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# Investigation into the protective effects of Naringenin in phthalates-induced reproductive damage

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**Abstract. – OBJECTIVE: Di-n-butyl phthalate (DBP) is a ubiquitous environmental pollutant, extensively used as a plasticizer in many products, including plastics, cosmetics, and medical devices. Naringenin (NAR) is a flavonoid belonging to the flavanones subclass. It is widely distributed in several citrus fruits, bergamot, tomatoes, and other fruits. It is also found in its glycoside form (mainly naringin). Several biological activities have been ascribed to this phytochemical: antioxidant, antitumor, antiviral, antibacterial, anti-inflammatory, antiadipogenic, and cardioprotective effects. This study hypothesized that phthalates' possible reproductive damage mechanism is oxidative attack, and naringenin could have a protective effect against radical forms in the body through its antioxidant properties.**

**MATERIALS AND METHODS: Thirty-two male rats were used in our study (n=8 each). Rats were randomly divided into four groups: Control, DBP, DBP +NAR and NAR. Phthalate (DBP) and NAR were administered through gastric oral gavage (phthalate group 500 mg/kg/day DBP; NAR group 50 mg/kg/day NAR). At the end of four weeks, testis tissue samples were taken under anesthesia. Testis tissue and blood samples were collected from the four groups in this study. Histological, biochemical and spermatological analyses were conducted.**

**RESULTS: Tissue samples from the control and NAR groups showed normal histological appearance on light microscopy. The DBP group exhibited deterioration in seminiferous tubules, vascular congestion in capsule, vascular congestion between the seminiferous tubules, edema in the intestinal area and vacuolization, arrested spermatocytes in different stages of division; sloughing of cells into the seminiferous tubular lumen was observed. It was also observed that NAR treatment significantly inhibited and prevented the histopathological damage caused by DBP. Tissue TBARS, antioxidant parameters, sperm motility, sperm density and abnormal spermatozoon ratios were determined. As a result, it was shown that DBP caused oxidative damage by increasing TBARS levels and decreasing antioxidant parameters, increased abnormal sperm rate and decreased sperm motility, and concentration and histopathological damage, so the antioxidant activity of naringenin inhibited this damage.**

**CONCLUSIONS: DBP had toxic effects in rat testis tissue; NAR treatment ameliorated these effects. Further studies are warranted to confirm our findings.**

*Key Words:*
Di-n-butyl phthalate, Naringenin, Oxidative stress, Spermiotoxicity, Testicular damage.

## Introduction

Phthalic acid esters (PAEs) are mainly used in the plastics industry as adhesives and plasticizers for polyvinyl chloride materials to improve flexibility and easy manufacturing. They are a kind of toxic persistent organic pollutants[1]. Phthalate diesters (phthalates) are broadly used in manufacturing plastics, solvents, sealants, paints, varnishes, personal care products, food processing, medical, and pharmaceutical industries[2,3]. Human exposure occurs by ingestion, absorption, and inhalation. All studies published to date have confirmed the presence of phthalate metabolites in human urine and some serum and breast milk samples, demonstrating the ubiquitous and unavoidable nature of these chemicals[3-6].

Di-n-butyl phthalate (DBP) is one of the most widely studied phthalate esters that disrupt the normal growth of reproductive organs. The most prominent effect of DBP is testicular atrophy[7,8]. Studies demonstrating that phthalates impair fetal rodent testis development reinforce epidemiological data indicating increased incidence of testicular dysgenesis in boys exposed to phthalates in

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utero. Because humans are continuously exposed to phthalates from gestation through adulthood, it is imperative to understand what threat phthalates pose at other life stages[9]. Many studies have shown[2,8-10,12] that phthalate esters cause reproductive toxicity. They act as an endocrine disrupter, affecting the breakdown of reproductive organs in animals and humans. Studies have shown[10] that the male reproductive system is more vulnerable to phthalates than is the female reproductive system. To date, several mechanisms have been proposed to explain the induction of testicular atrophy by DBP, such as the depletion of zinc[7], increased oxidative damage of proteins, lipids, DNA[10], and alteration of vimentin cytoskeleton organization or membrane alteration in Sertoli cells, leading to sloughing of spermatogenic cells[11]. Exposure to plasticizers DBP and diethylhexyl phthalate (DEHP) during sexual differentiation causes male reproductive tract malformations in rats and rabbits. These two phthalate esters decrease testosterone (T) production and insulin-like peptide 3 (insl3) gene expression in the fetal male rat, a hormone critical for gubernacular ligament development[12].

Naringenin is one of the most important naturally occurring flavonoids (4, 5, 7-trihydroxyflavone), predominantly found in some edible fruits, such as citrus species, tomatoes, and figs[13] (belonging to Smyrna-type Ficus carica). Naringenin is a bioflavonoid derived from grapefruit and related to citrus species. Naringin, or its metabolite naringenin, reportedly exhibits diverse biological and pharmacological properties, including anti-carcinogenic, lipid-lowering, superoxide-scavenging, anti-atherogenic, metal-chelating, and antioxidant activities. Recently, naringin has received considerable attention as a dietary supplement, and growing evidence has indicated that naringin or naringenin displays antioxidant effects both *in vitro* and *in vivo*[14]. Previous studies[15] have shown that naringenin prevents dyslipidaemia and hyperinsulinaemia, has a cholesterol-lowering effect in rabbits and rats[16] and exhibits antiviral activity[17]. The study conducted by Goldwasser et al[18] found that naringenin could replace the actions of fibrates (PPARα agonists), thiazolidinediones (PPARγ agonists), and statins in the treatment of type-2 diabetes or hyperlipidaemia.

