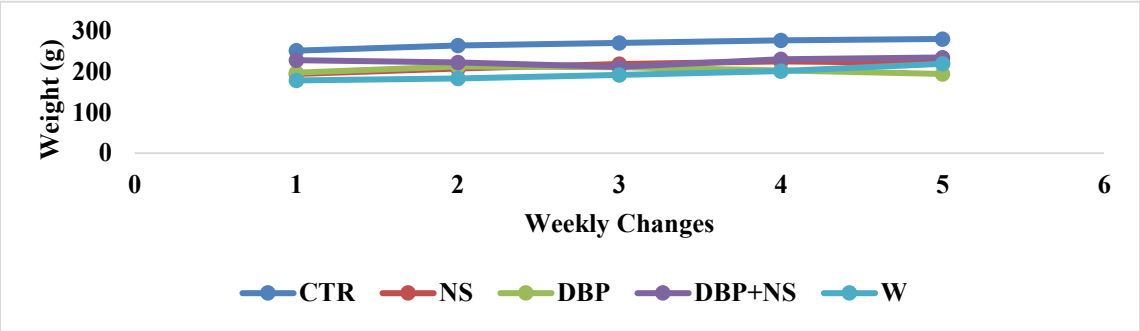

Figure 1: Effects of DBP and NSO on the Body Weights. CTR- control, NS- *Nigella sativa* oil, DBP- Dibutyl-phthalates, W- withdrawal.

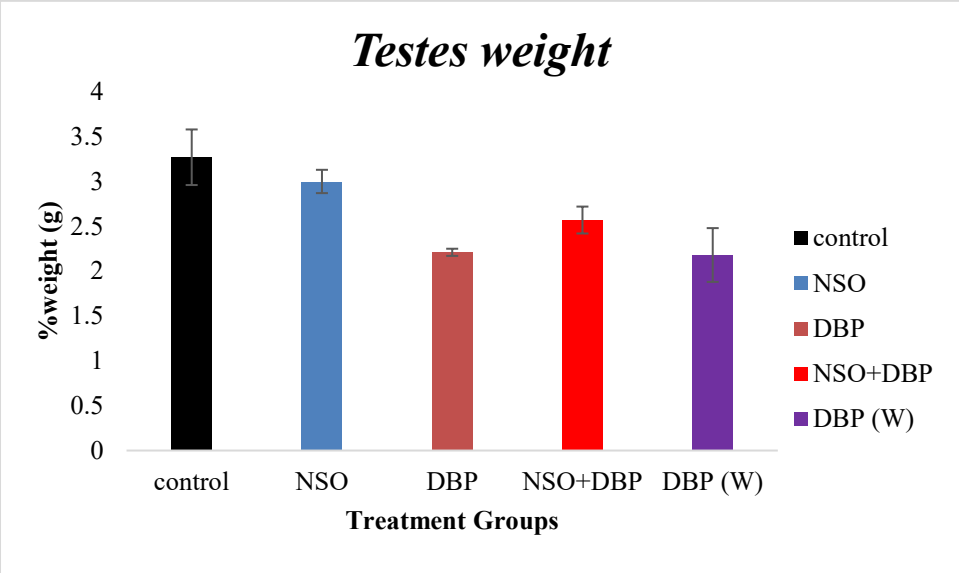

Figure 2: Effects of DBP and NSO on the Testicular Organ Weights. CTR- control, NS- *Nigella sativa* oil, DBP- Dibutyl-phthalates, W- withdrawal. *(P<0.05) compared to control group. ±(P<0.05) compared to the *Nigella sativa* group.

Co-administration of *Nigella sativa* oil significantly attenuated DBP-induced weight loss, as rats in the NSO + DBP group exhibited greater weight gain compared with the DBP-only group. The DBP withdrawal group exhibited initial weight loss followed by a rebound post-treatment, though still below control levels. Statistically significant differences in body weight were observed between the DBP-only group and the control, NSO-only, NSO+DBP, and DBP withdrawal groups. While co-administration of *Nigella sativa* oil with DBP significantly improved testicular mass, rats in the DBP withdrawal group, where DBP administration was discontinued after an initial exposure period and no therapeutic intervention was provided, failed to show a statistically significant recovery in testicular weight (Figure 2).

