Reproductive Toxicity Induced by Low Dose Bisphenol A(BPA) in Male Rats

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Abstract

Bisphenol A(BPA) an endocrine disruptor used in the manufacturing process of plastic. BPA low doses on long exposure periods had many hazardous effects. The aim of the present study was to assess the effect of lowest dose of BPA on the male induced orally on the at reproductive performance and changes in the testes that takes place histologically. P53 gene expression by Realtime PCR was also evaluated. Seventy- two albino male rats were divided into two groups, group I (control male rats receiving corn oil only). Group II (64 male rats) which was treated with oral doses of 50 µg/ kg/ day daily for 8 weeks. Every week 8 male rats were dissected and subjected to the investigated parameters. BPA treated groups presented decrease in sperms count, motility, progression and viability as well as a significant increase in sperms head or/and tail abnormalities were recognized. Histology of the testes showed abnormal and irregular organization of seminiferous tubules and decreased sperms in the testes lumen. Moreover, up-regulation in p53 expression was recorded after BPA exposure. In conclusion, these results showed that exposure to BPA at low dose 50µg/kg can cause many reproductive disorders which may impair fertility.

Keywords: Bisphenol A(BPA); endocrine disruptor; reproductive toxicity**.**

1. Introduction

Endocrine disrupting chemicals disruptors are chemicals that interfere with the natural hormone balance, thus recently great attention been paid to their harmful effects **[1]**. The biological effects of EDCs may vary significantly. Either it acts as antagonists or agonists that mimic hormonal action by binding to hormone receptors, estrogen receptors (ER) or androgen

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receptors (AR) it can also interfere with hormone synthesis and metabolism, ultimately affecting cell apoptosis, and differentiation causing cancer and inflammation **[2].**

BPA is an endocrine disruptor, capable of binding and activating estrogen receptor subtypes (ER) **[3].** It is also suggested that the normal functioning of ER receptors is disturbed, although it has less affinity to nuclear ERs than estradiol **[4]**. BPA may also disturb the androgenic signaling pathways that play an important function in the development of male sex organs and reproductive functions **[4,5]**.

BPA is involved in synthesis of some plastics; in some food and beverage containers, infant bottles **[6,7]** and epoxy resins that are used to coat food cans and pipes for water supply **[8]**. Some dental composites and sealants also can contribute to exposure to BPA **[9,10].** Exposing plastics to high temperatures or acidic environments can increase the amount of BPA that leaches from containers into food, and beverages **[6]**. More troubling is a recent study that found newborns in the intensive care unit are most likely exposed to higher levels of BPA than average via the medical device usage in the unit **[11]**.

Maternal blood, maternal urine, neonatal blood, placenta, cord blood, amniotic fluid, and breast milk have been found to contain BPA **[6,12]**. BPA is also transmitted through dermal route **[13]**. Individuals with frequent handling of thermal paper (receipts) or recycled paper, such as cashiers, are estimated to be exposed to levels of up to 71µg / day, significantly higher than those found in the normal population **[14,15].**

BPA leads to the induction of oxidative stress in the brain, kidney and liver **[16,17].** As well as in patients recorded with chronic kidney diseases, high BPA in the plasma content lead to the reduction in glomerular filtration rate **[18]**. Studies have stated a relation between the concentration of BPA in the urine and liver enzyme abnormalities, cardiovascular disease and type 2 diabetes **[19,20]**. Also, BPA is associated with neuro-behavioral disorders (e.g. autism), male's abnormal urethra/penile development, female early sexual maturation and increase in hormonally mediated cancers like breast and prostate cancers have also been reported **[21,22]**.

Sperms production is a complicated process of cell differentiation called spermatogenesis **[23]**, which requires the functional coordination of numerous endocrine and paracrine factors. For this reason, EDCs have been repeatedly linked to disruption of

spermatogenesis **[24,25]**. This occurs through impairment of steroidogenesis and oxidative stress inducing DNA damage and alteration of junctions of Sertoli cells **[26]**.

Numerous adverse effects of BPA on the reproductive system have been reported. At low dose exposure levels, BPA has an inhibitory effect on testicular spermatogenesis, possibly acting via the estrogen receptor. BPA also suppressed aromatase gene expression and biosynthesis of estradiol (E2**) [27].** In male germ cells, BPA can act as a mutagen and causes damage to DNA in sperms **[28].** BPA attacked Sertoli cells, and blocked germ cell meiotic progression **[29]**.

In adult 8-week male Sprague-Dawley rats exposed to 0.5 or 5mg/kg/ day (8weeks by gavage), lesions in Sertoli and Leydig cells, mainly in the endoplasmic reticulum, were observed in treated groups **[30],** which may cause a decrease in male fertility. In turn**, [31]** have shown that exposure to low-dose BPA (20 g kg^{-1} bw day^{-1}) by preimplantation embryos impairs the development of testes and disrupts testicular testosterone synthesis . recently, studies found that, maternal BPA exposure during pregnancy reduced the number of sperm, luteinizing hormone, testosterone, and testicular tissue structure was damaged in offspring. Which plays an important function in reproductive system development of male fetus **[32]**.

Therefore, this study aims to assess the effect of lowest oral dose of BPA on the male rat reproductive performance and histological study of the testes. Also, p53 gene expression was carried out.

2. Materials and Methods

2.1.Chemicals

Bisphenol A (~99% purity) was purchased from Sigma Chemical Co. (St Louis, MO) USA. Other chemicals used manufactured and purchased from El Gomhouria Co for trading chemicals and appliances, El Ameeria, Cairo, Egypt.

2.2.Animals

Male albino rats of Wistar strain weighing 200-220g of age 10–12 weeks were used for the present study. They were obtained from animal house, MASRI (Faculty of Medicine, Ain shams university, research institute). All animals were kept at temperature 22 ±2℃, 50–55%, humidity and natural light/day cycle in hygienic conditions in polypropylene cages. Standard pellet diet and water ad libitum. Water was provided in

glass bottles to avoid exposure to BPA. The animals were acclimatized one week before the examination. The ethical protocols for treatment of animal were followed and supervised by the animal facilities, National Research Center.