This study hypothesized that naringenin could prevent DBP-induced reproductive toxicity due to its antioxidant properties. Based on this hypothesis, this study investigated the oxidative stress parameters and histopathological changes in the testis tissue of rats administered with DBP and treated with naringenin.

## Materials and Methods

### Animals

Thirty-two Wistar albino rats (age 12-16 weeks; weight 280-320 g) were obtained from the Experimental Animals Unit of Inonu University (Malatya/Turkey). Rats were placed in polypropylene cages with a 12 h light/12 h dark cycle, at an ambient temperature of 21°C. Food and water were provided *ad libitum*. The study protocol was approved by the Inonu University Experimental Animals Ethics Committee (2014/A-02).

### Chemical

The rats were randomly divided into four groups (n=8 each): Control, DBP, DBP+NAR, NAR.

Group 1 - Control group: CMC (the solvents of NAR and DBP solutions) were administered to the animals with intraperitoneal (i.p.) and gastric gavage.

Group 2 - DBP group: DBP was administered intraperitoneally (i.p.) at a dose of 500 mg/kg/day.

Group 3 - (DBP+NAR group): DBP was administered with i.p. at a dose of 500 mg/kg/day and NAR at 50 mg/kg/day by gastric oral gavage.

Group 4 - (NAR group): NAR was administered at 50 mg/kg/day by gastric oral gavage.

DBP and naringenin were dissolved in Sodium Carboxy Methyl Cellulose (CMC) (CMC-CAS: 9004-32-4, TCI, Tokyo, Japan), and solutions were freshly prepared in such a way to be administered. DBP was obtained from ABCR company (Karlsruhe, Germany, CAS: 84-74-2), and naringenin (NAR) was purchased from alfa easer (Massachusetts, MA, USA, CAS: 67604-48-2).

After a four-week drug administration, rats were sacrified and testis samples were removed. Rat blood samples were collected from the left ventricle for biochemical analysis. The collected samples were used for histological, immunohistochemical, biochemical and spermatological evaluations.

### Biochemical Analysis

The homogenization of tissues was carried out in a Teflon glass homogenizer with 150 mm KCl (pH 7.4) to obtain a 1:10 (w/v) dilution of the whole homogenate[19-22]. The homogenates were centrifuged at 18,000 g (4°C) for 30 min to

determine thiobarbituric acid reactive substances (TBARS), glutathione (GSH) levels, and catalase (CAT) activities, and at 25,000 g for 50 min to determine glutathione peroxidase (GPx) levels and copper/zinc superoxide dismutase (CuZn-SOD) activities[23]. The levels of homogenized tissue TBARS, as an index of lipid peroxidation, were determined by thiobarbituric acid reaction, using the Yagi[24] method. The product was spectrophotometrically evaluated at 532 nm, and the results were expressed as nmol/g tissue. The superoxide dismutase (SOD) activity was measured by inhibiting nitroblue tetrazolium (NBT) reduction due to $O_2$ – generated by the xanthine/xanthine oxidase system. One unit of SOD activity was defined as the amount of protein, causing 50% inhibition of the NBT reduction rate. The product was spectrophotometrically evaluated at 560 nm. Results are expressed as IU per mg protein[25]. The enzymatic decomposition of $H_2O_2$ was directly followed by the decrease in absorbance at 240 nm, and the difference in absorbance per unit time was used as a measure of CAT activity[25]. The enzyme activities are given in per mg of protein. The GPx activity was measured according to a related previous study[26].

### Sperm Analysis

The sperm concentration in the right cauda epididymis was determined with a hemocytometer using a modified method developed by Ciftci et al[25]. The freshly isolated right cauda epididymis was used to assess sperm motility. The sperm motility rate was evaluated using a light microscope with heated stage[27]. The slides stained with eosin-nigrosin (1.67% eosin, 10% nigrosine, and 0.1 M sodium citrate) were prepared and viewed under a light microscope at 400 magnifications to determine the percentage of morphologically abnormal spermatozoa. In this study, 300 spermatozoa were examined on each slide (2,400 cells in each group), and the head, tail, and total abnormality rates of spermatozoa were defined as percentages[28].

