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Histological effects of bisphenol-A on the reproductive organs of the adult male albino rat

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SUMMARY

Bisphenol-A (BPA) is widely used in the environment. The objective of this study was to investigate the histological reproductive toxicity of BPA in the adult male albino rats. Forty-five adult male albino rats were divided into 3 groups. Group I received normal saline orally daily (negative control); Group II received corn oil (0.5 ml/day) orally daily (positive control, and Group III were treated with BPA in a dose of 25 mg/kg B.W dissolved in corn oil for 30 days orally. Specimens from the testis, epididymis, prostate and seminal vesicles were collected for light microscopic examination. Blood samples were collected for serum testosterone level assessment. BPA caused histopathological alterations in the epithelium and connective tissues of all organs. Results of the serum testosterone level statistical analysis showed a highly significant decrease in BPA-treated group (III) as compared to the control groups (I & II). The epithelium of the accessory sex glands (prostate and seminal vesicles) responds to BPA by proliferative differentiation. The mean area percentage of collagen fibers stained with Masson's trichrome measured in the stroma of all organs was significantly increased. Also, there was a significant increase in the area percentage of positive inducible nitric oxide synthase (iNOS) immunoreactions in all organs. In conclusion, BPA induced DNA oxidative damage via iNOS activity in all reproductive organs.

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Key words: Bisphenol A – Reproductive – Histology – Rat

INTRODUCTION

In the past few decades there has been an increased incidence of reproductive disorders attributed to substances known as endocrine disrupters that are able to disrupt the function of the endocrine system. Bisphenol-A (BPA) is an estrogenic endocrine disrupter which has been used in the manufacture of resins such as polycarbonate plastics and epoxy. The polycarbonate plastics and epoxy are mainly used for the interior coating of food and drink containers, baby bottles, some dental sealants, thermal receipt paper, dental fillings and detergents (Li et al., 2011; Bhan et al., 2014). Dietary exposure to BPA is the main route of exposure in humans, as BPA polymers can be hydrolyzed under acidic or basic conditions and dissolved in the food and drink containers (Welshons et al., 2006). BPA has been shown to induce different pathological insults such as neurological disorders, cancers or behavioral defects, and has a significant role in type 2 diabetes, cardiovascular disorders and liver enzyme abnormalities (Richter et al., 2007). Bisphenol-A was tested for its estrogenic properties, as it binds to classic estrogen receptors and has the capacity to activate the membrane estrogen receptors (Quesada et al., 2002). Bisphenol-A was associated with male reproductive injuries such as reduced sperm count, testicular lesions, sperm motility changes and sperm morphologic abnormalities. Therefore, much attention has been paid to the effects of the Bisphenol-A on male reproductive health (Wisniewski et al., 2015). BPA can generate reactive oxygen species resulting in damage in testes (Aydogan et al.,

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2010); reactive oxygen species formation from the BPA metabolites and from reduced mitochondrial fractions (Mourad and Khadrawy, 2012); production of nitric oxide from arginine mediated through stimulation of inducible nitric oxide synthase (iNOS). Nitric oxide is synthesized from the terminal guanidine nitrogen of the semi-essential amino acid L-arginine (Kaplan et al., 2012). The activity of the iNOS has been detected in different cell types. So, iNOS expression in immunohistochemical studies has been used as a marker of oxidative stress (Takimoto et al., 2007). The most important organ in the male reproductive system is the testis. It is characterized by two main functions, synthesis of steroid hormones and production of spermatozoa (Carreau et al., 2002). The male reproductive system includes the epididymis, which is an important tubular coiled structure connecting the testis with the vas deferens and consists of caput (head), corpus (body) and cauda (tail) (Cornwall, 2009). Spermatozoa maturation and storage occurs in the epididymis (Beu et al., 2009).The epididymis integrity is essential to keep the proper environment for male fertility and viability of the sperms (Schimming et al., 2001). The male accessory sex glands of mammals have an important role in the reproductive function; these glands include the seminal vesicles and the prostate gland (Adebayo et al., 2009). The prostate is a hormone-dependent male sex gland present in different mammals. It consists of three pairs of lobes distributed around the prostatic urethra (Untergasser et al., 2005; Favor and Cagnon, 2006). The weights of prostate, testes, epididymis and seminal vesicle are reduced by BPA (Akingbemi et al., 2004). Another study reported that the rats given BPA showed decreased weight of their prostate and seminal vesicles but the testis and epididymis weights were not decreased (Takahashi and Oishi, 2001). Testicular testosterone levels are reduced by BPA treatment in the mouse by affecting both testis and pituitary hormones (Nakamura et al., 2010). However, Takahashi and Oishi (2001) reported that the level of serum testosterone concentration was not decreased in BPA-treated animals. Therefore, there are controversial study reports on the effect of BPA on the reproductive system. The present study was designed to investigate the histopathologic effects of BPA on the reproductive organs of the adult albino rats due to the heavy use of BPA as an organic plasticizer.

MATERIALS AND METHODS

Chemicals

Bisphenol-A (CAS No. 80-05-7) in the form of crystalline white powder imported from Germany via Sigma–Aldrich Inc. Testosterone was assessed by an enzyme-linked immunosorbent assay kit (IB79174, IBL-America, Minneapolis, Minnesota, USA).

Experimental Design

Forty-five adult male albino rats six months old with average weight 220±30 grams. They were obtained from the Laboratory Animal Unit, Faculty of Medicine, Zagazig University. They were kept in fan-ventilated wide polypropylene animal cages with stainless-steel tops, under the prevailing environmental conditions at the room temperature (25±2ºC) and illumination (12 h-light/dark) under pathogen-free conditions. Their food was a balanced diet in the form of barely, lettuce, milk, carrots, bread and water. Before the experiment, rats were acclimatized to the experimental conditions for a period of ten days. All rats were handled accordance to the standard guide for the care and use of laboratory animals (Institute of Laboratory Animals Resources, 1996). Experimental protocols were approved by the ethical committee of the Faculty of Medicine, Zagazig University.

The experimental rats were divided into three equal groups, each group containing fifteen rats. Group I received ordinary food without any additives and received normal saline orally via intragastric tube daily (negative control); Group II received oral corn oil daily (0.5 ml/day) (positive control), and Group III were treated orally with Bisphenol-A in a dose of 25 mg/kg body weight for 30 days dissolved in corn oil (Wisniewski et al., 2015).

Histological and immunohistochemical study

After 30 days, the animals anesthetized using ether inhalation and experimental protocols were approved by the ethical committee of the Faculty of Medicine, Zagazig University. The specimens from the testis, epididymis, prostate and seminal vesicles were taken out and fixed in 10% buffered formalin 6 hours, processed to prepare 5 µm paraffin sections stained with hematoxylin and eosin (H&E) and Masson's trichrome stain for demonstration of collagen fibers for the light microscopic examination (Bancroft and Gamble, 2008). Inducible nitric oxide synthase (iNOS) immunohistochemistry (using the avidin-biotin peroxidase method), all specimens were processed routinely. Paraffin sections of 4-µm thickness were dewaxed in xylene, rehydrated in descending series of ethanol, and immersed in 0.3% H_2O_2 for 30 min to block endogenous peroxidase. Antigens were retrieved by microwaving for 15 min in citrate buffer (pH 6.0). Non-specific binding was blocked with 10% goat serum (Dako Ltd, Cambridgeshire, UK) for 30 min. Sections were incubated overnight at 4°C with iNOS antibody (rabbit polyclonal antibody, catalog no. 611473; Transduction Laboratories, San Diego, California, USA; at a dilution of 1:200). The sections were incubated with biotinylated anti-rabbit or anti-mouse immunoglobulins (Dako Ltd) for the corresponding primary antibody. Sections were incubated with the avidin-biotin horseradish peroxidase complex according to the manufactur-

er's instructions. Peroxides were visualized by incubating the sections in diaminobenzidine (Sigma Chemical Co., Poole, UK) and H_2O_2. Negative control sections were processed in the above-mentioned sequence but omitting the incubation with the primary antibody (Kiernan, 1999).

Morphometric study

Using a Leica Quin 500 Image Analyzer (Leica Ltd, Cambridge, UK) in the Image Analyzing Unit of the Pathology Department, Faculty of Dentists, Cairo University (Egypt), the area percentage of positive iNOS immunoreactivity in anti-iNOS immunostained sections was measured. In addition, the area percent demonstration of collagen fibers in the male reproductive system organs was done. The sections of the testis, epididymis, seminal vesicle and prostate were enclosed inside the standard measuring frame .Then the collagen fibers areas were masked by blue binary color via the colour cube selection to be measured. The area percentage and standard measuring frame of a standard area equal to 116,362.6 mm^2 were chosen from the parameters. All measurements were taken at x400 magnification in ten random fields from five sections for each rat in each groups using the interactive measure menu.