Dose preparation

BPA dissolved in small amount of pure ethyl alcohol and was then diluted with corn oil and administrated orally using oral tube at a dose of 50 µg/ kg/ day the lowest dose to cause effect, The dose was selected according to **[33]**, the oil was used as a vehicle control.

2.3.Experimental design

Animals were divided into 2 main groups, a control group (group I) and BPA group (group II).Group I contain 8 males rats which received corn oil only during the experiment period, while Group II contain 64 male rats administrated to oral dose of 50 µg/ kg/ day daily for 8 weeks , at the end of every week 8 animals were dissected and the following parameters were carried out.

2.4.Sperms motility, progression and count

By the end of each week, animals were sacrificed by cervical dislocation, the cauda epididymis was excised, placed in a sperms collection vial containing BSA-Hanks solution (2 mL). Then incubated at 37 ◦C for 10 min to allow the sperms to be released from the tubules. The number of non-motile sperms in the diluted fluid was determined using a hemocytometer as described by **[34]**.

The sperms motility percentage was calculated as follows

$$\boldsymbol{motile\ sperms\ rate}(\%)$$

$$= \frac{(\boldsymbol{number\ of\ sperms}) - (\boldsymbol{number\ of\ non-motile\ sperms})}{\boldsymbol{total\ number\ of\ sperms}} \times \mathbf{100}$$

The sperms that move in a forward manner is considered progressive, and those moving in circular motion are called non-progressive. 200 sperms were counted, and the progressive sperms percentage was calculated as follows:

$$\boldsymbol{sperms\ progression}\ (\%) = \frac{\boldsymbol{number\ of\ progressive\ sperms}}{\boldsymbol{total\ number\ of\ sperms}} \times \mathbf{100}$$

The diluted sample containing sperms was transferred using a micro pipette to Neuberger chamber, allowing the capillary action to fill the chamber. Total sperms count was taken using x10 under light microscope. **The total sperms count was calculated by counting in 5 large squares (L-shape) of the hemocytometer**. by the following equation **[35].**

$$sperm\ count = number\ of\ sperms \times 10{,}000 \times dil.factor$$

2.5.Determination of sperms vitality

Sperms vitality (alive/dead) was evaluated using an eosin-nigrosine morphology stain according to method **of [36]:** On clean, warmed glass (to avoid temperature shock) one drop of sperms (5 µl), two drops of eosine (10 µl) and four drops of nigrosine (20 µl) were put together, the solution was mixed then the smears were made for microscopic examination, using a phase contrast microscope at 10× and 40× magnifications. One hundred spermatozoa were counted. vitality was determined by stain, if the sperms are stained (unvital/dead) and if unstained it is recorded to be (vital/alive).

$$\boldsymbol{sperms\ viability}\ (\%) = \frac{\boldsymbol{number\ of\ live\ sperms}}{\boldsymbol{total\ number\ of\ sperms}} \times \mathbf{100}$$

2.6. Sperms abnormalities

The caudal epididymis was cut into pieces to collect the mature sperms in a petri dish containing 2 ml saline (0.9%NaCl) and mixed until it turns turbid. Onto a clean glass microscopic slide, sperms smears were made and let in the air to dry. Fixed in Absolut methanol for 20 min. Then stained using 1% Eosin Y, allowed to dry then examined under the light microscope (400X). 1000 sperms per animal are examined for either head or tail abnormalities or for both**. [37].**

2.7.Gene expression

2.7.1. Extraction of total RNA and synthesis of cDNA

RNA was isolated from processed liver tissue of rats (~15 mg tissue) using STRATEC Molecular Kit according to the manufacturer instructions. The tissues were disrupted and homogenized using 600µl RLT β-ME buffer mixture. Homogenizes was performed according

to manufacturer's instructions, STRATEC Molecular Kit (www.ceb-eg.com). Then the extracted RNA concentrations were measured using NanoDrop® ND 1000 spectrophotometer. Successful extracts were normalized for concentration as 100ng for each sample Total RNA was reverse transcribed (RT) into cDNA using the reverse transcriptase enzymatic reaction (High Capacity c-DNA Reverse Transcription Kit) according to the manufacturer manual., and cDNA samples were stored at –20◦C.

2.7.2. Primer design

The p53 primers were designed by the accession of the rat genome through the UCSC genome browser (https://genome.ucsc.edu) and viewing the DNA. All the primers were ordered and synthesized by Invitrogen, UK.

GAPDH-forward	
5'-3'	**AAGTTCAACGGCACAGTCAAGG**
GAPDH-reverse	
	CATACTCAGCACCAGCATCACC
P53- forward	
	CCCAAACTGCTAGCTCCCAT
P53- reverse	
	ACTACTCAGAGAGGGGGCTG

2.7.3. Real time PCR

In the assay, Syber green was used as reporter dye. Real-Time PCR reactions were performed via Green-2-GO qPCR. Total volume was 20 µL including 10 µL of SYBR Green Master Mix (TaKaRa, Japan), 1µL of forward and reverse primers,3 µL of cDNA template and 5 µL of nuclease free water. Thermal cycling program was performed for 10 min. at 95°C (for enzymatic activity) following 15 seconds at 95°C denaturation step and 60 seconds at 60°C for both annealing and extension steps for 40 cycles and melting curve analysis ramping from 65°C to 95°C and rinsing 1°C each step PCR reaction was carried out for all the samples and the house keeping gene. The slope is determined by drawing the standard curve by plotting the logarithmic input cDNA concentration against mean CT and the slope was evaluated. **Expression of target genes were calculated as follows using threshold cycle formula.**

$$\Delta Ct = Ct[\text{week 3 or control}] - Ct[GAPDH]$$

$$\Delta\Delta Ct = \Delta Ct \text{ (week 3 group)} - \Delta Ct \text{ (control group)}$$

$$\text{Relative gene expression} = 2^{-\Delta\Delta Ct}$$

2.8.Histological study

2.8.1. Transverse sections (T.S.) of the testes

Testis were fixed using 10% neutral formalin buffer. dehydrated by upgrading series of alcohol from 70 to 100% then to xylene each for 1 h. followed by making sections in paraffin blocks of 5µm thickness **[38].** Then stained by hematoxylin and eosin and examined under light microscope **(100 X and 400X)**

