

## BISPHENOL A INDUCED OXIDATIVE DAMAGE IN THE RAT REPRODUCTIVE ORGANS: A POSSIBLE CAUSE FOR DECLINE IN STEROIDOGENESIS

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Endocrine disrupting activities of bisphenol A (BPA) contribute to adverse effects on health and fertility. The current study was conducted to show the effect of BPA on male reproductive organs in prepuberty.

**Methods:** Forty prepubertal albino male rats were divided into 4 groups (10 rats each). The first was considered as control and the other three were injected subcutaneously with 11.4, 57.1, and 114.2 mg/kg of BPA dissolved in DMSO, respectively for 6 weeks. Histopathological examination for reproductive organs was performed in addition to biochemical assays of plasma sex hormones determined by ELISA to evaluate the effect of BPA on fertility. Moreover, oxidative stress indices in testis tissue were evaluated by colorimetric methods.

**Results:** The levels of testosterone and LH were significantly decreased in rats injected with BPA in a dose of 57.1, and 114.2 mg/kg/day compared to controls. BPA significantly increased the oxidative stress indices in testis tissues. The histopathological changes observed in the testis treated with BPA demonstrated its potentials to induce cytotoxic and endocrine disrupting effects on the spermatogenic, Sertoli and Leydig cells. Furthermore, the severity of BPA effects observed was dose dependent.

**Conclusion:** BPA caused dose dependent reduction in the plasma levels of testosterone and LH in male rats associated with oxidative stress and histological changes in the testis. The effect of BPA on the testis may be due to oxidative stress in testis or the effect of decreased testosterone level or both.

**Keywords:** Bisphenol A, hormones, reproductive organs, oxidative stress, fertility.

### INTRODUCTION

Endocrine disrupting chemicals (EDCs) are compounds that act as estrogen-like and/or anti-androgenic compounds. They disrupt and

interfere with hormones in the body responsible for the development and healthy maintenance of reproductive organs<sup>1</sup>. Data of epidemiological studies showed prevalence of diseases associated with EDCs, such as cancer

and infertility over the last decade. The action of EDCs is mediated through estrogen-related receptors which causes changes in metabolism of body hormones in addition to interfering with feedback regulation and neuroendocrine cells and epigenetic modifications including variations in DNA methylation or histone acetylation<sup>2</sup>.

Generally, male reproductive health is defined by the proper development and function maintenance of the reproductive organs. Androgens produced by the fetal testes drive male sexual differentiation so consequently, it is probable that EDCs will have a major effect on male developmental programming and maturation of the reproductive tract<sup>3</sup>.

Environmental pollution associated with plastic use is due to the large amount of waste produced in addition to the substances leaching out of plastic. Bisphenol A (BPA) is a key component used in plastics and is known to be released from plastic products. BPA is also known as an EDC owing to its ability to modulate the endocrine system. BPA is used in food and water containers, different types of bottles and linings of metal cans. Small amounts of BPA can migrate from polymers to food or water, especially when heated<sup>4</sup>.

Adverse health concerns demonstrated in laboratory animal models upon exposure to environmentally relevant doses of BPA, strongly support the idea that the endocrine disrupting activities of BPA contribute to adverse effects on human health since the doses tested correspond to those observed in humans<sup>5</sup>.

Several studies have documented that BPA can affect steroidogenesis and fertility<sup>5&6</sup>. Bisphenol A is one of the major causes in 77% of cases of the male infertility<sup>7</sup>. Exposure to BPA as an environmental toxicant has been shown to adversely affect spermatogenesis in rodents and humans, which can lead to low sperm count, abnormal sperm morphology and poor semen quality<sup>8&9</sup>. Moreover, BPA has been found to produce several defects in the embryo, such as atrophy of the testes and epididymides, increased prostate size and affects hypothalamic-pituitary-testicular axis by modulating hormone synthesis, expression, and function of respective receptors<sup>10</sup>.

Generally, regulation of spermatogenesis is mediated through the release of follicle-stimulating hormone (FSH) and testosterone from the Leydig cells in response to luteinizing hormone (LH). FSH increases the number of spermatogonia and enhances subsequent entry into meiosis, thus stimulating spermatogenesis<sup>11</sup>. Results from previous studies demonstrated that downregulation of FSH or testosterone can affect the process of spermatogenesis<sup>12</sup>. It is reported that spermatogenesis and Leydig cell steroidogenesis are both vulnerable to oxidative stress<sup>13</sup>. It is reported that BPA induces oxidative stress in the testis and epididymis, by inhibiting antioxidant enzymes activities and stimulating lipid peroxidation<sup>10</sup>.

Accordingly, in the current study, we investigated the effect of BPA on prepubertal male rat reproductive organs by histological examination of male reproductive organs, biochemical determination of the plasma levels of testosterone, LH, and FSH and oxidative stress markers in tissues of testis to evaluate the BPA toxicity on male fertility.

## MATERIALS AND METHODS

### Chemicals

BPA was purchased from Sigma-Aldrich Company (>99% purity; Sigma, St. Louis, MO, USA). Other chemicals used in the present study were of analytical grade and fine.