Biochemical study

The blood samples were collected from the retro-orbital plexuses and centrifuged for 15 min. The collected blood was used for serum testosterone hormone assay in the three groups**.** Testosterone was assessed by an enzyme-linked immunosorbent assay kit (IB79174, IBL-America, Minneapolis, Minnesota, USA). The values of testosterone were expressed as nanograms per milliliter of serum.

Statistical analysis

The measurements of serum testosterone hormone levels were statistically analyzed using ANOVA and LSD (least significant difference) tests which were done to compare between the groups. Data were expressed as mean ± SD and the results considered significant when P value < 0.05.

The statistical analysis of collagen and iNOS immunoreaction area percents in the Bisphenol-A treated group (group III) compared with the control groups (I&II) was made using the way analysis of variance test (ANOVA). Data were expressed as mean ± SD. Results were considered significant when P value < 0.05.

RESULTS

Biochemical results: serum testosterone level

The mean level of serum testosterone was statistically analyzed using one way analysis of variance test (ANOVA) and LSD (least significant difference) test. Group II (positive control) showed a non-significant difference (P>0.05) as compared to Group I (negative control). The mean level of serum testosterone of BPA-treated Group (III) showed highly significant decrease (P<0.001) as compared to Group I and Group II (Table1).

Histological results

Hematoxylin and Eosin stain

The histological appearance of the negative control Group (I) and the positive control Group (II) showed the same histological structure in all reproductive organs examined included the testis, epididymis, prostate and seminal vesicles. The testis of control groups (I and II) of the adult male albino rats showed a well-developed interstitium between regularly arranged seminiferous tubules arranged on intact basement membranes. The seminiferous tubules had lumina filled with sperms (Fig. 1A).

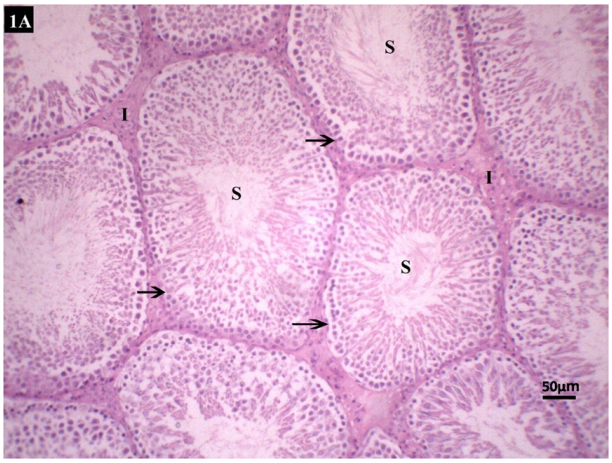


Fig 1. 1A: Section in the control testis showing a well-developed interstitium (I) between regularly arranged seminiferous tubules on intact basement membrane (arrows) with lumina filled with sperms (S).

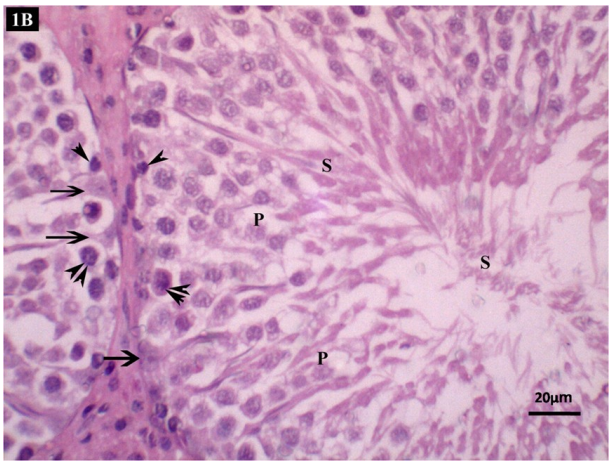
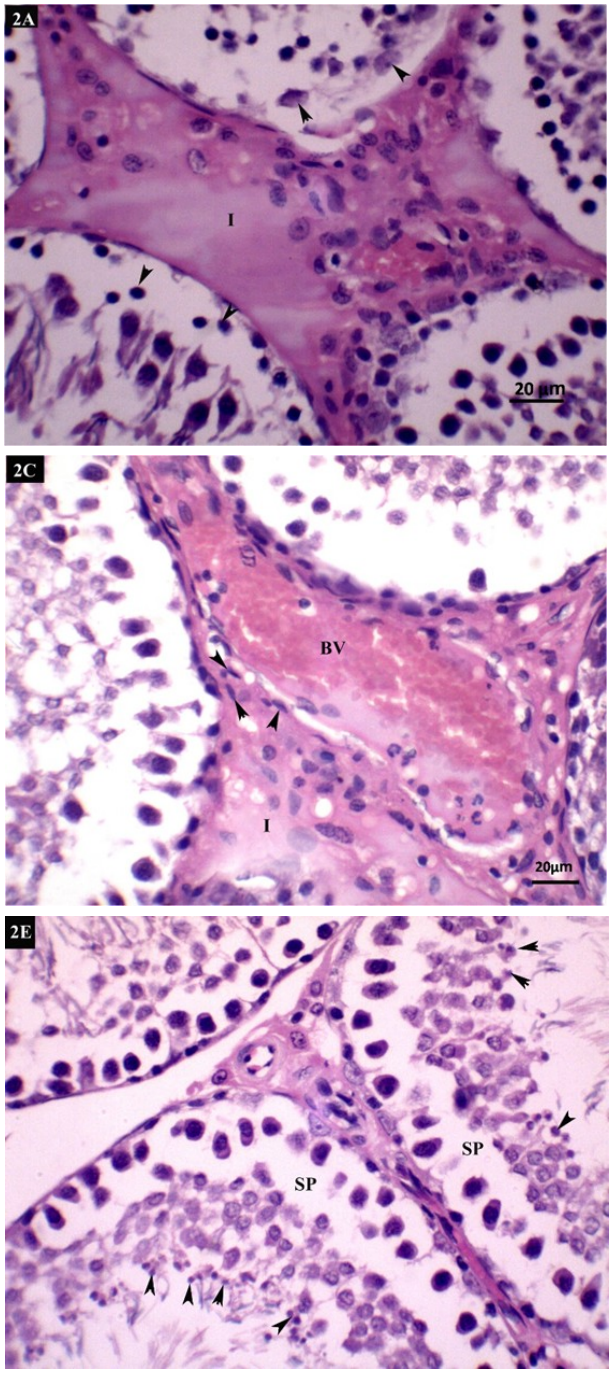


Fig 1. 1B: The lumen of the seminiferous tubules lined with stratified epithelium being formed of spermatogonia appeared as small rounded cells (arrow heads), primary spermatocyte with large rounded nuclei (double arrow heads). Early spermatids were small rounded cells with pale nuclei (P), sertoli cells had triangular nuclei attached to the basement membrane (arrows) and late spermatids (mature sperms) (S). 1A: Hx & E, X100. Scale bar=50 µm.; 1B: Hx & E, X400. Scale bar=20 µm.



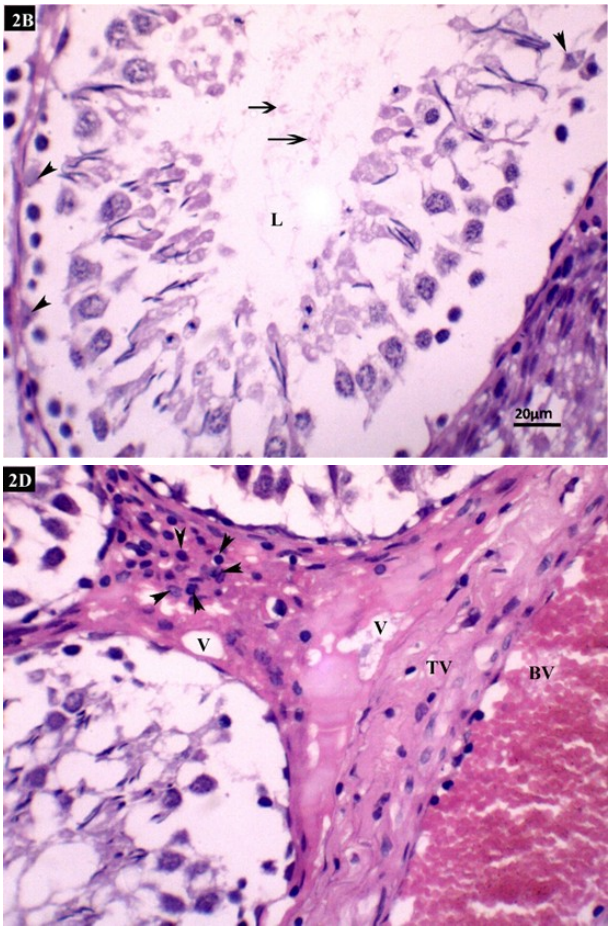


Fig 2. 2A: Section of the testis of Bisphenol-A treated (group III) showing widening of the interstitium (I) between the seminiferous tubules with sloughing germinal epithelium (arrow heads). **2B**: Little debris of spermatids (arrows) in the lumen (L) of the seminiferous tubules and degenerated Sertoli cells with pyknotic nuclei (arrow heads). **2C**: Widening of the intertubular interstitial spaces (I), dilated congested blood vessels (BV) surrounded with monocellular inflammatory cells (arrow heads). **2D**: Vacuolations (V) and monocellular inflammatory cells (arrow heads) in the interstitium and thick tunica vasculosa (TV) of the testicular capsule which contained severely congested dilated blood vessels (BV) **2E**: Wide spaces (SP) appeared between the degenerated germinal epithelium, which had pyknotic nuclei (arrow heads). 2 A -F: Hx & E, X400. Scale bars=20 µm.

