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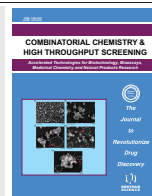
Nigella sativa Oil Alleviates Mouse Testis and Sperm Abnormalities Induced by BPA Potentially through Redox Homeostasis

Mohamed A. Al-Griw[1], Suliman M. Shalabi[1], Rabia O. Alghazeer[2,*], Abdul H. Elnfati[1], Soad A. Treesh[1], Ahmeda E. Benjama[1], Ghalia Shamlan[3], Mahmmoud M. Habibullah[4], Areej A. Eskandrani[5], Afnan M. Alnajeebi[6], Nouf A. Babteen[6] and Wafa S. Alansari[6]

[1]*Department of Histology and Genetics, Faculty of Medicine, University of Tripoli, Tripoli 13203, Libya;* [2]*Department of Chemistry, Faculty of Science, University of Tripoli, Tripoli 50676, Libya;* [3]*Department of Food Science and Nutrition, College of Food and Agriculture Sciences, King Saud University, Riyadh 11362, Saudi Arabia;* [4]*Medical Laboratory Technology Department, Faculty of Applied Medical Sciences, Jazan University, Jazan 45142, Saudi Arabia;* [5]*Department of Chemistry, Faculty of Science, Taibah University, Medina 30002, Saudi Arabia;* [6]*Department of Biochemistry, Faculty of Science, University of Jeddah, Jeddah 21577, Saudi Arabia*

Abstract: *Background & Aim*: Significant evidence indicates that endocrine disrupted bisphenol A (BPA) seriously endangers human health. In males, BPA affects testis architecture and sperm quality, and ultimately reduces fertility. This study explored the therapeutic potential of *Nigella sativa* (NS) seed extract on testis and sperm abnormalities in BPA-exposed mice and characterized the underlying mechanism.

Methods: Forty male Swiss albino mice (5.5 weeks old, N = 8 per group) were randomly divided into five groups: Group I, normal control, Group II, vehicle control (sterile corn oil); Group III, NS-exposed (oral 200 mg/kg); Group IV, BPA-exposed (oral 400 μg/kg body weight); Group V, BPA + NS-exposed mice. Animals were treated for 6 weeks and sacrificed for biochemical and histological examination.

Results: The results indicated that BPA exposure results in significant testis and sperm abnormalities. Specifically, BPA promoted a marked reduction in the body and testis compared with the control group. Histopathological findings showed that BPA caused a widespread degeneration of spermatogenic cells of the seminiferous epithelium, decreased sperm counts and motility, and augmented sperm abnormalities, and whereas little alteration to sperm DNA was observed. In addition, BPA increased the levels of the lipid peroxidation marker, malondialdehyde (MDA), and reduced the levels of the antioxidant marker, reducing glutathione (GSH). Treatment with NS oil extract during BPA exposure significantly alleviated testis and sperm abnormalities, reduced MDA levels, and enhanced GSH levels.

Conclusion: The results demonstrate that NS oil protects mice against BPA-induced sperm and testis abnormalities, likely by suppressing levels of the oxidative stress marker, MDA, and enhancing the levels of the antioxidant marker, GSH.

Keywords: BPA, testis, sperm, reproductive toxicity, oxidative stress, *Nigella sativa*, mice.

1. INTRODUCTION

Exposure to environmental insults, such as pollutants and toxicants, leads to transgenerational inheritance of phenotypic variations and diseases [1, 2]. Animal studies have shown that exposure to toxicants at the germ cell level or during intrauterine life, postnatal life, or early life, can cause detrimental effects on disease susceptibility and an altered phenotype later in life [3, 4].

For decades, male reproductive health has declined by reduced fertility and sperm quality, which has resulted in a significant economic and societal burden [5]. Since the 1970s, a decline of approximately 50% in sperm concentration has occurred in the population [5]. Environmental toxicants may contribute to this transgenerational inheritance of poor sperm quality and testis pathology because of their effects at the epigenetic level [5].

*Address correspondence to this author at the Department of Chemistry, Faculty of Science, University of Tripoli, Tripoli 50676, Libya;
E-mail: R.alghazeer@uot.edu.ly

Humans and animals are recurrently exposed to a variety of environmental toxicants [6]. Bisphenol A (BPA), is a compound used widely in polycarbonate beverages and food packaging materials. It is also the main ingredient in the production of plastic bottles for infants [7, 8]. It is among the most ubiquitous environmental chemical and is found in large amounts in food preparations. The mean dietary daily intake of BPA by the adult population was found to be between 0.4-1.4 µg/kg (FAO/WHO, 2010). Humans are at greater risk for BPA exposure as heating can cause it to leach out from containers into water and food [9]. Because of this leeching process, studies have found traces of BPA in human fluids, which may have a negative effect on human health [10, 11].

A close relationship exists between BPA exposure and certain pathological events, including cardiovascular disorders, type 2 diabetes, breast cancer [12], and liver enzyme abnormalities [13]. BPA interferes with hormone action, leading to non-monotonic dose responses, which are atypical exposure to a standard toxicant. The male reproductive system, in particular, is vulnerable to BPA, which negatively affects development and reproduction [14]. Similarly, the effects of BPA on hormone levels during developmental stages are more damaging because programming during these periods can be negatively impacted by hormonal changes. BPA is referred to as a xenoestrogen because of its imitation of estrogen and thus contributes to an array of adverse health effects, such as abnormal reproductive function [15]. BPA has also been shown to initiate oxidative stress in the liver, brain, kidney, and dysregulate cytokine expression [16, 17].

Since ancient times, plants have been widely used as therapeutics. Black seed (*Nigella sativa*) is used in traditional medicine in Northern Africa, Asia, Middle East, and the Far East to treat various ailments. *Nigella sativa* (NS) consists of 36–38% proteins, fixed oil, saponins, alkaloids, and 0.4–2.5% essential oils. The other main components include p-cymene, thymoquinone, carvacrol, t-anethole, 4-terpineol, and sesquiterpene longifolene [18]. NS also contains dithymoquinone, thymol, thymohydroquinone, nigellimine-N-oxide, nigellicine, alpha-hedrin, carvacrol, and nigellidine [19].