### Histopathological Examination

Tissue samples obtained for histopathological examinations were fixed in 10% formaldehyde. Routine tissue processing procedures were performed on the detected tissues, and tissue samples were embedded in paraffin blocks. Subsequently, 5-µm-thick sections were prepared. Hematoxylin and eosin staining was applied for immunohistochemical evaluation, using following protocol. Testis tissue samples were sectioned on poly-lysine-coated slides. The sections were placed in citrate buffer (pH 7.6) and heated in microwave for 20 min. They were then cooled at room temperature for 20 min, washed with phosphate-buffered saline (PBS) and incubated in 0.3% hydrogen peroxide for 7 min to block endogenous peroxidase activity. After being washed again with phosphate buffered saline, sections were incubated with polyclonal anti Caspase-3 antibody (Ab4051, Abcam, Cambridge, MA, USA) for 2 hours at room temperature. The 3-Amino-9-ethylcarbazole (AEC) chromogen/substrate was applied for 15 min, then sections were stained in Mayer's Hematoxylin solution and examined by light microscopy using the Leica DFC 280 light microscope.

Seminiferous tubule diameter (MSTD) and germinal epithelial cell thickness (GECT) were measured at 20x magnification in 20 randomly selected round tubules from each section. Leica DFC light microscope and a Leica Q Win Image Analysis system were used for measurements.

### Statistical Analysis

One-way ANOVA variance analysis was used in comparison of sperm characteristics and biochemical findings. The results were shown as mean ± standard error. A value of $p \leq 0.01$ was considered statistically significant for sperm characteristics and biochemical findings.

SPSS 13.0 and MedCalc 11.0 statistical programs (Chicago, IL, USA) were used for statistical evaluations. The results were shown as mean ± standard error. A value of $p \leq 0.0001$ was considered statistically significant for histological findings.

## Results

### Biochemical Findings

TBARS GSH, CAT, SOD and GPx enzyme levels are presented in Table I ($p \leq 0.01$). The results show that TBARS, an indicator of oxidative damage in rats given DBP, statistically increased compared to the Control and all other groups. However, NAR treatment significantly prevented the increase in TBARS levels caused by DBP. TBARS levels of the DBP+NAR group approached the control group. DBP administration statistically decreased levels of GSH, SOD, GPx, and CAT enzyme activities. Besides this, it was observed that in the DBP+NAR group, the

**Table I.** The levels of TBARS, GSH, CAT, SOD and GPx in testis tissue of rat (Mean±SE).

| Groups | TBARS (nmol/g tissue) | GSH (nmol/mg protein) | CAT (U/mg protein) | SOD (U/mg protein) | GPx (U/mg protein) |
|---|---|---|---|---|---|
| **Control** | 2.57±0.08[a] | 110.8±2.42[a] | 0.047±0.0018[a] | 32.47±1.38[a] | 93.53±5.80[a] |
| **DBP** | 3.74±0.24[b] | 95.43±1.35[b] | 0.038±0.0019[b] | 26.76±0.53[b] | 101.2±5.37[a] |
| **DBP+NAR** | 2.71±0.17[a] | 105.9±1.11[a] | 0.047±0.0019[a] | 31.58±1.21[a] | 109.3±2.69[a] |
| **NAR** | 2.87±0.19[a] | 106.5±3.6[a] | 0.051±0.0031[a] | 32.94±1.38[a] | 93.82±4.93[a] |

Values with different superscripts in the same column are statistically significantly different ($p \leq 0.01$).
(TBARS: thiobarbituric acid reactive substances, GSH: glutathione, CAT: catalase, SOD: superoxide dismutase, GPx: glutathione peroxidase).

NAR prevented the decrease of GSH content and SOD enzyme levels due to DBP. There was an observed increase in CAT and GPx enzyme levels in the same group, but it was not statistically significant. There was no statistically significant difference between the control and NAR groups regarding all parameters.

## Spermatological Findings

### Effects on the reproductive organ weights

Changes in the reproductive system organs weights (testis, epididymis, seminal vesicle, and prostate) are presented in Table II. As observed in the table, DBP or NAR applications did not significantly change the reproductive system organs weights statistically compared to the Control group.

### Semen quality

Sperm motility, abnormal sperm count rate, and sperm concentrations, which represent the characteristics of sperm, are given in Table III. DBP treatment caused in a decrease in sperm motility and concentration, compared with the control and NAR groups. In the DBP+NAR group, NAR improved this DBP-mediated decrease and led to significantly sperm motility and concentration, compared with DBP group. These values were close to the Control group, and there was no statistical difference between the DBP+NAR and the Control group. It was also observed that DBP administration caused an increase in the amounts of abnormal sperm (head, tail, total); these effects were improved by NAR treatment.

### Histopathological evaluation

The Control (Figure 1A) and NAR-treated (Figure 1B) testis specimens had a normal histological appearance, as assessed by light microscopy. Seminiferous tubules, interstitial connective tissue, spermatological serial cells and Leydig cells were observed normal histological appearance The DBP group exhibited the following findings in the testis such as: deterioration in seminiferous tubules (Figure 2A, 2B), vascular congestion in capsule (white arrows) (Figure 2A) and between the seminiferous tubules (white asterisks) (Figure 2B), oedema in the intestinal area (black asterisk), vacuolization (thin black arrows) (Figure 2C), cells that temporarily stopped dividing at any stage of division in the seminiferous tubule epithelium (black arrows) (Figure 2D), luminised cells in the seminiferous tubule (black arrow) (Figure 2E). Light microscopy analysis of the DBP+NAR group observed that histopathological damage was significantly decreased and spermatological cells were increased (Figure 3A, 3B, 3C) ($p \leq 0.0001$).