Histopathological assessment of testes

Histological assessment of testicular sections revealed marked group-dependent differences in seminiferous tubule architecture and spermatogenic activity. The control group exhibited normal testicular histology, characterized by intact seminiferous tubules with

well-organized germinal epithelium, preserved basement membranes, and complete spermatogenic series extending to mature spermatozoa. Similarly, rats treated with Nigella sativa oil (NSO) alone demonstrated preserved seminiferous tubular structure with dense germinal epithelium and mildly enhanced spermatogenic activity, suggesting no adverse effects of NSO on testicular integrity (Figure 3).

In contrast, the dibutyl phthalate (DBP)-only group showed severe histopathological alterations, including distortion of seminiferous tubules, thickening of the basement membrane, marked depletion of germ cells, reduced tubular lumen diameter, and features consistent with tubular atrophy. These changes indicate profound impairment of spermatogenesis following DBP exposure. Co-administration of NSO with DBP resulted in notable histological improvement, evidenced by partial restoration of seminiferous tubule architecture, improved epithelial organization, and reappearance of spermatogenic cells compared with the DBP-only group. However, complete normalization was not achieved. The DBP withdrawal group demonstrated incomplete recovery, with persistent narrowing of seminiferous tubules and thinning of the germinal epithelium, indicating that cessation of DBP exposure alone was insufficient to fully reverse testicular damage (Figure 4).

Hematoxylin and eosin–stained testicular sections examined at ×400 magnification (Figure 5) revealed distinct histological differences among groups. The control and NSO-only groups (Figure 5a–b) showed normal seminiferous tubule architecture with intact germinal epithelium and active spermatogenesis. In contrast, the DBP-only group (Figure 5c) exhibited marked testicular damage, including seminiferous tubular disorganization, basement membrane thickening, vacuolation, reduced spermatogenic cells, and tubular atrophy. Co-administration of NSO markedly ameliorated these alterations, as shown in Figure 5d, with improved tubular architecture and reappearance of spermatogenic cells. The DBP withdrawal group (Figure 5e) demonstrated partial but incomplete recovery, characterized by narrowed tubular lumens and persistent histopathological abnormalities.

