

Protective effects of *Nigella sativa* oil against bisphenol A-induced testicular toxicity in rats

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Summary

The study investigated the protective effect of *Nigella sativa* (NS) oil against testicular damage induced by bisphenol A (BPA). A total of 36 Wistar Albino rats were divided into 4 groups: control (1 ml olive oil), NS (5 ml/kg NS), BPA (100 mg/kg BPA in 1 ml olive oil), BPA+NS (100 mg/kg BPA + 5 ml/kg NS). During the 30-day experiment, all substances were administered by oral gavage. The levels of testosterone and LH in serum, as well as MDA, GSH, SOD, TNF- α , IL-6 and IL-10 levels in testicular tissue were measured by ELISA. Spermatological examinations and histopathological analyses of testicular tissue were also performed. BPA was found to raise LH while reducing the serum testosterone level. BPA caused a decrease in GSH, SOD, TNF- α and IL-6, but an increase in MDA and IL-10. BPA also reduced sperm motility, plasma membrane integrity, mitochondrial activity and acrosome membrane integrity. Oedema, hyperaemia, mononuclear cell infiltration in the interstitial tissue, as well as giant cell formations, degeneration and necrosis in the seminiferous tubules were seen in the BPA group. In addition, reductions in germinative epithelium, tubule diameter and the Johnson testicular score were noted. In rats receiving NS, hormonal profile, oxidative stress markers and cytokine levels were within normal limits. NS improved sperm motility, mitochondrial activity (JC1), plasma membrane integrity (SYBR-14) and partially acrosome membrane integrity (FITC-PNA). NS reduced testis inflammation, significantly ameliorated the testicular score and, to a lesser extent, increased germinative epithelium thickness and tubule diameter. It was concluded that NS has a protective effect against BPA-induced testicular toxicity.

Keywords: BPA, *Nigella sativa*, histopathology, testis, antioxidant

Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane), an endocrine disruptor (17), is widely used in plastic containers, toys and medical devices, as well as in baby feeding bottles and pacifiers. The global annual production of this substance is 6.8 million tons. It is widely employed in several industrial fields, such as synthetic polymers, polycarbonate plastic, epoxy resins and paper manufacturing (57). Recent epidemiological, clinical and experimental evidence indicates that BPA may be responsible for the rise in male reproductive dysfunction (39).

BPA may disrupt oxidative equilibrium by increasing oxidative mediators and decreasing antioxidant enzymes, impairing mitochondrial function, altering cell signalling pathways and inducing apoptosis (58, 62). Oxidative stress is caused by an increase in free radical and oxidant production as well as a weakening of the antioxidant defense system (such as glutathione (GSH), superoxidedismutase (SOD), and catalase

(CAT)) (23). In addition to oxidative damage, BPA has lately been linked to inflammatory indicators in humans (19). BPA exposure has been shown to increase the expression of interleukin (IL)-1, IL-6 and tumor-necrosis factor- α (TNF- α) in a variety of tissues and organs (33, 62). Furthermore, BPA binds to androgen receptors and acts as an agonist, which is the primary regulator of androgynous cell signalling and is required for the development of male reproductive function (4). It is claimed that exposure to BPA raises blood levels of luteinizing hormone (LH) while decreasing testosterone levels in males (32).

Nigella sativa L. (NS, black cumin), a member of the Ranunculaceae botanical family is widely used as a component of nutraceuticals. The term „nutraceuticals”, which arose from the strong association between optimal nutrition and life expectancy, has gained popularity among dieticians, nutritionists, food scientists, physicians and the food and pharmaceutical

businesses (23). NS is native to Turkey, Egypt, Iran, Greece, Syria, Albania, Saudi Arabia and other countries in the eastern Mediterranean, northern Africa, the Indian subcontinent and Southwest Asia. It is grown in several places, including India (24). NS includes pharmaceutically significant components, such as thymoquinone (TQ), thymohydroquinone, thymol, carvacrol, nigellidin, nigellisin and a-hederin, which are primarily responsible for its pharmacological and therapeutic actions (29). As a result, NS is a potential therapeutic agent for the treatment of several disorders (11, 37). NS has been shown to have a variety of effects, including anti-analgesic, anti-inflammatory, anti-oxidative and anti-asthmatic effects, as well as immunological regulation (29, 54). However, research on the preventive action of NS against the harmful effects of BPA on testicles is limited.

