

## THE EFFECT OF BISPHENOL A ON THE TESTIS OF ADULT MALE ALBINO RATS AND THE POSSIBLE PROTECTIVE EFFECT OF THYMOQUINONE: A HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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**Abstract:** *Bisphenol A (BPA) is an endocrine-disrupting molecule and can bind to  $\alpha$  and  $\beta$  estrogen receptors and exerts its harmful effects. It is widely used in epoxy resins and polycarbonate plastics. Exposure to BPA can be associated with many health problems as abnormalities of reproductive system, cardiovascular disease, metabolic disorders, cancer and neurobehavioral disorders. Thymoquinone (TQ) have antioxidant, anticonvulsant, antitumor and anti-inflammatory effects, and may protect organs from oxidative damage by free radical generating agent. The objective of this study is to evaluate the protective effect of thymoquinone against BPA induced germ cell toxicity of adult male albino rats testis. In this study, forty adult male rats were used. They were divided into four groups (10 rats for each): group I (control group), group II (thymoquinone treated group), group III (bisphenol A treated group), group IV (bisphenol A and thymoquinone treated group). Testicular tissue were used for histological and immunohistochemical studies. Blood plasma was used for hormonal analysis. Administration of BPA significantly reduced seminiferous tubules diameter and epithelial height with impaired spermatogenesis, decreased plasma testosterone, FSH and LH levels. BPA caused decreased androgen receptors. Administration of thymoquinone with bisphenol A led to amelioration of these toxic effects. Thus exposure to BPA induces toxic effects on the testis with an imbalance in the hormonal levels, while thymoquinone could ameliorate these toxic effects.*

**Key words:** Rat testis, Bisphenol A, Thymoquinone

### INTRODUCTION

Bisphenol A (BPA) is a synthetic estrogen that is used to form epoxy resins and polycarbonate plastics [1]. BPA is an endocrine-disrupting molecule and can bind to  $\alpha$  and  $\beta$  estrogen receptors and exerts its harmful effects [2]. The epoxy resins are used in the internal coating of food container. BPA is also used in dental sealants, water bottles, flooring, table ware, sports equipment, CDs, DVDs and in making thermal paper used in sale receipts [3]. The FDA has

ended its authorization of the use of BPA in baby bottles and infant formula packaging etc [4].

Humans are exposed to BPA as it was detected in dust, air particles and water. BPA can also be leach out from polycarbonate plastics and epoxy resins and become in contact with water and foods [5]. The canned food contribute to 15-40 % of the daily BPA intake [6]. In humans, the BPA is absorbed rapidly following ingestion and then converted to a number of metabolites in the liver mainly BPA glucuronide

[7]. BPA was found in 98% urine samples of school children in eastern china [8]. The high exposure to BPA can be associated with many health problems as abnormalities of reproductive system, cardiovascular disease [9], metabolic disorders, cancer [10] and neurobehavioral disorders [11]. These effects are attributed mainly to its estrogen-like action thus changing the hormonal balance of the body [12].

Several studies reported that BPA adversely affects the male reproductive system. It causes testicular atrophy, decreased sperm concentration and motility [13] and induce apoptosis in rat germ cells [14]. It is genotoxic and causes abnormalities in meiotic division in male germ cells [15].

*Nigella sativa* (NS) seeds and their oil have been used for centuries as traditional remedies worldwide. *N. Sativa* (black seed) plant has been investigated for its anti-inflammatory, antioxidant and anti-cancer activities in both in vitro and in vivo model since 1960s [16]. *N. sativa* crude extracts have been commonly used in traditional medicine as bronchodilators, appetite stimulants, liver tonics and analgesics and to treat multiple conditions like asthma, diabetes, hypertension, cardiovascular disease, kidney and liver diseases [17].

The black seeds extracts have an important role in the prevention and treatment of multiple non-infectious and infectious diseases [18]. The active component of the volatile oil of NS seeds is called thymoquinone (TQ) which have antioxidant, anticonvulsant, antitumor and anti-inflammatory effects, and may protect organs from oxidative damage by free radical generating agent [17]. The *Nigella sativa* contain phenols and alkaloids which can stimulate the secretion of follicle stimulating hormone and testosterone and thus increased sperm production [19]. Badary et al. (2003) have shown that TQ has strong antioxidants activities through scavenging ability of different free radicals in an in vitro model [20]. The objective of this study is to evaluate the protective effect of thymoquinone against bisphenol A induced germ cell toxicity in rat testis.

## MATERIALS AND METHODS

**Chemicals:** Bisphenol A was obtained from sigma-Aldrich chemical company (Saint Louis, MO,

USA). BPA was dissolved in corn oil which was also purchased from sigma-Aldrich and used as a vehicle. Thymoquinone (TQ) was obtained from Sigma Company. It was dissolved in Tween 80 (0.8%) and saline (NaCl) solution.

**Animals:** Forty adult male rats of average weight 150-250 grams, aged 8-9 weeks were used in this study. The animals were kept in healthy standard environmental conditions with 12-h Light/dark cycle, constant temperature 22 -24°C, humidity (55%-45%) and fed with basal diet and tap water. Rats were acclimatized to laboratory conditions one week before start of the study. All ethical protocols for animal treatment were followed. The experimental protocol was approved by the Ethical Committee of Menoufia faculty of medicine.