### Animals and experimental design

Forty male albino rats (4 weeks old), weighing 80-100 gm were housed in the animal facility, Faculty of Medicine, Assiut University. The experimental protocol followed the Guide for Care and Use of Laboratory Animals. Rats were maintained in stainless steel cages under controlled conditions (23±1°C, 12-h light-dark cycle, relative humidity of 50±10%) and had access to a standard rodent laboratory diet and tap water *ad libitum*. Rats were divided into 4 groups (10 rats each), one control received the vehicle (DMSO) and the other 3 groups were dosages with 11.4, 57.1 and 114.2 mg/kg/day BPA dissolved in DMSO injected subcutaneously daily, respectively for 6 weeks according to Nakamura *et al.*<sup>14</sup>. After 6 weeks of injection, the rats of all groups were anaesthetized by

diethylether and killed for collection of blood in heparin-containing tubes and reproductive organs (testis, epididymis, seminal vesicle and prostate). Plasma was separated by centrifugation of blood at 2500 rpm for 10 min at 4°C and kept in small aliquots at -80°C until assays. Small pieces of testis, epididymis, seminal vesicle and prostate were fixed for the histopathological examination and the remnants of organs were kept at -80°C until the biochemical assays.

### **Histopathological evaluation of reproductive organs**

Testes were fixed in Bouin's solution for two days then fixed in 10% neutralized formalin solution. Epididymis, seminal vesicle and prostate were fixed in neutralized-formalin solution. Tissue samples were embedded in paraffin and cut at 5µm thickness, stained with hematoxylin and eosin (H&E) and then examined under light microscope.

### **Determination of hormones levels**

The plasma levels of rat testosterone, LH, and FSH were determined by ELISA Kits (WEKEA MEDSUPPLIES CORP, USA) according to the guideline instruction.

### **Determination of oxidative stress markers in testis**

The tissues of testes of different groups were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4), using a Potter-Elvehjem homogenizer fitted with a taflon Plunger. Homogenates were centrifuged at 11,000g for 20 min and the resulting supernatants were divided into aliquots and stored at -80°C. Total proteins levels in tissue supernatant were determined by the method of Lowry *et al.*<sup>15</sup> using commercial kit (Sclavo Diagnostics, Italy). The levels of lipid peroxides were measured as thiobarbituric acid reactivity and product malondialdehyde (MDA) was measured as described by Thayer<sup>16</sup>. The level of nitric oxide (NO) was determined as total nitrite by Griess reagent, using sodium nitrite 10-100 µM as standard according to Ding *et al.*<sup>17</sup>. The level of glutathione was determined chemically as described by Dutta *et al.*<sup>18</sup>. The activity of superoxide dismutase (SOD) was assayed according to the procedure of Nishikimi *et al.*<sup>19</sup>.

### **Statistical analysis**

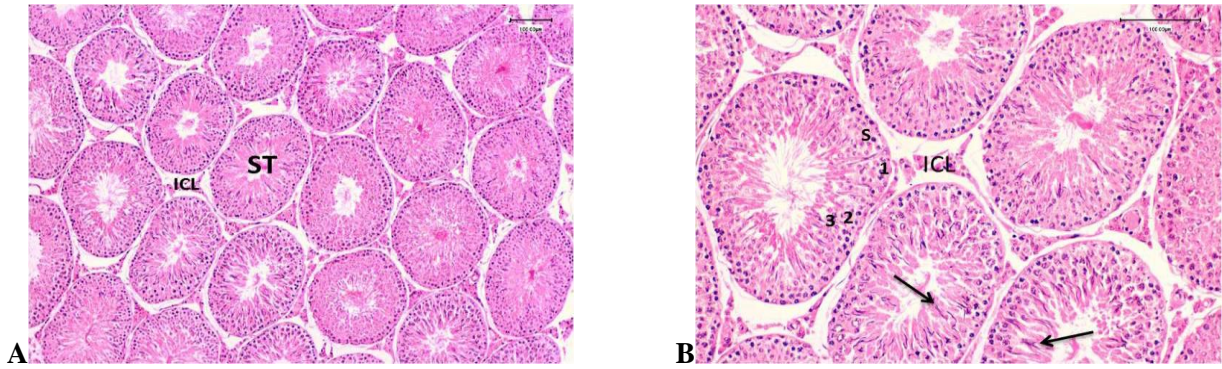
The results were expressed as mean ± standard error (SE). Differences between groups were assessed by one-way analysis of variance (Bonferroni test) using the Prism version 5 software package for Windows. *P* values less than 0.05 were regarded as statistically significance.