**Table II.** Testis weights, epididymal weights, seminal vesicle weight and prostate weights.

| Groups | Testes weight (g) | | Epididymis weight (g) | | Seminal vesicle Weight (g) | Prostate weight (g) |
|---|---|---|---|---|---|---|
| | Right | Left | Right | Left | | |
| **Control** | 1.450±0.69 | 1.397±0.66 | 0.599±0.03 | 0.579±0.02 | 1.403±0.10 | 0.513±0.05 |
| **DBP** | 1.303±0.54 | 1.365±0.56 | 0.557±0.03 | 0.566±0.02 | 1.307±0.05 | 0.458±0.04 |
| **DBP+NAR** | 1.383±0.35 | 1.384±0.03 | 0.601±0.03 | 0.552±0.02 | 1.301±0.10 | 0.537±0.05 |
| **NAR** | 1.426±0.54 | 1.417±0.77 | 0.595±0.02 | 0.541±0.02 | 1.488±0.07 | 0.509±0.03 |

**Table III.** Sperm motility, epididymal sperm concentration, abnormal sperm rate.

| Groups | Sperm motility (%) | Parameters Epididymal sperm concentration (million/g tissue) | Abnormal sperm rate (%) | | |
|---|---|---|---|---|---|
| **Control** | 91.83±1.06[a] | 241.71±5.98[a] | 3.71±028[a] | 3.42±0.29[a] | 7.14±0.45[a] |
| **DBP** | 61.40±3,54[b] | 162.28±1,61[b] | 10.14±0.88[b] | 8.42±0.68[b] | 18.57±0.29[b] |
| **DPB+NAR** | 82.52±3.29[a] | 239.64±3.09[ad] | 6.71±0.28[a] | 6.28±0.47[c] | 13.00±1.41[c] |
| **NAR** | 84.57±2.21[a] | 277.35±3.56[c] | 3.57±0.29[a] | 3.14±0.40[a] | 6.71±0.42[a] |

[a]There was no difference between Control and NAR. [b]Shows that DBP differs from all groups. [c]Shows that DBP+NAR differs from all groups.

### *Immunohistochemical Findings*

We compared all the groups for Caspase-3 positive stained spermatological cells. Caspase-3 immunoreactivity was not observed in Control (Figure 4A) and NAR (Figure 4D) groups. In the DBP group (Figure 4B), both the number of Caspase-3 positive stained cells and the staining intensity were quite significant. In the DBP+NAR group (Figure 4C), there were significantly reductions in Caspase-3 positive stained cells and their intensity.

The effects of DBP and NAR on MSTD and GECT are shown in Table IV. As seen in Table IV, the tubular diameter (MSTD) and tubular epithelial thickness (GECT) in the DBP group were significantly smaller than the control group. In the DBP+NAR group, MSTD and GECT values showed a significant increase compared to the DBP group, indicating that NAR partially inhibited the damage of DBP. In the light of this information, the findings suggest that DBP may cause severe damage to the testicular tissue and that naringenin has a partially protective effect against this damage.

## Discussion

DBP belongs to a group of compounds known as phthalate esters-diesters of 1,2-dicarboxylic acid. DBP is a special plasticizer used in many consumer products, including food packaging materials and cosmetics[29,30]. Phthalates are high-production-volume chemicals used to soften and add flexibility to plastic consumer products, including intravenous fluid bags and infusion sets, blood