2.9.Statistical Analysis

All data were analyzed using SPSS for windows software, version 16.0. one way analysis of variance (ANOVA) was performed to test for any significant differences among groups and independent sample T-test was used to calculate statistical significant between (the control group and each treated group). The p value was considered significant at $p \leq 0.05$ for all tests **[39].**

3. Results

3.1.Reproductive performance

3.1.1. Sperms motility

The percentage of motile sperms in the treated animal weeks (1-8) are illustrated in table (1). The results showed that animals exposed to 1 or 8 weeks 97.2% and 91.4% respectively, didn't show any significant change compared to the control. On the other hand rats which were sacrificed after 2-7 weeks showed significant ($P<0.05$) decline in the sperms motility (64,15.1, 75, 62.2, 76.8 and 46.18%) respectively compared with the control group 95.5%.

3.1.2. Sperms progression

The progressive sperms are those moving in a forward manner. The percentage of progressive sperms in weeks (2-7) 58.7%, 14.5%, 71.3%, 59%, 73.1% and 45.5% respectively showed significant decrease ($P<0.05$) compared with the control group 95%. Unlike weeks (1 and 8) 94.1% and 90% respectively that didn't show any significant change compared with the control

group. Week (3) showed the least percent 14.5% of progressive sperms among all the subgroups as illustrated table (1).

3.1.3. Sperms count:

Statistically significant decrease ($P < 0.05$) in the sperms counts of all the treated weeks (1-8) 34.8, 23.1, 22.5, 26.1, 31.8, 26.7, 29.1 and 26.1* 10^6 respectively compared with the control 51.9* 10^6 as shown in table (1). week (3) showed the lowest sperms count mean value 22.5*10^6 among all the subgroups.

3.1.4. Sperms viability

The percentage of viable live sperms in the weeks (1,2,3,4,6 and 7) 84%, 23.1%, 8.6%, 58.9%, 73% and 33% respectively, showed significant decrease ($P<0.05$) compared with the control group 94.3%. Unlike week (5 and 8) 90% and 93% respectively that didn't show any significant change compared with the control group. Week (3) showed the least percent 8.6% of viable live sperms among all the subgroups as illustrated in table (1).

3.1.5. Sperms abnormalities

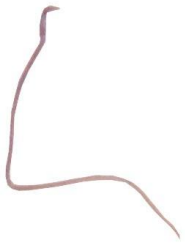
The observed results indicate a significant increase ($P< 0.05$) in the head, tail and the total sperms abnormalities as illustrated in table (2) and fig. (1). With the highest defects found in the 3 parameters in the 3rd week ,46.8, 46.4 and 92.8 respectively compared with the control group 2.4, 5.8 and 8.2 respectively. Therefore, the total number of normal sperms was significantly decreased in all the treated weeks. Week (3) showed the least normal sperms count 99.8 compared with the control 193 average count out of 200 sperms per animal.

(a)

(b)

(C)

(d)



(e)

(f)

Fig. (1): photomicrographs for sperms smears X 400 magnification showing (a) normal sperm, (b)loop tail, (c) and (d)bent tail, (e) amorphous head and (f) double head.

3.2. P53 gene expression

Real-time RT-PCR results revealed an upregulation of the p53 gene with -0.2 value. The Ct average values of p53 was significantly increased ($p<0.05$) using one sample t test in male rats of the 3rd week treated with BPA 50 µg/ kg/ day daily for 3 weeks showing 29.7 cycle threshold means compared with the control groups received corn oil only 24.4.

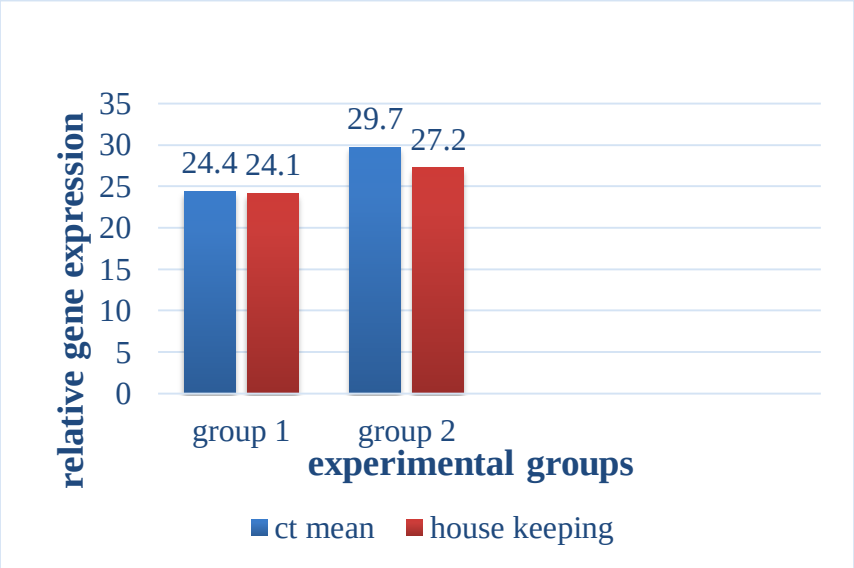
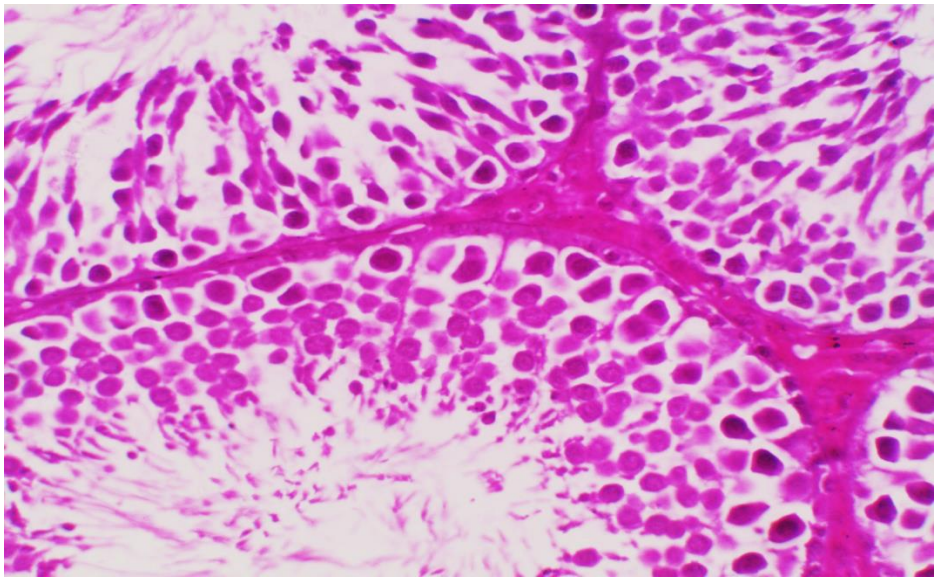