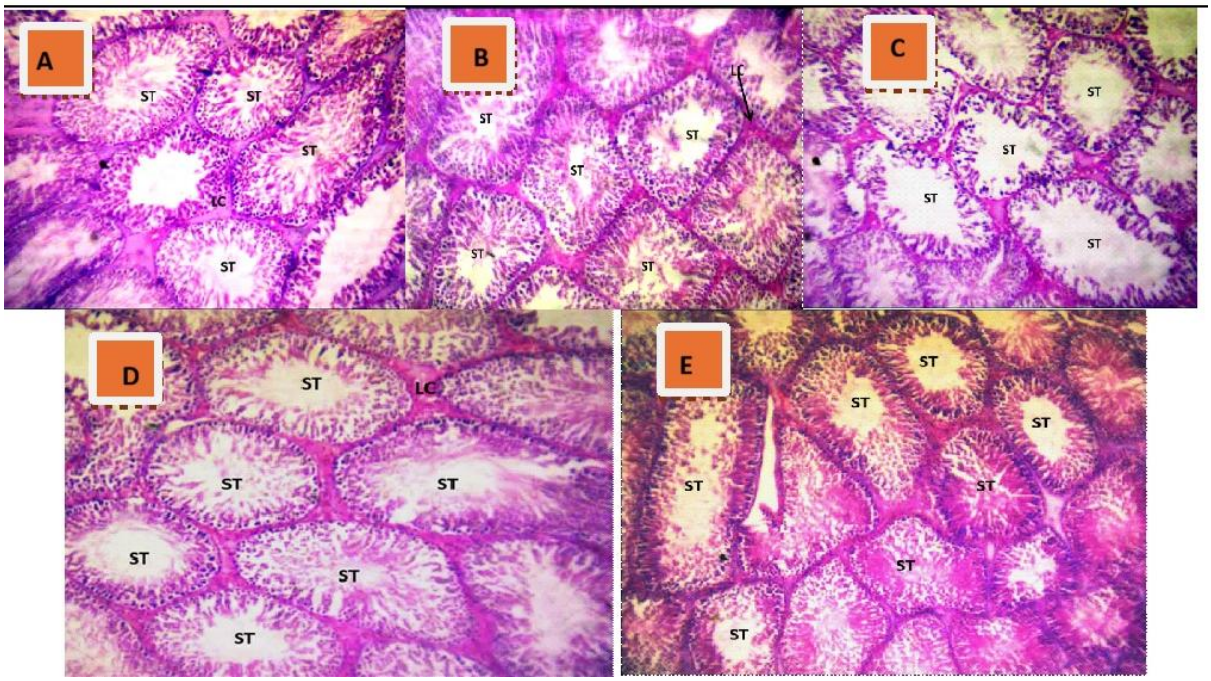

Figure 3: Representative Periodic Acid–Schiff–stained photomicrographs of rat testicular sections across experimental groups (×100). (a) Control, (b) NSO-only, (c) DBP-only, (d) NSO + DBP, and (e) DBP withdrawal groups. DBP: dibutyl phthalate; NSO: Nigella sativa oil; ST: seminiferous tubules; SG: spermatogonium; PSM: primary spermatocyte; SSM: secondary spermatocyte; S: spermatids; SZ: spermatozoa; SC: Sertoli cells; ICL: interstitial cells of Leydig.