Recent clinical and experimental studies have documented various therapeutic effects of NS, including antioxidant [18-20], anti-microbial, anti-inflammatory [21], anti-helminthic [22], anti-tumor [23], anti-diabetic [24], and anti-ulcerogenic [25] activity. Studies suggest that NS is effective against various pathogens, including standard and nosocomial microorganisms, such as Staphylococcus aureus, Candida albicans, and Pseudomonas aeruginosa [26]. In a study of 24 different bacteria, the antibacterial effects of NS oil were attributed to [27] its p-cymene, thymoquinone, and carvacrol components. In another *in vitro* study, NS effectively inhibited the growth [28] of eight dermatophyte varieties, demonstrating the anti-dermatophyte properties of the plant, which contribute to the treatment of fungal infections of the skin. In the present study, we analyzed the effects of NS on testis and sperm abnormalities in adult male mice during early-life BPA exposure. In the present study, we evaluated the therapeutic potential of NS seed oil extract against the adverse effects of BPA-induced mouse testis and sperm abnormalities and examined the underlying molecular mechanism(s).

2. MATERIALS AND METHODS

Bisphenol A (BPA) was purchased from Sigma (Germany) and *NS* was purchased from a spice shop in Tripoli, Libya, in November 2019.

2.1. Collection of Plant Material

The collected plant material was authenticated and established to be that of *Nigella sativa* in the herbarium section of the Department of Botany, Faculty of Sciences, University of Tripoli, and processed and extracted in the Department of Chemistry, Faculty of Sciences.

2.2. Extract Preparation

Extracting oil from *N. Sativa* L. seed was based on a previously published cool water extraction protocol with [29]. Briefly, NS seeds were crushed with a pestle and mortar. Approximately 100 g of the powder was mixed with 50 ml of distilled water. The mixture was divided into several molds and dried in a desiccator for 15 minutes. After that, each mold was wrapped and tied with a white piece of cloth and then subjected to pressure using a hydraulic machine. The resulting oil was stored in air-tight containers in the refrigerator until used.

2.3. Animals

Male Swiss albino mice were housed in the animal care facility under a 12-hour light/dark cycle and standard temperature (26 ± 2°C) conditions. Animals were provided free access to food and water. The study was approved by the Research Ethics Committee, Biotechnology Research Center, University of Tripoli (Reference BEC-BTRC 8-2019).

2.4. Experimental Design

Forty adult 5.5 week-old male Swiss albino mice, weighing 17.35 ± 2.14 g, were randomly and equally divided into the following five groups: Group I, control (received no treatment); Group II, treated with vehicle control (corn oil); Group III, treated with NS oil (200 mg/kg); Group IV, treated with BPA (400 µg/kg); Group V, treated with BPA+NS. All treatments were administrated orally for 6 weeks. The BPA dose was selected based on previous studies regarding the adverse effects of BPA and its environmental relevance [30, 31]. The NS dose was chosen based on a previously published study [32, 33].

Six weeks following treatment, two males from each group were randomly selected for fertility examination. Males were housed with fertile control females. Mating was established by the presence of vaginal plugs. The day the vaginal plug appeared was designated gestation day 1 (GD-1). During the copulation period, the weight of the female mice was measured twice per week. To further confirm pregnancy, F0 pregnant dams were observed and their body weight was recorded daily. After the appearance of the plug, females were weighed and caged individually. The dams were allowed to deliver naturally and the day of delivery was designated as postnatal day 0 (PND-0). After the 2-week mating period, unmated males were considered infertile. After delivery, the percentage of fertile males, number of pups, alive/dead pups, and pup weights were recorded on
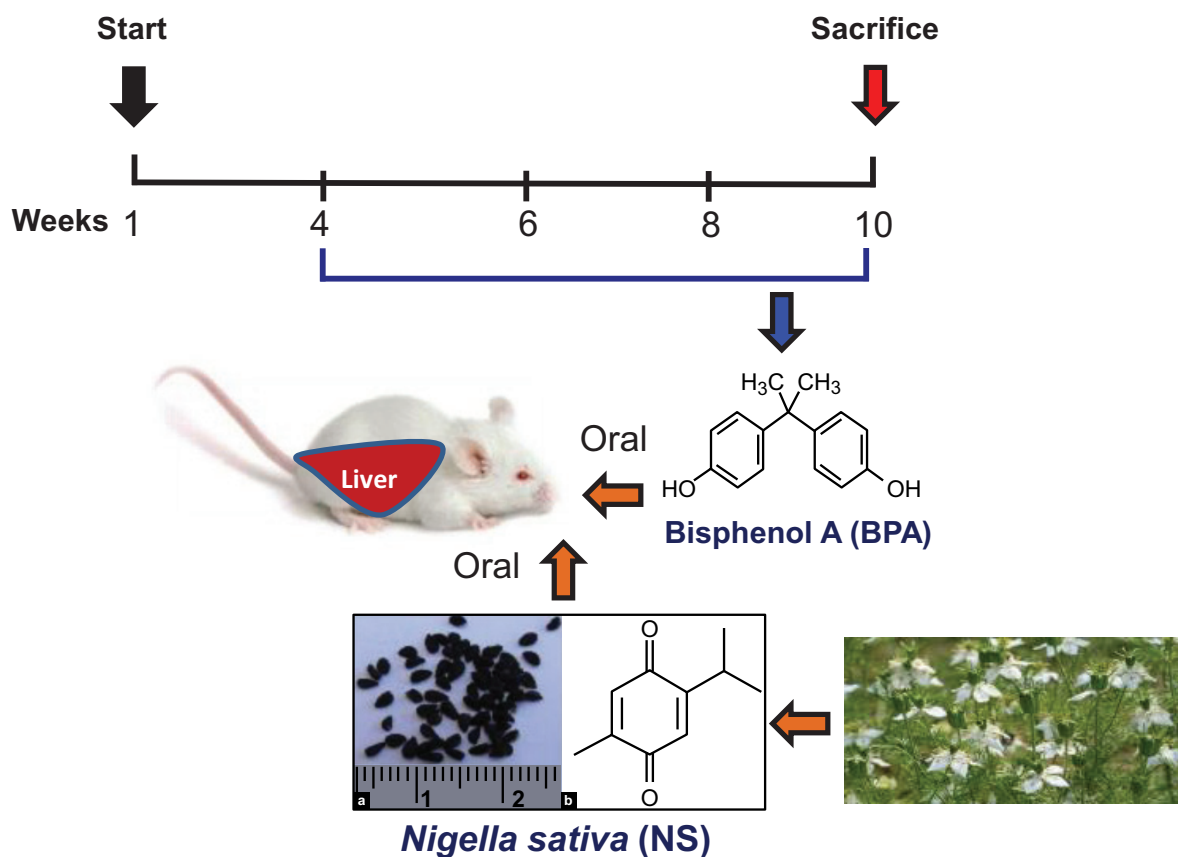
Fig. (1). Schematic of the animal treatment protocol. Animals were treated with vehicle control, NS, BPA, or BPA+NS. BPA and NS were administered orally *via* gavage at a dose of 400 µg/kg and 200 mg/kg, respectively, for 6 weeks. At 11.5 weeks, the mice were sacrificed and tissues were collected.