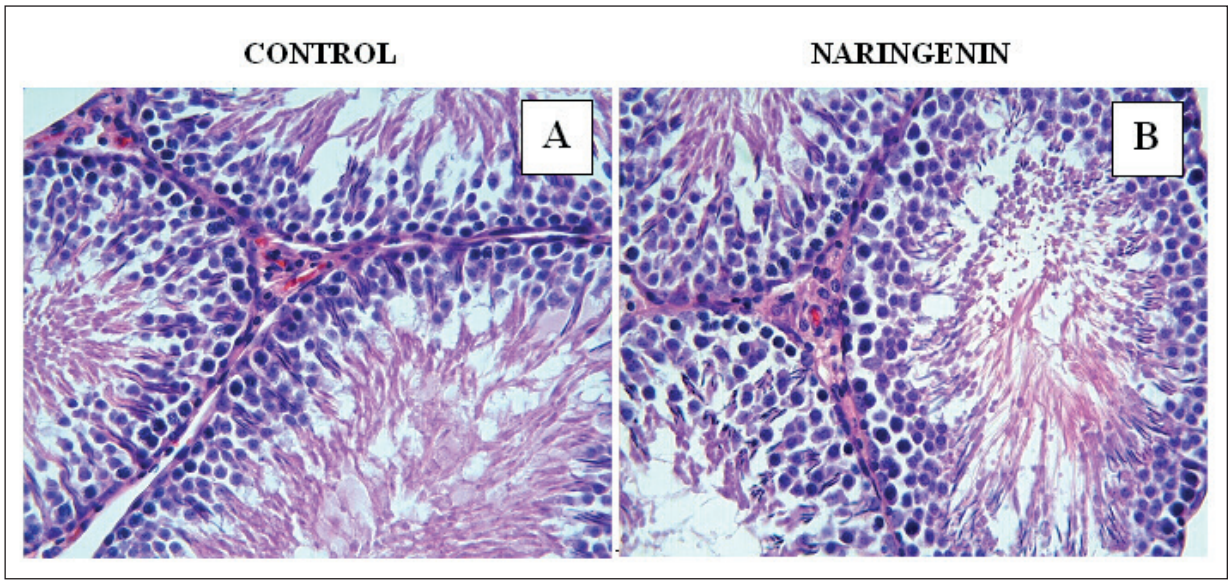


**Figure 1.** Control and Naringenin groups. Testis tissue showed normal histological appearance in Control (**A**) and Naringenin (NAR) (**B**) groups. Arrangement of germinal epithelium, seminiferous tubules and interstitial cells were normal histological appearance. **A, B**: H-E; X40.

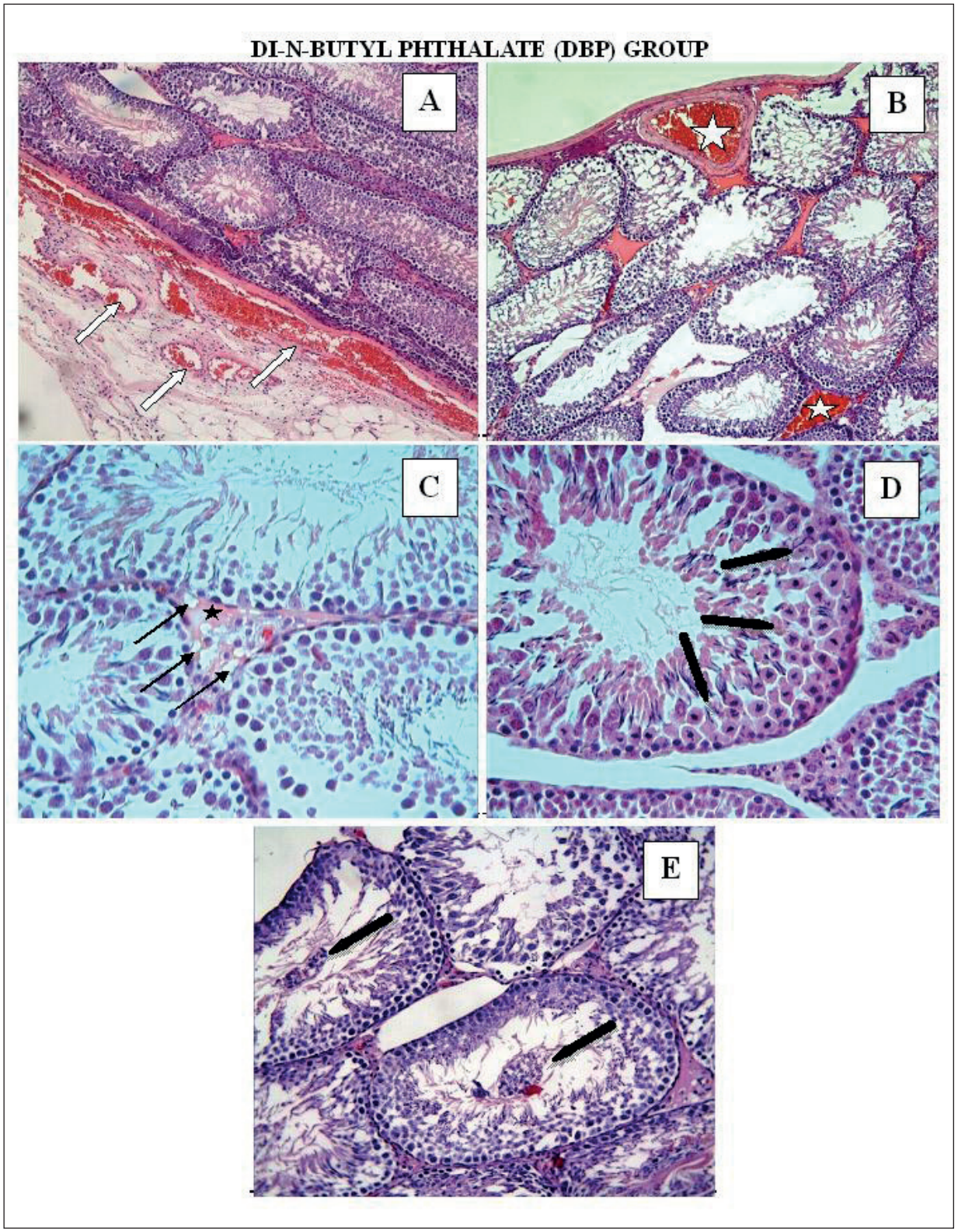


**Figure 2.** DBP group. Deterioration in seminiferous tubules (**A**, **B**), vascular congestion in capsule (white arrows) (**A**), vascular congestion between the seminiferous tubules (white asterisk) (**B**), edema in the intestinal area (black asterisk) and vacuolization (thin black arrows) (**C**), arrested spermatocytes in different stage in division (white arrows) (**D**) and sloughing of cells into the seminiferous tubular lumen (black arrow) (**E**) were observed in DBP group. **A**, **E**: H-E; x20, **B**: H-E; X10, **C, D**: H-E; X40.