There are significant data suggesting that BPA, an endocrine disruptor, is harmful to human and animal health. BPA may impair fertility in males by harming the testicles and lowering sperm quality. The purpose of this research was to look into the possible effects of NS, a nutraceutical, on testes, sperm abnormalities, oxidative stress and cytokine levels in rats exposed to BPA.

Material and methods

Chemicals and other reagents. BPA was obtained from Sigma Chemical Company (St. Louis, Mo, USA). *Nigella sativa* seed oil (NSO) was supplied by Botalife (Isparta, Turkey). Malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and enzyme-linked immunosorbent assay (ELISA) kits were purchased from Bioassay Technology Laboratory (Shanghai, China). Tumour necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and interleukin 10 (IL-10) ELISA kits were purchased from Thermo Fisher Scientific Inc. (USA). Testosterone and LH ELISA kits were purchased from Cayman Chemical Company (USA).

Animals and diets. Animal experiments were carried out at the Selcuk University Experimental Application and Research Center (Turkey). The ethics committee of the Experimental Animal Production and Research Centre at the Veterinary Faculty of the Selcuk University approved the study protocol (approval no. 2022/34). All experimental procedures were carried out in accordance with the European Economic Community Directives on animal welfare (86/609/CEE and 2010/63/EU). This study used 36 male adult Wistar albino rats weighing 400–450 g. Before beginning the study, the overall health of the animals was evaluated. During the 30 days of research, the rats were housed in plastic rat cages in an environment with 12/12 day-night light cycles, a room temperature of $22 \pm 2^\circ\text{C}$, and a humidity of $50 \pm 10\%$. After 7 days of acclimatization, the rats were divided into four groups based on their mean body weight: control, BPA, NS and BPA+NS. Throughout the study, the control group received 1 ml of olive oil, the BPA and BPA+NS groups received 100 mg/kg body weight

of BPA (dissolved in 1 ml of olive oil), and the NS and BPA+NS groups received 5 ml/kg of *N. sativa* containing 0.6% percent thymoquinone (12). NS was administered at a dose equivalent to 1.25 percent of the daily food ration for 30 days. During the trial period, BPA was freshly prepared each day directly before administration. An experienced researcher used ball-type gastric feeding needles (Greatlth, China) to administer BPA and NS to all animals via gavage in equal and full doses. Feeding needles attached to syringes were carefully inserted through the rats' mouths into the lower esophagus. These needles were used to prevent the needle from entering the trachea and to avoid trauma to the oral cavity and esophagus.

Determination of serum testosterone and LH values.

At the end of the 31st day, blood was collected from the hearts of all animals via cardiac puncture under general anesthesia (Xylazine 10 mg/kg and Ketamine 5 mg/kg). Blood was collected in serum tubes (BD Vacutainer SSTTM II Advance-367953) and centrifuged at 4500 rpm (Hettich Universal 32R) for 10 minutes at $+4^\circ\text{C}$. Serum samples were kept at -80°C in Eppendorf tubes until hormone analysis. Serum testosterone and LH levels were determined using ELISA kits by Voller et al. (61).

Evaluation of oxidative/antioxidant indices. The rats were sacrificed by cervical dislocation under general anaesthesia, and their testicles and epididymes were taken for analyses. Right testicles were cleaned with cold saline solution, individually chopped and homogenized (10% w/v) in a Potter-Elvehjem homogenizer in an ice-cold sodium potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl. Tissue homogenates were then centrifuged at 5000 rpm for 10 minutes at 4°C , and aliquots of the supernatant were separated and stored at -80°C until further testing of lipid peroxidation, antioxidant and cytokine parameters (42). Tissue oxidative stress and antioxidant indicators were assessed. MDA was determined in testis tissue homogenates according to a procedure described by Ohkawa et al. (49). Testis GSH concentration and SOD activities were determined in testis tissue homogenates according to Beutler et al. (13) and Nishikimi et al. (47), respectively.

Evaluation of cytokine levels. In tissue samples, cytokines were quantified using commercial ELISA kits. TNF- α , IL-6 and IL-10 levels were measured with anti-rat ELISA kits according to the manufacturer's instructions as mentioned above (48).

Evaluation of sperm motility. The epididymes were rapidly transferred to the laboratory for fluorescent staining in saline. After collecting semen by puncture of the cauda epididymis, semen was diluted with PBS (pH: 7.4) to 100×10^6 spermatozoa/ml. As much as a pinhead of it was transferred to a pre-warmed slide (37°C) and covered with a preheated coverslip (37°C), and at least 7 regions were examined under a phase contrast microscope with a $20 \times$ objective. Motility was determined subjectively.