Fig. (2): Bar chart showing the cycle threshold means of the the p53 gene and the house keeping gene (GADPH) in male of control and treated groups.

3.3. Histology sections of the testes

Testicular histopathology revealed that the treated week (3) fig. (6), administered BPA 50 µg/ kg/ day daily, showed deviation from the control group (fig. 3). resulted in dysregulation of spermatogenesis, the architectural arrangement of the testes, intertubular space and thin epithelium with decreased spermatogenesis and mature sperms in the lumen. Testes sections of all the treated weeks show disruption and changes in the architecture, more or less subgroup 8 is in the closest form to the control group, that the sperms reappeared again in the lumen but with some changes in the spermatogenic cells. However, week 3 is the most affected group compared with the control (fig. 4-11).

(a)



(b)

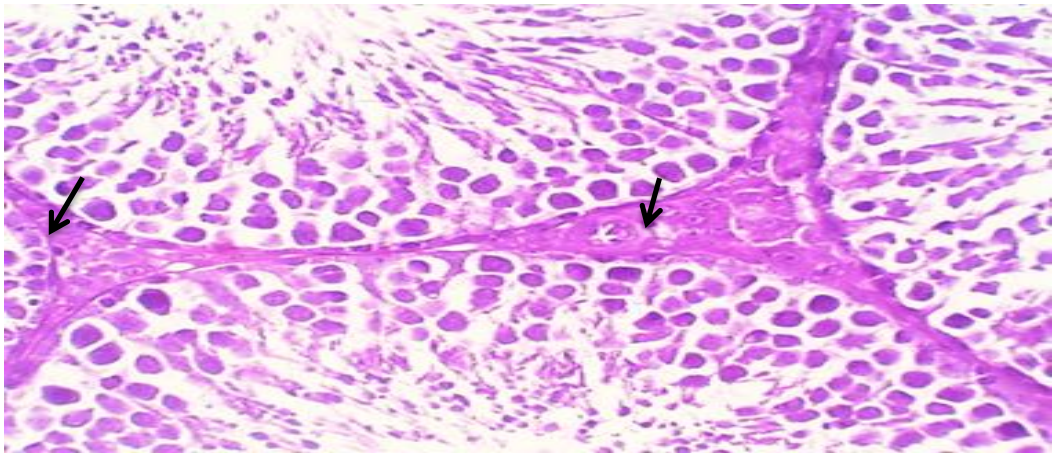


Fig. (3): (a) and (b) The Photomicrograph of control (group 1) rat testis showing normal seminiferous tubules that are hexagonal in shape and normal interstitial tissue and cells lined with series of spermatogenic cells; the primordial germ cells, the spermatogonia, primary and secondary spermatocytes and spermatids. Also, Sertoli cells are seen with attached sperms for nourishment. Tubules are surrounded by primordial germ cells and Sertoli cells. The interstitial spaces contain interstitial cells and Leydig cells (←). (X 400)

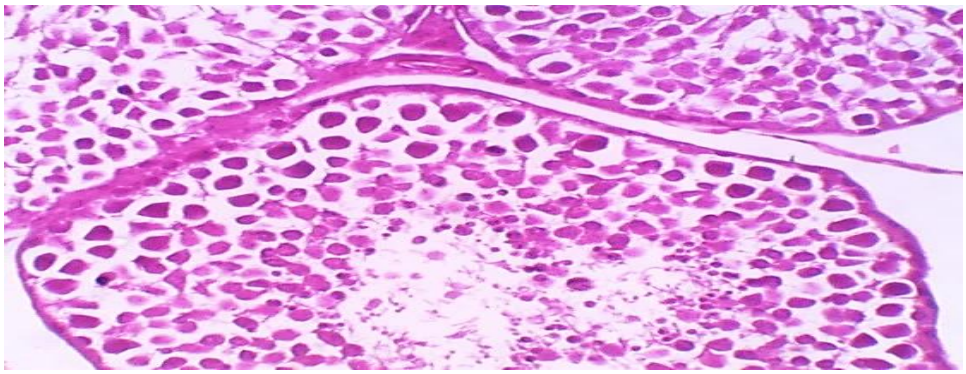


Fig. (4): After one week the photomicrograph of treated rat testis of group 2 showing disturbance in spermatogenic cells with a decrease in the number of sperms (X 400).