**Experimental procedure:** The rats were randomly divided into four groups included 10 animals for each.

**Group I:** (control group): The animals of this group were provided with normal saline

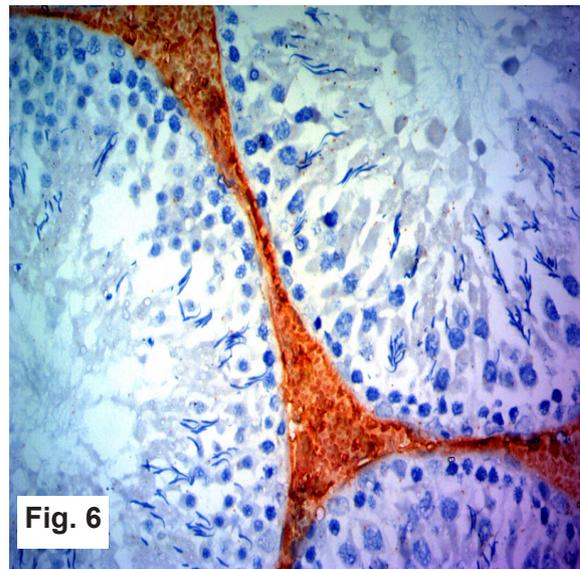
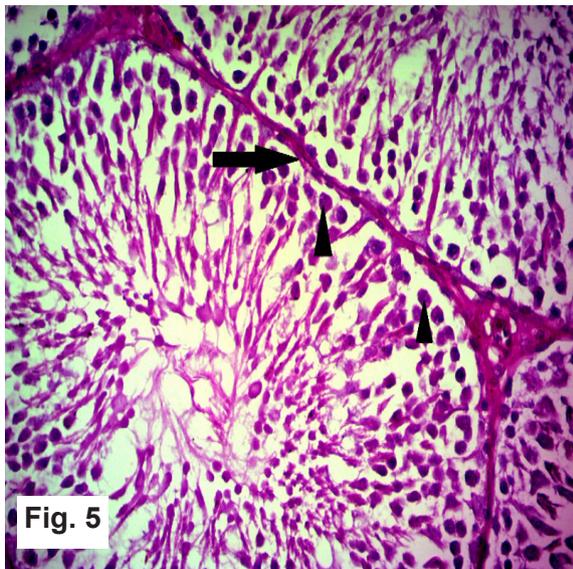
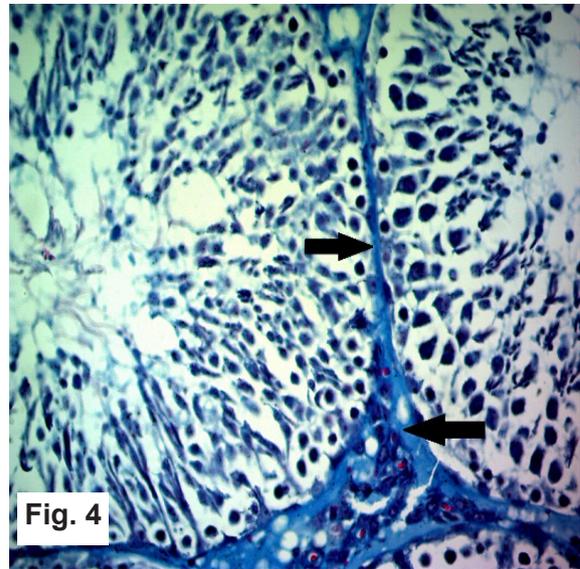
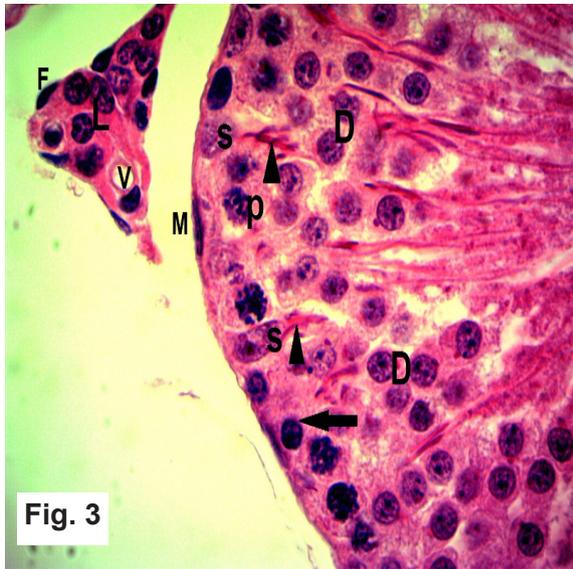
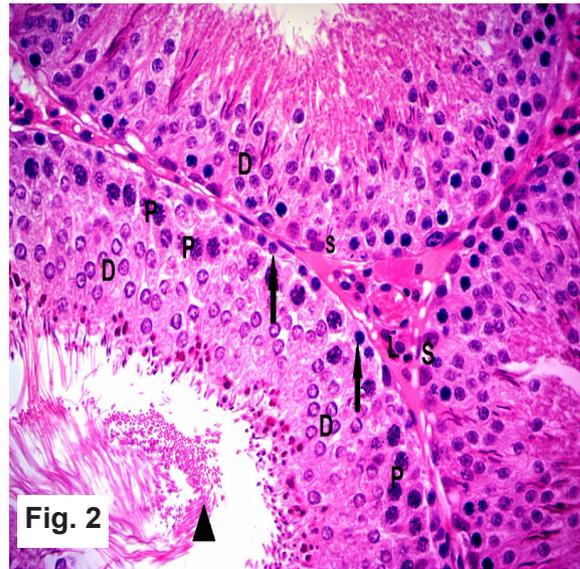
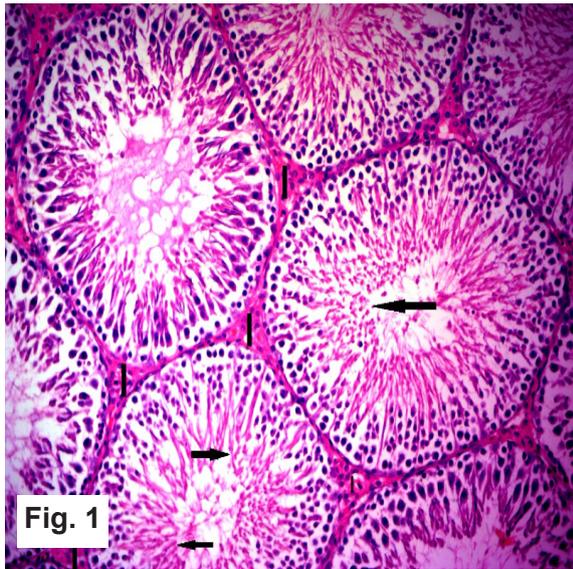
**Group II:** (Thymoquinone treated group): The animals received thymoquinone (TQ) 5 mg/kg/d by gastric tube for eight weeks [21].

**Group III:** (bisphenol A treated group): The animals received bisphenol A 50 mg/kg dissolved in corn oil by gastric tube daily for eight weeks [22].

**Group IV:** (bisphenol A and TQ treated group): The animals received bisphenol A and thymoquinone an hour prior to bisphenol A daily for eight weeks via the same route and doses as previously described.

24 h after the last dose, the animals were scarified by cervical decapitation. The blood was collected to measure the levels of sex hormones and the right testes were removed and cleaned by normal saline. The specimens were subjected to the following studies.

**Histological study:** The testes were fixed in Bouin's fluid, washed and processed for paraffin sections of about 5-6 µm thickness. Sections were obtained and stained with hematoxylin and eosin (Hx&E) to show the histological details & Mallory's trichrome stain [23] to detect the collagen fibers.



**Histochemical study:** Testes were processed as above and stained for Periodic acid-Schiff (PAS) for detection of glycogen [24].

**Immunohistochemical study:**

**1. Androgen receptor immunostaining:** The testes were fixed and processed as above. The sections were stained immunohistochemically for androgen receptor. The epitope retrieval solution was put on the section (IHC World, USA) at 90°C for 15 min and allowed them to cool for 20 min at room temperature. Then, the sections was carried out with a peroxidase blocking solution (IHC World, USA) for 10 min at room temperature and then rinsed with washing buffer. Next, the sections were blocked with avidin/biotin blocking solution (IHC World, USA) for 30 min at room temperature and rinsed with PBS. Thereafter, the sections were incubated with the anti-androgen receptor rabbit monoclonal antibody (1:250, Abcam Inc., USA) for 50 min and washed with the buffer. After that, sections were incubated with biotinylated goat anti rabbit IgG (Abcam Inc., USA) at 37°C for 60 min and rinsed with washing buffer. These sections were incubated with Streptavidin HRP protein (1:5000, Abcam Inc., USA) at room temperature and rinsed with buffer. Finally sections were counterstained with Meyer's hematoxylin. (Merck, Germany) [25].