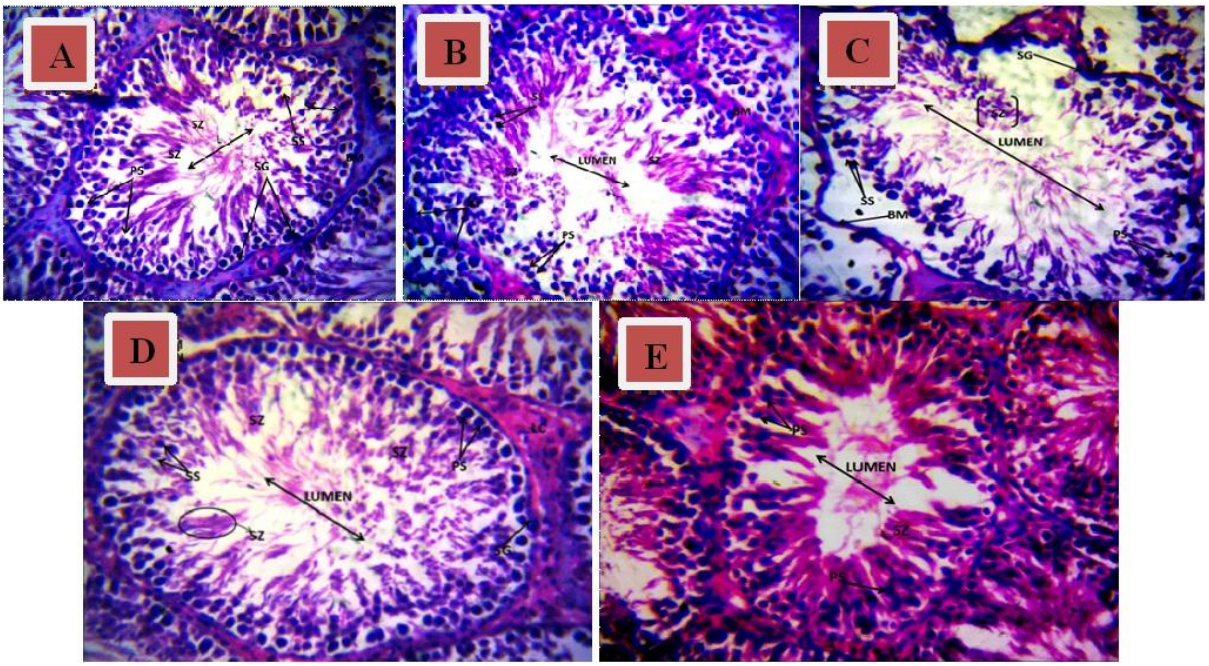

Figure 4: Light microscopic sections of testes from various examined groups: control (a), NSO only 2ml/kg bw (b), DBP only 500mg/kg bw (c), NSO+DBP 2ml/kg+500mg/kg bw (d), DBP withdrawal 500mg/kg bw (e) for five weeks. Sections (5 mm) were stained with Periodic Acid Schiff (x400). DBP: Dibutyl-phthalate; NSO: *Nigella sativa* oil, ST: Seminiferous Tubules, SG: Spermatogonium, SSM: Secondary spermatocyte, PSM: Primary spermatocyte, BM: Basement membrane, S: Spermatids, SZ: Spermatozoa, SC: Sertoli cells, ICL-Instertitial cells of Leydig.

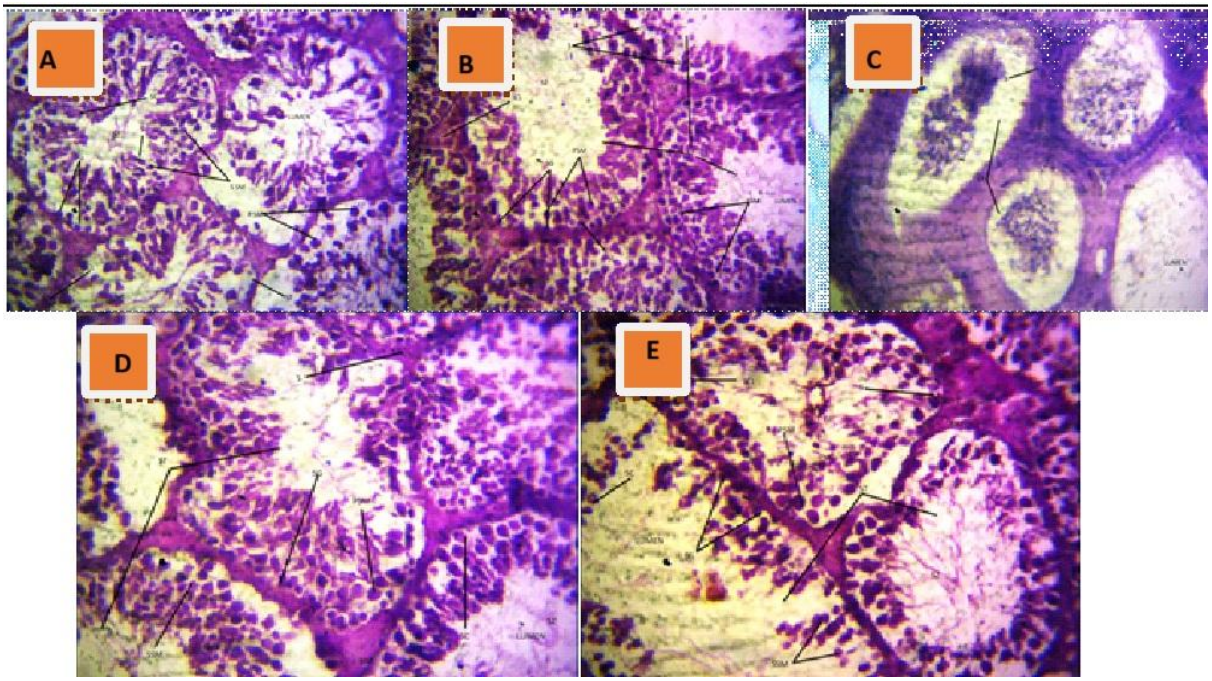

Figure 5: Representative hematoxylin and eosin–stained photomicrographs of rat testicular sections across experimental groups (×400). (a) Control, (b) NSO-only, (c) DBP-only, (d) NSO + DBP, and (e) DBP withdrawal. DBP: dibutyl phthalate; NSO: Nigella sativa oil; ST: seminiferous tubules; BM: basement membrane.