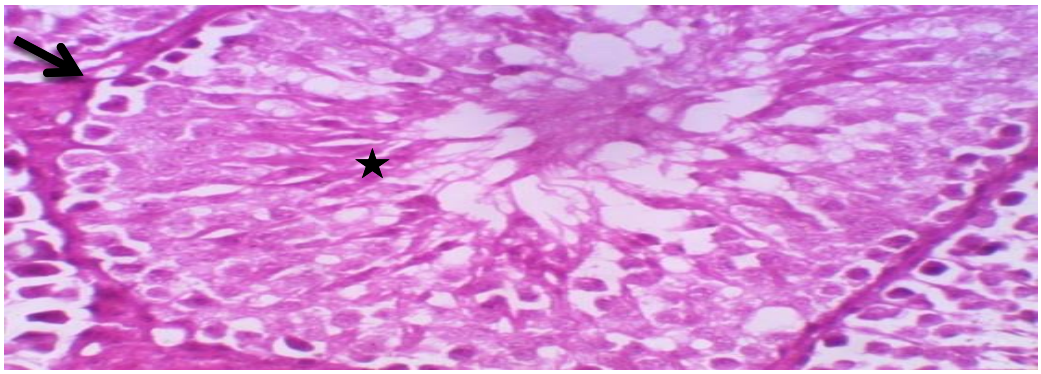


Fig. (5): After two weeks the photomicrograph of treated rat testis of group 2 showing some vacuoles(★) in seminiferous tubules and abnormal primordial germ cells (→).(X400)

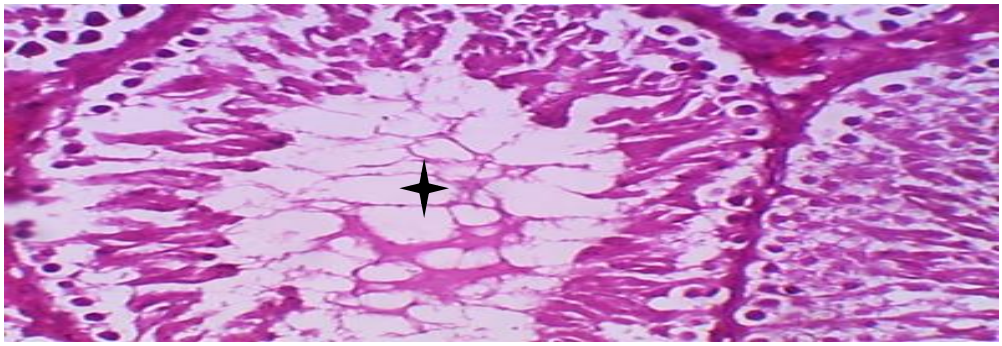


Fig. (6): After three weeks the photomicrograph of testis of group 2 showing degenerative change and the atrophy of the tubules (✦). Absence of defined cells in atrophied tubules. (X400)

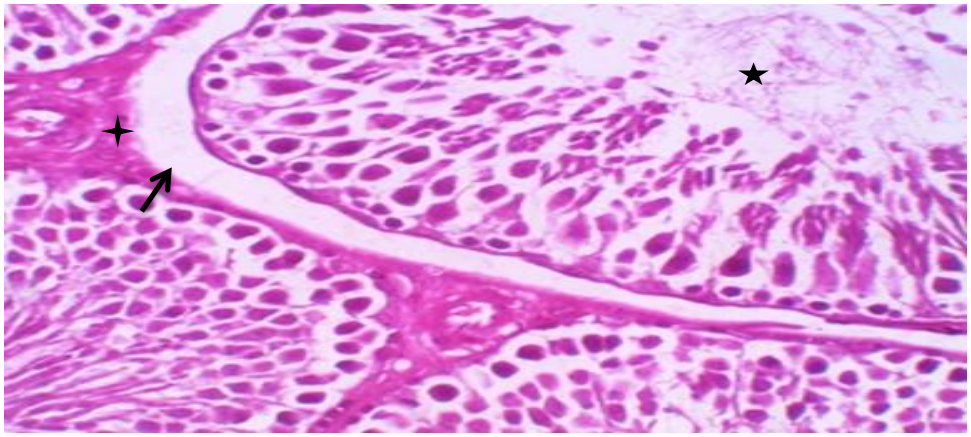


Fig. (7): After four weeks the photomicrograph of testis of group 2 showing decrease in the sperms (★), abnormal basement membrane (↗) and enlarged and vacancy of the intertubular space (✦).(X400)

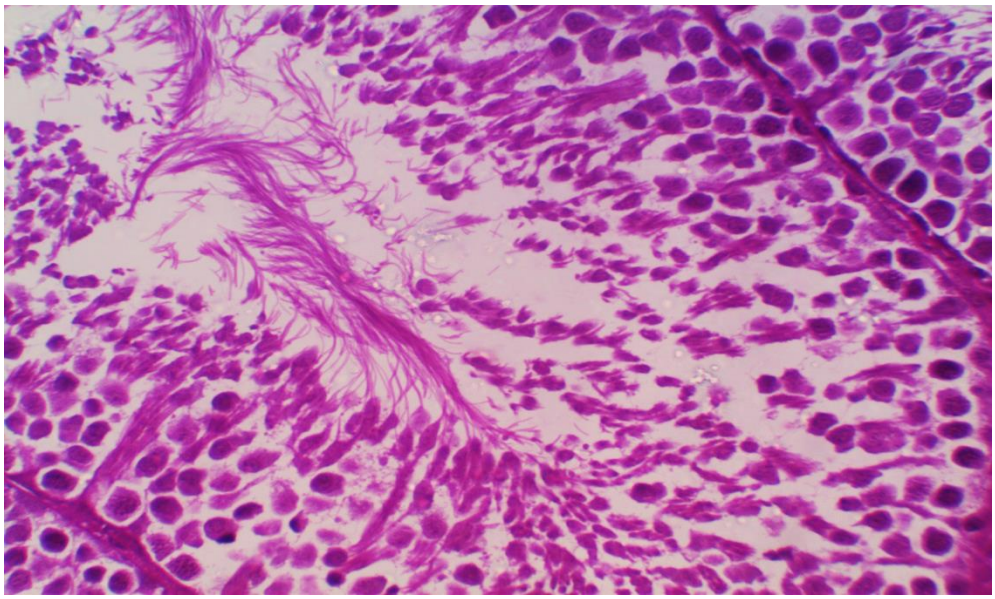


Fig. (8): After five weeks the photomicrograph of testis of treated group2 showing improved tubules arrangement, seminiferous epithelium with spermatogenesis cells, presence of spermatozoa in tubules and less interstitial space. (X400)