The lumen of the seminiferous tubule lined with stratified epithelium being formed of spermatogonia appeared as small rounded cells with rounded nuclei; primary spermatocyte appeared large in size with large rounded nuclei arranged in one or two layers. Early spermatids were small, rounded cells with pale nuclei and late spermatids (mature sperms) inside the lumen. The Sertoli cells had triangular nuclei inside acidophilic cytoplasm and attached to the basement membrane in between the layers of spermatogonia and spermatocytes (Fig. 1B). The testis of the adult male albino rats treated with Bisphenol-A (group III) showed loss of the normal arrangement of the seminiferous tubules due to widening of the interstitium and sloughing of the germinal epithelium lined with the seminiferous tubules (Fig. 2A). There was little debris of spermatids in the lumen of the seminiferous tubules and degenerated Sertoli cells with pyknotic nuclei (Fig. 2B). There was widening of the intertubular interstitial spaces due to dilated congested blood vessels and invaded with monocellular inflammatory cells (Fig. 2C). The seminiferous tubules separated from vacuolated interstium and covered by thick tunica vasculosa of the testicular capsule which contained sever congested dilated blood vessels (Fig. 2D). There were wide spaces appearing between the degenerated germinal epi-

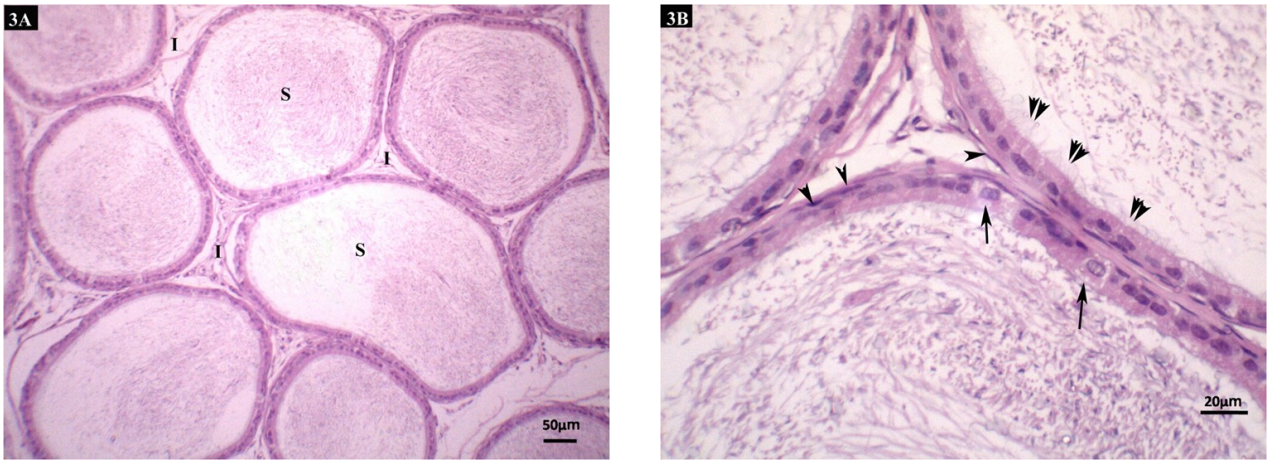


Fig 3. 3A: Section of a control cauda epididymis showing circular tubules containing large number of sperms in their lumina (S) and separated by interstitial tissues (I). **3B**: The principal cells with stereocilia (double arrow heads), the basal cells rested on the basement membrane (arrow heads) and clear cells with pale cytoplasm (arrows). 3A: Hx & E, X100. Scale bar=50 µm.; 3B: Hx & E, X400. Scale bar=20 µm.

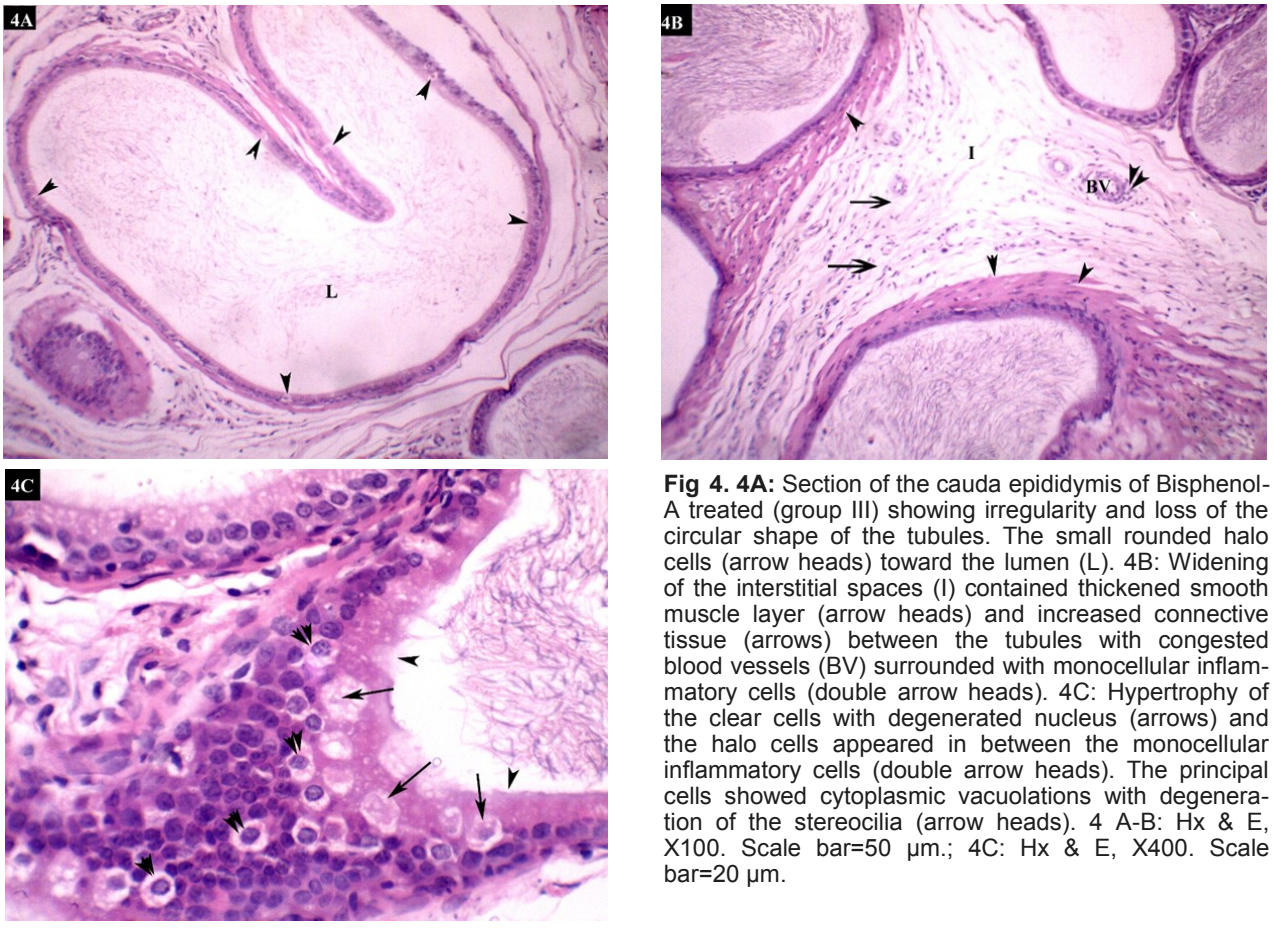


Fig 4. 4A: Section of the cauda epididymis of Bisphenol-A treated (group III) showing irregularity and loss of the circular shape of the tubules. The small rounded halo cells (arrow heads) toward the lumen (L). 4B: Widening of the interstitial spaces (I) contained thickened smooth muscle layer (arrow heads) and increased connective tissue (arrows) between the tubules with congested blood vessels (BV) surrounded with monocellular inflammatory cells (double arrow heads). 4C: Hypertrophy of the clear cells with degenerated nucleus (arrows) and the halo cells appeared in between the monocellular inflammatory cells (double arrow heads). The principal cells showed cytoplasmic vacuolations with degeneration of the stereocilia (arrow heads). 4 A-B: Hx & E, X100. Scale bar=50 µm.; 4C: Hx & E, X400. Scale bar=20 µm.

thelium, which separated from the basement membrane and were replaced by wide spaces inside the seminiferous tubules (Fig. 2E).