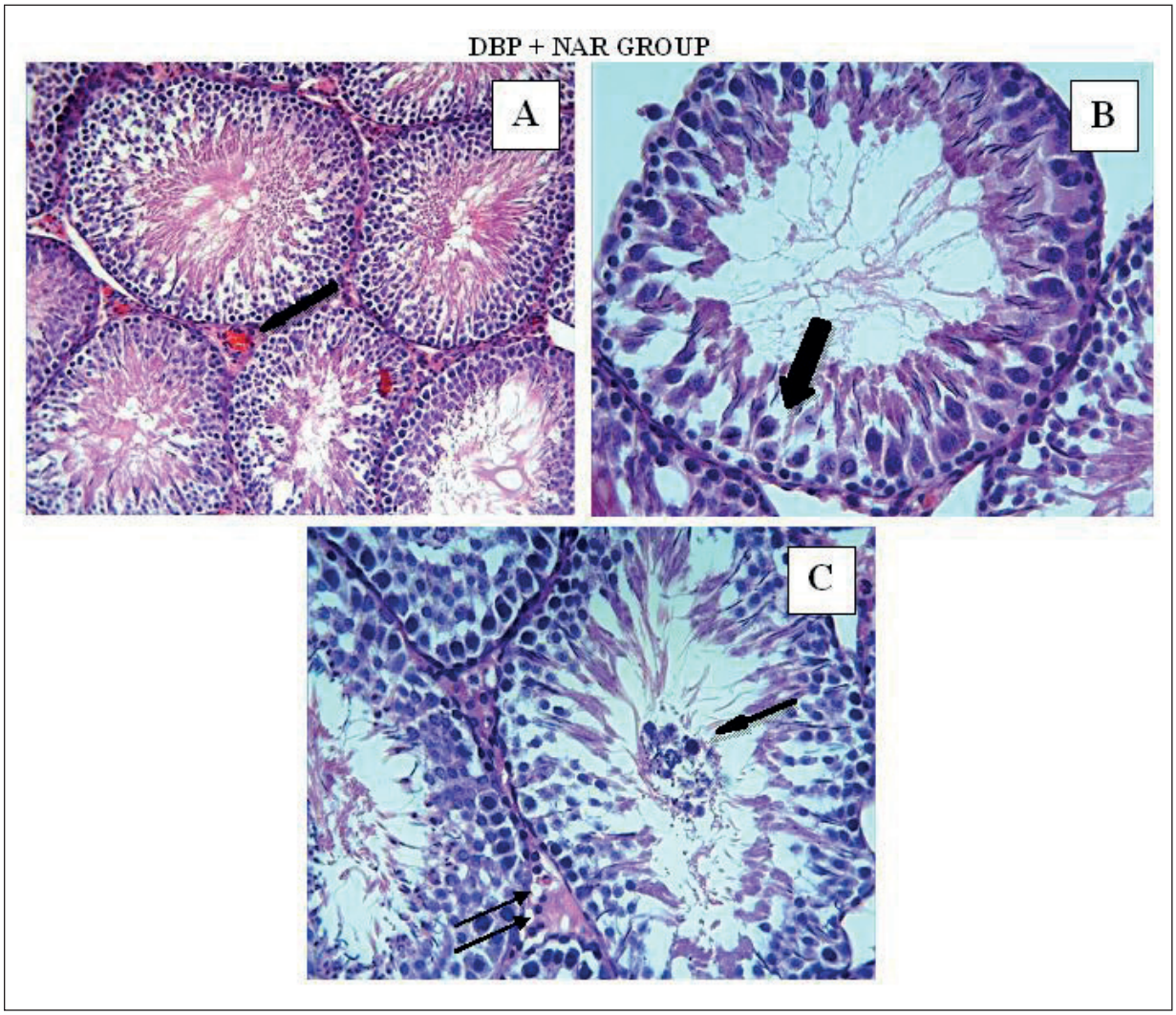


**Figure 3.** DBP+NAR group. Decreased vascular congestion (black arrow) (**A**), arrested spermatocytes in different stage in division (black arrow) (**B**), a small amount of sloughing of cells into the seminiferous tubular lumen (black arrow) (**C**) and vacuolization (thin black arrows) were observed. **A**: H-E; X20, **B**, **C**: H-E; X40.

bags, children's toys and plastic curtains[31]. These chemicals are also present in solvents, lubricating oils, fixatives, and detergents used in residential and commercial construction and the automotive industry. DBP is found in personal care products, such as hair spray, nail polish, perfumes, and skin emollients[32]. Phthalates cause leakage of substances that disrupt the blood testicle barrier into the seminiferous tubes. That produces reactive oxygen radicals, which lead to impaired spermatogenesis, low testosterone levels, and testicular atrophy[33]. One of the mechanisms of this damage is oxidative stress[34].