postnatal day 1 (PND-1). At 13.5 weeks old, the mice were sacrificed for biochemical and histological examinations (Fig. **1**).

2.5. Clinical Assessment

The survival of the animals was monitored throughout the study. The animals were observed midmorning and late afternoon for behavioral and clinical signs that may have resulted from toxicity. Additionally, deaths occurring overnight were recorded the next morning. The cause of death was confirmed by three independent observers to rule out mortality unrelated to the treatment.

2.6. Body and Testis Weight

Changes in body weight were determined before and after the end of the experiment. At the end of treatment, testes were excised and the relative weight (%) was determined.

2.7. Sample Collection

The animals were sacrificed and the testes and epididymis were removed using a scalpel blade and washed using phosphate buffered saline (PBS). After that, the testis was homogenized with a high-speed mortar for 2 min with 50 mL of normal saline (0.9% sodium chloride, NaCl). The homogenate was rinsed again with normal saline and collected. Homogenized testes was used for the measurement of

oxidative stress and other parameters. A fraction of the tissue was preserved in 10% formalin solution for histological examination.

2.8. Measurement of Sperm Count, Motility, and Morphology

Quality of epididymal sperms (counts, motility, and morphology) was measured as previously described Al-Griw *et al*. [33, 34]. Briefly, epididymis was removed and placed in a Petri-plate containing 1 ml of normal saline at 37ºC. The epididymis was cut into small portions to allow the sperm to swim out. The solution containing the sperms was centrifuged at 1000 rpm for 3 minutes. After centrifugation, the seminal plasma (supernatant) was frozen without preservatives and stored at −70°C until further assay for malondialdehyde (MDA), a lipid oxidative stress marker, total protein isolation, and reduced glutathione (GSH).

The examination of epididymal sperm count and motility was carried out by dropping a drop of solution containing the sperms (sperm suspension) on a counting chamber (Neubauer's haemocytometer, Germany). After the sperms had settled on the grid, they were counted in the 5 squares using objective lens 40 after the semen had been diluted 1:10. The number of sperms in five squares was multiplied by 106 to determine the number of sperms per milliliter. The sperm count was expressed as sthe number of sperm/ ml of solution containing sperm.

The examination of sperm head morphology was carried out by dropping 0.5 ml of sperm suspension and 0.5 ml of 2% eosin Y solution were mixed and kept for 60 minutes to stain the sperm. Smear was prepared using 2-3 drops of the above solution then air-dried and fixed with absolute methanol for 3 minutes. 200 sperm per animal was examined to determine the morphological abnormalities under oil immersion in light microscope (Leica, Germany). Data was shown in terms of % of abnormal sperm.

2.9. DNA Fragmentation Test

Sperm DNA fragmentation analysis was done as described by Kim *et al.* [35]. Briefly, the slides were smeared with 10 μL of raw semen, air-dried, and then fixed in methanol and acetone solution (1:1) at room temperature for 5 min. The slides were hydrolyzed in 0.1% HCl for 2 min, and then they were stained with toluidine blue (Sigma-Aldrich, Germany) for 10 min. After which, the slides were rinsed in phosphate-buffered saline (PBS) for 1 minute and air dried. Each slide was examined by light microscopy at X1.000 magnification. Sperm that stained dark blue with eosin counterstain was considered abnormal. The percentage of abnormal chromatin condensation in the sperm was determined as the ratio of the number of dark-blue sperms to the total number analyzed.

2.10. Lipid Peroxidation Assay

Malondialdehyde (MDA) was measured as a thiobarbituric acid reactive substrate as described previously [36, 37]. Briefly, testicular tissue was homogenized using a tissue homogenizer (IKA, RW 20.n, Germany) in PBS. The homogenate was centrifuged and 0.5 ml of the supernatant was added to 2 ml of TBA–HCL-TCA mixture (0.37%, 0.24 N, and 15%, respectively) and boiled at 100°C for 15 min. The solution was allowed to cool and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was read at 532 nm. A standard curve was prepared using 1, 1, 3, 3-tetramethoxypropane and the content of TBA-MDA adducts was inferred. MDA levels are presented as nmol/ml.

2.11. Total Protein Isolation and Measurement

Total protein in testicular tissue was measured as described previously [38, 39]. Briefly, the testes were homogenized in sodium phosphate buffer (pH 7.4) and centrifuged for 15 min at 6000 × g. The resulting clear supernatant was used to determine protein concentration using BSA as a standard.