The cauda epididymis of control groups (I and II) of the adult male albino rats consisted of circular tubules which contained large number of sperms in their lumina and were separated by interstitial tissues between them containing smooth muscle fibers and connective tissue cells (Fig. 3A). The epididymal tubules lined with pseudostratified epithelium that had 3 types of cells: principal cells, basal cells, and clear cells. The principal cells were columnar to cubical cells characterized by the presence of long microvilli known as stereocilia extended into the lumina of the tubules, while the basal cells were lodged between the bases of the principal cells and had oval nuclei and rested on the basement membrane, the cytoplasm of the basal cells occupied by oval indented nuclei. The clear cells were characterized by pale cytoplasm with central nucleus (Fig. 3B). The cauda epididymis of the adult male albino rats treated with Bi-

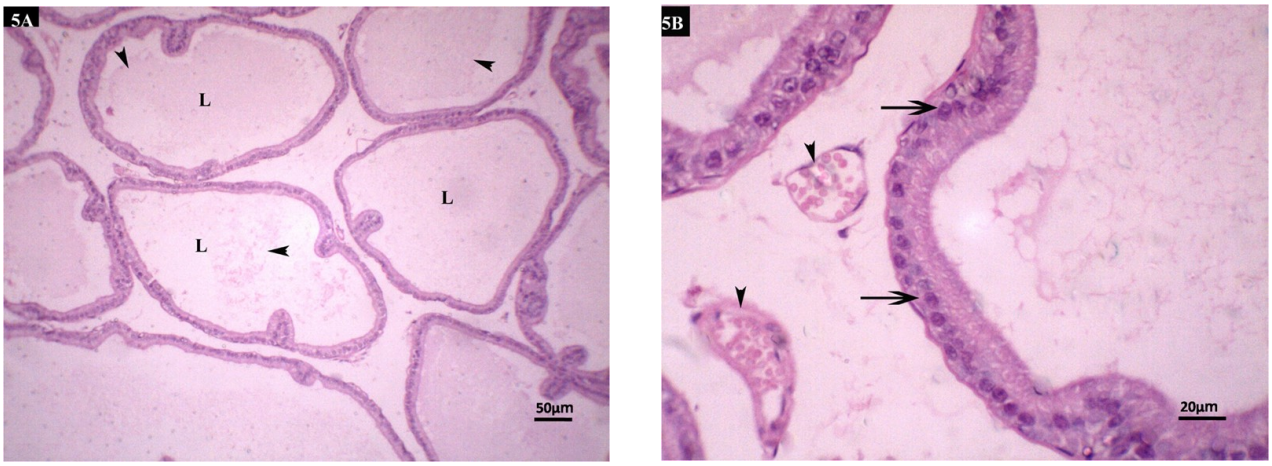


Fig 5. 5A: Section of control prostate showing regular size acini. The acini contains prostatic secretions (arrow heads) in the lumen (L). **5B**: The acini lined with simple columnar epithelium (arrows) embedded in a fibromuscular stroma contained blood capillaries (arrow heads). 5A: Hx & E, X100. Scale bar=50µm.; 5B: Hx & E, X400. Scale bar=20 µm.

sphenol-A (group III) showed irregularity and loss of the circular shape of the tubules. The epithelium altered from columnar to flat cells with appearance of the halo cells toward the lumen, which were small rounded cells with pale cytoplasm containing central rounded slightly indented nuclei (Fig. 4A). There was widening of the interstitial spaces containing thickened smooth muscle layer and increased connective tissue between the tubules with congested blood vessels surrounded with monocellular inflammatory cells (Fig. 4B). There were hypertrophy of halo cells and the principal cells characterized by cytoplasmic vacuolations with degeneration of the stereocilia and clear cell hypertrophy (Fig. 4C). The ventral prostate of control groups (I & II) of the adult male albino rats were formed by acini of regular size and shapes which were embedded in a fibromuscular stroma. The lumen of the acini contains prostatic secretions (Fig. 5A). The epithelial lining of the acini was formed mainly by simple columnar epithelial cells with basal nuclei. The height of lining epithelium varied according to the functional state of the gland that covered the folds. The fibromuscular stroma between the acini contained blood capillaries (Fig. 5B).

The histological structure of the ventral prostate of the adult male albino rats treated with BPA (group III) showed disturbance in the shape and the size of prostatic acini. The acini showed epithelial prostatic hyperplasia and were surrounded with stromal hyperplasia when compared with the control groups. The epithelial hyperplasia looked likes an arborization (Fig. 6A). There was thickened proliferative fibromuscular stroma surrounding the hyperplastic acini forming nodules suggesting the appearance of benign nodular prostatic hyperplasia. So, benign prostatic hyperplasia involved both the epithelium of the gland and the stroma (Fig. 6B). In some sections, there was acute prostatitis because the prostatic acini showed severe infiltration with small dark blue monocellular inflammatory cells inside the acinar lumen and around the acini (Fig. 6C). The fibromuscular stroma showed various pathological lesions. In some sections, severe pink hyaline proteinous plaques invaded with monocellular inflammatory cells. There were hemorrhages and congested blood vessels in the prostatic capsule (Fig. 6D). In other sections, the fibromuscular stroma showed wide vacuolations and spaces appearing in between the proliferative connective tissue (Fig. 6E). In some sections, the epithelial lining of the acini was markedly increased in thickness forming papillary folds covered with pseudostratified columnar cells lining the acini and disappearance of the prostatic secretions (Fig. 6F).

The histological structure of the seminal vesicles of control groups (I & II) of the adult male albino rats showed branched acini containing smooth muscular tissue in between the branches of the acini (Fig. 7A). The epithelial lining of these acini consisted of a pseudostratified columnar epithelium with highly vesicular nuclei and foamy cytoplasm and the cavity of the vesicle contains secretions of these acini (Fig. 7B). The epithelial lining of the seminal vesicle acini of the (group III) albino rats treated with BPA were markedly increased in thickness and number forming a highly coiled hyperplastic epithelial nodules (Fig. 8A). The smooth muscular tissue in between the branches of the seminal vesicle acini showed edema, congested blood vessels and hemorrhage surrounded with monocellular inflammatory cells (Fig. 8B). The seminal vesicle of the (group III) albino rats treated with BPA showed little secretions inside the acini of the seminal vesicles and other acini had no secretions. The acini of the seminal vesicles lined with vacuolated epithelium with atypical shapes of the large irregular hyperchromatic nuclei (Fig. 8C). The hyperplastic epithelium was characterized by large hyperchromatic nuclei with prominent nucleoli (Fig. 8D).