**2. Caspase-3 immunostaining:** Paraffin sections of 4 µm thickness were mounted on glass slides coated on pol-L-lysine, deparaffinized, dehydrated and then put in 10 M sodium citrate buffer. Then,

sections were put in a microwave oven at 60°C for 15 minutes. Endogenous peroxidase were inactivated. Then sections were incubated with primary antibody overnight at 4°C. Activated caspase-3 expression was assessed using a peroxidase-conjugated rabbit monoclonal antibody IgG (Cell signaling Technology, Ipswich, MA) (dilution 1:200). After 3 rinses with phosphate-buffered saline, the sections were incubated with a commercial kit (Pic-Ture™, Zymed and South San Francisco, CA) for visualization of immunoreactions. Finally, the sections were counter stained with Mayer's hematoxylin. Normal lymphoid tissue was used as positive control. Negative control was performed by omitting primary antibody step consequently no immune-staining was found [26].

**3. Sex hormone assay:** Blood samples were obtained and centrifuged, then serum was separated to measure the levels of sex hormones according to the protocol of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone enzyme-linked immunosorbent assay (ELISA) kits [27].

**Morphometric study:** Using Hx&E stained sections, morphometric measurements in the form of diameter of seminiferous tubules and germinal epithelial height were done. From each rat, 10 microscopic fields were used. At least 10 tubular diameter were measured from various areas in each animal [28]. The thickness of the epithelium of the seminiferous tubules was also measured [29].

**Statistical analysis:** The testis weight, tubular

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**Explanation of figures:**

**Fig. 1:** A photomicrograph of a control adult testis showing rounded to oval seminiferous tubules lined with spermatogenic cells and bounded by basal lamina, in between tubules there are interstitium (I) . Notice, sperms (arrows) are detected in the lumen of the tubules. Hx&E X 200

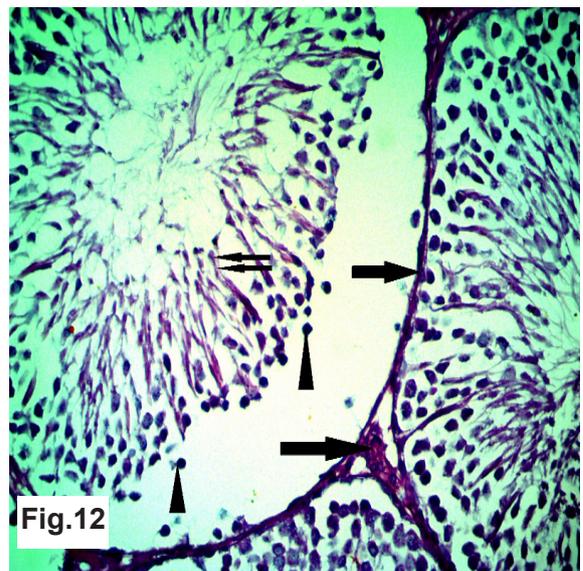
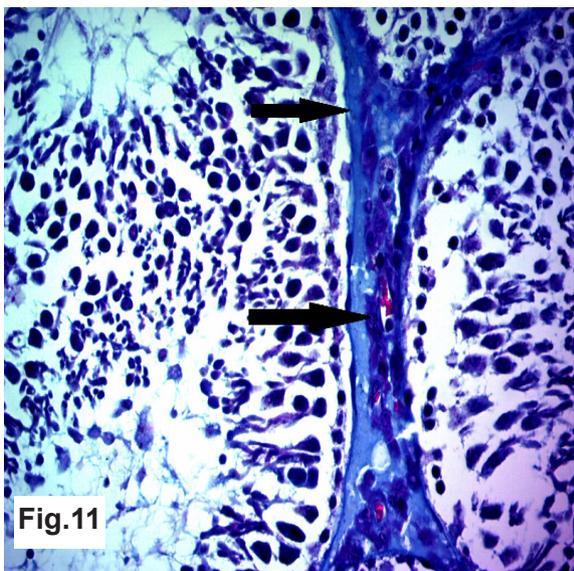
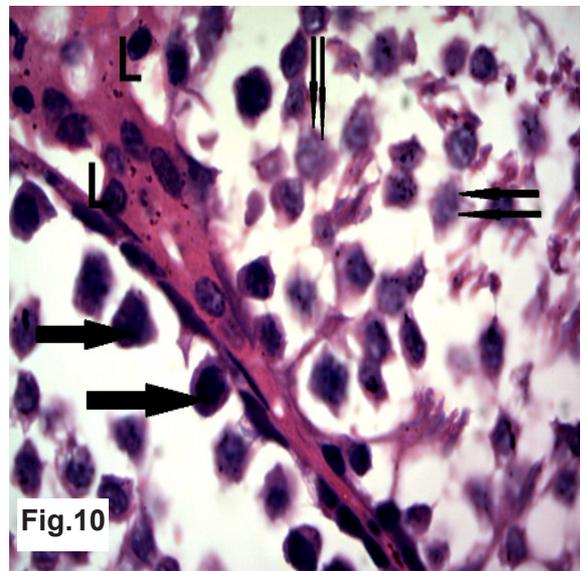
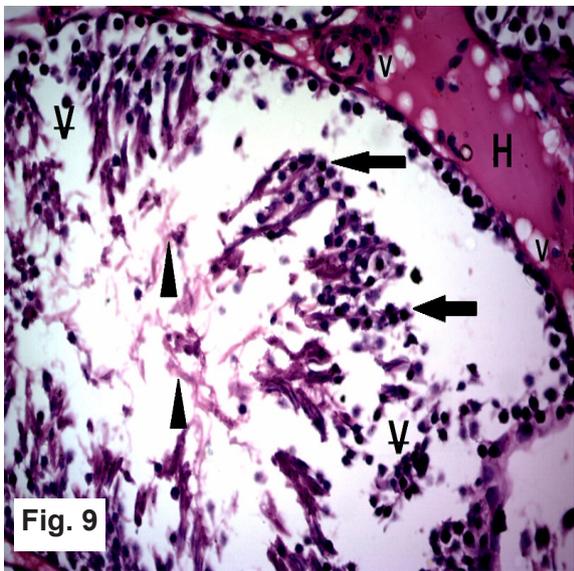
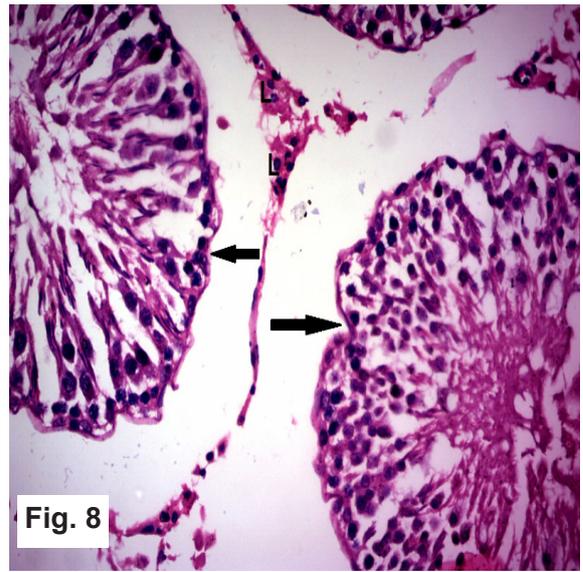
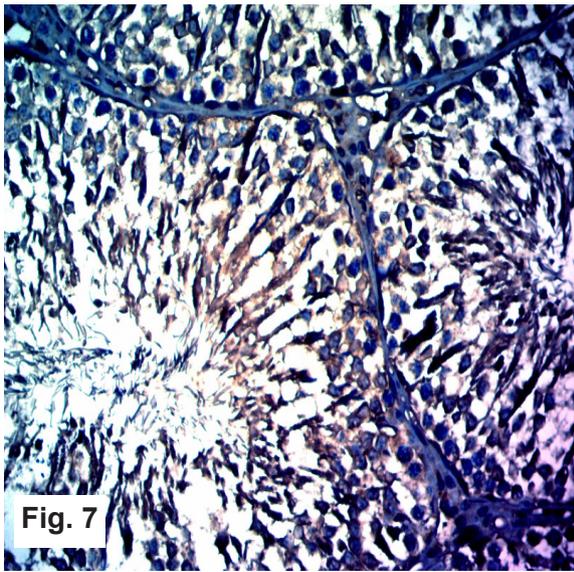
**Fig. 2 :** A photomicrograph of a control adult testis showing three adjacent seminiferous tubules lined with spermatogonia (arrows), primary spermatocytes (P), spermatids (D) and sertoli cells (S) with leydig cells (L) in between tubules. Sperms are observed in the lumen of seminiferous tubules (arrow head). Hx&E X 400