2.12. Reduced Glutathione (GSH) Measurement

Reduced glutathione (GSH) levels were quantitated using Ellman's reagent [5, 5-dithiobis-(2-nitrobenzoic acid)] (DTNB) as previously described [40]. Tissue homogenates were mixed with TCA (25%) to precipitate protein and centrifuged at 4,000 rpm for 5 min. The resulting supernatants were incubated with phosphate buffer (pH 7.4) and DTNB for 10 min. The absorbance of the mixture was measured at 412 nm. The amount of glutathione is presented as nmol/mg protein.

2.13. Histopathological Analysis

The testicular tissues were processed for histological examination as described previously [41]. Formalin-fixed tissue was embedded in paraffin and 5 μm thick sections were cut. Sections were deparaffinized, hydrated, and stained with H&E. Tissue sections were visualized under a light microscope (Leica, Germany). Histological quantification was based on a scoring scale as described previously [42]. The scale was set from 0 to 10, where scores 10,9,8,7,6,5,4,3,2,1 indicated full spermatogenesis, disorganized tubular epithelium, few late spermatids, no late spermatids, few early spermatids, no spermatids, few spermatocytes, spermatogonia only, Sertoli cells only no germ cells, and no seminiferous epithelial cells, respectively. For each testis tissue, the average of 8 scores was considered a replicate, whereas for each tissue section, the average of ten scores was considered a replicate. Tissue sections from each animal were assessed blindly by a histopathologist for histological anomalies. The tissue architecture of the mouse testis was imaged using low- and high-power objective lenses under a light microscope (Leica, Germany).

2.14. Statistical Analysis

Findings were analyzed using the GraphPad Prism software version 7.0. The data were expressed as the mean ± SEM. Normality was assessed using the computerized Kolmogorov-Smirnov test. For data with normal distributions, multiple comparisons were made with one-way ANOVA followed by a post-hoc test Dunnett's to determine which differences were significant. P values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Effects of BPA and NS on Animal Survival

The survival and mortality rate of the mice from all groups was compared. Based on regular monitoring and clinical examination, no mortality was observed in any group throughout the study period. Similarly, no signs of acute toxicity were observed.

3.2. Effects of NS on Motor Activity and Swimming Ability following BPA Exposure

There was a significant decrease in motor activity and swimming in the BPA-treated animals compared with that in control mice ($p < 0.046$; Fig. **2**). However, the data was comparable between control and vehicle-treated mice. In contrast, a significant improvement in motor activity and swimming was observed in the BPA+NS treated mice compared with mice treated only with BPA.

3.3. NS Preserves Body and Testis Weight of Mice Following BPA Exposure

The total body weight (TBW) of all animals was recorded beginning at birth and throughout the study, whereas testis weight was noted at the time of necropsy, as shown in Fig. (**3**). The results indicated that the mean of TBW was significantly elevated in the BPA group compared with the control group ($p < 0.05$; Fig. **3**), whereas the TBWs were
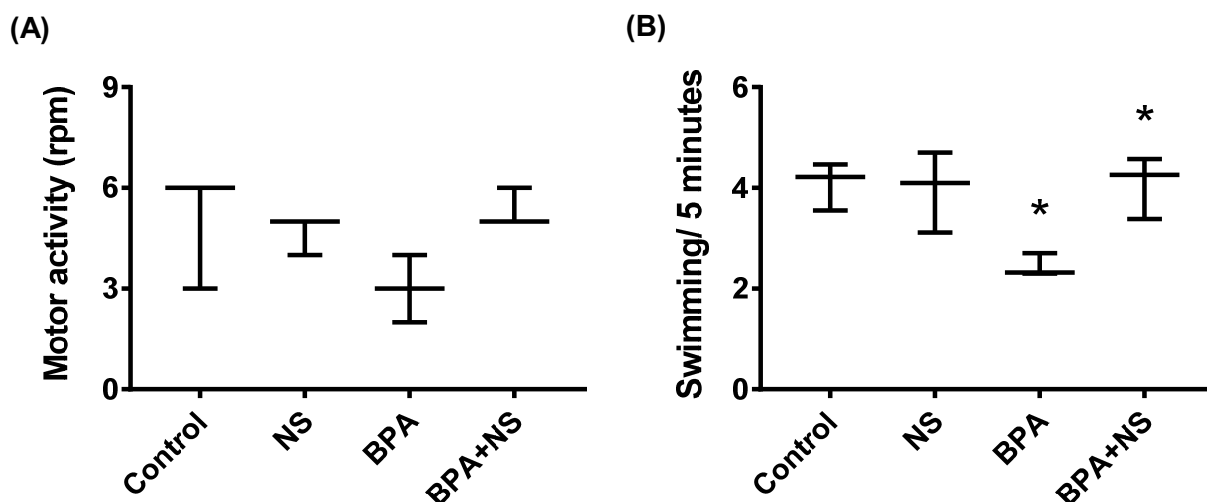
(A)

(B)