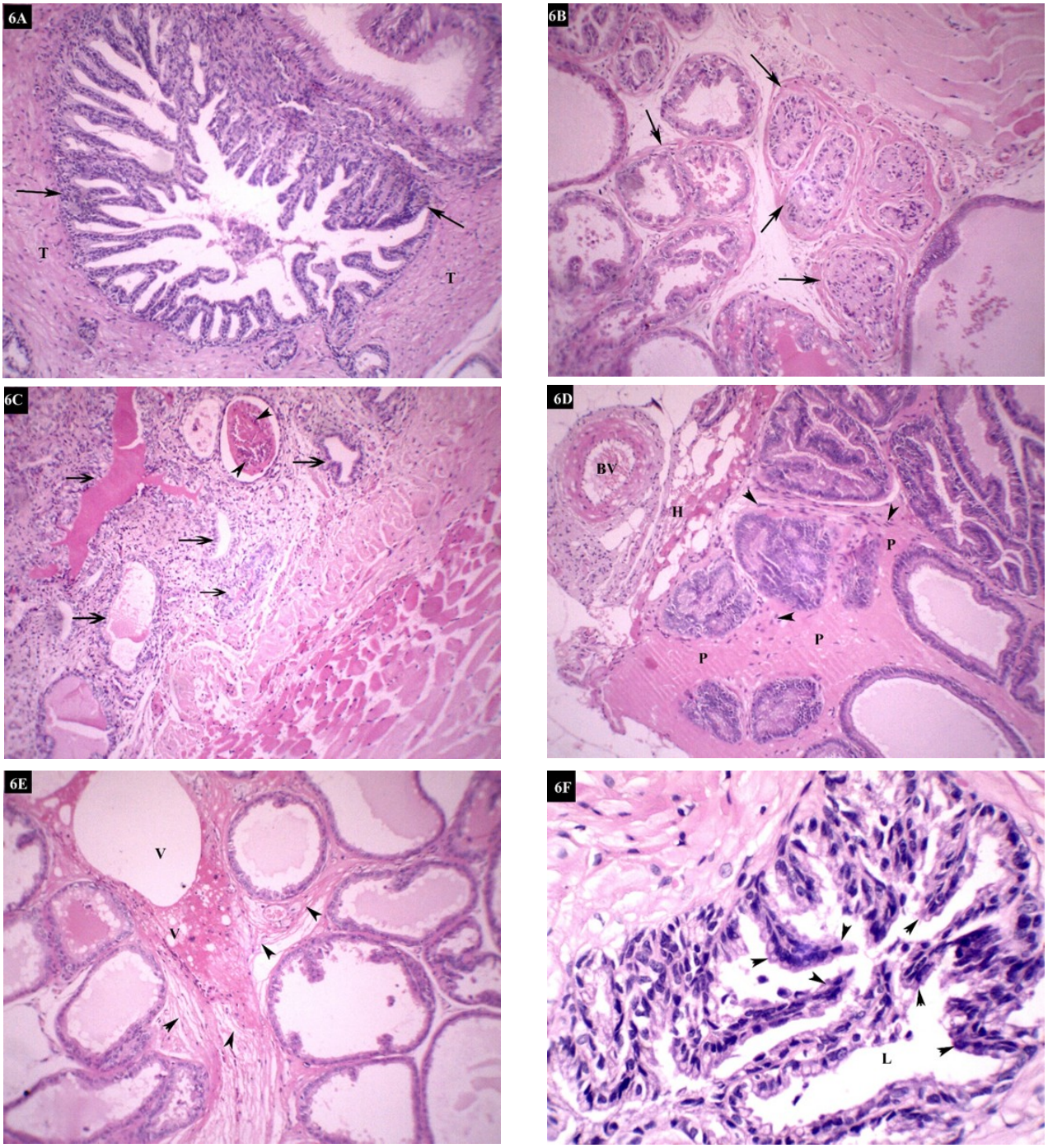


Fig 6. 6A: Section of the prostate of Bisphenol-A treated (group III) showing the prostatic acinar hyperplasia looks like an arborization (arrows). There is thick stroma (T) surround the acini. **6B**: The disturbance in the shape and the size of prostatic acini and benign nodular prostatic hyperplasia (arrows). **6C**: Acute prostatitis with small dark blue monocellular inflammatory cells inside the acinar lumen (arrow heads) and around the acini (arrows). **6D**: Sever pink hyaline proteinous plaques (P) invaded with monocellular inflammatory cells (arrow heads). There are hemorrhages (H) and congested blood vessels (BV) in the prostatic capsule. **6E**: The fibromuscular stroma with vacuolations (V) appear in between the proliferative connective tissue (arrow heads). **6F**: The papillary folds covered with pseudostratified columnar cells lining the acini (arrow heads) and disappearance of the prostatic secretions in the lumen (L). 6 A-E: Hx & E, X100. Scale bar=50 µm.; 6F: Hx & E, X400. Scale bar=20 µm.

Masson's trichrome stain

The histological appearance of the Masson's trichrome stained sections of the negative control group (I) and the positive control group (II) showed the same few amount of the collagen fibers which were appeared blue colour in the interstitial spaces around the blood vessels of reproductive organs included the testis (Fig. 9A), epididymis (Fig. 9B), prostate (Fig. 9C) and seminal vesicles (Fig. 9D). The Masson's trichrome stained sections of the Bisphenol-A treated (group III) showed great amount of the collagen fibers around the dilated congested blood vessels in the testis (Fig. 10A), epididymis (Fig. 10B), prostate (Fig. 10C) and

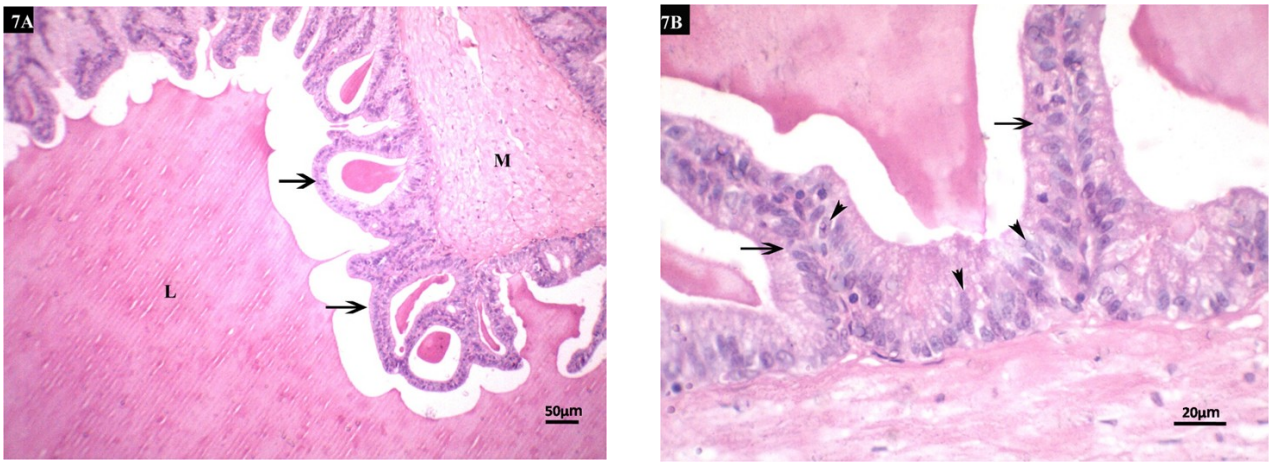


Fig 7. 7A: Section of control seminal vesicles showing branched acini (arrows) containing smooth muscle tissue (M) in between the branches of the acini. The lumen (L) of the vesicle contains secretions of these acini. **7B**: The epithelial lining of these acini consists of a pseudostratified columnar epithelium with foamy cytoplasm (arrows) and highly vesicular nuclei (arrow heads). 7A: Hx & E, X100. Scale bar=50 µm.; 7B: Hx & E, X400. Scale bar=20 µm.

