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Effects of Pre-and Postnatal Exposure to Bisphenol- A on the Reproductive Efficacy in Male Albino Rats

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Abstract

This study was carried out at the college of Veterinary Medicine, Kerbala University to determine the effect of pre- and postnatal exposure to Bisphenol A (BPA) on serum reproductive hormones levels (Testosterone" T",Luteinizing Hormone "LH" and follicle- stimulating Hormone" FSH"), relative organ weight and histopathology of (Liver, Kidney, Testis and Prostate) , as well as semen analysis(sperm concentration, viability and abnormality) .

Thirty six pregnant female rats (F0) were gavage three doses of BPA suspended in corn oil (50 µg, 50 mg, 250 mg/kg/BW) or only corn oil as control from gestational day (GD) 6 through postnatal day (PND) 21. The weanlings (F1) from all dose groups(6 of each group) were still administered postnatally (after weaning) with the samedoses that given for their dams daily till maturity, then were subjected to necropsy 3 months of age. The results of statistical analysis showed significant decreased ($P<0.05$) in serum testosterone levels, and (LH), but not FSH in all treated groups compared with control group. The results also revealed significant increase in relative weight of prostate in all treated groups, of liver at doses (50 mg and 250 mg/kg/BW) and of kidney at highest dose only, while the relative weight of testis was significantly reduced in all treated group compared with control.

Sperm concentration and viability were significantly decreased and abnormality was increased due to BPA treatment.Histopathologic effectsof Bisphenol A on liver of male rats showed that thetreatment with all doses of BPA resultedin deleterious effects in liver and kidney.

Histology of rat's testes pre and postnatal exposed to all doses of BPA showed disarrangement and sever sloughing of the germinal epithelium and destruction the wall of some seminiferous tubules. There is little number of spermatids in the lumen of seminiferous tubules, necrosis the some germinal epithelia was present in high dose group only, on the other hand prostate histology indicate to presence of hyperplasia cell lining acini and decrease of prostatic secretions.From the present study it has been revealed that thexenoestrogen BPA adversely affect animal reproduction thereby itsaction on gonadal steroidogenesis

الملخص :

اجريت الدراسة الحالية في البيت الحيواني لكلية الطب البيطري- جامعة كربلاء لدراسة مدى التاثير السمي نتيجة التعرض لمادة البسفينول – أ اثناءمدة الحمل وبعد الولادة(حتى سن البلوغ)على خصوبة ذكور الفئران البالغة وذلك من خلال دراسة مستويات هرمونات التكاثر(الشحمون الخصوي, الهرمون الليوتينيو الهرمون المحفز للجريب)ومن خلال الاوزان النسبيه والتغيرات النسجية لكل من الكبد ,الكلية ,الخصية والبروستات بالاضافة الى دراسة تحليل السائل المنوي (تركيز الحيامن ,الحيوية و النسبه المئوية لتشوهات الحيامن).

استخدمت في هذه الدراسة 36 انثى جرذ حامل قسمت بالتساوي الى اربعة مجاميع, المجموعة الاولى اعطيت زيت الذره النقي فقط عن طريق الفم واعتبرت كمجموعة سيطرهاما المجاميع الثلاث الاخرى جرعت عن طريق الفم مادة البسفينول – أ المعلقة بمحلول زيت الذره وبالجرع الاتية 50 مايكروغرم ,50 ملغرام و 250 ملغم لكل كغم من وزن الجسم ابتداءا من اليوم السادس من الحمل وصولا الى عمر الفطام ثم تم فصل الجيل الول من ضغار الجرذان الى ذكور واناث واستمر تجريع الذكور بعد الفطام حتى سن البلوغ (عمر 90 يوم) حيث تم سحب الدم من الحيوانات وقتلها لغرض دراسة الاعضاء الداخلية المذكوره اعلاه.

وقد اضهرت النتائج وجود انخفاض معنوي في مستوى هرموني الشحمون الخصوي و اللوتيني في المجاميع المعامله مقارنة بمجموعة السيطره غير ان الهرمون المحفز للجريب لم يتأثر بالتعرض لمادة البسفينول.كما بينت نتائج الدراسة ايضا وجود ارتفاع معنوي في الاوزان النسبية لكل من البروستات في كل مجاميع المعاملة و الكبد في مجموعتي المعامله ب50 ملغم