## **RESULTS**

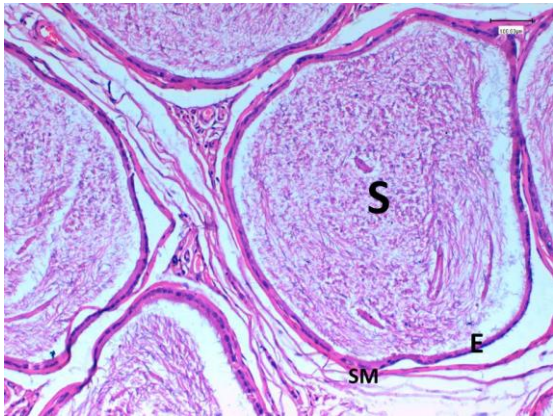
### **Histological results**

Light microscopic examination of the control rat testis showed the normal organization of normal sized seminiferous tubules with narrow interstitial spaces in between occupied by clusters of normal interstitial cells of Leydig. Each tubule was surrounded by flat nuclei of the myoid cells. The tubules were lined by stratified epithelium of closely packed and regularly arranged spermatogenic cells in different phases. They included the spermatogonia occupying the basal compartment of the tubules, followed by the large spermatocytes and then the spermatids. Individual Sertoli cells were identified by their triangular outline, basophilic cytoplasm and their position immediately overlying the tubular basal lamina. A large number of maturing sperm heads and tails were also seen. The normal Leydig cells appeared as aggregation of polyhedral cells closely associated to the interstitial blood capillaries. Each cell showed an eosinophilic vacuolated cytoplasm and showed a large vesicular nucleus (Fig. 1.a&b).

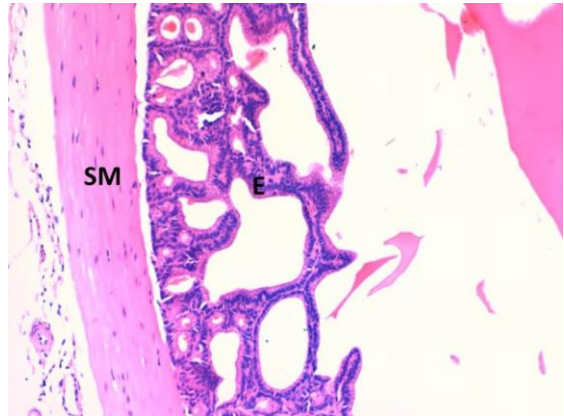
The tube of the epididymis was lined with pseudostratified columnar epithelium and bearing stereocilia which was surrounded by muscular wall. The lumen was filled with sperms (Fig. 2). The seminal vesicle was lined with pseudostratified tall columnar epithelium with outer muscular wall (Fig. 3). The prostate gland was composed of glands and stroma. The glands showed convoluted pattern with the epithelium thrown up into folds. The epithelium was tall columnar with prominent rounded basal nuclei and pale staining cytoplasm. The glands contain secretions on the luminal surface. The supporting stroma was a mixture of collagenous fibrous tissue and smooth muscle fibers (Fig. 4).



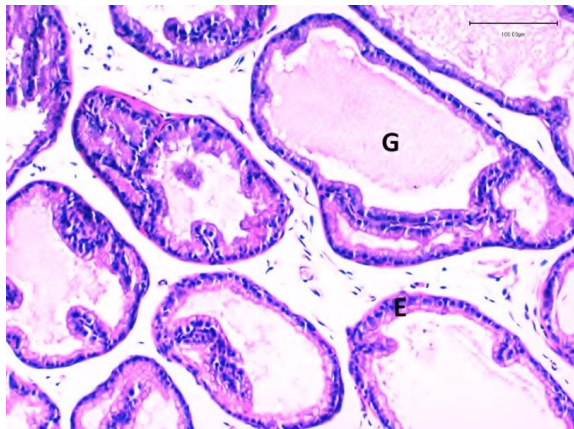
**Fig. 1a&b:** Light photomicrographs of control rat testis (GI) demonstrating cross section of normal seminiferous tubules (ST) lined with regular layers of spermatogenic cells. (1) spermatogonia, (2) primary spermatocytes, (3) different phases of spermatids, sperms. (S), Sertoli cells and (ICL) interstitial cells of Lyedig. (H&E stain). Mic Mag. a X200, b X400.



**Fig. 2:** Light photomicrograph of control rat epididymis (GI) demonstrating cross section of the epididymis tubes lined with pseudostratified columnar epithelium (E) bearing stereocilia. The epithelium is surrounded with a smooth muscle wall (SM). The lumen is filled with sperms (S). (H&E stain). Mic Mag. X400.



**Fig. 3:** Light photomicrograph of cross section of the control rat seminal vesicle (GI) demonstrating the epithelial lining of pseudostratified tall columner type (E). The epithelium is surrounded with a smooth muscle wall (SM). (H&E stain). Mic Mag. X400.



**Fig. 4:** Light photomicrograph of cross section of the control rat prostate (GI) demonstrating the prostatic gland acini (G) lined with tall columnar epithelium (E). Note the presence of supporting stroma (SS). (H&E stain). Mic Mag. X400.