**Fig. 3:** A photomicrograph of a control adult testis showing seminiferous tubule lined with spermatogonia (arrow), primary spermatocyte (P) with mitotic figures, rounded pale spermatids (D) and triangular pale sertoli cells (S) resting on the basal lamina with sperms attached to its apical parts (arrow heads).The interstitial tissue contains group of leydig cells (L), fibroblasts (F) and blood vessel (B).Notice, spindle shaped myoid cell (M) outside the basal lamina. Hx&E X 1000

**Fig. 4:** A photomicrograph of a control adult testis showing moderate amount of collagen fibers around basal lamina and in the interstitium (arrows). Mallory's trichrome X 400

**Fig. 5:** A photomicrograph of a control adult testis showing strong PAS positive reaction in a well circumscribed basal lamina (arrow) and spermatogenic epithelium (arrow heads). PAS X 400

**Fig. 6:** A photomicrograph of a control adult testis showing strong androgen receptor nuclear immunoreaction in the leydig cells and lack of immunoreaction in seminiferous tubules. AR X 400



diameter and germinal epithelium height data were expressed as mean  $\pm$  SD. The student t-test was used to evaluate the significant change in each parameter in the experimental groups when compared to the control group. The statistical analysis of data was carried out using Excel and statistical package for the social science software, version 11. The significance of differences between data was assumed at  $P < 0.05$  [30].

## RESULTS

### Histological, histochemical and immunohistochemical results of different groups:

#### A. Control group (Group I):

**Haematoxylin and eosin stain:** Sections of the adult testis of control group showed round to oval seminiferous tubules with interstitium in between them. Each tubule is lined with spermatogenic cells with different stages of maturation and supporting sertoli cells (Fig. 1). Spermatogenic cells include spermatogonia, primary spermatocytes, spermatids and sperms (Fig. 2). Spermatogonia are resting on the basal lamina with single oval nucleus. Primary spermatocyte are the largest cells seen in the tubule that showed mitotic figures. Spermatids are seen close to lumen of the tubules, containing pale vesicular nuclei. Sertoli cells are seen at interval in between spermatogenic cells. They are triangular in shape with irregular apices resting on the basal lamina (Fig. 3). The interstitium appears as triangular area between seminiferous tubules and

contains large pale leydig cells, fibroblast cells and blood vessels (Fig 3).

**Mallory's trichrome stain:** Mallory's trichrome stained sections showed moderate amount of collagen fibers around basal lamina and in the interstitium (Fig. 4).

**Periodic Acid Chief's reaction (PAS):** Periodic Acid Chief's staining of the testis showed strong positive reaction in a well circumscribed basal lamina and spermatogenic epithelium (Fig. 5).

**Androgen receptor immunostaining:** Androgen receptor immunostaining of testis showing strong nuclear reaction in the interstitial cells and lack of immunoreaction in seminiferous tubules (Fig. 6).

**Caspase-3 immunostaining:** Testicular section from the control group revealed weak cytoplasmic immunoreactivity for caspase-3 in the germinal epithelium of the seminiferous tubules and interstitial tissue (Fig. 7).

**B. Thymoquinone treated group (Group II):** Thymoquinone treated group of rats showed the same histological and immunohistochemical appearance like control group.

**C. Bisphenol A treated group (Group III):** Haematoxylin and eosin stain: Testicular sections from this group stained with Hx & E showed shrunken seminiferous tubules with corrugated basal lamina and the interstitial tissue were enlarged

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### Explanation of figures:

**Fig. 7:** A photomicrograph of a control adult testis showing weak cytoplasmic immunoreactivity for caspase-3 in the germinal epithelium of the seminiferous tubules and interstitial cells. Caspase-3 X400

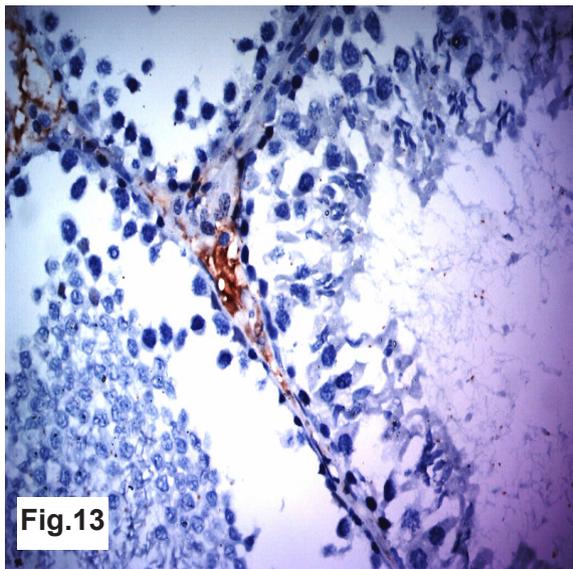
**Fig. 8:** A photomicrograph of adult testis of bisphenol A treated group showing shrunken seminiferous tubules with corrugated basal lamina (arrows). Leydig cells (L) in the widened interstitium are observed. Hx&E X400

**Fig. 9:** A photomicrograph of adult testis of bisphenol A treated group showing seminiferous tubule with detachment and exfoliation of spermatogenic cells (arrows) leaving vacuoles inside tubule (V) with apparent decreased number of sperms (arrow heads). Notice, hyalinization (H) and vacuolation (V) in the interstitium. Hx& E X 400