و250 ملغم لكل كغم من وزن الجسم والكليةفي مجموعة الجرعة العالية فقط, غير ان الوزن النسبي للخصية انخفض معنويا في كل المجاميع المعرضة للبسفينول – أ مقارنة بمجموعة السيطره.
تحليل السائل المنوي للفئران المعرضة للبسفينول بين وجود انخفاضا معنويا في كل من معدلات تركيز الحيامن والنسبة المئوية للحيوية فيما ارتفعت معنوية نسبة الحيامن المشوهه وفي جميع التراكيز المدروسة.
كما اضهرت الدراسة النسجية ان البسفينول – أ ادى الى حصول اذى في كل من الكبد والكلية اما المقاطع النسجية للخصية اشرت وجودعدم انتظام ترتيب وانسلاخ في الضهارة الجرثومية اضافة الى تحطم في جدران بعض النبيبات المنوية مع وجود اعداد قليلة من النطف في تجويفها في كل المجاميع المعاملة مع حدوث تنخر في خلايا الضهارة الجرثومية عند فئران الجرع العالية فقط غير ان الدراسة النسجية للبروستات بينت وجود فرط تنسج للخلايا المبطنة للاسناخ مغ قلة في كمية افرازات البروستات. وقد خلصت الدراسة الحالية الى مادة البسفينول – أ لها تأثير ضار على الجهاز التناسلي لذكور الجيل الاول التي سبق لها التعرض لهذه الماده خلال مرحلتي الحملوالرضاعة وصولا للبلوغ .

Introduction:

The increasing incidence of reproductive disorders observed over the past few decades has raised concern about the role of substances known as endocrine disrupters (EDs) that are capable of modulating or disrupting the function of the endocrine system. One such estrogenic ED, known for its ubiquitous exposure is Bisphenol A (BPA), which is become ubiquitous in the environment within the past 80 years because of its presence in a multitude of products including food and beverage packaging, flame retardants, adhesives, building materials, electronic components, and paper coatings (**1**). BPA is one of the highest volume chemicals produced and global consumption of BPA in 2011 was predicted to exceed 5.5 million metric tons (**2**).Exposure occurs because when BPA molecules are polymerized, they are linked by ester bonds that are subject to hydrolysis, which is accelerated as temperature increases and in response to contact with acidic or basic substances. The consequence is that as polycarbonate products are repeatedly washed, or polycarbonate plastic or metal cans are exposed to heat and/or acidic or basic conditions, significant leaching of BPA due to hydrolysis of the ester bond occurs (**3** and **4**).

Bisphenol A (BPA) was first synthesized by A.P. Dianin in 1891 and was later investigated in the 1930s during the search for synthetic estrogens. At that time, it was tested for its estrogenic properties but abandoned for pharmaceutical use when diethylstilbestrol (DES) was determined to be much more potent .Thus, until recently; BPA was considered a weak environmental estrogen because of its relatively low affinity for estrogen receptors compared to estradiol (**5**and **6**). However, results from recent studies have revealed a variety of pathways through which BPA can stimulate cellular responses at very low concentrations, below the levels where BPA is expected to bind to estrogen receptors (**7**).

Natural estrogens bind estrogen receptors and they in turn bind to estrogen responsive elements and induce the expression of genes in their target cells. These cells include those in the reproductive organs (vagina, uterus, oviduct, ovary, cervix, testis and epididymis), the mammary gland, the brain and pituitary, the thyroid gland, and the skeletal and cardiovascular systems, among others (**8**). As a synthetic estrogen with the capability of binding to estrogen receptors, BPA also has the potential to alter development at various levels of organization (**9**).

High doses of BPA may mediate its effects through mechanism other than those regulated by estrogen receptors (ERs). Soxenoestrogens such as BPA must be investigated for another possiblemechanisms of action such as their apoptosis inducing and enhancing/ suppressing activities specially on male reproductive organsparticularly testis. There were various studies lonely investigated theeffect of BPA on male reproduction either through prenatal orpostnatal exposure, meanwhile there was paucity of informationconcerning the effect of both pre- and postnatal exposure to BPAespecially at low dose level (50 µg and 50 mg /kg / day) according to**(10**) and high dose level (250 mg/kg /day) (1/20 LD50)according to (**11**).

To our knowledge there is lack of researches about (PBA) in Iraq. So the current study aimed to investigate effects of long term BPA treatment during prenatal and postnatal periods on reproductive performance of male albino rat.

Materials and Methods:

Bisphenol A (BPA, CAS 80-05-7, > 99% pure) was purchased from Sigma Aldrich Company(USA) via OMA company (Iraq).Tocopherol-stripped corn oil (ICN Biomedicals Inc., Aurora, OH) served as the vehicle and control substance.Appropriate amounts of BPA were mixed with corn oil to achieve the desired concentrations. Fresh solutions were prepared weekly for each concentration and stored in glass containers. Based on the body weight of pregnant rats dose was administered to each one.

The present study included 36 mature female and 12 mature male albino rats, kept under hygienic conditions, housed in metal cages to avoid bisphenol exposure from old polycarbonate cages with hard wood shaving as bedding. Tab water were provided via glass bottles, and feed were giving ad –libitum throughout the experimental period.

Females were examined daily using vaginal smear technique to ensure that they were in regular estrous cycle **(12).** Female proved to be in estrous phase were mated with mature male rat in a separate cage. After mating a vaginal smear was taken. The presence of sperms indicated zero day of gestation **(13)**. The pregnant female albino rats (36 females) were separated from the stud then divided into four main groups:

Control Group: Nine pregnant female rats served as control group which received corn oil only as vehicle.