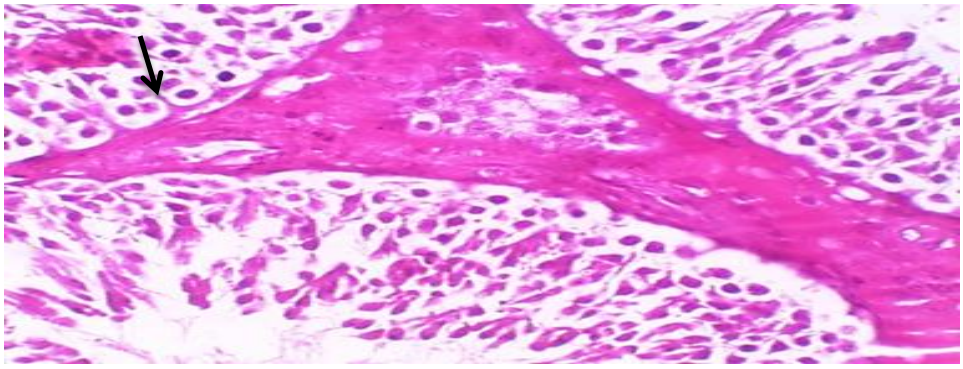


Fig. (9): After six weeks the photomicrograph of rat testis of the treated group 2 showing edema and enlargement of primordial germ cells (←)and interstitial tissue. (X400)

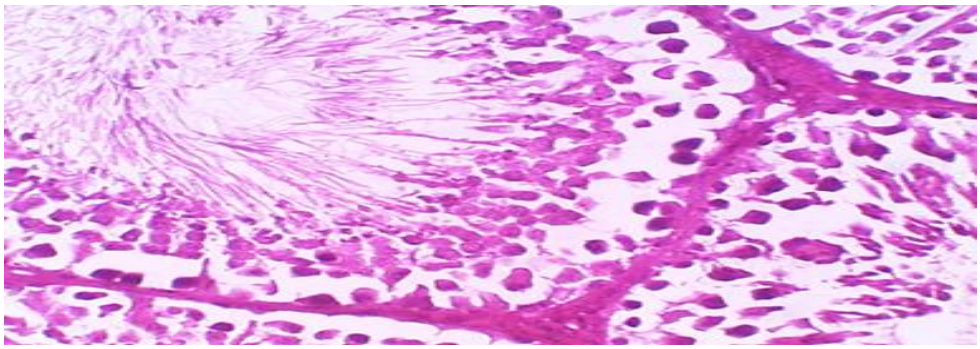


Fig. (10): After seven weeks the photomicrograph of testis of treated group 2 showing disruption in spermatogenic cells, thickness of the primordial germ cells with presence of sperms in the tubules. (X400)

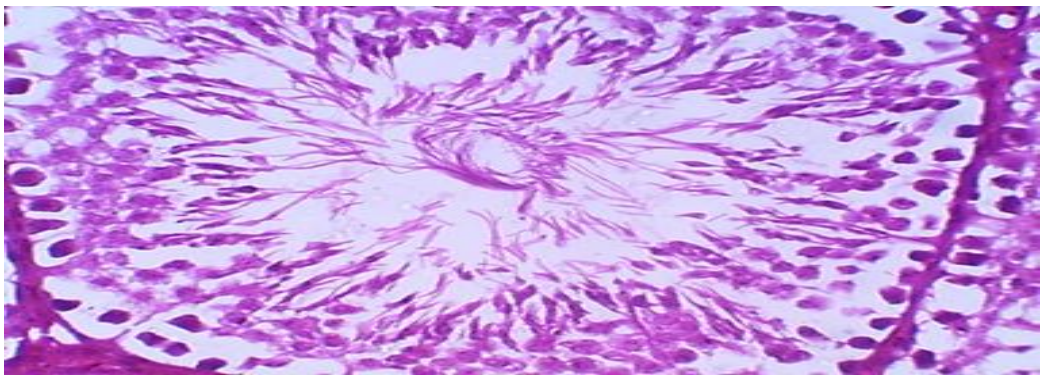


Fig. (11): After eight weeks the photomicrograph of testis of treated group 2 showing more or less normal architecture in spermatogenic cells with presence of sperms in tubules. (X400)

4. Discussion

One of the main healthcare issues about reproductive disorders is male infertility. This type of reproductive disorder has been linked to estrogenic compounds such as BPA **[40]**. Studies have reported multiple adverse effects of low-dose BPA in animals, many of these endpoints are present in concern to humans due to BPA large environmental availability **[41]**. BPA interferes with production, of androgens which had a great function in the activation of the Sertoli cell and production of sex hormones that are vital in spermatogenesis **[32,42,43].**

Therefore, we investigated the toxicity of BPA at dose 50µg/kg b.w. on the adult male rat reproductive system, via the investigation of sperms quality and count. The present results showed decrease in the sperms motility, sperms progression and count at the lowest dose of BPA (50µg / kg b.w.), the most effect appeared after 3 weeks of exposure. This was in an agreement with **[44]**, that prenatal and BPA exposure in neonates also affects testicular development and spermatogenesis of the offspring during adulthood. Similar studies showed that BPA has several effects on male reproductive systems. Also, **[28]** examined the effect of BPA on sperms count and its efficiency in the testes, resulted in a reduction in sperms productivity and quality. Also, **[45]** showed that male rats upon BPA exposure recorded a decreased in sperms motility and count. **[46]** reported that decreased sperms count via reduction in the spermatogonia, spermatocytes and spermatids because of the oxidative stress generated upon BPA exposure.