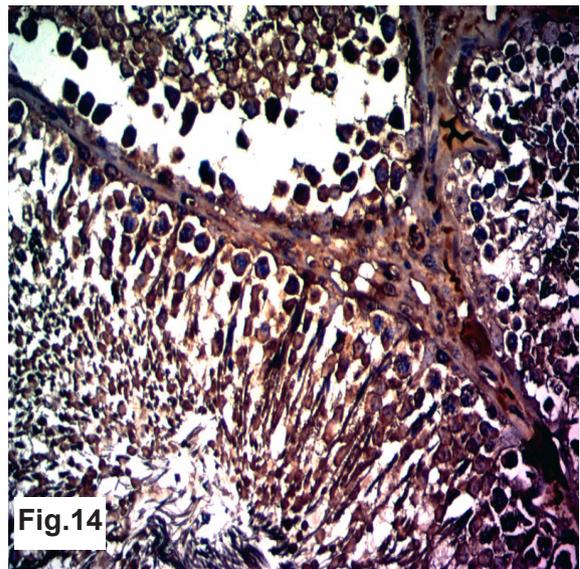
**Fig. 10:** A photomicrograph of adult testis of bisphenol A treated group showing seminiferous tubules with disorganization of spermatogenic cells, some with dark nuclei, large in size (arrows) and others with faint degenerated nuclei (double rows). Some Leydig cells are small with dark nuclei (L) in the interstitium. There is distortion of part of the basal lamina (arrow head). Hx&E X 1000

**Fig. 11:** A photomicrograph of adult testis of bisphenol A treated group showing massive amount of collagen fibers around basal lamina and in the interstitium (arrows). Mallory's trichrome X 400

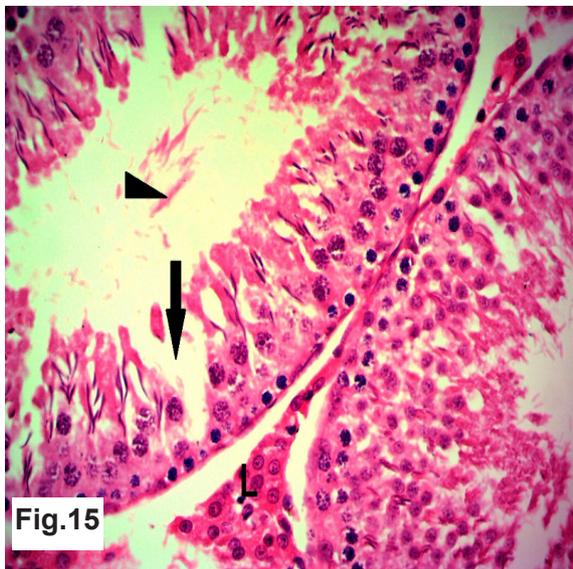
**Fig. 12:** A photomicrograph of adult testis of bisphenol A treated group showing moderate PAS reaction in the basal lamina and interstitium (arrows), weak reaction in sperms (double arrow) and no reaction in the spermatogenic cells (arrow heads). PAS X 400



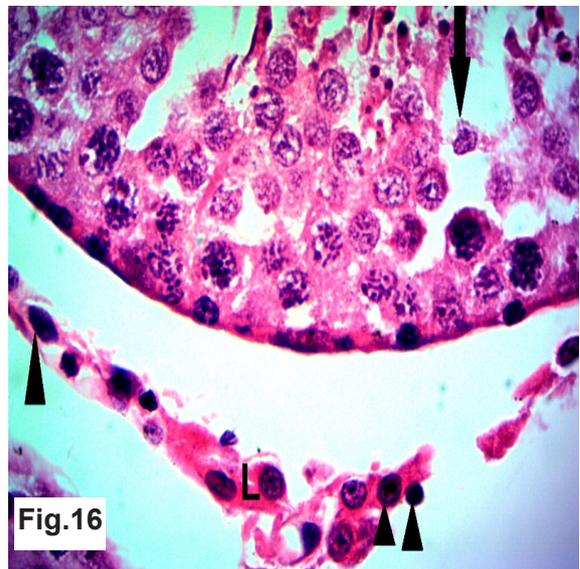
**Fig.13**



**Fig.14**



**Fig.15**



**Fig.16**

**Fig. 13:** A photomicrograph of adult testis of bisphenol A treated group showing weak immunoreactivity of androgen receptors in the Leydig cells. AR immunostaining X 400

**Fig. 14:** A photomicrograph of adult testis of bisphenol A treated group showing strong cytoplasmic immunoreactivity for caspase-3 in the germinal epithelium and interstitial cells. Caspase-3 X 400

**Fig. 15:** A photomicrograph of adult testis of bisphenol A and TQ treated group showing two seminiferous tubules, the lower one appeared normal and the upper one showed reduced height of the germinal epithelium with some areas of distortion (arrow). Notice, few sperms in the lumen (arrow head). Leydig cells (L) are observed in the interstitium. Hx&E X 400

**Fig. 16:** A photomicrograph of adult testis of bisphenol A and TQ treated group showing nearly normal appearance of spermatogenic cells with some areas of distortion with sloughed cells (arrow). Some Leydig cells are pale (L) and others appear small with dark nuclei (arrow heads) in the interstitium. Hx&E X 1000

(Fig. 8). In some tubules, the spermatogenic cells were detached and exfoliated in the lumen of the tubules leaving vacuoles with apparent decreased number of sperms and hyaline material deposition with vacuolation in the interstitium (Fig. 9). There was distortion of part of basal lamina. Some spermatogenic cells showed dark large size nuclei and while others showed faint nuclei. Leydig cells were seen in the interstitium, some were pale and others were small with dark nuclei (Fig. 10).

**Mallory's trichrome stain:** Mallory's trichrome stained section showed massive amount of collagen fibers around basal lamina and in the interstitium (Fig. 11).

**Periodic Acid Chief's reaction (PAS):** Section from this group showed moderate PAS reaction in the basal lamina and interstitium, weak reaction in sperms and no reaction in the spermatogenic cells (Fig. 12).

**Androgen receptor immunostaining:** Weak immunoexpression of androgen receptors in the leydig cells was observed (Fig. 13).

**Caspase-3 immunostaining:** Testis sections from this group showed strong cytoplasmic immunoreactivity for caspase-3 in the germinal epithelium and interstitial cells (Fig. 14).

#### **D. Bisphenol A and Thymoquinone treated group (Group IV):**

**Haematoxylin and eosin stain:** Testis sections from this group revealed some seminiferous tubules with normal general architecture, but others showed reduced height of the germinal epithelium with few sperms in the lumen (Fig. 15). Some areas of seminiferous tubules were distorted (Figs. 15,16) with sloughed cells (Fig. 16). Some leydig cells appeared pale and others became small in size with dark nuclei (Fig. 16).

**Mallory's trichrome stain:** Sections from this group showed moderate amount of collagen fibers around basal lamina and interstitium (Fig. 17).