Evaluation of plasma membrane integrity. The plasma membrane integrity of spermatozoa was assessed using a modified SYBR-14 fluorescent dye developed by Garner and Johnson (20). SYBR-14 stock solution was diluted

1 : 10 with DMSO and stored at -20°C in Eppendorf tubes. Sperm samples were diluted 1 : 3 with Tris without egg yolk and glycerol, mixed with 30 μL of the sperm sample, 6 μL SYBR-14 and 2.5 μL PI and then incubated for 20 minutes at 37°C . At the end of the incubation, 10 μL of Hancock solution was added, and semen movement was halted. 2.5 μL of the sample was collected and placed on the slide, which was then covered with a coverslip and monitored. At least 200 spermatozoa were counted using a fluorescence microscope at $400\times$ magnification. If the spermatozoon's head was red, orange or yellow, it was classified as dead; if the spermatozoon's head was green, it was evaluated as alive.

Evaluation of mitochondrial activity. JC-1/PI The mitochondrial activity of spermatozoa was assessed using a fluorescent dye modified from Garner et al. (21). With DMSO solution, 5,5', 6,6' tetrachloro-1,1', 3,3' tetraethyl-benzimidazolecarbocyanine (1.53 mM) (T3168 JC-1, Invitrogen, Carlsbad, CA) was prepared, filtered and transferred to Eppendorf tubes as 100 μL . It was then kept at -20°C . Tris without egg yolk and glycerol was used to dilute the semen 1 : 3. 300 μL of diluted sperm was gently mixed with 2.5 μL of JC-1 and 2.5 μL of PI before incubating at 37°C for 20 minutes. At the end of incubation, 10 μL of Hancock solution was added, and semen movement was halted. 2.5 μL of the sample was collected and placed on a slide, which was then covered with a coverslip and monitored. At least 200 spermatozoa were counted using a fluorescence microscope at $400\times$ magnification. If the spermatozoon's tail is orange, yellow or bright green, there is activity; if the spermatozoon's tail is dull/pale green, there is no activity.

Evaluation of acrosome membrane integrity. Modified FITCH-PNA/PI (Fluorescein isothiocyanate conjugated to Arachis hypogaea/propidium iodide) fluorescent dye from Nagy et al. according to the method (46). 120 μL of FITCH-PNA was diluted with 1 ml of PBS. It was then filtered and transferred to Eppendorf tubes as 100 μL and stored at -20°C . After diluting the semen sample 1 : 3 with Tris without egg yolk and glycerol, 60 μL of the sample was mixed with 10 μL FITCH-PNA and 2.5 μL PI and incubated at 37°C for 20 minutes. At the end of incubation, 10 μL of Hancock solution was added, and the movement of sperm was stopped. 2.5 μL of the sample was placed on a slide, covered with a coverslip and monitored. At least 200 spermatozoa were counted under a fluorescence microscope at $400\times$ magnification. If the acrosome part of the spermatozoa is red, the acrosome is damaged; if the acrosome part is green, the acrosome is considered intact.

Histopathological investigation. Left testis samples were fixed in 10% formaldehyde and Bouin's solution for 48 hours. Then, the tissues were

washed in running water for 12 hours and taken to a tissue tracking device (Leica TP 1020), and routine histopathological tissue processing was performed. Then, 5 μm thick sections were taken with a microtome (Leica RM 2125RT)

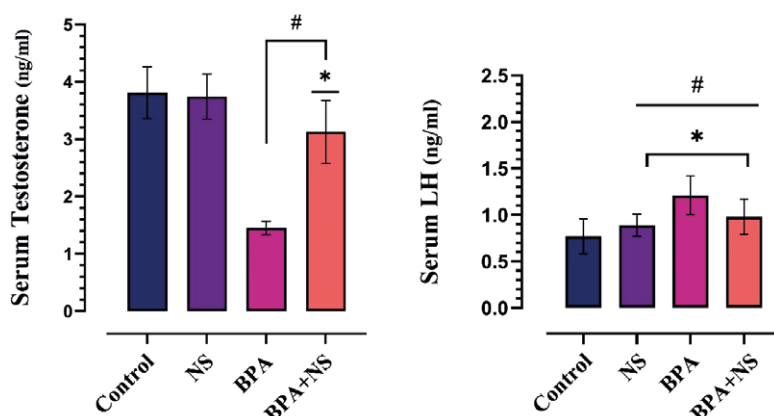


Fig. 1. Testosterone and LH levels in serum

Explanations: # – a significant difference as compared to the control group ($p < 0.05$); * – a significant difference as compared to the BPA group