Several studies have found associations between phthalate exposure and defects related to human reproductive health. DBP is a known endocrine disruptor. It is a developmental toxicant that has pronounced adverse effects on the male reproductive tract. Exposure to DBP (400–500 mg/kg/day) during reproductive development in rodent and nonrodent species results in a variety of malformations of the male reproductive tract, including aplastic or absent epididymis and accessory sex glands, reduced anogenital distance, testicular atrophy, a decline in the number of ejaculated sperm, an increase in the number of abnormal sperm, cryptorchidism and hypospadias[35]. Helmy et al[33] showed that hesperidin application improves testicular functions following phthalate-induced damage.

In rats, male reproductive tract development is disrupted by DBP exposure[36]. Exposure to DBP

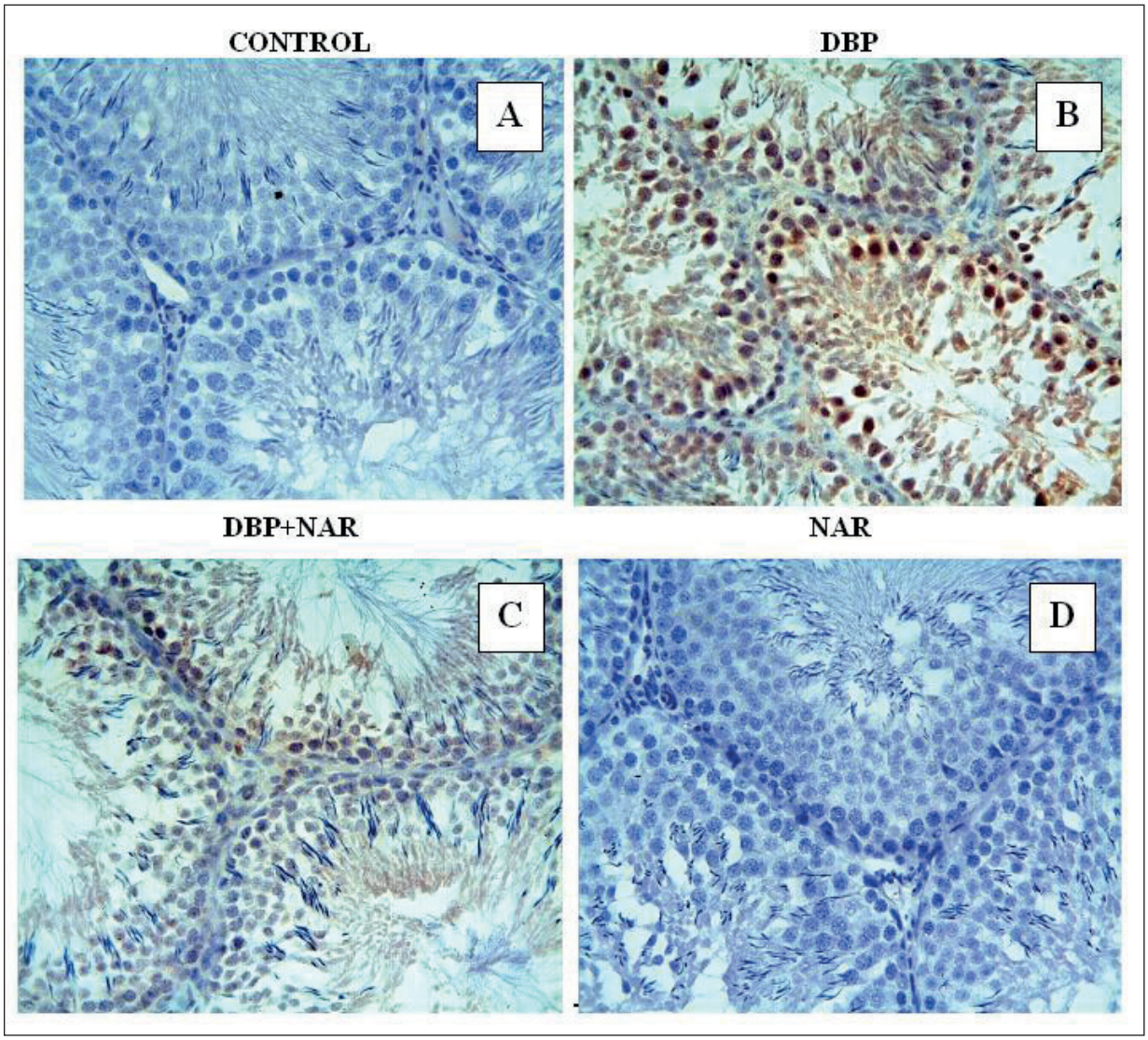


**Figure 4.** Immunohistochemical expression of Caspase-3 in Control (***A***), DBP (**B**), DBP+NAR (**C**) and NAR (**D**) groups. The number of stained (positive) cells decreased in DBP+NAR group (C) compared with DBP+NAR group (**B**). **A-D**: Caspase-3; X40.

on gestation days (gd) 12-18 significantly lowered levels of fetal testicular testosterone[37,38] and altered testosterone- and dihydrotestosterone-dependent tissue development. Rats exposed to DBP *in utero* showed decreased anogenital distance, retained nipples, delayed preputial separation, underdeveloped or absent epididymides, hypospadias and cryptorchidism[39-41].