**Periodic Acid Chief's reaction (PAS):** Testis sections stained with PAS showed moderate reaction in basal lamina and spermatogenic cells (Fig. 18).

**Androgen receptor immunostaining:** Testis sections from this group of animals demonstrated moderate immunoreaction in androgen receptor in nuclei of leydig cells (Fig. 19).

**Caspase-3 immunostaining:** Section from this group showed moderate cytoplasmic immunoreactivity for caspase-3 in the germinal epithelium of seminiferous tubules (Fig. 20).

**Morphometric results:** Exposure of adult male rats to bisphenol A led to highly significant reduction in the testicular weight when compared to the control group ( $P<0.001$ ). Administration of bisphenol A in association with thymoquinone improve the testicular weight (Table 1 and Fig. 21).

Group III (bisphenol A treated group) showed marked highly significant reduction in the tubular diameter and epithelial height when compared to the control group ( $P<0.001$ ). Administration of thymoquinone with bisphenol A improve tubular diameter and epithelial height, but still there is significant difference when compared to the control group ( $P<0.05$ ) as evident in Table 1 and figure 22.

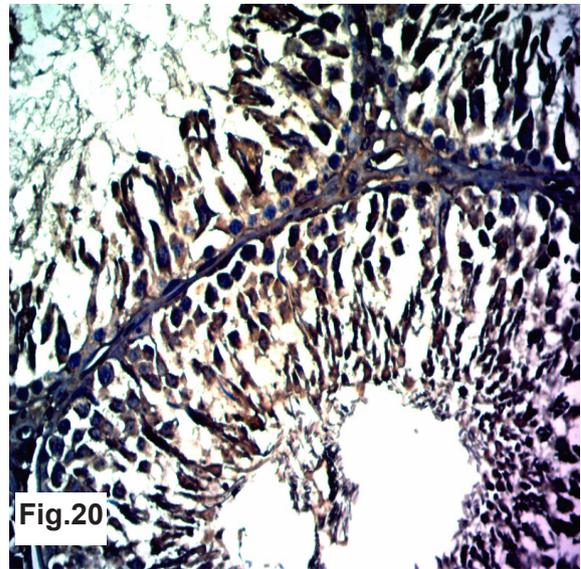
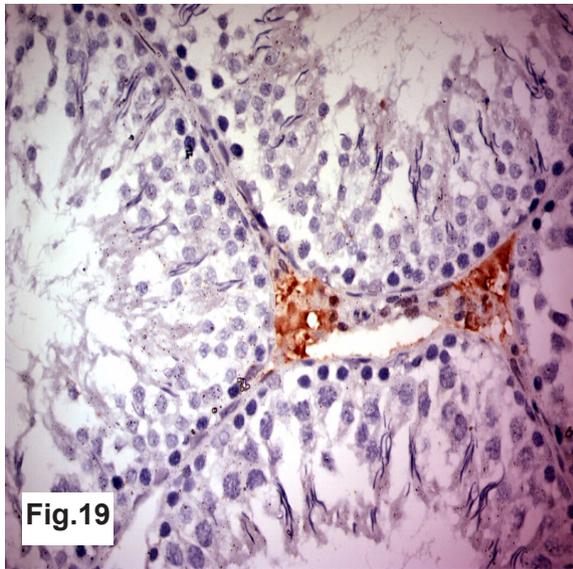
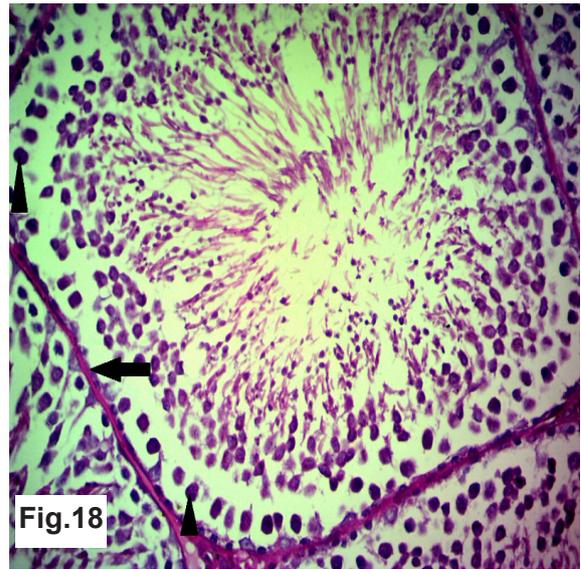
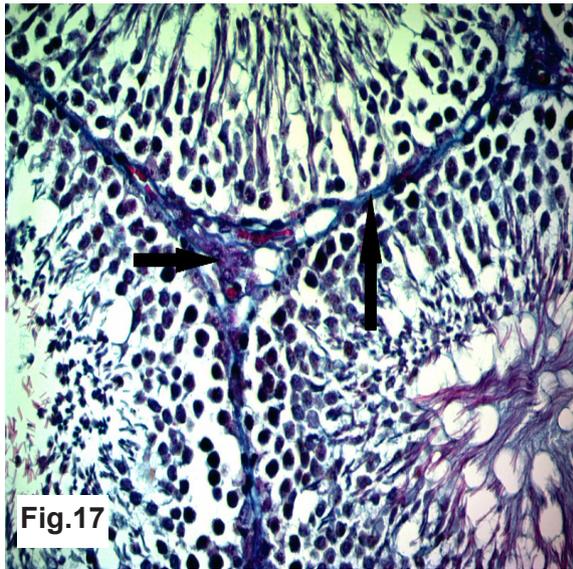
**Sex hormone assay:** Bisphenol A administration resulted in a highly significant reduction in the serum level of FSH when compared with the control group ( $P<0.001$ ). Administration of thymoquinone with bisphenol A improves FSH level, but still there is significant difference when compared to the control group ( $P<0.05$ ) as seen in Table 2 and figure 23.

Bisphenol A treated group showed significant reduction in the serum level of LH when compared to the control group ( $P<0.05$ ). Supplementation of thymoquinone with bisphenol A improve this parameter too (Table 2 and Fig. 24).

Bisphenol A exposure results in highly significant reduction in the serum level of testosterone when compared with the control group ( $P<0.01$ ). Administration of thymoquinone with bisphenol A improve testosterone level, but still there is significant difference when compared to the control group ( $P<0.05$ ) (Table 2 and Fig. 25).

## **DISCUSSION**

Bisphenol A (BPA) is a synthetic estrogen that is



**Fig. 17:** A photomicrograph of adult testis of bisphenol A and TQ treated group showing moderate amount of collagen fibers around basal lamina and interstitium (arrows). Mallory's trichrome X 400

**Fig. 18:** A photomicrograph of adult testis of bisphenol A and TQ treated group showing moderate PAS reaction in basal lamina (arrow) and spermatogenic cells (arrow heads). PAS X 400

**Fig. 19:** A photomicrograph of adult testis of bisphenol A and TQ treated group showing moderate androgen receptor nuclear immunorexpression in the leydig cells. AR immunostaining X 400

**Fig. 20:** A photomicrograph of adult testis of bisphenol A and TQ treated group showing moderate cytoplasmic immunoreactivity for caspase-3 in the germinal epithelium of seminiferous tubules. Caspase-3 X 400

**Table 1:**

	Control Mean ± SD	Bisphenol A Mean ± SD	Bisphenol A and TQ Mean ± SD	P.value1	P.value2
Mean testicular weigh/gm	1.45±0.19	1.03±0.03	1.37±0.15	0.00** <0.001	0.31 >0.05
Mean seminiferous tubular diameter	356.5±0.26	221.4±0.22	354.4±0.19	0.00** <0.001	0.014 <0.05
Mean seminiferous epithelial height/mm	248.44±0.24	109.6±0.21	246.6±2.2	0.00** <0.001	0.026 <0.05

P1 Comparison was done between control group (group I) and bisphenol A treated group (group III).