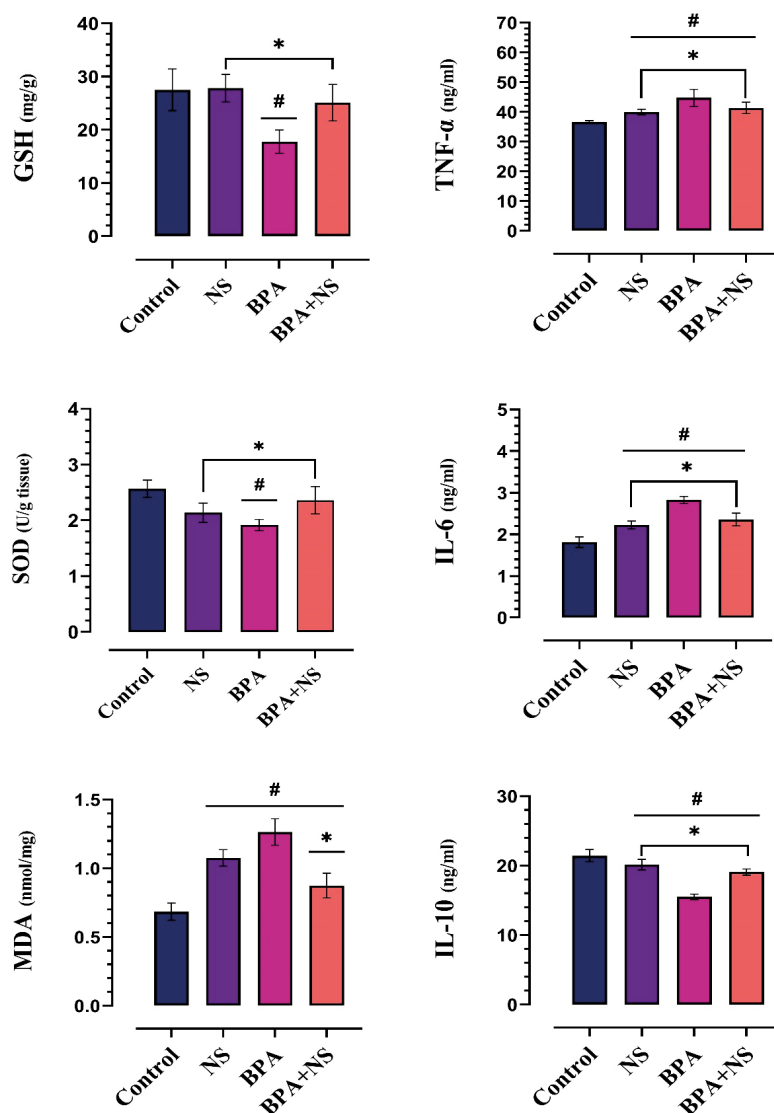


Fig. 2. Testicular antioxidant enzyme capacity, lipid peroxidation product and pro/anti-inflammatory cytokine levels between groups

Explanations: # – a significant difference as compared to the control group ($p < 0.05$); * – a significant difference as compared to the BPA group

from the tissues blocked in paraffin. All sections were stained with Haematoxylin-Eosin (HE) and examined under a light microscope (Olympus BX51, Tokyo, Japan) (34). Histopathological findings were recorded separately, and photographs of characteristic findings (Olympus, EP50) were taken. In order to determine the diameter of seminiferous tubules (ST), measurements made from two different points (width–length) of 10 random STs at $20\times$ magnification were averaged in μm . Similarly, the average of measurements made from two different points of the germ cell layer thickness of selected STs was taken (in μm). The Johnson testicular score was obtained by giving each ST a numerical score ranging from 1 to 10 according to a previously reported method for evaluating testicular damage (55).

Statistics analysis. Normal distribution analyses of serum testosterone ve luteinizing hormone, testicular andioxidant enzyme capacity, lipid peroxidation product, pro/anti-inflammatory cytokine levels, microscopic sperm parameters and histopathological scoring were done with the Kolmogorov-Smirnov test. The homogeneity of variances was controlled by Levene's test. All data were evaluated by the Duncan analysis following one-way ANOVA (SPSS® program). Statistical importance was assumed as a value of $p < 0.05$.

Results and discussion

Results of hormone analyses. Figure 1 shows that the serum testosterone level in the BPA group was statistically significantly lower ($p < 0.05$), whereas the LH level was statistically significantly higher ($p < 0.05$). As seen in the BPA+NS group, NS significantly limited changes in the concentration of both hormones induced by BPA. Furthermore, NS administration alone significantly increased LH levels compared to those in the control group ($p < 0.05$) (Fig. 1).