Group 1: Nine pregnant female albino rats administered BPA (50 µg/kg BW. /day) dissolved in corn oil via gavage as Tolerable Daily Intake dose (TDI) for human according to (**10).**

Group 2: Ninepregnant female albino rats administered BPA (50 mg/kg b.wt /day) dissolved in corn oil via gavage as Lowest Observed Adverse Effect Level (LOAEL) according to (**10**)

Group 3: Nine pregnant female albino rats, orally administer BPA 250mg/kg b.wt./day (1/20 LD50) dissolved in corn oil via gavage as high dose (**11**).

The pregnant females (dams) dosed BPA daily according to their groups from Day six of pregnancy, through gestation, during lactation till weaning of their offspring.The weaned rat offspring of four groups (6 male)of each treated group and of control group are still administered postnatally daily with the same doses that given for their dams till maturity (90 days old).

At the end of experiment mature six F1 male rats offspring of each group were weighted and then sacrificed, followed by collection of blood to perform hormonal, hormonal and histopathological studies.

Six F1male rats of each group were sacrificed at the end of Day 90 of age, the rats before sacrifice were first weighed and then anaesthetized by placing them in a closed jar containing cotton sucked with chloroform anesthesia. The blood samples were collected via heart puncture,the blood sample were drops directly from the heart by using 5 ml disposable syringe and put in plane tube to be centrifuged (3000 rpm for 15 minutes) to obtain the serum which is then transferred to epndrofe tubes, for hormones measurement and then abdominal cavity was opened up through a midline abdominal incision to take the samples which include Liver, prostate, testes, and kidneys were removed and trimmed of their lipids. All were weighed with an electronic analytical and precision balance. The two testes of each male rat and two kidneys of each animal were measured and the average value obtained of each of two organs was regarded as one measurement. The organs were fixed in 10 % formalin for histological examination, the tail of epididymus was put in a petry dish contain 5 ml normal saline to be used for total sperm count and sperm availability and abnormality.

Parameters of study:

1- Relative organ weights:

2- Hormonal assay:

A- Estimation of serum testosterone level according to Wheeler (**14**).

B- Estimation of serum luteinizing hormone (LH) andFollicles-stimulating hormone (FSH) level according to(**15**).

3- Semen evaluation (Epididymal Spermatozoal Examination)

4- Histopathological studies:

The results were expressed as mean ± SD. The comparisons between groups were performed with analysis of variance (ANOVA) by using computerized SPSS program (Statistical Program for Social Sciences). P<0.05 was considered to be lest limit of significance. Least significant different test (LSD) was calculated to test difference between means (groups) for (ANOVA) (**16**).

Results:

As shown in table (1), the weight of liver increased significantly($p \leq 0.05$)due to BPA with high dose 250 mg and 50 mg /kg B.W compared with control group and other treated groups. It seems that the liver weight was not affected by low doses of 50 mg and 50µg /kg B.W BPA compared with control.

The oral dosage of 250 mg/Kg BW BPA seems to cause significant increase ($p \leq 0.05$) in kidney weight of treated male rats compared with control group, while the other two treated groups have non-significant differences in compared with control group. The kidney weights of treated males with different doses of BPA were not significantly different from each other's.

Concerning the means of testis weight of mature F1 male offsprings which pre- and postnatally exposed to (250, 50mg and 50µg/Kg B.W/day50) of BPA. There was significant decrease in testis weight of mature F1 male offsprings pre- and postnatally exposed to BPA at all dose levels when compared with the control group without any significant differences among treated groups.A significant increase ($p \leq 0.05$) was shown in prostate weight of male ratspre and postnatally exposed to BPA 250, 50mg and 50 µg /kg B.W (0.361, 0.337 and 0.310) respectively compared with control males (0.147). There were no significant differences in prostate weight among male rats exposed to different doses BPA.

Table (1)The Effect of Pre and Postnatal Exposure to BPA on Some Organs Relative Weight (g\100 g of BW) in F1 Mature male Rats (mean ± SD)

Parameters / Groups	Liver	Kidney	Testes	prostate
Control	B 3.57±0.29	B 0.734±0.17	A 0.825±0.19	B 0.147±0.24
Group 1 (50µg/kg B.W.)	B 3.64±0.26	AB 0.791±0.19	B 0.639±0.26	A 0.310±0.31
Group 2 (50mg/kg B.W.)	A 4.19±0.25	AB 0838±0.08	B 0.617±0.16	A 0.337±0.16
Group 3 (250mg/kg.B.W.)	A 4.22±0.39	A 0.962±0.10	B 0.611±0.32	A 0.361±0.35
LSD	0.435	0.215	0.179	0.155

N=6

Different letters represent significant difference at ($p \leq 0.05$).

The result of the present study revealed a significant decrease in serum testosterone level in mature F1 male offspring pre-and postnatally exposed to 250 mg , 50 mg and 50 µg /kg B.W./day of BPA when compared with control group (1.773±0.105, 2.273±0.163 ,2.368±0.151and 5.611±0.245) respectively. Table (2), and the group exposed to highest dose of BPA was also significantly decreased ($p \leq 0.05$) in compare with that exposed lowest dose.