P2 comparison was done between control group (group I) and bisphenol A and TQ treated group (group IV)

P < 0.001 means highly significant, P < 0.05 means significant, P > 0.05 means NS.

**Table 2:**

	Control Mean ± SD	Bisphenol A Mean ± SD	Bisphenol A and TQ Mean ± SD	P.value1	P.value2
FSH	251.3±3.5	205.5±13.2	226.3±11.5	0.00 <0.001	0.04 <0.05
LH	5.25±0.59	4.57±0.6	5.07±0.28	0.01 <0.05	0.42 >0.05
Testosterone	221.6±7.4	198.0±21.99	203.3±19.2	0.005 <0.01	0.026 <0.05

P1 Comparison was done between control group (group I) and bisphenolA treated group (group III).

P2 comparison was done between control group (group I) and bisphenol A and TQ treated group (group IV).

\*\*p<0.001, \*p< 0.01, \*p< 0.05, P>0.05 NS.

utilized to form epoxy resins and polycarbonate plastics [1]. BPA is associated with many health problems as metabolic disorders, cardiovascular and reproductive diseases [10]. BPA is able to separate from the containers into foods and from dental sealants into saliva of patients after application and can cross the human placenta into fetus [5]. BPA is completely absorbed in the intestine and metabolized in the liver by glucuronidation with appearance of metabolites in urine [7]. BPA is also considered to be weak carcinogen [31].

The aim of the present study is to clarify the possible protective effect of thymoquinone against BPA induced testicular germ cell toxicity. In the present study, the levels of FSH, LH and testosterone hormones were decreased by exposure to BPA. The androgen produced in response to LH and the pituitary hormone FSH are responsible for spermatogenesis [32]. BPA decrease quality of sperm by interrupting the hypothalamic-pituitary-testicular axis [33].

The decreased level of testosterone may be due to P450 cytochrome 17 blocking and decreased activity of the leydig cells [34]. Mendiola et al. [35]

found that environmental exposure to BPA would decrease testosterone level in adult male rats. When adult male rats, administrated BPA 2 mg/kg, FSH levels and the testosterone decreased while level LH of blood increased.

In the present study, BPA treated group showed significant morphometric alterations when compared to the control group. There were decrease in the seminiferous tubule diameter and the germinal epithelial height. These results confirmed by histopathological changes including shrunken seminiferous tubules with corrugated basal lamina and the spermatogenic cells were detached and exfoliated with apparent decreased number of sperms. These results are consistent with those of Takahashi and Oishi [36] who reported that spermatogenesis delay and disorganization of germinal epithelium in rats exposed to high dose of BPA. Santoro et al. [37] reported that injurious agents stimulate myoid cells to produce more collagen fibers around basal lamina which became irregular and corrugated in shape and thickened.

It was reported that administration of BPA in high dose decreased the antioxidant gene expression in rat

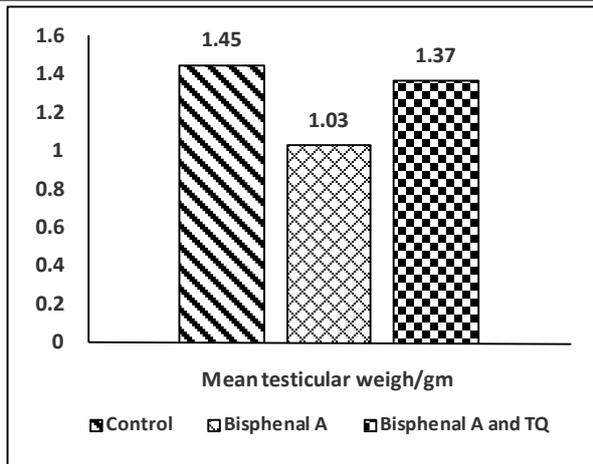


Fig. 21: Mean testicular weight in control group, bisphenol A treated group and bisphenol A and TQ treated group.

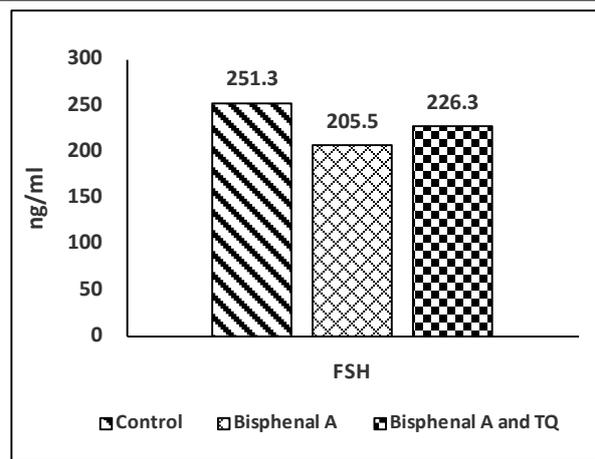


Fig. 23: Mean FSH level in control group, bisphenol A treated group and bisphenol A and TQ treated group.

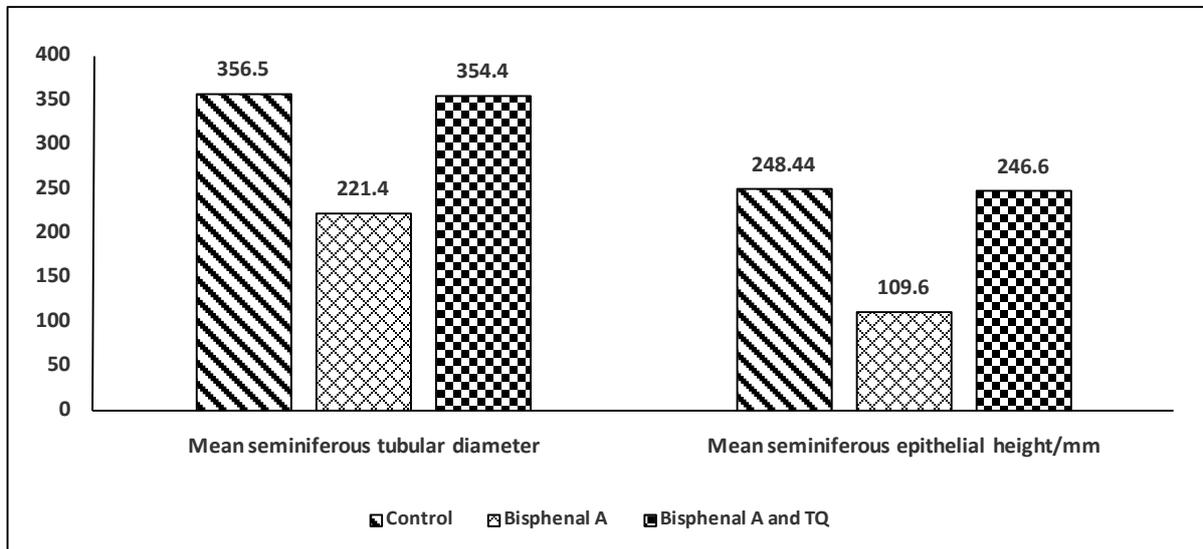


Fig. 22: Mean tubular diameter and epithelial height in control group, bisphenol A treated group and bisphenol A and TQ treated group.