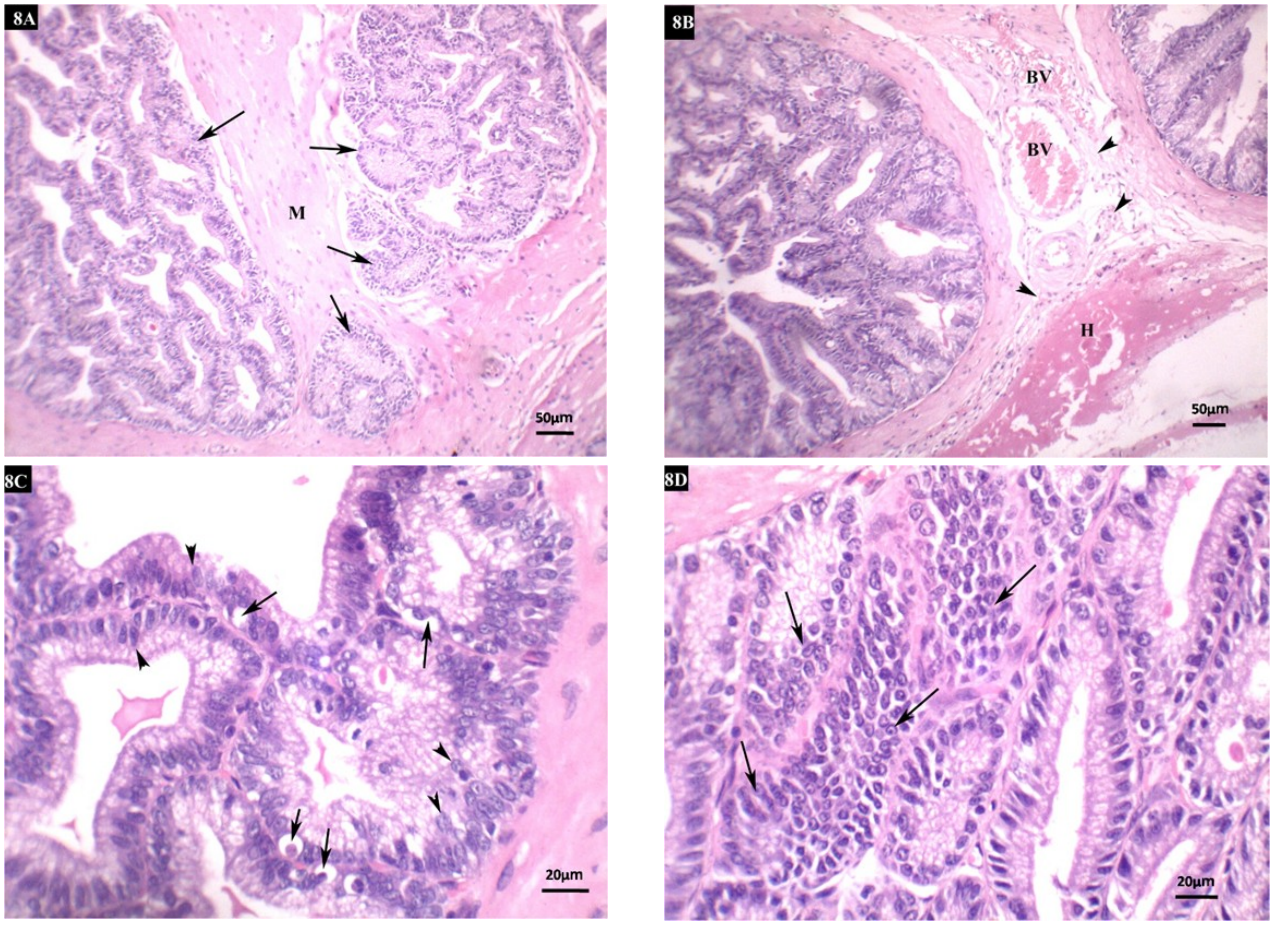


Fig 8. 8A: Section of the seminal vesicle of Bisphenol-A treated (group III) showing the epithelial lining of the seminal vesicle markedly increased in thickness and number, forming a highly coiled hyperplastic epithelial nodules (arrows) in between the smooth muscle tissue (M). **8B**: The smooth muscular tissue in between the seminal vesicle nodules shows edema with monocellular inflammatory cells (arrow heads) surrounded the congested blood vessels (BV) and hemorrhage (H). **8C**: The seminal vesicles lined with vacuolated epithelium (arrows) with atypical shapes of the large irregular hyperchromatic nuclei (arrow heads). **8D**: The hyperplastic epithelium is characterized by large hyperchromatic nuclei with prominent nucleoli (arrows). 8 A-B: Hx & E, X100. Scale bar=50 µm.; 8 C-D: Hx & E, X400. Scale bar=20 µm.

seminal vesicles (Fig. 10D).

Immunohistochemical stain for inducible nitric oxide synthase (iNOS)

Immunohistochemical stained sections showed negative immune reaction for inducible nitric oxide synthase in the negative control group (I) and the positive control group (II) of all the reproductive organs, including the testis (Fig. 11A), epididymis

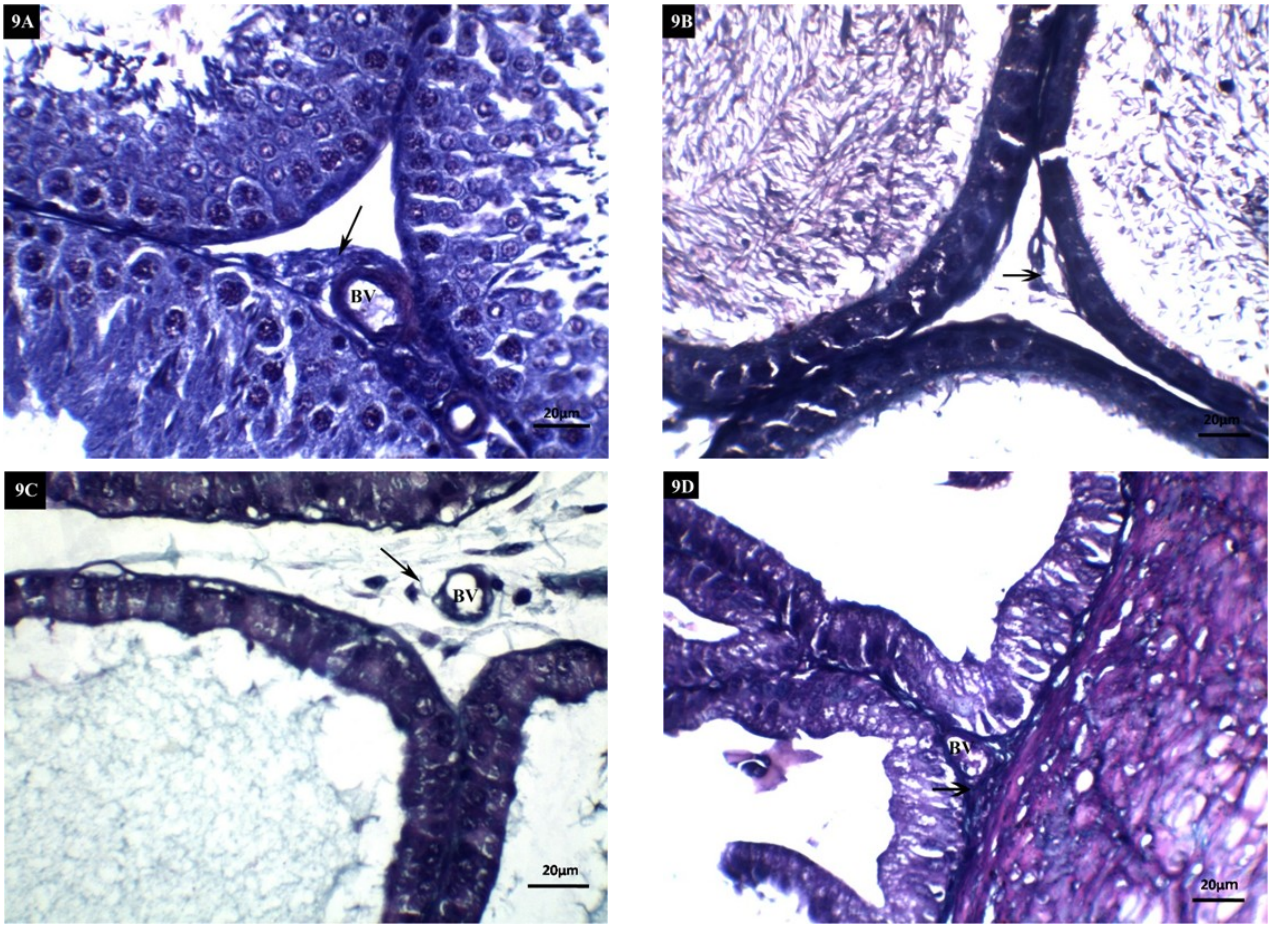


Fig 9. Sections of the control testis (9A), control epididymis (9B), control prostate (9C) and control seminal vesicle (9D) showing little amount of the collagen fibers (arrow) in the interstitial spaces around the blood vessels (BV). 9 A-D: Masson's trichrome, X400. Scale bar=20 µm.

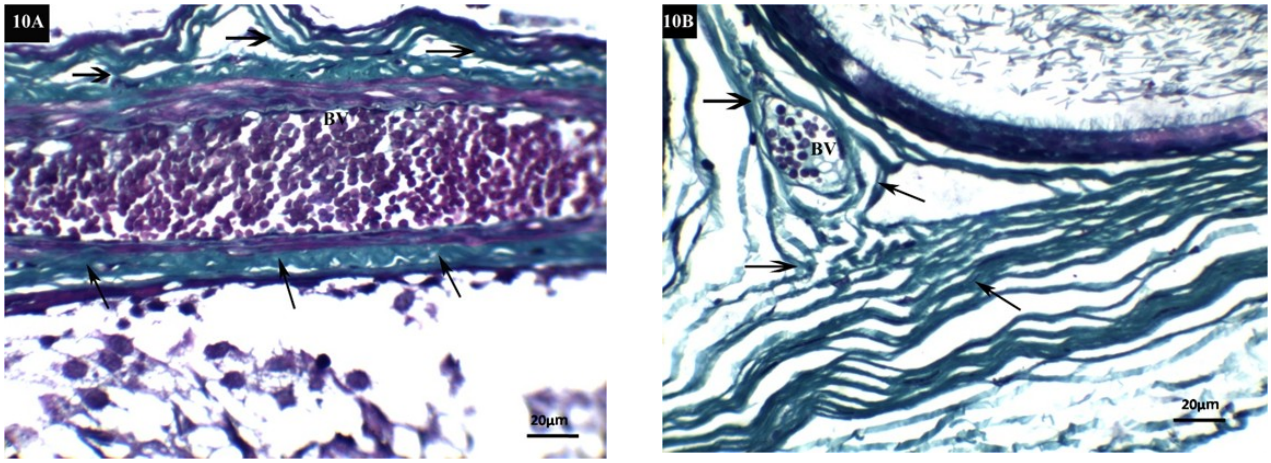


Fig 10. Photomicrographs of the Bisphenol-A treated (group III) showing abundant amount of the collagen fibers (arrows) around the congested dilated blood vessels (BV) in the testis (10A), epididymis (10B), prostate (10C) and seminal vesicle (10D). 10 A-D: Masson's trichrome, X400. Scale bar=20 µm.