The LH level in F1 mature male rats pre and postnatallytreated with BPA250 mg , 50 mg and 50 µg /kg B.W./daywere significantly decreased ($p \leq 0.05$) compared with LH level of control males table (2),also the group treated with250 mg /kg B.W was significantly decreased compared with the group treated with 50 mg/ Kg/ B.W.

.There was no significant difference ($p \geq 0.05$) in FSH level among all male groups was recorded.

Table (2)The Effect of Pre and Postnatal Exposure to BPA on SomeSerum Hormones Levels in F1 Mature Male Rats(Means ± SE)

Parameters / Groups	Testosterone ng/ml	LH µIU/ml	FSH µIU/ml
Control	A 5.611±0.245	A 1.598±0.145	A 1.640±0.122
Group 1 (50µg/kg B.W.)	B 2.368±0.151	BC 0.494±0.088	A 1.455±0.143
Group 2 (50mg/kg B.W.)	BC 2.273±0.163	B 0.780±0.081	A 1.377±0.099
Group 3 (250mg/Kg.B.W.)	C 1.773±0.105	C 0.363±0.057	A 1.522±0.100
LSD	0.5631	0.3167	0.2975

N=6

Different letters represent significant difference at ($p \leq 0.05$).

Table (3) revealed that the sperm concentration in male rats pre- and postnataly treated with BPA250 and50 mg /kg B.W were significantly decreased ($p \leq 0.05$) compared with that of control male rats ,while in the lowest dose`s group the decrease doesn't reach the significance($p \geq 0.05$) in compared with control group (table 3). Moreover, there were no significant changes ($p \geq 0.05$) among all three treated groups were observed.

The results in table(3) indicated a significant increase ($p \leq 0.05$) in sperm viability percentage of male rats pre and postnataly exposed to BPA 250,50 mg and 50 µg /kg B.W /day compared with control group . No significant differences among treated groups were shown.

Regarding the sperm cell abnormalities, the result of the present study showed a significant increase in the percentage of sperm cell abnormalities in mature F1 male offsprings exposed to BPA(250 ,50 mg and 50 µg/Kg BW/day when compared with control group but the increase of dose was had no significant($p \geq 0.05$) effect on sperm cell abnormalities (table3)

Table (3)The Effect of Pre and Postnatal Exposure to BPA on Seminal Analyses (means ± SE)

Parameters / Groups	Sperm concentration $X10^6/mm^3$	Viability%	Abnormality%
Control	A 2.391±0.510	A 69.638±2.685	B 9.016±1.895
Group 1 (50µg/kg B.W.)	AB 1.933±0.257	B 49.650±2.597	A 28.416±4.099
Group 2 (50mg/kg B.W.)	B 1.256±0.103	B 48.233±2.943	A 27.533±2.753
Group 3 (250mg/kg B.W.)	B 1.316±0.108	B 46.950±2.852	A 30.566±2.869
LSD	0.8036	9.7381	7.2731

N=6

Different letters represent significant difference at ($p \leq 0.05$).

The liver tissue examination of the control group has shown histological structure of the liver, the hepatic lobules that consisted of hepatocytes arranged in hepatic cords radiating from the central vein to the periphery of the lobule. The cellular cords were separated by sinusoids (Fig 1).

Liver sections of the exposed F1 male rats to dose of 50 µg/kg B.W. of BPA have revealed damages included clear enlarged central veins and cytoplasmic vacuolation(fig-2) .Liver sections in

animals exposed to the dose 50 mg/kg B.W. of BPA have shown irregular arrangement of hepatocyte, enlargement of sinusoid spaces and congestion of central vein (Fig-3). The high dose (250 mg / kg B. W.) in male rats causes clear congestion of central vein ,the hepatocytes were larger and flattened with clear enlarged pyknotic nuclei .In addition, liver appeared irregular irradiation structure and obvious sinusoidal spaces.(fig-4).

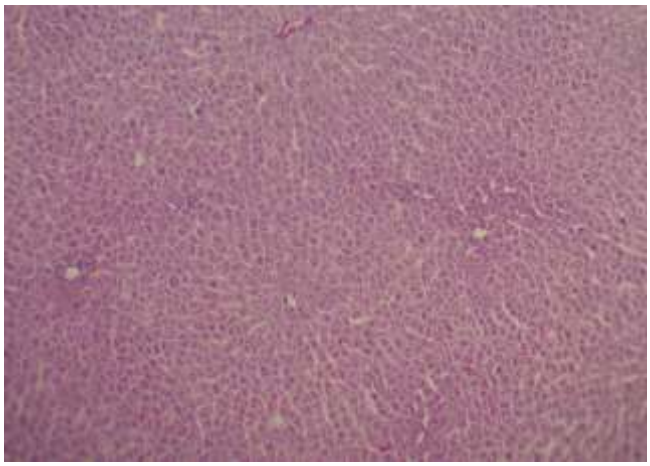

Figure -1: Light micrograph shows the histological liver structure of control rats.. The tissues appear to be normal with the presence of a small amount of vacuoles (fatty infiltration). H&E, 40x.