Effects of BPA and NS on antioxidant status in testis tissue. When compared to those in the control group, testicular GSH and SOD levels in the BPA group were statistically significantly lower ($p < 0.05$), while MDA levels were statistically significantly higher ($p < 0.05$). As compared to those in the BPA group, testicular GSH and SOD levels were statistically higher in the BPA+NS group ($p < 0.05$), while MDA levels were statistically lower with a simultaneous application of NS. (Fig. 2)

Effects of BPA and NS on TNF- α , IL-6 and IL-10 in testis tissue. In the NS, BPA and BPA+NS groups (Fig. 2), a significant increase in the concentration of TNF- α and IL-6 and a decrease in the concentration of IL-10 were found ($p < 0.05$). These effects were most pronounced in the BPA group and were statistically different from those in the NS and BPA+NS groups ($p < 0.05$).

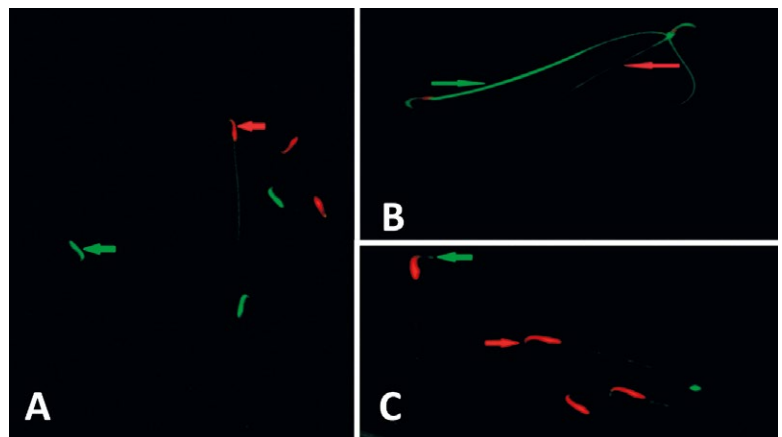


Fig. 3. A. Determination of Plasma Membrane Integrity. SYBR-14/PI Staining. $400\times$ magnification. Those with green heads are spermatozoa with intact plasma membranes (Green Arrow). Those with red heads are spermatozoa with damaged plasma membranes (Red Arrow). **B. Mitochondrial Membrane Integrity Detection.** JC1/PI Staining. $400\times$ magnification. Bright green bodies indicate mitochondrial activity (Green Arrow). Pale green bodies mean no mitochondrial activity (Red Arrow). **C. Acrosomal Membrane Integrity Detection.** FITC-PNA/PI Staining. $400\times$ magnification. Those with green acrosomes are spermatozoa with damaged acrosome membranes (Green Arrow). Those whose acrosomes do not receive green dye are spermatozoa with intact acrosome membranes (Red Arrow)

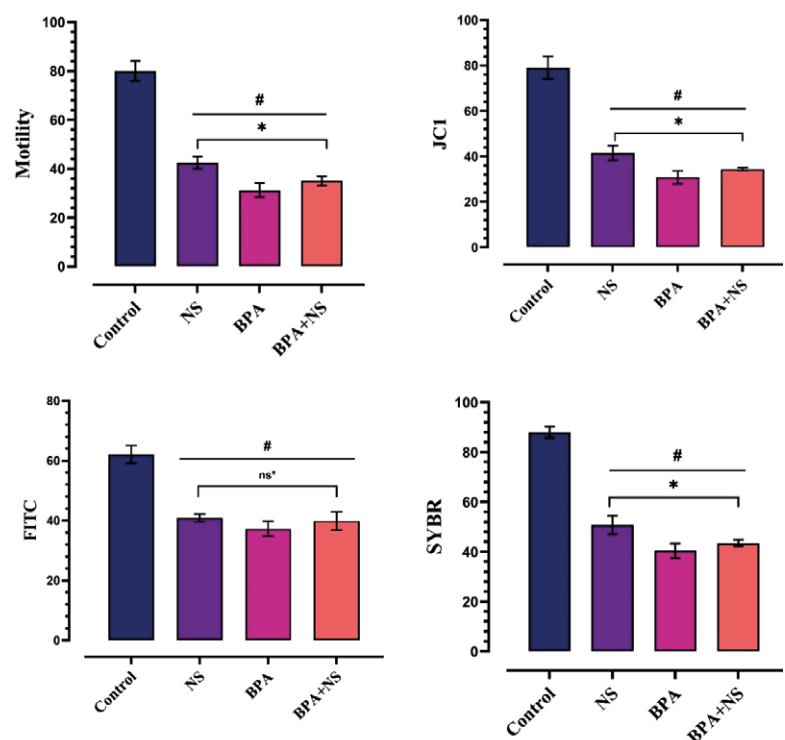


Fig. 4. Graphs of motility, mitochondrial activity (JC1), acrosome integrity (FITC-PNA) and viability (SYBR-14) of rat sperm supplemented with BPA and NS