**Group II:** BPA treated group in a dose of 11.4 mg/kg BW/day

The light microscopic examination of the H&E sections of the testis of rats in this group showed normal spermatogenic cells in most tubules. Average mass of the interstitial cells of Lyedig was apparent in most of the examined sections. The detection of spermatogenic cells was also found in most of the cross sections of seminiferous tubules of this group. However, some sections showed unusual widening of the spaces between the seminiferous tubules associated with congested blood capillaries (Fig. 5a&b). There were no changes in the microscopic structure of the epididymis, seminal vesicle and prostate gland.

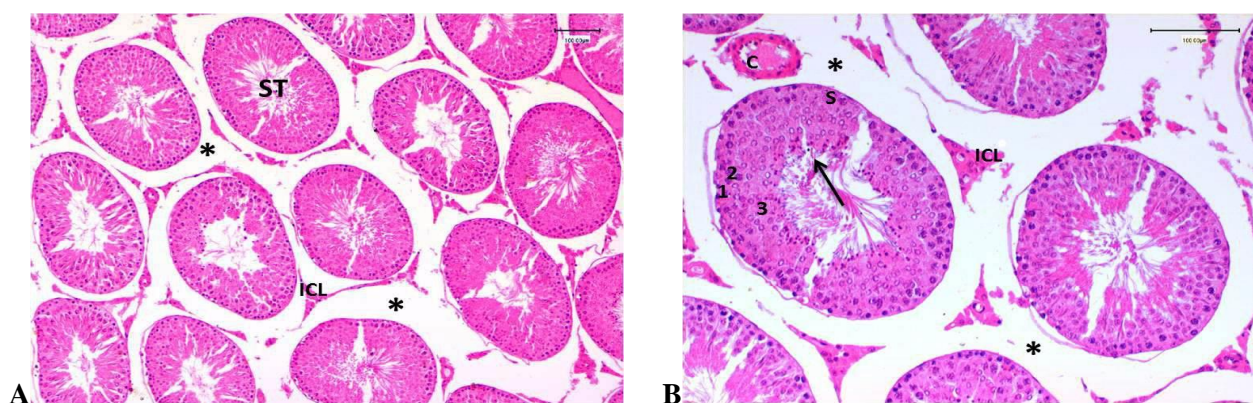
**Group III:** BPA treated group in a dose of 57.1 mg/kg BW/day

The light microscopic examination of the H&E sections of the testis of rats in this group showed histological changes both in testicular seminiferous tubules and Lyedig cells. Tissue oedema was apparent in the space between the seminiferous tubules. There was thinning out of the layers of lining spermatogenic cells in some seminiferous tubules. There were many congested blood capillaries in the space

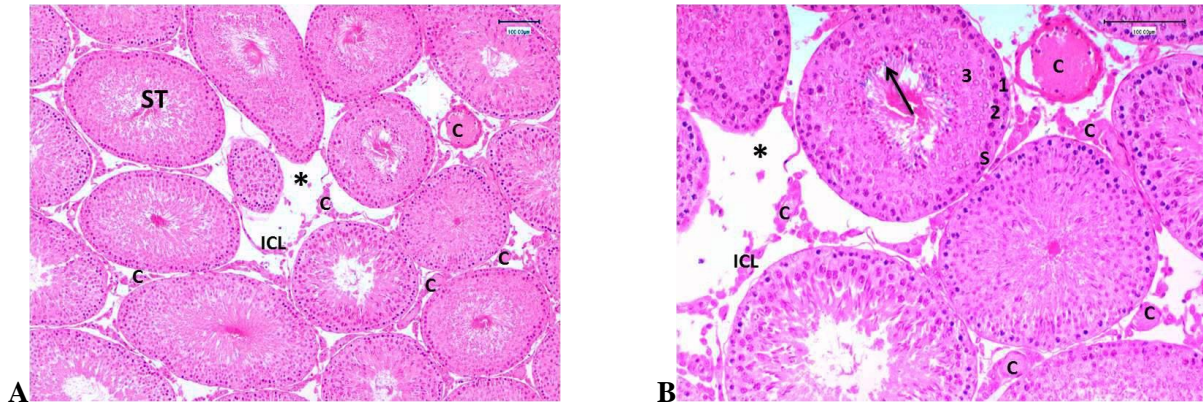
between the seminiferous tubules with marked reduction in the number of interstitial cells of Leydig (Fig. 6.a&b). The only change in the epididymis was the decrease in the content of the sperms in the lumen (Fig. 7). There were no changes observed in the microscopic structure of the seminal vesicle and prostate gland.

**Group IV:** BPA treated group in a dose of 114.2 mg/kg BW/day

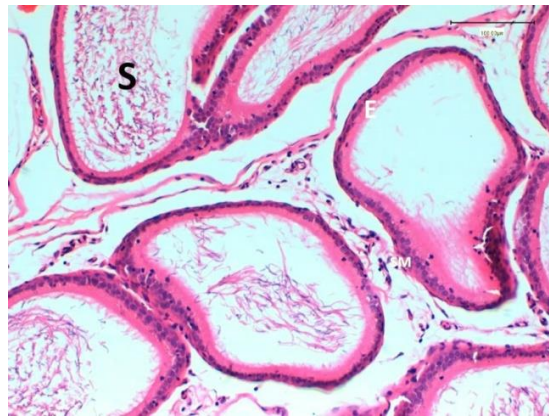
Many seminiferous tubules were distorted with evident intertubular exudation and vascular congestion in most of the examined sections. The oedema was seen in the wall of the seminiferous tubules with loosening and detachment of the spermatogenic cells with evident destruction and loss of the spermatogenic cell number involving either individual cells or segment of the seminiferous tubules. Spermatozoa were also missing in the lumen of the seminiferous tubules indicating a profound alteration of spermiogenesis process (Fig. 8). The lumen of the epididymis was mostly empty with no sperms in it (Fig. 9). There were no changes in the microscopic structure of the seminal vesicle and prostate gland (Figs. 10&11).