Also, BPA caused reduction in epididymal sperms motility and count in adult male [**47]**. Moreover, **[48]** confirmed that sperms development in offspring was impaired by gestational exposure to low doses of BPA in mice, also **[49]** reviewed The formation of Sertoli and germ cells resulting from developmental BPA exposures starts in the fetal period and alters the differentiation of the male gonad. However, BPA exposure prenatally decreases sperms counts and/or motility and disturbed the production of germ cells as an irregular distribution of spermatogenesis stages in F3 generation **[46]. [50]** indicated that BPA does not only alter the quality of sperms cells but also significantly reduced sperms cells quantity in mice during spermatogenesis. Reduction of sperms quantity in these mice may be due to the damaging ability of BPA on Sertoli cells whose population is a determining factor of the sperms cells volume that will be produced.

The decrease in daily sperms count might be due to spermatid degeneration as observed with the male Fischer rats exposed to BPA **[51]** and / or because of the Leydig cell apoptosis which then induces germ cell apoptosis through Fas / FasL and caspase-3 upregulation **[52].** Also, male infertility might be due to the decrease in the quantity of viable sperms **[53]**. Moreover, sperms concentration is an indicator of male infertility **[54]**, and sperms motility is important to deliver sperms through the reproductive tract **[55].**

Anomalies in sperms represent point mutation in germ cells, which may have resulted in structural changes in cell organelles causing head and tail malformation **[56].** Spermatogenesis is a well-organized process, it is highly sensitive to environmental pollutants, which can cause sperms damage or lead to infertility **[57].**

The present study further revealed that BPA induced several types of sperms abnormality such as sperms head and/or tail defects. The defects in sperms head reported in this study might be because of BPA effect on genes which function in expressing acrosome characteristics **[58].**

However, abnormalities of sperms heads could occur as a result of genetic mechanisms or testicular DNA alterations from cytotoxic agents, which ultimately alters events leading to spermatozoa differentiation **[59].** The sperms tail defects (short tail, wrong tail attachment and folded tail) might be an indication of aging of sperms. Sperms tail defects mostly occur during storage of sperms, maturation and epididymal transit during which motility of spermatozoa develop **[60]**.

A variety of target genes, which play prominent roles in cell cycle arrest, repair and apoptosis, are activated by p53 **[61].** We revealed down regulation of the P53 expression after in liver tissue after exposure to BPA at a dose (50µg/ kg b.w.) for 3 consecutive weeks .This results was in accordance with **[62]**, who found down regulation of the P53 protein levels in prostate epithelial cells(RWPE-1) after exposed to BPA for 24 h.

Exposure to BPA (10, 103 and 105 nM) disrupts microRNA and gene expression in endometrial cancer. The researchers also found that BPA exposure caused down-regulation of DNA repair gene, p53 and upregulated cyclin E2 interrupting to the cell cycle. These results suggested that BPA exposure could affect the p53 pathway leading to arrest the cell cycle, also

causes cancer in the endometrium and metastasis via decreasing expressions of genes responsible for apoptosis and increasing cyclins **[63]**.

In our study, several histopathological lesions were spotted in testes tissue due to BPA exposure, such changes included separation of germinal epithelia, obliteration in the wall of some seminiferous tubules, and eosinophilic material between seminiferous tubules. Also, loss of spermatozoa in the testes lumen. This finding was coinciding with the study that showed damage to the spermatogonia cells at various levels of exposure to BPA from few low-dose damages to extreme high-dose damage to male mice **[64]**.

Respectively, degeneration in seminiferous tubules and in Leydig cells. The seminiferous tubules are avascular, all oxygen and nutrients must pass out of the interstitial space, then to of the Sertoli cells to reach the germ cells. This position positions them at the edge of hypoxia and can render them very vulnerable to BPA **[65]**. Leydig cells synthesize androgens that promote spermatogenesis as well as maintain secondary sexual characteristics and sexual function after getting signals from luteinizing hormone. During spermatogenesis, androgens promote the meiosis and sperms differentiation **[66]**. The degenerative changes in Leydig cells can interfere with normal function and cause decline in the androgens production level which can further negatively affects spermatogenesis.

It is suggested that BPA exposure is related to decrease in cell proliferation, increased ROS-mediated damage and increased apoptosis of male gametes by the inhibition of anti-apoptotic pathways such as Bcl-2 and activation of pro-apoptotic signaling like Caspase 3 **[67].** Also, BPA can disrupt the hormones responsible for spermatogenesis of primordial germ in seminiferous. BPA itself is an Androgen Receptor antagonist that acts by interfering with AR binding sites, leading to reduction in AR translocation **[68].** Moreover, BPA can interfere with testicular steroid formation **[69].**

Conclusion

The findings of this study are essential as the effects of low-dose environmental factors of BPA on adult male fertility. In conclusion, the present study suggests that exposure of adult male rats to BP A (50 µg /kg/b.w.) for 8 weeks causes harmful effects on the spermatogenesis of primordial germ cells in seminiferous tubules and causes histological changes in the architecture of the testes. The observed impairment in sperms production, quality and motility suggests that BPA exposure impairs male fertility in rats.