Fig. (2). NS preserves motor activity and swimming in BPA-exposed mice. Mice were treated with vehicle, NS, BPA, or BPA+NS. (**A**) Measurement of motor activity. (**B**) Measurement of swimming ability. Data shown are the mean ± SEM (*n* = 8) *indicates *p* < 0.05.
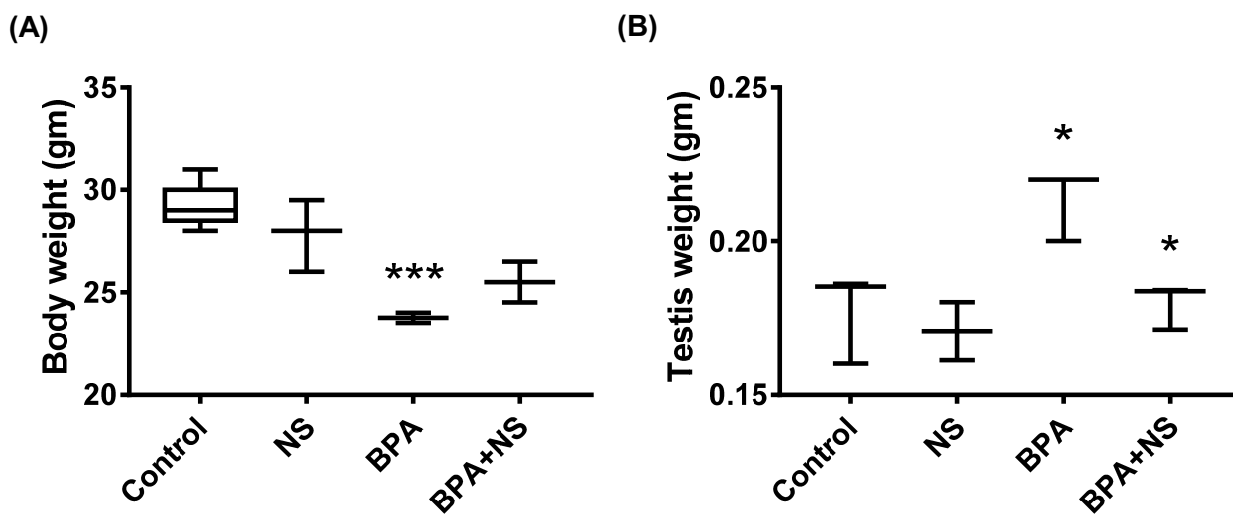
(A)

(B)

Fig. (3). NS preserves body and testis weight in BPA-exposed mice. Mice were treated with vehicle, NS, BPA, or BPA+NS. (**A**) measurement of TBW. (**B**) Measurement of testis weight. Data are the mean ± SEM (*n* = 8 per group). (*) indicates *p* < 0.05.

comparable between the control and vehicle groups (data not shown). Treatment of mice with NS following BPA exposure enhanced TBW compared with BPA-treated mice but did not reach statistical significance (Fig. **3A**). There was an increase in the mean testis weight of the BPA-exposed mice compared with control mice (*p* < 0.029; Fig. **3B**). In contrast, NS treatment upon BPA exposure was effective against BPA-induced testis weight increase (*p* < 0.04; Fig. **3B**), which was restored to near normal levels.

3.4. NS Enhances Sperm Quality upon BPA Exposure

Regarding the cauda epididymal sperm quality, BPA exposure resulted in harmful effects on motility, count, and head morphology compared with the control group (Fig. **4**). Specifically, BPA exposure promoted a marked decline in sperm count and increased the percentage of sperm immotility and head abnormalities (Fig. **4A-C**), whereas treatment with NS preserved sperm count and motility (Figs. **4A** and **4B**). With respect to the morphology of the cauda epididy-

mal sperms, NS treatment reduced sperm abnormalities compared with the controls (Fig. **4C**).

3.5. NS did not Prevent Sperm DNA Fragmentation Induced by BPA Exposure

The percentage of abnormal sperm DNA in all experimental mouse groups was compared. No statistically significant differences were observed between the experimental groups (data not shown).

3.6. NS Attenuates Testis Abnormalities in Mice upon BPA Exposure

The testis tissues of the control group displayed normal seminiferous tubules and spermatogenesis (panel i; Fig. **5A**). The tissues from BPA-exposed mice showed attenuation of spermatogenesis at various levels (panel ii, Fig. **5B**). In contrast, the testis tissues of mice treated with NS upon BPA exposure exhibited improvement with the regeneration of spermatogenesis to normal levels (panel iii, Fig. **5C**).
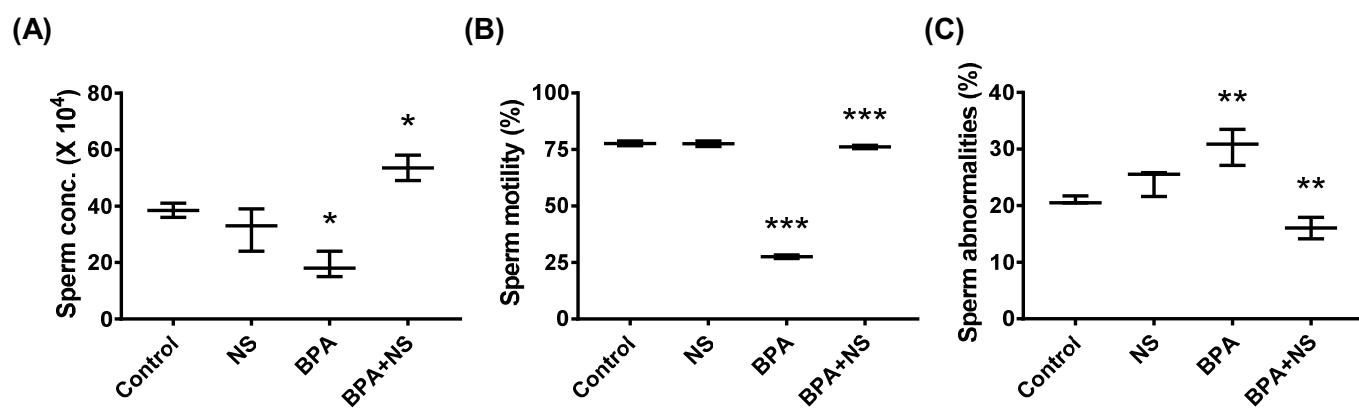
(A)

(B)

(C)