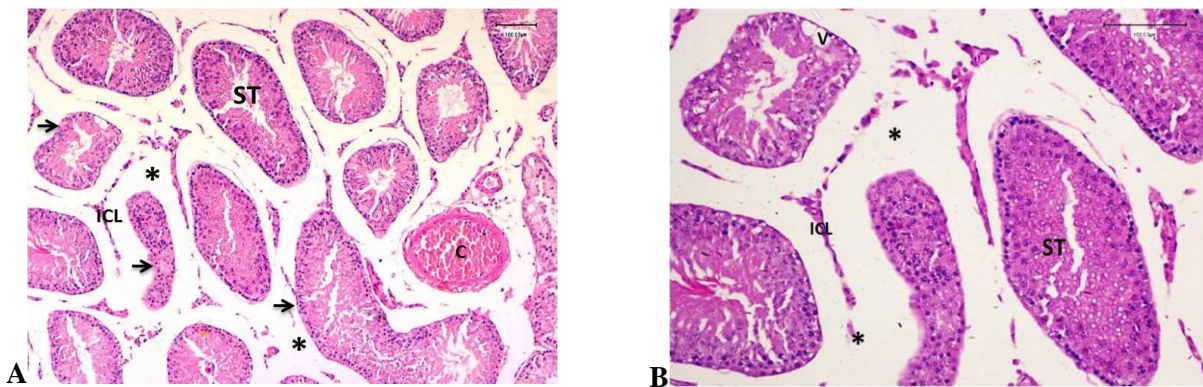
**Fig. 5a&b:** Light photomicrographs of rat testis treated with BPA (GII) demonstrating a. cross section of seminiferous tubules (ST) with widened intertubular spaces (\*). b. High power view showing apparently unaltered spermatogenic epithelium lining the seminiferous tubules. (1) spermatogonia, (2) primary spermatocytes, (3) different phases of spermatids, (S) Sertoli cells. The vascular interstitium is congested (C). (ICL): interstitial cells of Lyedig. (H&E stain). Mic Mag. a X200, b X400.



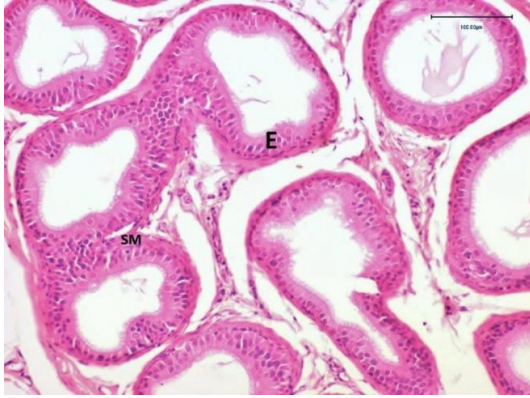
**Fig. 6a&b:** Light photomicrographs of rat testis treated with BPA (GIII) demonstrating a. cross section of seminiferous tubules (ST) with widened intertubular spaces (\*). b. High power view showing apparently unaltered spermatogenic epithelium lining the seminiferous tubules. The vascular interstitium is occupied by highly congested blood capillaries (C). Interstitial cells of Lyedig (ICL) are markedly reduced. (H&E stain). Mic Mag. a X200, b X400.



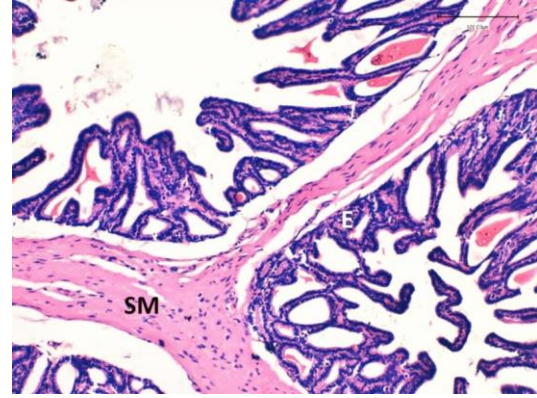
**Fig. 7:** Light photomicrograph of adult rat epididymis treated with BPA (GIII) demonstrating cross section of the epididymis tubes lined with pseudostratified columnar epithelium (E) bearing stereocilia. The epithelium is surrounded with a smooth muscle wall (SM). The mass of sperm (S) is decreased in the lumen. (H&E stain). Mic Mag. X400.