(Fig. 11B), prostate (Fig. 11C) and seminal vesicles (Fig. 11D). Examination of the immunohistochemical stained sections of the Bisphenol-A treated (group III) showed positive immune reaction for inducible nitric oxide synthase in the cytoplasm of the Sertoli cells and the interstitial Leydig cells of the testis (Fig. 12A), epididymis (Fig. 12B), prostate (Fig. 12C) and seminal vesicles (Fig. 12D).

Statistical analysis of area percentage of collagen fibers and iNOS immunoreaction

The statistical analysis of the area percentage of the collagen fibers and iNOS immunoreaction were done using one-way analysis of variance test (ANOVA). The group II (positive control) showed a non-significant difference (P>0.05) as compared to group I (negative control). The area percentage of

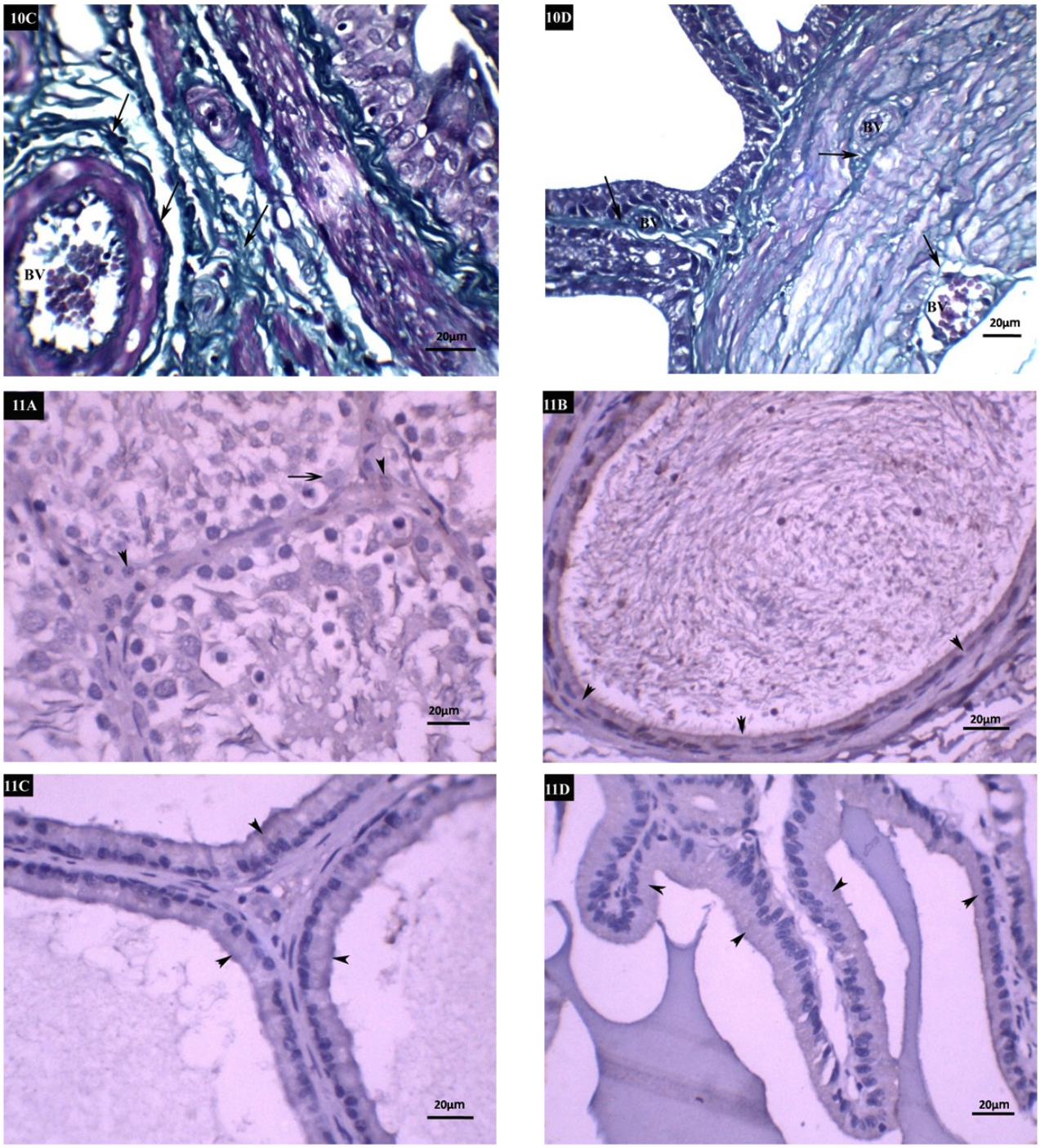


Fig 11. 11A: Section of a control testis showing negative iNOS immunoexpression in the cytoplasm of the Sertoli cells (arrow) and the interstitial Leydig cells (arrow heads). **11B**: The control epididymis shows negative iNOS immunoexpression in the cytoplasm of the lining epithelium of the tubules (arrow heads). **11C**: Negative iNOS immunoexpression in the cytoplasm of the lining epithelium of the prostatic acini (arrow heads) and the lining epithelium of the seminal vesicle (arrow heads) (**11D**). 11 A-D: Immunoperoxidase technique for iNOS, X400. Scale bar=20 µm.

collagen fibers of BPA treated (group III) revealed significant increase as compared to group I (negative control) and group II (positive control) in the testis, epididymis, prostate and seminal vesicles sections (Tables 2, 3).

DISCUSSION

Many factors affect the spermatogenesis in the environmental pollution such as endocrine disrupter and toxic elements (Vandenberg et al., 2010). The present study demonstrated that 30-day oral administration of BPA in adult male albino rats had multiple toxic histological effects on the reproductive system and significantly reduced plasma testosterone levels. In the current study, the testis of the BPA-treated (group III) showed irregularity with a loss of the normal arrangement of the seminifer-