Fig. (4). NS enhanced sperm quality in BPA-exposed mice. Mice were exposed to various conditions: control (sham and vehicle), NS, BPA, or BPA+NS. (**A**) Quantitation of sperm counts. (**B**) Quantification of sperm motility. (**C**) Quantification of sperm morphology. Data are the mean ± SEM (***n*** = 8 per group). *indicates $p < 0.05$.
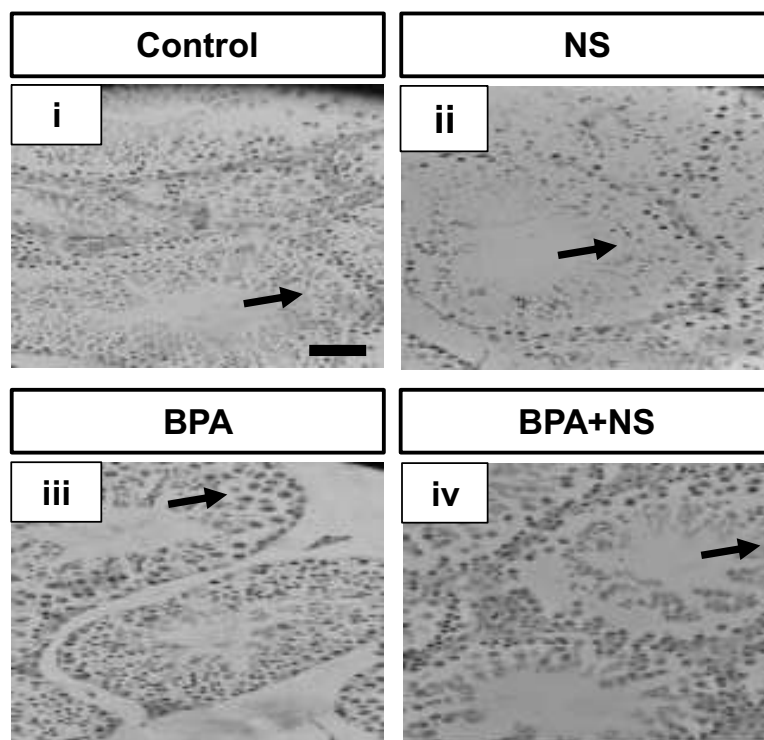

Fig. (5). Photomicrographs of H&E–stained testis tissue sections of control, NS, BPA, and BPA+NS-exposed mice. Histopathological features of the testis tissues of control- and NS-exposed mice showed normal seminiferous tubules and spermatogenesis (arrows, panels **i** and **ii**). Testis tissues of BPA-exposed mice showed the arrest of spermatogenesis at different levels (arrows, panel **iii**). Testis tissues of BPA+NS-exposed mice showed regeneration of spermatogenesis in some seminiferous tubules to normal levels (arrows, panel **iv**). Scale bar = 100 μm, 40X.

3.7. NS Modulates Oxidative and Antioxidative Status in Mice Following BPA Exposure

BPA exposure significantly increased lipid peroxidation, as evidenced by increased malondialdehyde (MDA) levels in the testicular tissue compared with control mice ($p < 0.01$; Fig. **6A**). In contrast, MDA levels in the testicular tissues of BPA-treated mice were significantly reduced after treating BPA-exposed mice with NS ($p < 0.0096$; Fig. **6A**), which restored MDA to near normal levels.

We next assessed the effect of NS on the antioxidative status in mice upon BPA exposure. GSH levels in the testis tissues of all experimental groups were measured and compared. The results showed that the levels of the antioxidant marker, reduced GSH, in the testis tissues of BPA-exposed mice were lower compared with that in control-exposed mice ($p < 0.0001$; Fig. **6B**). Treatment with NS enhanced GSH levels compared with untreated BPA-exposed mice ($p < 0.0042$; Fig. **6B**).
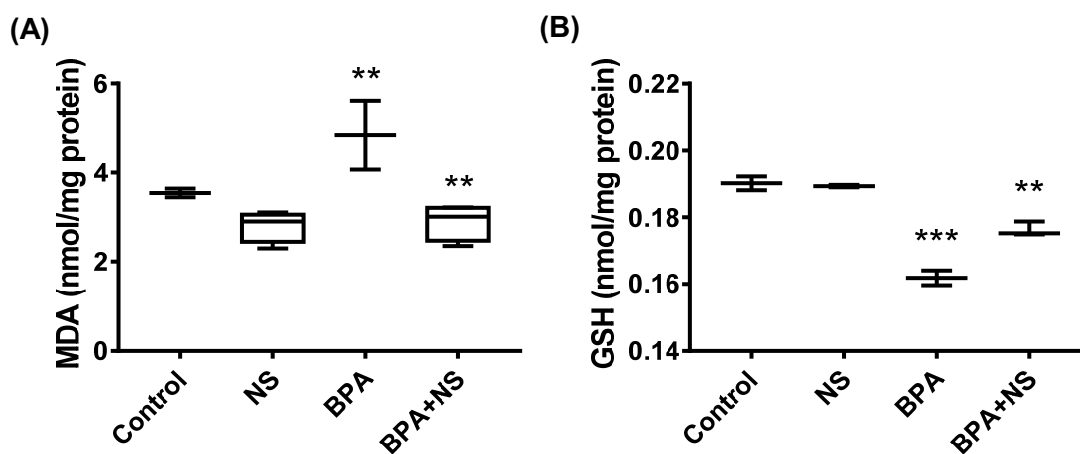
Fig. (6). NS reduced MDA levels and enhanced GSH levels in the testis tissues of BPA-exposed mice. Mice were treated with vehicle, NS, BPA, or BPA+NS. Measurement of (**A**) MDA levels (nmol/ml) (**B**) and GSH (nmol/mg) in testicular tissues. Data are the mean ± SEM ($n = 8$). *indicates $p < 0.05$, **indicates $p < 0.01$, and ***indicates $p < 0.001$.
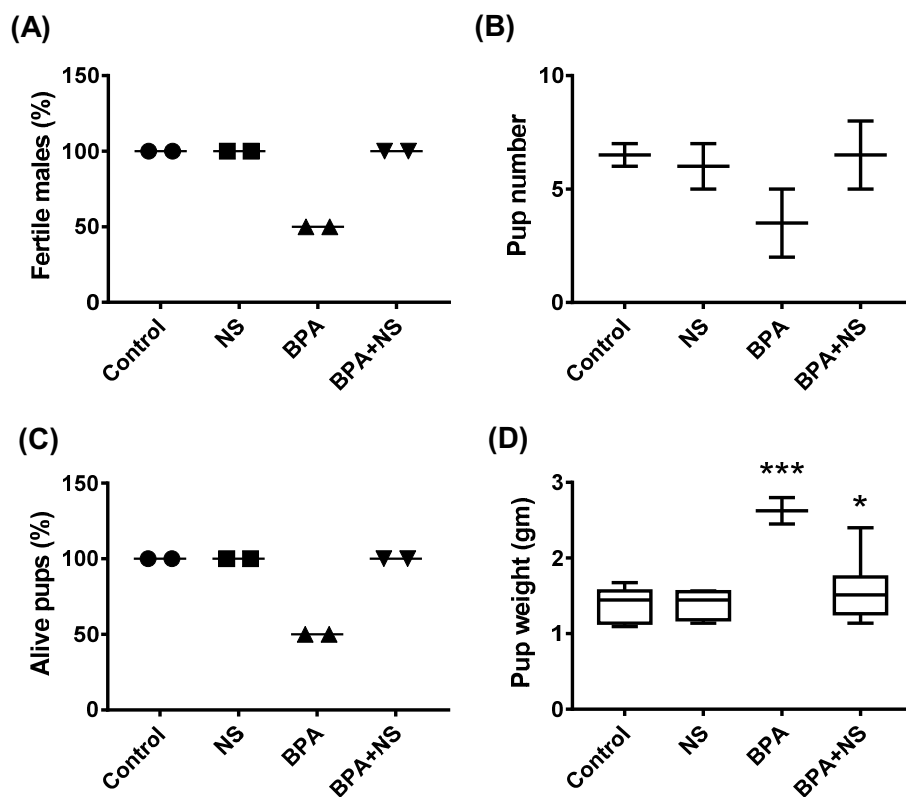