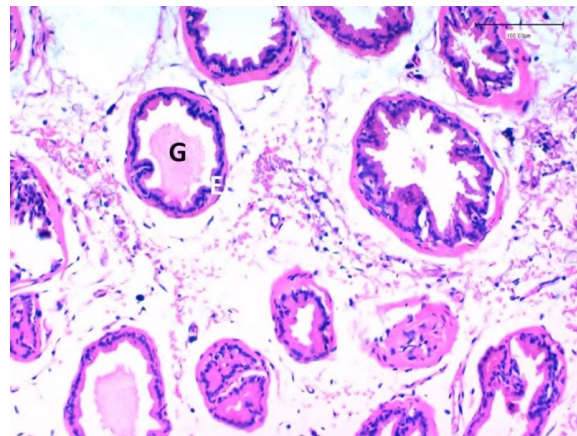
**Fig. 8a&b:** Light photomicrographs of rat testis treated with BPA (G IV) demonstrating a. variable forms of distortion of seminiferous tubules (ST) with extensively widened intertubular spaces (\*). The vascular interstitium is occupied by highly congested blood capillary (C). An apparent reduction in the mass of interstitial cells of Lyedig (ICL) is noticed. b. High power view showing segmental loss of different spermatogenic cells associated with intertubular empty oedematous gaps (V). (H&E stain). Mic Mag. a X200, b X400.



**Fig. 9:** Light photomicrograph of rat epididymis treated with BPA (GIV) demonstrating cross section of the epididymis tubes lined with pseudostratified columnar epithelium (E) bearing stereocilia. The epithelium is surrounded with a smooth muscle wall (SM). There are no sperms in the lumen. (H&E stain). Mic Mag. X400.



**Fig. 10:** Light photomicrograph of cross section of the adult rat seminal vesicle treated with BPA (GIV) demonstrating the epithelial lining of pseudostratified tall columnar type (E). The epithelium is surrounded with a smooth muscle wall (SM). (H&E stain). Mic Mag. X400.



**Fig. 11:** Light photomicrograph of cross section of the rat prostate treated with BPA (GIV) demonstrating the prostatic glands (G) lined with tall columnar epithelium (E). The glands contain secretions on the luminal surface. (H&E stain). Mic Mag. X400.

### Hormones levels in plasma

The levels of testosterone and LH were significantly decreased in rats injected with 57.1, and 114.2 mg/kg/day in comparison with controls. The rats injected with 11.4 mg/kg/day did not show significant changes in plasma levels of testosterone and LH in comparison with controls. The levels of FSH did not show significant changes in different treated rat groups in comparison with controls (Table 1).

### Oxidative indices levels in testis tissue homogenate

The levels of MDA and NO were significantly increased in testis tissue homogenate of rats injected with 11.4, 57.1, and 114.2 mg/kg/day in comparison with controls. The levels of GSH in testis tissue homogenate of rats injected with 11.4, 57.1, and 114.2 mg/kg/day were significantly decreased in comparison with controls. The SOD activities in testis tissue homogenate were significantly decreased in rats injected with 57.1, and 114.2 mg/kg/day in comparison with controls (Table 2).

**Table 1:** Effect of different doses of BPA on the plasma levels of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in male rats.

Parameters	Control group	Treated groups with BPA		
		11.4 mg/kg	57.1 mg/kg	114.2 mg/kg
Testosterone (ng/ml)	2.832 ± 0.214	2.542 ± 0.285	1.816 ± 0.164**	1.571 ± 0.106***
FSH (ng/ml)	7.202 ± 0.469	5.470 ± 0.763	6.625 ± 1.258	8.628 ± 1.495
LH (ng/ml)	63.19 ± 3.595	45.94 ± 7.872	39.91 ± 6.561**	33.78 ± 5.206 ***

Values are means ± SE for 10 rats. P-values are shown as \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  for comparison of treated groups versus controls.

**Table 2:** Effect of different doses of bisphenol A on the testis tissue homogenate levels (Mean±SE) of oxidative stress indices in different studied groups of male rats.

Parameters	Control group	Treated groups with BPA		
		11.4 mg/kg	57.1 mg/kg	114.2 mg/kg
MDA (nmol/mg protein)	1.53 ± 0.138	2.19 ± 0.110**	2.21 ± 0.15*	2.71 ± 0.226***
NO (nmol/mg protein)	1.46 ± 0.074	2.28 ± 0.299*	2.96 ± 0.035***	3.59 ± 0.27***
GSH (nmol/mg protein)	3.71 ± 0.153	3.21 ± 0.140*	3.25 ± 0.13*	3.17 ± 0.14**
SOD (nmol/mg protein)	2.10 ± 0.076	1.91 ± 0.189	1.45 ± 0.195*	1.33 ± 0.13***

Values are means ± SE for 10 rats. P-values are shown as \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  for comparison of treated groups versus controls. MDA: Malondialdehyde; NO: Nitric oxide; GSH: Glutathione; SOD: Superoxide dismutase.