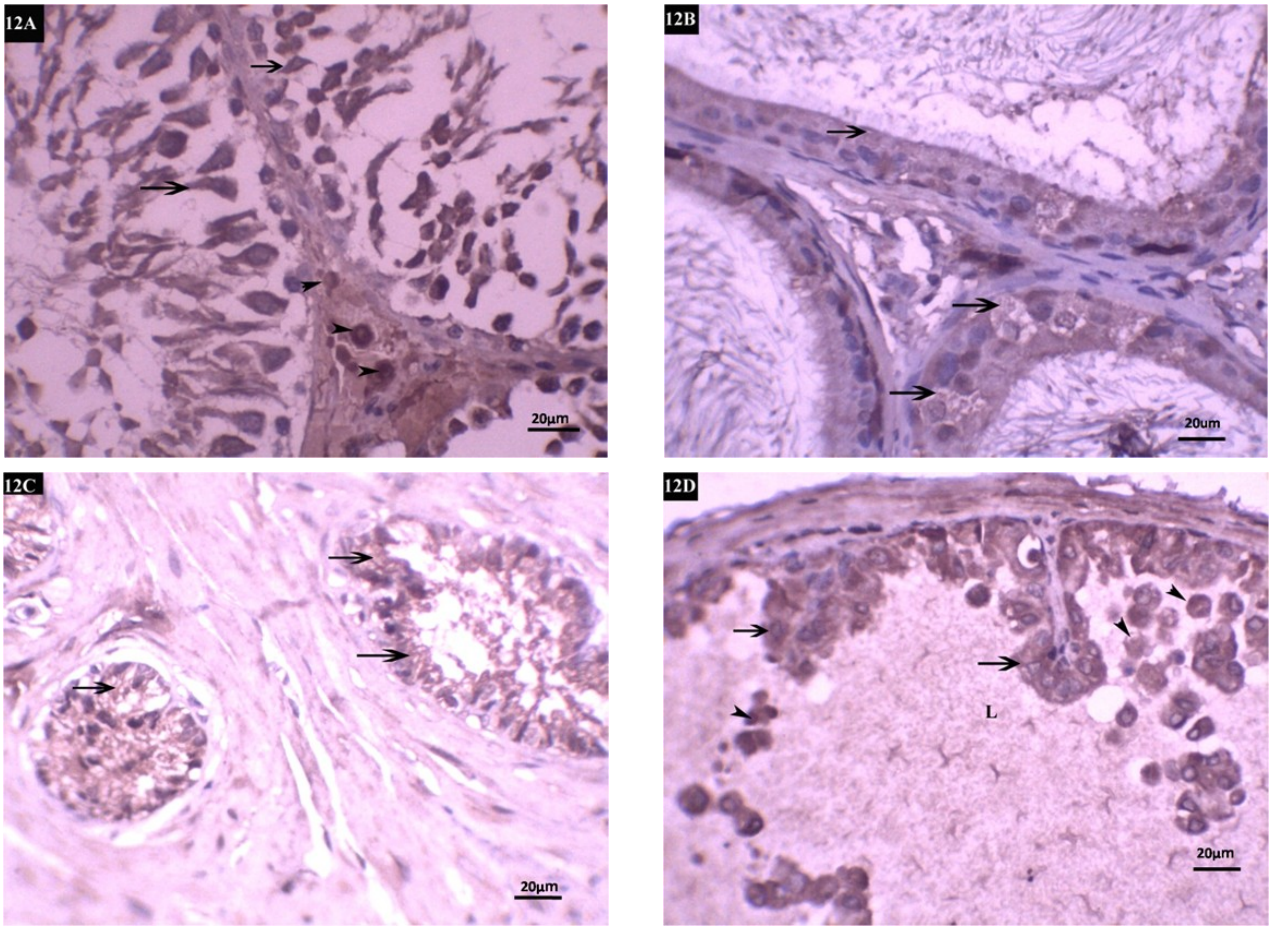


Fig 12. 12A: Image of a Bisphenol-A treated (group III) showing positive iNOS immunoexpression in the cytoplasm of the Sertoli cells (arrows) and the interstitial Leydig cells of the testis (arrow heads). **12B**: Positive iNOS immunoexpression (arrows) in the cytoplasm of the lining epithelium of the epididymis. **12C**: The cytoplasm of the lining epithelium of the prostatic acini shows positive iNOS immunoexpression (arrows). **12D**: The cytoplasm of the epithelium of the branched acini (arrows) and the exfoliated epithelium (arrow heads) in the seminal vesicle lumen (L). 12 A-D: Immunoperoxidase technique for iNOS, X400. Scale bar=20 µm.

ous tubules and sloughing of the germinal epithelium from the basement membranes. The same findings were found by Zhang et al. (2013). The epithelium of the male reproductive tubular organs may respond to injury by sloughing away from the basement membrane due to the increased movement of the smooth muscle cells as a result of the inflammation. The epithelial response depends on the degree of inflammation as the *acute inflammatory response* may cause excessive stretch of the ducts epithelium by the released chemotactic substance which attracts the neutrophils and other inflammatory cells (Nakai et al., 1993). In the current study, the albino rats treated with Bisphenol-A showed significantly decreased testosterone hormone level. Several studies have confirmed the decrease of testosterone hormone with BPA treatment as (Wisniewski et al., 2015; Zang et al., 2016). However, Kim et al. (2002) mentioned that fertility was reduced by BPA without a change in the testosterone hormone.

In this study, there was an irregularity of the tubules with degenerative changes in the stereocilia of the hypertrophied principal cells of the cauda epididymis of the group III treated with BPA. The epithelium altered from columnar to flat cells with appearance of the halo cells toward the lumen. There was hypertrophy of the halo cells and clear cell of the cauda epididymis. These results in agreement with El-ghazzawy et al. (2011), who reported that the degenerated stereocilia of the hypertrophied principal cells confirmed the role of the principal cells in the defense mechanism in the epididymis during the BPA exposure, because the stereocilia had a large surface area for molecular interaction and they contained different transport systems and membrane pumps to keep the proper volume of the epididymal lumen. Also, Ahmed et al. (2009) concluded that the principal cells may have a role in phagocytosis of the cellular debris and abnormal particles within the epididymis. The halo cells assumed to be lymphocytes that had a role in the male reproductive immunological function (Palacios et al., 1993). The clear cells are responsible for scavenging the immature damaged sperms and make absorption of the droplet of the sperm cytoplasm (Andrade et al., 2006).

Light microscopic examination of the prostate

Table 1. Comparison of serum testosterone level among studied groups by ANOVA & LSD tests. “a” group I vs group II, “b” group I versus group III, “c” group II versus group III. SD: Standard deviation, * Significant.

Test variable	group I **N=15**	group II **N=15**	group III **N=15**	**ANOVA F**	P	***LSD***
Mean ± SD	3.66± 0.30	3.58 ± 0.35	2.16±0.36	2.8 1	**0.000*** 0.31	**<0.001** [b,c] >0.05 [a]

Table 2. Comparison of area percentage (%) of the collagen fibers among studied groups by ANOVA & LSD tests. “a” group I vs group II, “b” group I versus group III, “c” group II versus group III. SD: Standard deviation, * Significant.

Mean ± SD	group I **N=15**	group II **N=15**	group III **N=15**	**ANOVA F**	P	***LSD***
Testis	0.183±0.113	0.262±0.192	37.721±10.712	183.735	0.000*	0.169[a] **<0.001*[b]** **<0.001*[c]**
Epididymis	0.15±0.058	0.18±0.051	22.872±3.291	712.404	0.000*	0.148 [a] **<0.001*[b]** **<0.001*[c]**
Prostate	0.017±0.008	0.022±0.010	33.323±13.082	97.252	0.000*	0.142 [a] **<0.001*[b]** **<0.001*[c]**
Seminal vesicles	0.212±0.047	0.189±0.052	36.763±25.981	29.641	0.000*	0.214 [a] **<0.001*[b]** **<0.001*[c]**

Table 3. Comparison of area percentage (%) of the iNOS immunoreaction among studied groups by ANOVA & LSD tests. “a” group I vs group II, “b” group I versus group III, “c” group II versus group III. SD: Standard deviation, * Significant.

Mean ± SD	group I **N=15**	group II **N=15**	group III **N=15**	**ANOVA F**	P	***LSD***
Testis	3.059±0.223	2.932±0.204	14.903±7.949	33.614	0.000*	0.115 [a] **<0.001*[b]** **<0.001*[c]**
Epididymis	0.187±0.065	0.205±0.049	36.125±6.348	480.440	0.000*	0.399 [a] **<0.001*[b]** **<0.001*[c]**
Prostate	1.029±0.491	1.181±0.552	35.938±9.633	194.989	0.000*	0.432 [a] **<0.001*[b]** **<0.001*[c]**
Seminal vesicles	0.885±0.469	1.015±0.521	13.872±5.155	92.548	0.000*	0.479 [a] **<0.001*[b]** **<0.001*[c]**

sections of Bisphenol-A treated (group III) revealed hemorrhage in the prostatic capsule and benign nodular prostatic hyperplasia. This prostatic hyperplasia is explained by Huang et al. (2004), who reported that the prostate gland is sensitive to estrogen, and that exposure to synthetic estrogens alters prostate development and differentiation. Estrogens which act in association with the androgens control prostate proliferation both in rats and human (Imamov et al., 2005). The seminal vesicles of the (group III) treated with Bisphenol-A showed congested blood vessels and hemorrhage. The epithelial lining of the seminal vesicles acini were markedly increased in thickness and number, forming a highly coiled hyperplastic epithelial nodules. The epithelial hyperplasia explained by Nakai et al. (1993), who argues that the epithelial injury of the male reproductive system undergoes differentiation by mitosis, and growth of a new hyperplastic epithelium occurs.

Dilatation and congestion of the interstitial blood vessels in the abundant connective tissue invaded with monocellular inflammatory cells and hemorrhage were one of the constant pathological changes that detected in all examined organs of the reproductive system. The mechanism of the blood vessels dilatation is explained by Kathryn and McCance, (2008), who state that the increase in the ratio of oxygen need to the oxygen supply causes an increased adenosine production rate. This lead to dilatation of the vessels and increased blood flow in order to restore the oxygen ratio to

the normal level.

In the current study, collagen fiber deposition increased in BPA-treated group if compared with the control groups. It has been reported that BPA could induce fibroblast hyperplasia in the prostate (Ramos et al., 2001). Oxidative stress is found to play an important role in various reproductive pathological conditions including fibrosis and excessive production of collagen. The reactive oxygen species may play a role in the activation of transcription factors such as nuclear transcription factor κB. The unfavorable consequences of these cellular events include inflammation and fibrosis (Barnes and Karin, 1997). The current study showed oxidative stress effect of BPA through reactive oxygen species. It was assessed by a significant increase in the area percentage of positive immunoreaction for iNOS in BPA-treated rats compared with the control. These results are in agreement with Chouhan et al. (2015), who reported that BPA induces oxidative stress by altering the expression of iNOS, which consequently leads to the down regulation of androgen receptors expression in the testis of male mouse. In conclusion, this study demonstrated that Bisphenol-A has a different histological effects on the tissues of the male reproductive organs. BPA induces epithelial proliferation in the accessory sex glands (prostate and seminal vesicles) and collagen fibers proliferation in all reproductive organs. BPA induced DNA oxidative damage via iNOS activity in all reproductive organs.

Ethical statement

The study has been approved by the Faculty of Medicine, Zagazig University Institutional 11 Review Board (ZU-IRB) and performed in accordance with the ethical standards for experimental animal rights.

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