Hormonal assays

Serum testosterone levels differed markedly across groups (Figure 6). The DBP group showed significantly reduced testosterone (~1.5 ng/mL) compared to the control group (~2.0 ng/mL). The NSO-only group exhibited elevated levels (~2.5 ng/mL) relative to the control. Co-administration of NSO with DBP resulted in the highest testosterone concentrations (~3.0 ng/mL). The withdrawal group displayed partial recovery (~2.2 ng/mL), approaching control values.

Luteinizing hormone (LH) levels (Figure 7) and follicle-stimulating hormone (FSH) levels (Figure 8) remained unchanged across all groups, with LH at ~0.06–0.1 IU/mL and FSH at ~0.25–0.3 IU/mL. Notably, the DBP-induced decline in testosterone was not accompanied by elevations in LH or FSH. These results indicate that DBP exerted a direct suppressive effect on testicular testosterone production without altering pituitary gonadotropin secretion. At the same time, NSO effectively prevented DBP-induced hypogonadism and independently increased testosterone levels.

Assessment of oxidative stress and inflammation

DBP exposure markedly increases oxidative stress in the testes, as evidenced by elevated MDA levels. In the DBP + NSO group, MDA levels are lower. Interestingly, the DBP withdrawal group shows even higher MDA levels than the DBP-only group. Glutathione peroxidase (GPx) is a crucial antioxidant enzyme that reduces peroxides to protect cells from oxidative damage. The varying GPx levels across groups indicate different oxidative stress responses. The DBP group shows significantly increased GPx levels. In the NSO-only group, GPx levels are lower. However, the DBP + NSO group shows lower GPx levels than all groups, as the DBP withdrawal group shows elevated GPx. DBP exposure significantly altered oxidative and inflammatory markers in male Wistar rats. GSH levels were markedly elevated in the DBP group, while NSO mildly enhanced GSH, and while co-treatment (DBP + NSO) elevated GSH, it was less effective than DBP alone. The withdrawal group's GSH levels remained low relative to the control group. IL-10, an anti-inflammatory cytokine, decreased significantly in the DBP group. NSO slightly increased IL-10, and co-treatment restored IL-10 closer to baseline. However, the withdrawal group still had lower IL-10 than the control. MPO levels, a marker of neutropil-mediated inflammation, were significantly increased in DBP-exposed rats. NSO alone caused only a slight MPO increase, while DBP + NSO lowered MPO compared to DBP alone. Withdrawal led to a reduction but not to a return to baseline MPO.