Fig. (7). NS enhances male reproductive performance and outcome. Mice were treated with vehicle, NS, BPA, or BPA+NS. (**A**) Quantification of the percent of fertile males. (**B**) Quantification of pup numbers. (**C**) Quantification of the percentage of live pups. (**D**) Quantification of pup weights. Data are the mean ± SEM ($n = 8$ per group). (*) indicates $p < 0.05$ and (**) indicates $p < 0.01$.

3.8. NS Enhances Male Reproductive Performance and Outcome Following BPA Exposure

Next, we determined whether the NS enhanced sperm quality after BPA exposure involved changes in male reproductive performance and outcome (reproductive capacity). Therefore, we measured fertility/ reproductive indices, including pregnancy rate and the percentage of fertile males as described in the Methods section. The results indicated that BPA exposure caused a reduction in the percentage of fertile males (44.4%) compared with controls ($p < 0.001$; Fig. **7A**).

Treatment of BPA mice with NS enhanced the percentage of fertile males compared with untreated BPA mice ($p < 0.01$; Fig. **7A**).

We next determined the reproductive outcome of males in response to NS following BPA exposure. The number of live pups and the weight of the pups were counted and compared. The results indicated that BPA significantly reduced the pup number compared with the control group ($p < 0.05$; Fig. **7B**). Furthermore, BPA mice exhibited a very low percentage of live pups compared with control mice, however,

our sample size was too small for statistical analysis ($n = 2$; Fig. **7B**). In contrast, treatment of BPA mice with NS significantly increased the mean number of pups as well as the percentage of live pups ($p < 0.05$; Fig. **7B**).

4. DISCUSSION

Environmental toxicants, such as BPA, remain a major public health concern worldwide as exposure can result in diseases and phenotypic variations [10, 36, 43]. Thus, exposure to environmental toxicants has a negative impact on health in early and later life [43-47]. The results of the present study support previous findings by our group that BPA markedly promotes testis and sperm abnormalities, as evidenced by biochemical and histological analyses. Specifically, BPA mice showed a marked decrease in body weight and an increase in testis weight compared with control mice. BPA increased sperm injury scores and MDA levels. Moreover, a reduction in the levels of antioxidant markers, such as reduced GSH, was observed. These detrimental effects were significantly attenuated by treatment with NS seed oil extract. Our findings suggest an important role for NS in BPA-induced testis and sperm abnormalities. A marked improvement in testis tissue architecture and sperm quality was observed following NS treatment. In addition, reduced MDA oxidative stress marker levels and enhanced GSH levels may have contributed to the protection provided by NS treatment in this model.

NS oil or its active agents are known to possess several pharmacological properties, including cytoprotective, antibacterial, anti-parasitic, antioxidant, and anti-inflammatory activity [19]. Thymoquinone has been shown to prevent oxidative stress-induced organ damage in various animal models [20]. It was reported that thymoquinone is an antioxidant and inhibits lipid peroxidation of tissue membranes [48, 49]. Antioxidant effects of thymoquinone appear to be mediated through the inhibition of leukotrienes B4 and thromboxane B2 eicosanoid generation by attenuating 5-lipooxygenase and cyclooxygenase enzyme activities, respectively [50]. NS oil also decreases total serum lipids and body weight [51] and decreases fasting plasma glucose [52]. Recently, it was shown that NS protects the liver from tetracholorocarbon toxicity [49]. However, whether BPA damages testis architecture and sperm quality remains elusive.

The protective effects of NS have been reported for its antioxidant activity, including superoxide anion scavenger, direct cytoprotective effects, and indirect androgen and antioxidant activities that protect semen and sperm components from testicular free radicals [53]. We found that BPA decreases GSH levels and increases the oxidative marker, MDA [54, 55]. Other studies demonstrated that treatment with NS oil reduced the levels of oxidative stress biomarkers (MDA and protein carbonyl) and enhanced GSH levels in many organs [49, 31, 56]. Similarly, we found that MDA levels were increased as a result of BPA exposure compared with the control group, whereas they decreased as a result of treating BPA-exposed mice with NS. In addition, we found that NS treatment increased the GSH levels. Taken together, these findings suggest that BPA causes a disturbance in the ROS and antioxidant defense balance and damage to mouse testes, which may be alleviated by NS treatment.