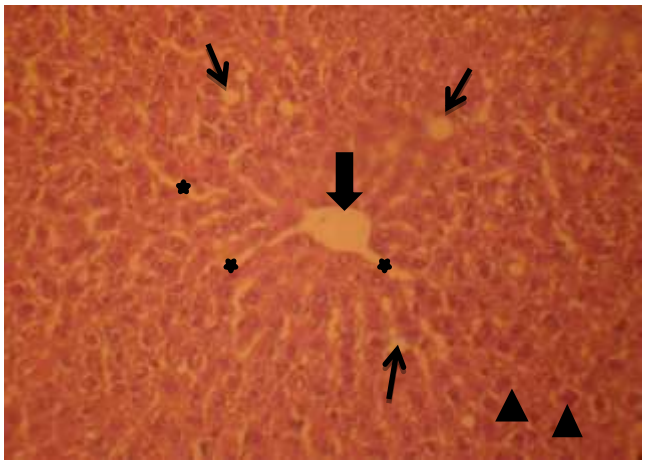

Figure-2: Light micrographof histological changesof liver of male rat pre and postnatal exposed to 50µg/kg B.W. of BPA showsvacuolization (arrow) ,enlarged central vein (thick arrow)),enlarged sinusoid space(stars) and pyknotic nuclei(arrow head) . H&E, 100x.

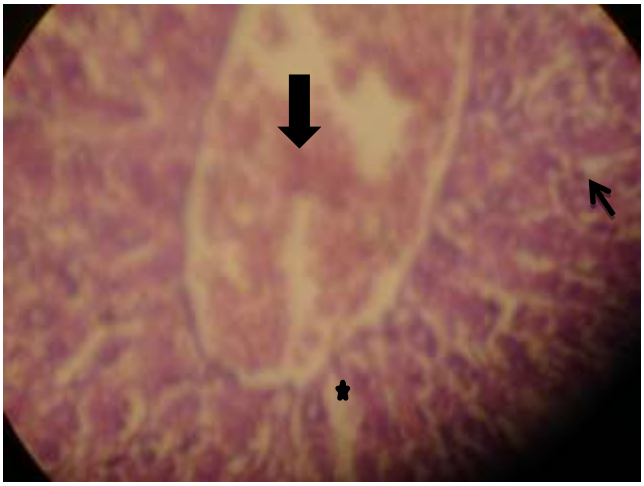

Figure-3: Lightmicrograph of histological changesof liver ofmale rat pre and postnatal exposed to 50mg/kg B.W. of BPA showscongested central vein(thick arrow),enlarged sinusoid space(stars) and vaculation(arrow). H&E.400x

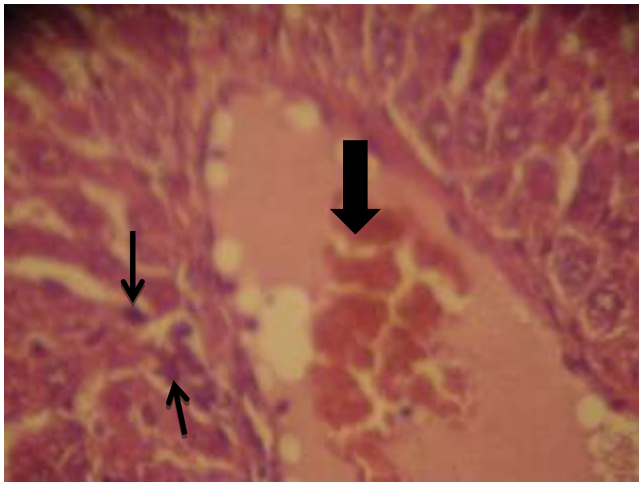

Figure-4.: Light micrograph of histological changesof liver of male rat pre and postnatal exposed to 250mg/kg B.W. of BPA shows pyknotic nuclei (arrow),Congested central vein(thick arrow). H&E, 400x.

The kidney tissue examination of the control group has shown histological structure revealed glomeruli with thin glomerular basement membrane, cellularity and patent capsular space surrounding proximal and distal convoluted tubules (fig. 5).Kidney section from F1 male rats exposed 50 µg / kg B.W. showed dilatation of Bowman's space also enlarged cells lining renal tubules, decrease lumen space and degeneration of lining cell of renal tubules (figure 6) .The previously exposed male and female rats with this 50 mg/kg/BW. result in obvious histological

changes include in renal tubules and in glomeruli . The epithelial cells lining renal tubule were degenerated and necrotic in addition to hemorrhage in the interstitial tissue and narrowing of tubular lumen (star shape) .in addition to presence necrosis in the cells lining glomerular capsule, and sloughing of tubular epithelial cells in tubular lumen (figure 7).Pre and postnatal exposed F1 male and F1 female rats with high dose250 mg/kg/BW. led to more deleterious histological changes in kidney represented in massive hemorrhagic areas also there were infiltration of the inflammatory cells surrounding thickened blood vessels.(figure 8)