Explanations: # – a significant difference ($p < 0.05$) as compared to the control group; * – a significant difference ($p < 0.05$) as compared to the BPA group; ns – an insignificant difference as compared to the BPA group ($p > 0.05$)

Spermatological findings. As shown in Figures 3 and 4, the highest motility, plasma membrane integrity, mitochondrial activity and acrosome membrane integrity rates were obtained in the control group

($p < 0.05$). The NS group showed significantly more motility and mitochondrial activity and higher plasma membrane integrity rates than did the BPA and BPA+NS groups ($p < 0.05$). But there were no significant differences between the NS, BPA and BPA+NS groups ($p > 0.05$) in terms of the acrosome integrity rate.

Histopathological results.

Control group testes were found to have a normal architecture. A decrease in the germinative epithelium of the seminiferous tubules (ST) of testicles was observed in rats from the BPA group ($p < 0.05$). Although the germinative epithelium in the BPA+NS group was reduced, this reduction was not found to be statistically significant ($p > 0.05$). It was observed that the atrophy ($p < 0.05$) in the BPA group was partially decreased by the addition of NS ($p > 0.05$), according to the tubulus diameter measurement media. The BPA group showed oedema, hyperaemia and thickening in certain vessel walls, mononuclear cell infiltration in interstitial tissue and giant cell formations, as well as degeneration and necrosis in seminiferous tubules. A significant decrease in the Johnson testicular score was observed in the BPA group ($p < 0.05$). A statistically significant amelioration of this score was found in the BPA+NS group ($p > 0.05$) (Fig. 5).

BPA is an endocrine disrupting compound that can alter human and animal reproductive function. Increased oxidative stress is known to play a major role in the detrimental effects of BPA on cells and tissues (40). Therefore, this study was aimed at evaluating the protective effects of NS, which is a natural antioxidant and anti-inflammatory, on testicles exposed to BPA (51). The rat testis/sperm model was employed in this research because of the strong phenotypic/physiological similarity of rats to humans (44).

BPA is known to disrupt the interplay between endogenous reproductive hormones, resulting in negative consequences to oestrogen- and androgen-sensitive tissues. In male adult rats exposed to BPA, it has been