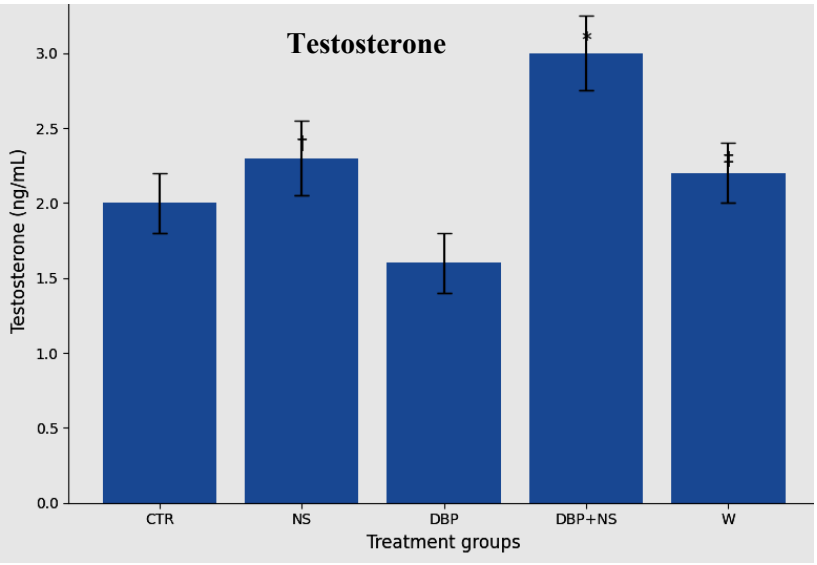

Figure 6: Testosterone levels across all experimental groups. CTR- control, NS- *Nigella sativa* oil, DBP- Dibutyl-phthalates, W- withdrawal. *(P<0.05) compared to control group. ±(P<0.05) compared to the *Nigella sativa* group.

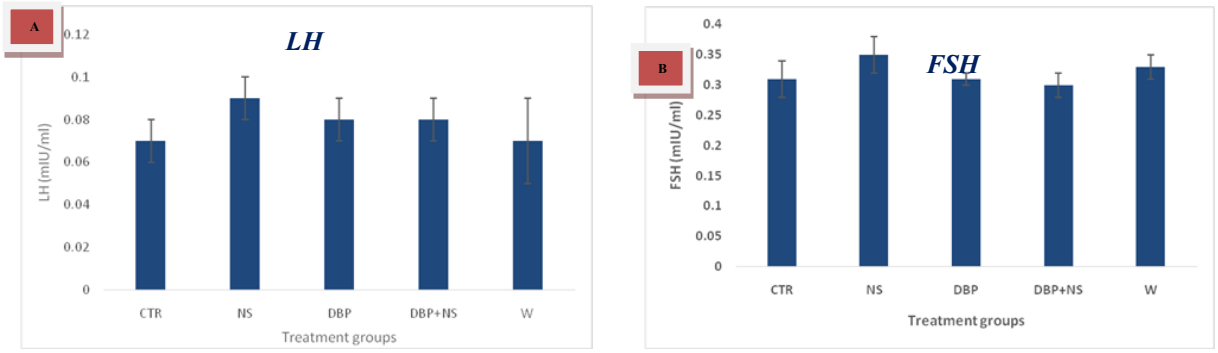

Figure 7: (a) Luteinizing Hormone (LH) levels across all experimental groups. (b) Follicle-stimulating hormone (FSH) levels across all experimental groups. CTR- control, NS- *Nigella sativa* oil, DBP- Dibutyl-phthalates, W-withdrawal. *(P<0.05) compared to control group. ±(P<0.05) compared to the *Nigella sativa* group**.**