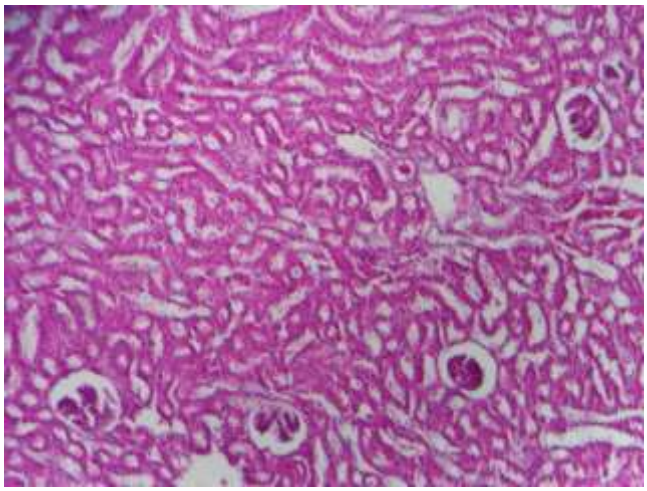

Figure-5: Lightmicrograph for kidney histological changes in control male rat shows normal renal glomeruliand tubules. H&E, 100x

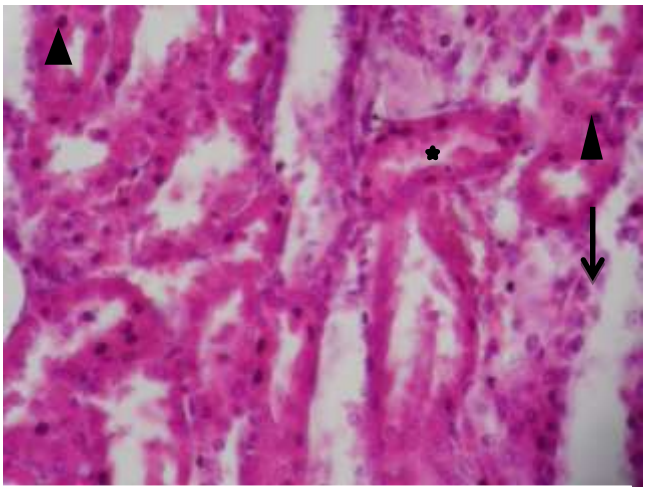

Figure -6 Light micrograph of histological changes of kidney of male rat pre and postnatal exposed to 50µg/kg B.W. of BPA shows enlarged cells lining renal tubules (arrow head),decrease lumen space (star)and degeneration of lining cell of renal tubules (arrow) . H&E, 400x

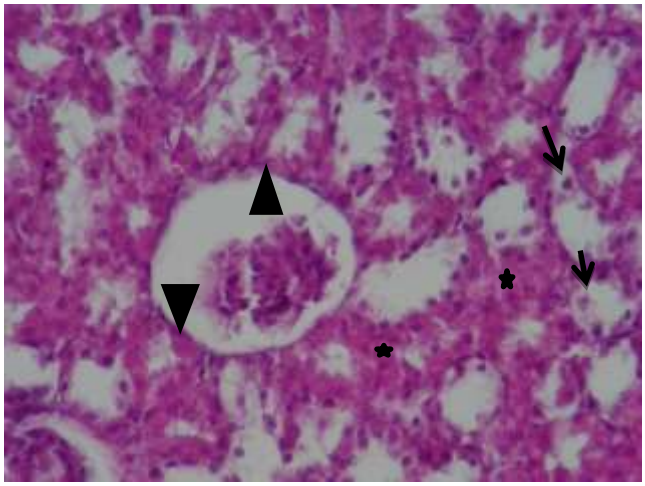

Figure -7: Light micrograph for kidney histological changes in male rat pre and postnatal exposed to 50mg/kg B.W. of BPA shows necrosis of cells lining glomerular capsule (arrow head) ,hemorrhagic areas (stars)and sloughing of a tubular epithelial cells in tubular lumen (arrows) . H&E, 400x

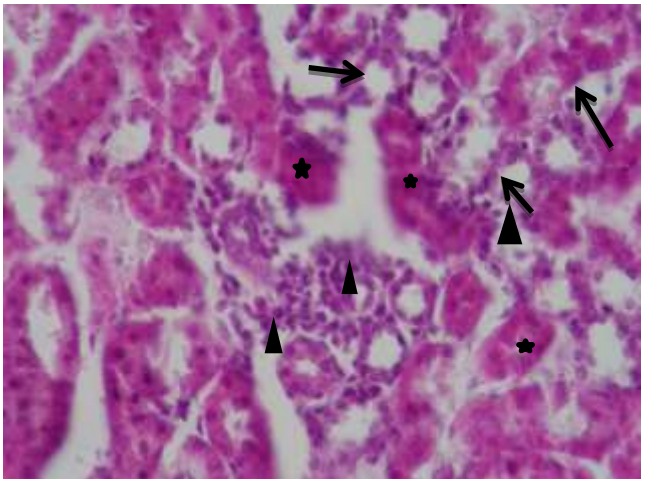

Figure -8: Light micrograph for kidney histological changes in male rat pre and postnatal exposed to 250mg/kg B.W. of BPA shows necrosis in the cells lining atrophied renal tubules (arrow) , hemorrhage (stars) and infiltration of inflammatory cells (arrow head) . H&E, 400x