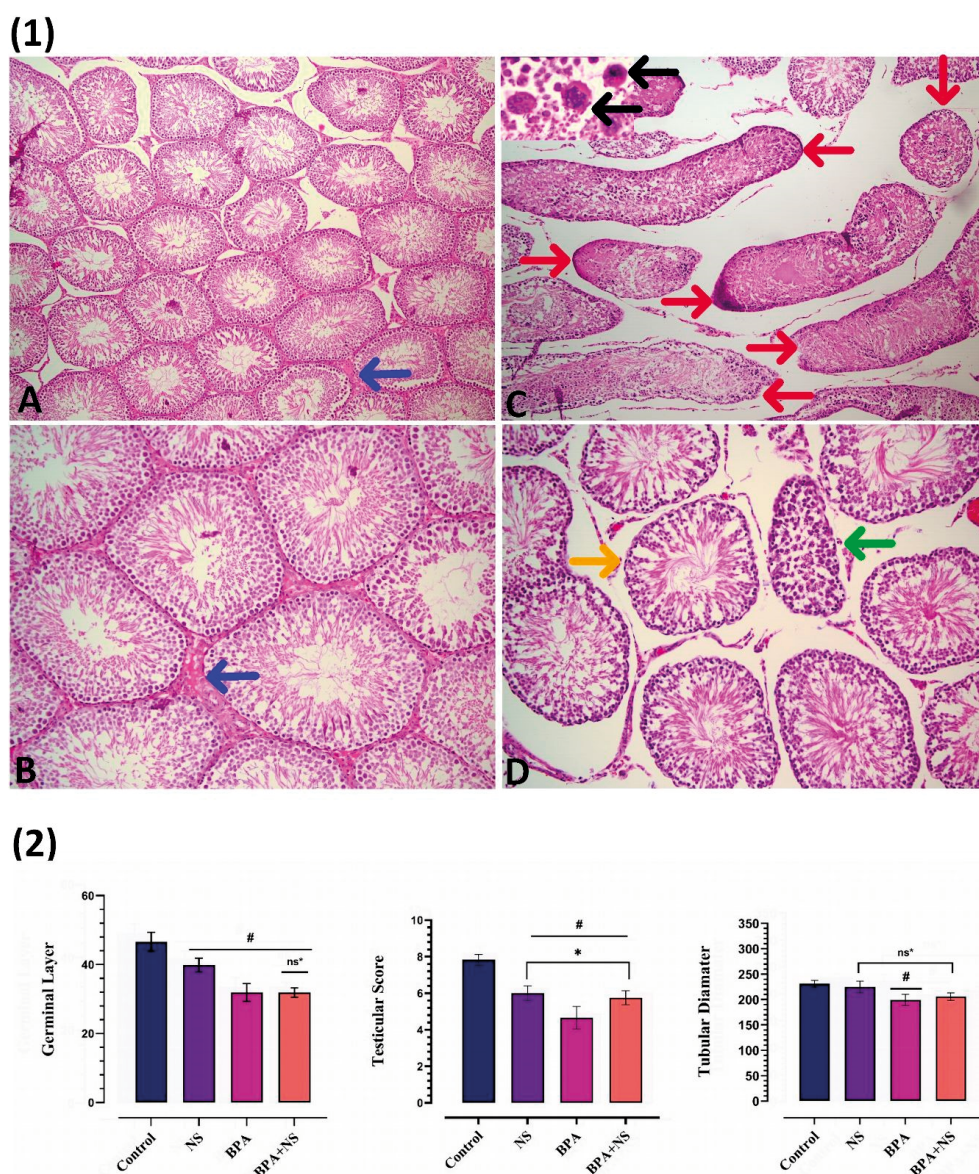


Fig. 5. (1) Photomicrographs of histopathological analyses. A. Control group, testis, HE, 10 ×; B. NS group, testis, HE, 20 ×; C. BPA group, testis, HE, 20 ×; D. BPA+NS group, testis, HE, 20 ×. Blue arrows: oedema, Black arrows: multinucleated giant cell formation in seminiferous tubules (ST), Red arrows: necrosis of ST, Green arrow: Atrophy of ST, Yellow arrow: degeneration in germinal epithelium. (2) Graphical representation of histopathological results

Explanations: # – a significant difference ($p < 0.05$) as compared to the control group; * – a significant difference ($p < 0.05$) as compared to the BPA group; ns * – an insignificant difference as compared to the BPA group ($p > 0.05$)

reported that the plasma testosterone level decreases, but LH concentration increases. Similarly, our results showed that BPA decreased testosterone levels in rats, which was accompanied by an increase in LH levels (41, 59). In the present study, BPA-induced tissue damage in testes, particularly in Leydig cells, resulted in insufficient testosterone synthesis. Thus, the negative feedback mechanism in the GnRH-LH-testosterone axis lost its functionality and caused LH elevation. NS reduced these negative effects of BPA and improved serum testosterone and LH levels (Fig. 1).

Testis have lower oxygen levels than other cells/tissues because the testicular artery is functionally weak. Testicular cells also need more oxygen to maintain the