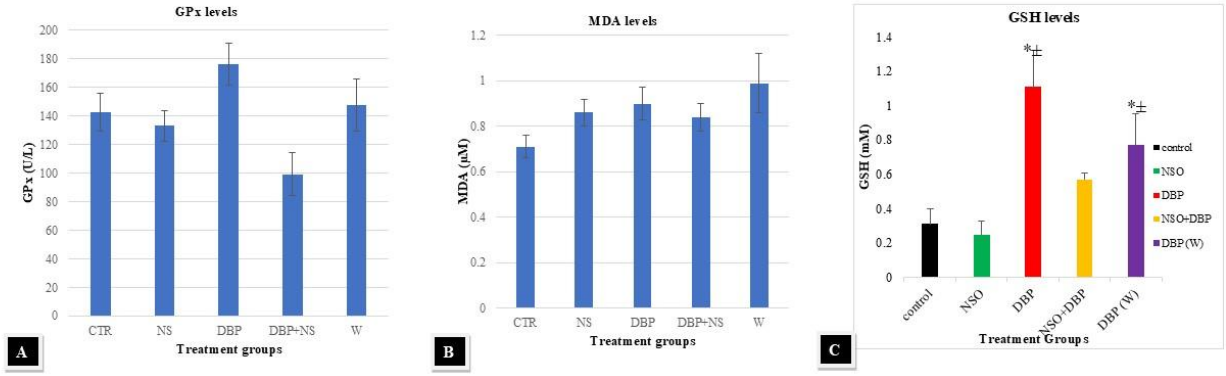

Figure 8. Testicular oxidative stress and antioxidant markers across experimental groups. (a) Glutathione peroxidase (GPx) activity, (b) Malondialdehyde (MDA) levels, and (c) Reduced glutathione (GSH) content in testicular homogenates. Groups: CTR (control), NS (*Nigella sativa* oil alone), DBP (dibutyl phthalate alone), DBP+NS (dibutyl phthalate + *Nigella sativa* oil co-treatment), W (withdrawal after DBP exposure). Data are presented as mean ± standard error. *P < 0.05 compared to control group; ±P < 0.05 compared to NS group.

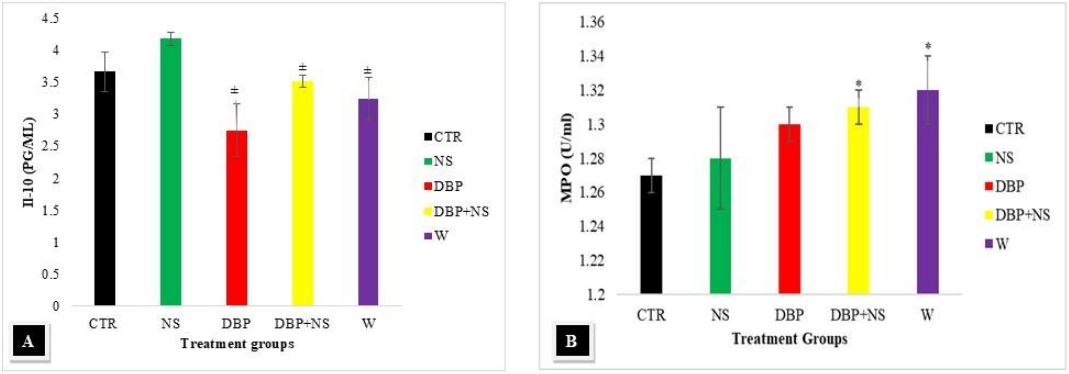

Figure 9. Testicular inflammatory markers across experimental groups. (a) Myeloperoxidase (MPO) activity and (b) Interleukin-10 (IL-10) levels in testicular homogenates. Groups: CTR (control), NS (*Nigella sativa* oil alone), DBP (dibutyl phthalate alone), DBP+NS (dibutyl phthalate + *Nigella sativa* oil co-treatment), W (withdrawal after DBP exposure). Data are presented as mean ± standard error. *P < 0.05 compared to control group; ±P < 0.05 compared to NS group.

DISCUSSION

The present study clearly establishes dibutyl phthalate (DBP) as a potent reproductive toxicant in male Wistar rats while demonstrating the remarkable protective efficacy of *Nigella sativa* oil (NSO). Exposure to DBP induced significant reductions in body and absolute testicular weights, reflecting its well-documented interference with anabolic processes and gonadal physiology through oxidative stress and endocrine disruption[11,12]. Although body weight partially recovered after DBP withdrawal, testicular weight remained depressed, indicating persistent damage to the germinal epithelium[13].

These microscopic changes were mirrored by profound histopathology in the DBP-only group, characterized by seminiferous tubule atrophy, germ cell depletion, thickened basement membranes, and poorly discernible Sertoli and Leydig cells, which are classic hallmarks of phthalate-induced testicular toxicity[14]. Co-administration of NSO largely prevented these lesions, preserving tubular architecture and cellular organization, whereas the NSO-only group even showed enhanced spermatogenic indices, consistent with its reported pro-fertility effects[15].