Table (1): Data of male rats treated daily with 50µg/ kg. / B.W. and each week a group was dissected and subjected to the following parameters:

	Group	Sperms count	Sperms motility %	Sperms progression %	Sperms viability %
Group1	**control**	$51.9*10^6$	95.5	95	94.3
Group 2	**Week 1**	$34.8*10^{6\,**}$	97.2	94.1	84^{**}
	Week 2	$23.1*10^{6**}$	64^{**}	58.7^{**}	23.1^{**}
	Week 3	$22.5*10^{6**}$	15.1^{**}	14.5^{**}	8.6^{**}
	Week4	$26.1*10^{6**}$	75^{**}	71.3^{**}	58.9^{**}
	Week 5	$31.8*10^{6**}$	62.2^{**}	59^{**}	90
	Week 6	$26.7*10^{6**}$	76.8^{**}	73.1^{**}	73^{**}
	Week 7	$29.1*10^{6**}$	46.18^{**}	45.4^{**}	33^{**}
	Week 8	$26.1*10^{6**}$	91.4	90	93

- Data expressed as percentage (%)
- *= significant at P< 0.05

Table (2): Data showing the sperms morphology abnormalities including total number of normal sperm, head abnormalities, tail abnormalities and the total abnormalities male rats treated daily with 50µg/ kg. / B.W. and each week a group was dissected and subjected to the following parameters:

	Group	Total no. of normal sperms	Total head abnormalities	Total tail abnormalities	Total abnormalities
Group 1	**Control**	193±2.5	2.4±-0.5	5.8±1.9	8.4±2.1
Group 2	**Week 1**	147 ± 2.6*	26.6±2.8*	30±3*	54.2± 5.7*
	Week 2	141.6±9.1*	30±4*	24±5.6*	56.5±3.2*
	Week 3	99.8±8*	46.8±2*	46.4±4.6*	92.8±4.8*
	Week4	168.4±3.5*	19.8±1.7*	32.8±5*	40.2±2.4*
	Week 5	167.4±5*	22±3*	18±2*	39.6±4.7*
	Week 6	175±1.4*	13±2.4*	21±1.2*	33.8±3.6*
	Week 7	177±4.9*	18.2±3.3*	14.6±4*	28.2±1.7*
	Week 8	171±1.1*	11.8±2*	24±1.4*	37±3.2*

- Data expressed as the mean ± standard deviation.
- *= significant at P< 0.05

List of Abbreviations:

BPA	Bisphenol A
µg	Microgram
EDCs	Endocrine disruptor chemicals
ER	Estrogen receptor
AR	Androgen receptor
DNA	Deoxy ribonucleic acid
Mg	Milli gram
BW	Body weight
p53	Tumor protein p53
NaCl	Sodium chloride
BSA	Balanced salt solution
RNA	Riboxy nucleic acid
cDNA	complementary DNA
RLT	lysis buffer
β-ME	Beta-mercaptoethanol
Ng	nanogram
RT	Reverse transcribed
UCSC	University of California Santa Cruz-genome browser
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase(house keeping gene)
Ct	Cycle threshold
Δ	delta
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of variance
Fas	type-II transmembrane protein that belongs to the tumor necrosis factor
FasL	Fas ligand
qPCR	Quantitative Polymerase chain reaction
Bcl-2	B-cell lymphoma 2

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الملخص العربي

السمية التناسلية الناتجة عن التعرض لجرعة منخفضة من مادة بيسفينول (أ) فى ذكور الفئران

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تعتبر مادة البسفينول (أ) احدي المواد التي تسبب خلل علي مستوي الغدد الصماء ,حيث انها تستخدم في عمليه تصنيع البلاستيك .أن التعرض لفترات طويله لجرعات صغيره من مادة البسفينول (أ) له العديد من التاثيرات الضاره .وعليه تهدف هذة الدراسه الي تقييم التأثير الناتج عن التعرض الي جرعه منخفضه من البسفينول (أ) (50ميكروجرام/ كجم من وزن الجسم) عن طريق الفم علي القدره الانجابيه لذكور الجرذان و كذلك ملاحظه التغيرات النسيجيه التي قد تطرأ علي بواسطه تقنيه تفاعل (p53) نسيج الخصيه,كما تم تقييم التعبير الجيني لاحد الجينات الخاصه بعمليه الموت المبرمج للخليه . البلمره المتسلسل

أجريت هذه الدراسه علي عدد (72) من ذكور الجزذان تم تقسيمها الي مجموعتين , المجموعه الاولي هي المجموعه . الضابطه و التي تحتوي علي 8 حيوانات تم تجريعها بزيت الذرة فقط لمدة 8 اسابيع

المجموعه الثانيه و التي كانت تحتوي علي 64 حيوان تم تجريعها بجرعه من البسفينول (أ) (50ميكروجرام/ كجم من وزن الجسم) مذابه في زيت الذرة لمده 8 اسابيع متتاليه . في نهايه كل اسبوع من فتره التجربه تم تشريح 8 حيوانات و . اخضاعها لعدد من الاختبارات و التي من خلالها تم تقييم معدل الخصوبه في تلك الحيوانات

أظهرت النتائج ان التعرض الي مادة البسفينول (أ) بجرعه 50ميمروجرام/ كجم أدي الي حدوث انخفاض في عدد الحيوانات المنويه و ضعف في الحركه و قله في حيويه تلك الحيوانات المنويه . كما لوحظ ايضا زيادة التشوهات . المورفولوجيه للحيوانات المنويه سواء في الرأس او الذيل

أوضحت الدراسه النسيجيه لنسيج الخصيه فقدان البنيه النسيجيه الطبيعيه و عدم انتظام شكل الانيبيات المنويه و كذلك خلوها من الحيوانات المنويه الناضجه .و علاوة علي ذلك فقد تم تسجيل ارتفاع ملحوظ في معدل التعبير الجيني الخاص ب p53 جين . و أجمالا, يمكن القول بأن التعرض لمادة البسفينول (أ) و لو بجرعه صغيرة فانه سوف يتسبب في العديد من . الاضرابات التناسليه و التي بدورها تؤدي الي انخفاض معدل الخصوبه