## DISCUSSION

Bisphenol A (BPA) is one of the potential environmental endocrine disruptors which has been shown to disrupt testosterone biosynthesis and inhibits its production by Leydig cells<sup>20</sup>. Previously, Nanjappa *et al.*<sup>21</sup> reported that BPA is a potential anti-androgen that mediates its action through its binding to the androgen receptors and inhibits the action of androgen. In addition, BPA decreased serum testosterone and LH levels due to alteration in LH synthesis and secretion at the pituitary level<sup>22</sup>. Another mechanism of BPA is the alteration of hormone receptor gene activity in target tissues<sup>23</sup> and selective modulation of estrogen receptor<sup>24</sup>. The present study demonstrated that BPA significantly reduced plasma testosterone and LH levels in a dose dependent manner. This was in accordance with the findings of many investigators<sup>14&22</sup>. Tohei *et al.*<sup>25</sup> found that BPA in a dose of 3mg/kg/day decreased the plasma testosterone level with increased in LH level of rats. The authors interpreted the increased level of plasma LH to the reduction in the negative

feedback regulation by testosterone. Other investigators found that plasma testosterone level was decreased following treatment of male mice with 12 mg/kg/day of BPA for 8 weeks compared with control group<sup>26</sup>. Moreover, Mendiola *et al.*<sup>27</sup> found that testosterone was reduced at early puberty of rats treated with BPA (40 g /kg/day) and this decrement persisted in the adults. Nakamura *et al.*<sup>14</sup> found that BPA significantly decreased Leydig cell numbers in the rat testis and effect on the pituitary gland. In addition, Nakamura *et al.*<sup>14</sup> demonstrated that BPA dose dependently decreased the expressions of cholesterol carrier protein (StAR), and steroidogenic enzymes such as P450<sub>sc</sub>. Therefore, the down-regulation of the steroidogenic enzymes and StAR could be associated with the decreased testosterone levels by BPA treatments. The authors also stated that the decrease in numbers of Leydig cells in the testis of the rats treated with BPA might in part be a result of the reduction of testosterone production.



Furthermore, in rodents, postnatal exposure to BPA decreased serum testosterone production and sperm production<sup>28&29</sup>. Akingbemi *et al.*<sup>22</sup> found that administration of BPA via oral route (2.4 µg/kg/day) for 2 weeks downregulates testosterone production through decreasing the expression of enzymes such as 17α-hydroxylase or through increasing the activity of aromatase enzyme<sup>30</sup>.

Generally, testosterone biosynthesis is regulated by LH secreted from pituitary. In the current study, the high dose of BPA caused significant reduction in plasma LH levels. Zhang *et al.*<sup>31</sup> reported that LH receptor knockout mice represented significantly lower expressions of cholesterol carrier protein (StAR), and steroidogenic enzymes compared to the wild-type mice. In accordance, the biosynthetic ability of testosterone was also significantly halted in the knockout mice. This data suggests that the reduction in plasma LH associated with high-doses of BPA might deteriorate the testosterone biosynthesis through negatively impacting the expressions of cholesterol carrier protein or steroidogenic enzymes. In other meaning, these dosages of BPA might have adverse effects on the pituitary gland. Male rats born to mothers exposed to 2.4 g/kg/day of BPA via feeding, had decreased levels of testicular testosterone, which was not due to a decline in serum LH levels but was consistent with a decline in the steroidogenic capacity of Leydig cells in the BPA exposed males<sup>22</sup>. Nakamura *et al.*<sup>14</sup> found that middle dose of BPA (100 mg/kg) significantly decreased steroidogenic enzymes and cholesterol carrier protein (StAR) expression with plasma testosterone reduction, but without significant reduction of plasma LH. Another study demonstrated that male rats administered 1mg/kg BPA for 2weeks showed decreased plasma testosterone levels and increased in plasma LH levels. These results suggested that BPA may directly affect enzyme expression in the Leydig cells of testis as well<sup>25</sup>. Collectively, BPA causes adverse effects in both testis and pituitary gland with a more pronounced effect on the testis. However, the reason behind the weaker effect of the low dose on the testosterone level in the current study is still unclear.

The histological changes observed in the testis of rats treated with BPA also

demonstrated its potentials to induce cytotoxic effects on the spermatogenic, Sertoli and Leydig cells. The disturbed hemodynamics and venous drainage of organs leading to vascular congestion, which was a histological finding in most of the examined sections of the testis in all groups treated with BPA. However, the severity of BPA damaging potentials appeared to be dependent on the dose of BPA. Some sections of rats treated with low dose of BPA (11.4 mg/kg) showed oedema in the inter-tubular spaces associated with congested blood capillaries. However, testes of the rats treated with high dose of BPA (114.2 mg/kg) showed severe damaging effect of BPA on the testicular tissue such as intertubular exudation and vascular congestion, intra-seminiferous tubules oedema with destruction and loss of the spermatogenic cell mass involving either individual cells or segment of the seminiferous tubules. The lumen of the epididymis was mostly empty with no sperms in it. Gillebert *et al.*<sup>32</sup> reported that persistent congestion cause a rise in the capillary filtration pressure and increased exudation and tissue oedema. BPA induce testicular injury via their initial actions at the blood testis barrier to elicit subsequent damage to germ-cell adhesion, thereby leading to germ-cell loss, reduced sperm count, and male infertility or subfertility<sup>33</sup>. It is obvious that BPA imposes a clear detrimental effect in the testis via disruption of cell junctions between testicular cells due to increase in oxidative stress<sup>34&35</sup>. These may illustrate the detachment of spermatogenic cells and missing of the sperms in the seminiferous tubules and epididymis.