At the endocrine level, DBP markedly suppressed serum testosterone without eliciting the expected compensatory rise in LH or FSH. This pattern, although initially counterintuitive, is a recognized feature of phthalate toxicity: DBP and its analogs disrupt the hypothalamic–pituitary–gonadal axis at multiple sites, including inhibition of GnRH pulsatility and reduced pituitary responsiveness, thereby blunting gonadotropin secretion despite low circulating androgen levels[20–22]. Numerous rodent studies with DBP and DEHP have reported exactly this dissociation: significant testosterone decline with unchanged or even lowered LH/FSH[23]. Thus, the absence of gonadotropin elevation in the present work reflects targeted Leydig cell injury coupled with impaired central feedback rather than simple primary hypogonadism. NSO completely reversed DBP-induced hypotestosteronemia and, in both NSO-only and DBP+NSO groups, drove testosterone well above control values. Such supra-normal elevation is not uncommon when potent antioxidants neutralize toxicant-induced ROS, removing inhibitory brakes on steroidogenic enzymes (StAR, CYP11A1, 3β-HSD) and allowing rebound or enhanced activity[18,19].

Oxidative stress parameters further illuminated the mechanistic interplay. Contrary to some literature showing massive lipid peroxidation after phthalate exposure[31,32], MDA levels in the present study remained largely unaltered by DBP, suggesting that the dose and duration produced sub-catastrophic oxidative insult sufficient to impair function but not to overwhelm membrane defenses. Glutathione (GSH) content, however, was significantly increased in the DBP group, an adaptive response frequently observed during moderate oxidative challenge before eventual

depletion sets in at higher or more prolonged exposures[37,38]. The markedly lower GPx activity in the DBP+NSO group does not indicate antioxidant failure but rather successful ROS quenching by NSO's thymoquinone and related compounds, thereby reducing the need for peroxidase mobilization[36]. Higher GPx in DBP-only and withdrawal groups accordingly represents a compensatory enzymatic effort against lingering oxidants.

Inflammatory markers displayed equally nuanced patterns. The highest MPO activity in the NSO-only group likely reflects physiological immune surveillance enhancement by NSO rather than pathology. In contrast, the surprisingly low MPO in the DBP-only group may indicate impaired neutrophil recruitment or selective immunomodulation induced by phthalates[42,43]. Co-treatment normalized MPO toward control values, and IL-10, a key anti-inflammatory cytokine, was preserved or elevated by NSO, countering the mild suppression seen with DBP alone[39,40]. The withdrawal group trended toward normalization of both markers, confirming reversibility.

Overall, DBP elicited integrated reproductive toxicity manifesting as growth impairment, testicular atrophy, Leydig cell dysfunction with low testosterone, adaptive antioxidant responses, and subtle immune alterations; all without activating classic gonadotropin feedback, a signature of phthalate action on the entire HPG axis. Concurrent administration of *Nigella sativa* oil consistently ameliorated or overcompensated nearly every adverse endpoint through its multifaceted antioxidant, anti-inflammatory, and steroidogenesis-promoting properties. The occasional deviations from some established patterns, such as unchanged LH/FSH, modest MDA, high GSH, and variable MPO, are context-dependent reflections of dose, duration, and the remarkable efficacy of NSO in preempting severe oxidative and inflammatory cascades. These findings strongly support the therapeutic potential of *Nigella sativa* oil as a natural, safe agent for mitigating phthalate-induced male reproductive toxicity.

CONCLUSION

Di-n-butyl phthalate (DBP) exposure causes significant oxidative, inflammatory, and hormonal disturbances in the testes, compromising male reproductive function. *Nigella sativa* oil (NSO) confers partial protection against these toxic effects, highlighting its promise as a natural antioxidant and anti-inflammatory intervention, while emphasizing the need for stricter regulation of phthalate exposure.

Conflict of Interest: The authors declare no conflict of interest.

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Author's contribution:

ATA: research concept, result interpretation, and critical review of manuscript; **AA:** data collection, data analysis, result interpretation and manuscript drafting; **AOA:** result interpretation and manuscript review; **MI** and **YMA:** data collection and analysis**;** **IAL:** result interpretation and manuscript review**;** **FOH:** methodology and critical review of manuscript; **KOO:** methodology and result interpretation; **SOI** and **SME:** methodology and result interpretation; **BJD**: result interpretation.

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