Infertility is a major health concern affecting human life. Exposure to environmental toxicants is a significant major contributor to male infertility. A number of animal studies have reported the negative effects of BPA on fertility [14, 57]. For example, female Sprague–Dawley rats exposed neonatally to BPA delivered a reduced number of pups after mating and the effect continued for several generations [57, 58]. Rodents treated with BPA perinatally showed reduced fertility [59], gestational index [58], and sexual development [60]. Male rats treated with BPA showed abnormalities in the male metabolic processes and reproductive system [61, 62]. Because of limited information concerning the impact of BPA exposure on male reproductive capacity, the current study determined whether BPA affects male reproductive performance and outcome. Free radicals can negatively affect various sperm parameters such as motility, count, and morphology, thus, they can reduce sperm fertility [63, 64]. We found that BPA-exposed mice showed reduced male reproductive performance and outcome compared with control mice. Collectively, our data suggest that BPA affects reproductive performance and outcome in male mice. Our findings also showed that treatment of BPA-exposed mice with NS enhanced male reproductive performance and outcome compared with untreated BPA mice.

In a clinical trial, daily use of NS oil (2.5 ml) by 68 infertile men for 8 weeks resulted in an improvement in sperm morphology, count and motility, and semen volume [65]. In different animal models, the positive effects of NS oil were observed on male reproduction and fertility. Most of the improvements include the increased weight of the testis and epididymis, sperm count, sperm production, improvement of serum level of luteinizing and testosterone hormones, and reproduction performance [66, 67]. To improve fertility quality in an obese rat model, NS oil was administrated at doses of 200 mg of NS200 (kg/day) and 400 mg of NS400 (kg/day). NS200 and NS400 improved TBW and testosterone compared with the obese group, whereas NS400 improved sperm concentration and mitochondrial membrane potential. No change in sperm viability and motility was observed in the remaining experimental groups [68].

In a recent systematic review by Darand *et al.* [69] regarding the effects of NS on infertility in men and women, the possible mechanisms by which NS improves male fertility are discussed. The antioxidant components of NS can enhance steroid hormone synthesis, sperm production, and semen quality. These antioxidants include flavonoids, anthocyanin, carotene, isothiocyanate, and carotenoids [70]. Similarly, NS oil contains linoleic acid and oleic acid, which have been reported to effectively improve sperm count, motility, and normality [71]. Moreover, phenolic and alkaloid compounds play important roles in increasing the levels of follicular stimulating hormone and testosterone in males [66, 72].

Recently, it was reported that BPA damages sperm genetic material [73] and reduces the integrity of chromatin [74]. During spermatogenesis, BPA exposure resulted in an increase in sperm DNA fragmentation and enhanced DNA repair activity in zebra fish embryos [64] and decreased sperm integrity was observed in mice treated with a higher BPA dose (2000 μg/kg) [75]. Sperm DNA damage resulting

from environmental factors may be either repaired or passed on to subsequent generations [76]. In this study, BPA exposure caused minimal sperm DNA fragmentation compared with the control group and did not reach statistical significance.

Physiologically, the weight and size of the epididymis and testis increase in the presence of vitamins, zinc, magnesium, and copper, which are components of NS. These elements also increase the secretion and activity of steroid hormones and many metabolic enzymes [77, 78]. In the present study, BPA caused testicular toxicity. The histopathological findings in the testes of BPA-exposed mice showed the arrest of spermatogenesis at different levels and a reduction in the size and thickness of the germinative cell layer of seminiferous tubules, indicating spermiotoxicity from oxidative damage to membrane biomolecules. It was reported that treatment with NS significantly attenuated the detrimental effects of chemical substances in different organs of rats [20, 31]. Interestingly, we found that treatment of BPA-exposed mice with NS significantly attenuated the detrimental effects of BPA on mouse testes. Specifically, we found that the testis architecture of the NS-administrated mice exhibited improvement with regeneration of spermatogenesis in some seminiferous tubules, which were restored to normal.

At the molecular level, there is no evidence in the literature describing the activity of specific NS components on the male reproductive system and germ cells. However, in a recent study of female mice with polycystic ovary syndrome, the results showed that NS improved the epigenetic modification of many genes. The expression of genes involved in epigenetic regulation as well as maternally derived genes was found to be upregulated in the oocytes of NS treated mice [76].

CONCLUSION

Based on the findings of this study, we concluded that early BPA exposure has a detrimental impact on testis tissue architecture and sperm quality of adult male mice. This work also showed that BPA significantly promoted testicular oxidative damage by disturbing oxidant/antioxidant status, and ultimately led to testis abnormalities as evidenced by biochemical tests and histological alterations. These findings shed light on the possible underlying mechanisms for the development of a range of abnormalities caused by BPA. Importantly, concurrent treatment with NS had protective effects on the testis tissue structure and afforded protection against BPA toxicity. However, we were unable to identify the precise mechanism through which NS exerts its effects. Nonetheless, our findings indicate some of the activity may be mediated by suppression of MDA levels and an increase of GSH levels following BPA-mediated testicular toxicity. Nevertheless, our findings suggest that NS provides effective treatment for BPA-induced testis and sperm abnormalities. Further studies delineating the underlying mechanism of NS action are warranted.

AUTHORS' CONTRIBUTIONS

Conceptualization: M.A.A. and R.A. Data curation: S.M.S., S.A.T., A.A.E., A.M.A., N.A.B., and W.S.A. Formal analysis: M.M.H., A.A.E., G.S., A.A.E., A.M.A., N.A.B., and W.S.A. Investigation: M.A.A., R.A., S.M.S. and S.A.T. Methodology: M.A.A., R.A., M.M.H. and A.A.E. The paper was written with input from all authors and they read and approved the final manuscript.

LIST OF ABBREVIATIONS

BPA   =   Bisphenol A

NS   =   *Nigella sativa*

MDA   =   Malondialdehyde

PBS   =   Phosphate Buffered Saline

TBW   =   Total Body Weight

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