Oxidative stress associated with the use of environmental toxicants including BPA is a key contributing element in male infertility<sup>36&37</sup>. In the present study, BPA treatment caused a significant increase in the lipid peroxides and NO and a significant decrease in the GSH and SOD in testicular tissue in dose dependent manner. Similarly, Mourad and Khadrawy<sup>38</sup> found that a significant elevation in lipid peroxidation that was accompanied by a significant decrease in GSH levels in the testis of rats treated with BPA for 6 weeks. This may be due to the richness of the testicular membranes with polyenoic fatty acids that undergo oxidative decomposition<sup>39</sup>. BPA increases oxidative stress by down-regulating

the production of antioxidant enzymes<sup>40</sup> and causing disruption of cell junctions between Sertoli–Sertoli cells and/or Sertoli–germ cells<sup>41</sup>. Free radicals are generated in response to gonadotrophin withdrawal, this involves electron leakage from the inhibited steroidogenic pathway of the Leydig cells. These free radicals then attack the germ cells within the seminiferous tubules leading to extensive apoptosis and spermatogenesis disruption<sup>42</sup>. Oxidative stress in testis is capable of disrupting the steroidogenic capacity of Leydig cells<sup>13</sup> as well as the capacity of the germinal epithelium to differentiate normal spermatozoa<sup>43</sup>. Moreover, it was reported that oxidative stress created in the Leydig cells due to chronic ethanol exposure reduced the steroidogenic enzymes activities of the testes and lowering blood testosterone levels<sup>44</sup>.

### Conclusion

The BPA causes a dose dependent reduction of plasma levels of testosterone and LH in male rats. The impaired hormones levels were associated with oxidative stress in the testicular tissue and histological changes in the reproductive organs. The effect of BPA on the testis may be due to oxidative stress and inhibition of the steroidogenic pathway of the Leydig cells.

### Conflict of interest

Authors declare no conflict of interest.

### Acknowledgment

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## البسفينول أ مستحث التلف المؤكسد في الأعضاء التناسلية للفئران: سبب محتمل لانخفاض تكون الستيرويد

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الأنشطة المضطربة للغدد الصماء للبسفينول أ (BPA) تساهم في حدوث آثار ضارة على الصحة والخصوبة. أجريت الدراسة الحالية لتوضيح تأثير مادة BPA على الأعضاء التناسلية الذكرية في مرحلة ما قبل البلوغ.

**الطريقة:** تم تقسيم أربعين من ذكور الجرذان البيضاء إلى ٤ مجموعات (١٠ جرذان لكل مجموعة). تم اعتبار الأول كعنصر تحكم وتم حقن الثلاثة الأخرى تحت الجلد بـ ١١.٤ و ٥٧.١ و ١١٤.٢ مجم/كجم من BPA مذاب في DMSO، على التوالي لمدة ٦ أسابيع. تم إجراء فحص الأنسجة للأعضاء التناسلية بالإضافة إلى القياسات البيوكيميائية لهرمونات الجنسية في البلازما بواسطة طريقة الاليزا وذلك لتقييم تأثير BPA على الخصوبة. علاوة على ذلك، تم تقييم مؤشرات الإجهاد التأكسدي في أنسجة الخصية بالطرق اللونية.

**النتائج:** مستويات هرمون التستوستيرون وهرمون ملوتن (LH) انخفضت بشكل ملحوظ في الجرذان المحقونة بـ BPA بجرعة ٥٧.١ و ١١٤.٢ مجم/كجم/يوم مقارنة بالضوابط. BPA زاد بشكل ملحوظ من مؤشرات الإجهاد التأكسدي في أنسجة الخصية. أظهرت التغيرات النسيجية المرضية التي لوحظت في الخصية المعالجة بـ BPA قدرتها على إحداث تأثيرات سامة للخلايا واضطراب الغدد الصماء على الخلايا المنوية وخلايا Sertoli و Leydig. علاوة على ذلك، كانت شدة تأثيرات BPA التي لوحظت تعتمد على الجرعة.

**الخلاصة:** تسبب BPA في انخفاض يعتمد على الجرعة في مستويات البلازما لهرمون التستوستيرون وهرمون ملوتن (LH) في ذكور الجرذان المرتبطة بالإجهاد التأكسدي والتغيرات النسيجية في الخصية. قد يكون تأثير BPA على الخصية ربما بسبب الإجهاد التأكسدي في الخصية أو تأثير انخفاض مستوى هرمون التستوستيرون أو كليهما.