The microscopic finding of testes of control rat includes the normal structures of seminiferous tubules shows spermatogenic cells and supporting cells. (fig. 9).The microscopic finding of rat' s testes pre and postnatal treated with BPA50 µg /kg B.W. shows disruption of germinal epithelium which is irregularly placed on the basement membrane with few number of spermatogonia ,sloughing in germinal epithelia also accumulation of pinkish edematous fluid between seminiferous tubules (fig.10).Histology of rat' s testes pre and postnatal exposed to 50 mg /kg B. W. of BPA shows disarrangement and more sever sloughing of the germinal epithelium and destruction the wall of some seminiferous tubules. There is little number of spermatids in the lumen of seminiferous tubules.(fig.11).Microscopic examination of testes of rats treated withBPA250 mg /kg B. W shows atrophy of seminiferous tubules indicated by increased the interstitial space between the seminiferous tubules and destruction of these tubules ,necrosis and sloughing in the some germinal epithelia. No mature spermatozoa were seen in some the lumens of the seminiferous tubule,(fig.12).

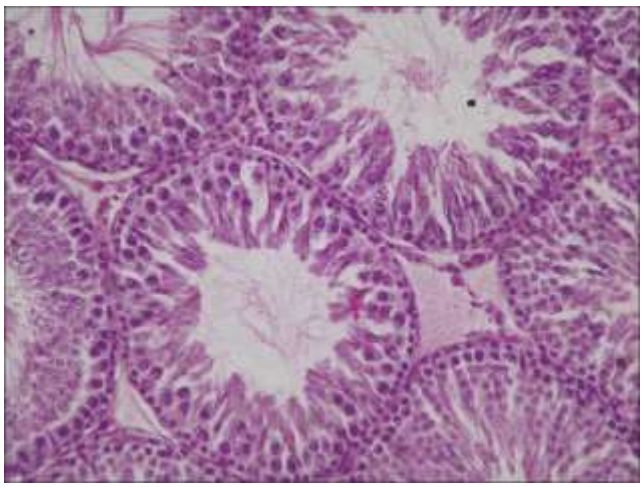

Figure -9: Light micrograph of testes of control rat notice the normal structures of seminiferous tubules shows spermatogenic cells. H&E, 100x

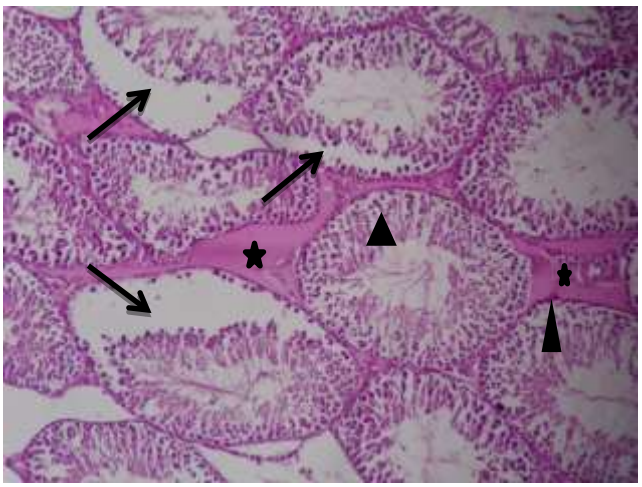

Figure -10 Light micrograph of histological changes in testes of rat pre and postnatal exposed to 50µg/kg B.W. of BPA shows sloughing germinal epithelia of seminiferous tubules into the lumen(arrows) ,few number of spermatogonia (arrow head) and pinkish edematous fluid between seminiferous tubules (stars),. H&E, 100x

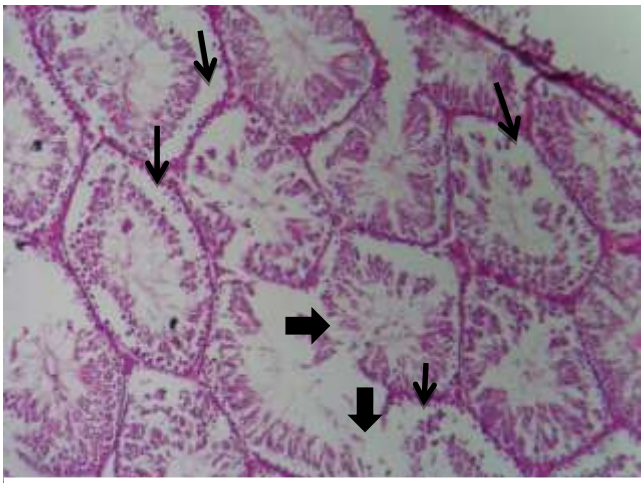

Figure-11 Light micrograph of histological changes in testes of rat pre and postnatal exposed to 50mg/kg B.W. of BPA shows sloughing germinal epithelia of most seminiferous tubules into the lumen (thin arrows) and destruction in wall of some seminiferous tubules(thick arrows). H&E, 100x