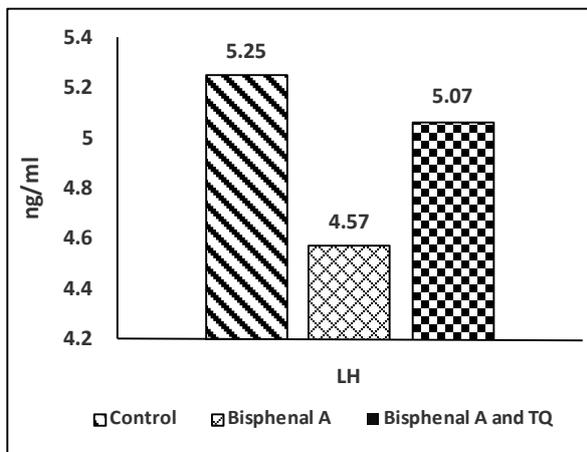


Fig. 24: Mean LH level in control group, bisphenol A treated group and bisphenol A and TQ treated group.

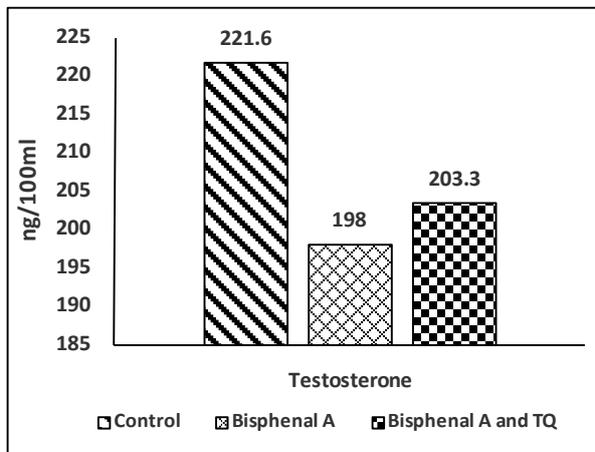


Fig. 25: Mean testosterone level in control group, bisphenol A treated group and bisphenol A and TQ treated group.

model and caused oxidative damage in reproductive organs, liver and kidney [38] by disturbing the balance between antioxidants defense mechanism and ROS [39]. The exposure to high dose of BPA could increase lipid peroxidation, decreased glutathione reductase, glutathione peroxidase and glutathione S-transferase [39]. Thus the decreased testosterone level could decrease concentration of sperms [40].

BPA exposure could increase ROS and cause DNA damage which lead to either arrest of cell cycle for DNA repair or induction of cellular apoptosis [41]. ROS are normally produced in living cells during their metabolic processes including spermatozoa, marked production of ROS can induce the production of toxic lipid peroxides. Cells exhibit defense mechanisms using multiple antioxidants [42]. ROS can bind with many intra cellular structures, especially Trans-membrane protein and unsaturated fatty acids. The oxidation of these molecules can cause disturbance in permeability of cell membrane [43].

Moreover, sperm lipid peroxidation could destroy the structure of the lipid matrix in spermatozal membrane with rapid decrease of intracellular ATP that leads to decreased sperm viability [43]. Leydig cells secrete the primary male sex hormone testosterone, which is responsible for spermatogenesis and male fertility. In the present study, in bisphenol A treated group, there was weak immunoexpression of androgen receptors in the leydig cells. Strong cytoplasmic immunoreactivity for caspase-3 in the germinal epithelium and interstitial cells was also detected. Akingbemi et al. [44] reported that BPA acts as a mitogen in the leydig cells, BPA suppressed protein expression of the luteinizing hormone receptor and the 17-beta-hydroxy steroid dehydrogenase enzyme thereby, decreasing androgen secretion by leydig cells. The side effects of BPA exposure on the function of leydig cell indicate that BPA has the potential to interfere with spermatogenesis and reduce male fertility.

FSH and testosterone can reduce germ cell death by suppressing apoptosis [32]. Liu et al. [45] found that FSH and testosterone can inhibit autophagy in sertoli cells and ovarian cancer cells. Billig et al. [46] found that the apoptotic index is markedly increased in a

dose dependent manner and spermatocytes were the main cell type undergoing apoptosis after treatment with a gonadotropin releasing hormone antagonist.

The germinal epithelial death, especially caused by chemical toxicants, is more probably associated with a decreased testosterone level from leydig cells or a lack of growth factors or nutrients provided by sertoli cells. Direct action of BPA at high doses on germ cell apoptosis cannot be excluded [46]. BPA causes its cellular effect through binding to estrogen receptors at low doses, and interacting with androgen and thyroid hormone receptors at higher doses. It has cytotoxic actions on various cells and tissue through multiple signaling pathway [47].

Group IV (bisphenol A and Thymoquinone treated group) showed marked improvement of morphometric parameters and enhancement of testosterone, FSH and LH blood level. These hormonal results consistent with previous studies that reported that *Nigella sativa* significantly increased blood level of testosterone, reduced testis melandialdehyde and increased level of antioxidant enzymes [48].

Administration of thymoquinone with bisphenol A showed marked improvement of the histological appearance, the seminiferous tubules start to regain its normal architecture, some tubules appeared normal and other showed area of distortion with sloughed cells with decreased cytoplasmic immunoreactivity of caspase-3. This protective effect of thymoquinone (TQ) may be due to the antioxidant and anti-inflammatory effect [49]. TQ has been considered as the main and active ingredient of *Nigella sativa* by pharmacological studies [50]. *Nigella sativa* could increase sperm motility from the epididymis due to its effect on oxidative phosphorylation enzymes [51].

The potent anti-inflammatory effect could be due to suppression of mRNA expression of multiple pro-inflammatory mediators as IL-1B, IL-8 and monocyte. The antioxidant activity could be related to the ability to improve the tissue and blood glutathione, reduce ROS and lipid peroxides production and improve the activity of multiple antioxidant enzymes [52]. TQ could inhibit apoptosis in seminiferous tubules through affecting mRNA expressing levels of P53, Bax/Bcl-2 ratio,

caspase-3 and 8 [52]. Previous study showed that combination of TQ and chemotherapeutic agents could produce higher therapeutic effect and reduce their toxicity [53].

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