amounts of cell division required for the formation and maturation of germ cells. They may occasionally compete with one another to receive adequate oxygen as an outcome (10). Therefore, testicular cells and the spermatogenesis cycle are especially vulnerable to oxidative stress, which can lead to aberrant sperm generation and formation of morphologically abnormal spermatozoa (53). Because of this sensitivity, oxidative stress is thought to be the primary cause of male infertility (15). Indeed, the decrease in GSH and SOD levels and the increase in MDA levels in the BPA-exposed group, compared to the control, in the current study suggest the development of oxidative stress through the production of reactive oxygen species (ROS) in testicular tissue, including superoxide anion and hydrogen peroxide (Fig. 2). Wu et al. (63) suggest that exposure to BPA results in increased production of ROS and decreased antioxidant capacity in testicular tissue. In the current study, severe degeneration and necrosis observed histopathologically in the seminiferous tubules is an indication that the testicles are susceptible to oxidative stress. As a matter of fact, increased oxidative stress triggering inflammation with plasma membrane injury and mitochondrial damage may result in degeneration and necrosis of testicular cells. According to Kaur et al. (27), an 8 week exposure of male mice to BPA resulted in a substantial rise in testicular ROS and lipid peroxidation, as well as a decrease in sperm concentration and motility. Consistent with these findings, it is thought that the deterioration in sperm membrane integrity and the decrease in motility observed in the BPA group in the present study may have occurred as a result of damage caused by increased ROS in testicular tissue (Fig. 2, 4 and 5). A recent study found that exposing pregnant mice to BPA for 7 days increased oxidative stress in spermatozoa, even in mature F1 male mice (52). Increased oxidative stress strongly effects DNA, lipids and antioxidant enzymes in germ cells, inducing apoptosis and worsening sperm parameters related to male sterility (5). These findings suggest that BPA, in addition to its endocrine disrupting properties, disrupts the pro-oxidant and antioxidant balance in testicular cells and reduces sperm quality, thus leading to infertility. The improvement in GSH and SOD levels and the decrease in MDA levels in the group in which BPA and NS were administered simultaneously (BPA+NS) reveal the antioxidant potential of NS for quenching free radicals. Consistent with the findings of our study, there are other studies showing the protective efficacy of NS against various xenobiotics in testicular tissue (1, 7, 8, 16, 30).

Endocrine disrupting chemicals, such as BPA, can cause immune systems to malfunction (38). Cytokines are regulatory proteins involved in inflammation and immune responses. Cytokines have direct effects on testicular cell functions (25). In the present study, it

is noteworthy that BPA increased pro-inflammatory cytokine levels (TNF- α and IL-6) and decreased anti-inflammatory cytokine levels (IL-10) in testicular tissue (Fig. 2). In point of fact, it is thought that BPA, an estrogenic steroid, can induce an immune response through specific receptors, such as estrogen receptors (ERs) (31, 45). Exposure to estrogenic compounds can activate or inhibit the expression of nuclear factor kappa B (NF- κ B), resulting in alteration of inflammatory mediators, resulting in cytokine secretion (14). BPA was also reported to induce non-physiological polarisation of T cells and cytokines by influencing dendritic cell development, which might lead to immune system activation that is inappropriate (50). Furthermore, BPA was shown to impact lipopolysaccharide (LPS) activation directly and may play a role in aberrant immunological reactivity (26). In the current study, it was demonstrated histopathologically that BPA stimulated the immune response characterized by mononuclear cell infiltration, edema and hyperemia in testicular tissue as a reflection of this fluctuation in cytokine levels. According to reports, NS possesses potent anti-inflammatory properties (2, 28, 36). In the current study, it is noteworthy and consistent with previous studies that the abnormal response in cytokine levels caused by BPA was alleviated by NS (Fig. 2). This is probably due to the levels and types of phytochemicals found in NS, which have important anti-inflammatory and antioxidant properties (9).

Sperm motility is a key indicator of male fertility, and optimum sperm motility and motility kinematics are important for successful fertilization (56). Antioxidant supplementation has been shown to have prophylactic effects on sperm quality, including the viability, motility and morphology of spermatozoa (43). Some antioxidants, such as GSH, vitamin C and vitamin E, have been shown to improve semen function and fertilisation capacity in *in vitro* experimental settings (18, 51, 60). Similarly, NS has been reported to increase sperm count, mobility and motility, and to improve antioxidant activity in the testis (35). Consistent with previous findings, the results of the present study show that sperm motility reduced by BPA was mostly restored by NS (Fig. 3, 4). In addition, we found that BPA exposure caused morphological abnormalities in the head and tail of the sperm. These anomalies were significantly fewer in the BPA+NS group compared to the BPA group (Fig. 3, 4). The sperm cell membrane contains substantial quantities of polyunsaturated fatty acids and phospholipids, which are sensitive to oxidative stress (6, 22). DNA damage, lipid peroxidation, as well as protein and biomembrane damage may occur in sperm after an increase in free radicals (5). Agents with antioxidant properties, such as NS, can transfer electrons to oxidizing agents and inhibit free radical production and sperm damage (3, 5).

Based on the findings of the present study, it was concluded that BPA is a highly toxic substance that disrupts the cellular redox balance in the testis and sperm, damages the testicular structure and function, thus causing endocrine imbalance and abnormal sperm formation. The protective effect of NS against BPA-induced testicular damage was shown to be due to improvements in endocrine, oxidative stress and cytokine levels, as well as histopathological scores and sperm parameters. It should be noted, however, that these results are limited to the animal used in the study, and more research is needed to determine the proper dosage of NS and a treatment plan.

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