DBP also induces histopathological changes within seminiferous epithelium in rats and rabbits, including widespread germ cell loss, vacuolization of Sertoli cell cytoplasm, Leydig cell hyperplasia, presence of giant cells, dysgenetic seminiferous tubules and atypical germ cells resembling carcinoma *in situ* cells[40,42]. In another study[43], improvements were shown in oxidative parameters and histopathological changes in which phthalate-induced testicular damage was treated with alpha-lipoic acid, whose antioxidant properties are known.

In this study, DBP caused significant histological changes in testicular tissue. Deterioration in seminiferous tubules, vascular congestion in capsule, vascular congestion between the seminiferous tubules, edema in the intestinal area, vacuolization, arrested spermatocytes in a different stage in the division, and sloughing of cells into the seminiferous tubular lumen were observed in

**Table IV.** The effects of NAR on changes in MSTD and GECT formed by DBP.

| Groups | Tubule diameter (MSTD) | Tubular epithelium thickness (GECT) |
|---|---|---|
| **Control** | 281.86 ± 2.70[a] | 69.37 ± 1.12[a] |
| **DBP** | 236.69 ± 2.47[b] | 38.67 ± 0.72[b] |
| **DBP + NAR** | 258.89 ± 2.98[c] | 57.27 ± 0.99[c] |
| **NAR** | 270.38 ± 3.44[a] | 64.85 ± 1.04[a] |

The mean differences of the values bearing different superscript letters within the same column are statistically significant. ($p \leq 0{,}0001$). SE: Standard Error.

the DBP group. NAR treatment significantly inhibited and prevented the histopathological damage caused by DBP.

The decreased activity of testicular antioxidants caused by DBP, and increased lipid peroxidation (LPO) may explain the observed decrease in total antioxidant capacity (T-AOC). This finding indicates that DBP-induced oxidative stress in rat testes with remarkably increased TBARS concentration may be due to reactive oxygen species (ROS) overproduction[44].

High levels of ROS are associated with poor sperm quality, since ROS induce excessive apoptosis of spermatogenic cells and sperm by disrupting the inner and outer mitochondrial membranes or affecting the balance between pro- and anti-apoptosis systems[45]. Oxidative stress can be defined as a mechanism by which an imbalance between antioxidant enzymes and antioxidants of excess ROS production occurs. One of the main indicators of oxidative stress is the lipid peroxidation product TBARS. ROS may lead to irreversible cell damage and the inactivation of many enzymes. The testis is the main target organ of oxidative stress, since it contains cells consisting of polyunsaturated lipids and is equipped with an antioxidant system[9].

In this study, the findings showed that the TBARS level had increased, and SOD, CAT, GSH-Px, and GSH levels have decreased significantly with DBP administration. The oxidant system has exceeded the antioxidant defense system, causing tissue damage. In the DBP+NAR group, while the TBARS level has decreased, SOD, CAT, GSH-Px, and GSH levels have increased significantly, and the damage of DBP was prevented by naringenin.

Aly et al[46] demonstrated that both sperm count, and motility significantly decreased, in a dose-related manner, in response to DBP (200, 400, or 600 mg/kg, respectively) compared to the corresponding control group. DBP decreased epididymal sperm concentration, sperm motility, organ weights, and increased abnormal sperm count in this study. The decrease in organ weights was not statistically significant. In the DBP+NAR group, a significant increase in sperm quality was observed compared to the DBP group, abnormal sperm rate decreased, and a strong increase in sperm concentration and motility occurred. In the light of these findings, it was inferred that naringenin (NG) significantly blocked gonadal toxicity. Another study[46] showed that NG significantly blocked CP- and DX-induced gonadal toxicity in male rats, most probably by inhibiting oxidative/nitrosative stress, inflammatory responses, and apoptosis. NG maintained the normal histology of testicular tissue, significantly decreased testicular injury score, and significantly preserved spermatogenesis in CP- and DX-challenged rats.

As shown in Table IV, the tubular diameter (MSTD) and tubular epithelial thickness (GECT) in the DBP group are significantly smaller than the control group. In the DBP+NAR group, MSTD and GECT values showed a significant increase than the DBP group, indicating that NAR partially inhibited the damage of DBP. DBP causes important damages to the testicular tissue, and NAR treatment in conjunction with the DBP administration resulted in a marked improvement in histological damage in testis tissue.

## Conclusions

This study confirmed the toxic effects of DBP at a dose of 500 mg/kg/day on testicular (increased oxidative stress and histological changes in tissue) and spermatological (decreased sperm motility) damage in rats. Also, it was shown that NAR supplementation (50 mg/kg/day), in combination with DBP, generally reversed the toxicity of DBP to the reproductive system. The beneficial effects of NAR against DBP-induced reproductive damage may be due to its antioxidant properties. Thus, the findings obtained in this study suggest that NAR supplementation may attenuate the DBP-induced reproductive system toxicity.

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### Conflict of Interest

The authors reported no conflict of interest.

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