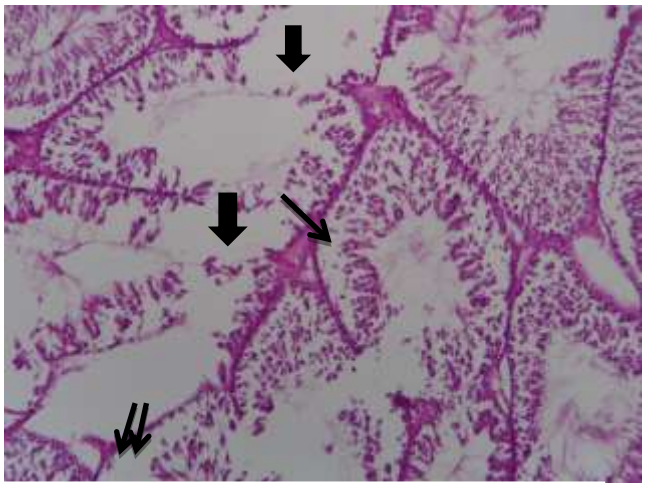

Figure -12 Light micrograph of histological changes in testes of rat pre and postnatal exposed to 250mg/kg B.W. of BPA shows destruction of walls of seminiferous tubules (thick arrows), necrosis (thin arrow) and sloughing in germinal epithelia (double thin arrows)). H&E, 100x

Microscopic examination of prostate of control rats shows normal, there are trabeculay from capsule dividing the gland into lobules containing mucous secretory units (acini) (fig.13).Very little secretion was founded in light micrograph of prostate removed from rat pre and postnatal exposed to BPA50µg /kg B. W. There is also degeneration of epithelial cell and sloughing of some epithelia. (fig. 14) . BPA led to hyperplasia of lining epithelium and papillary projections toward alveolar lumen with no secretion of prostatic fluid (fig. 15). The microscopic finding of rat's prostate pre and postnatal treated with BPA 250 mg /kg B. W. shows sever hyperplasia found in most of acini ,the mass of hyperplasia closed the lumen of some alveoli in addition to presence of papillary projections in other alveoli(fig16).

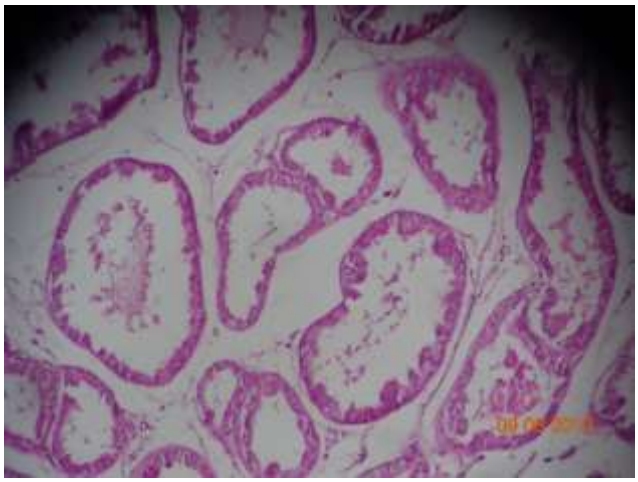

Figure -13: Light micrograph of prostate from control rats shows normal shape and size of lobule and alveoli. H&E, 100x

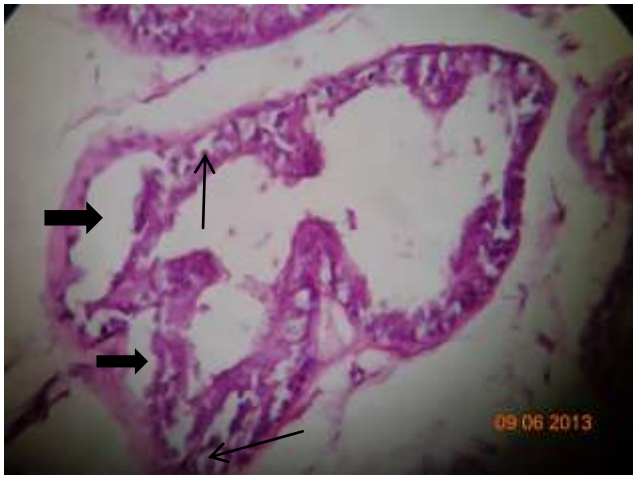

Figure -14: Light micrograph of prostate from rat pre and postnatal expose do BPA50 µg/kg B. W. shows of degeneration epithelial cell (thin arrows)and sloughing of epithelia (thick arrows) H&E, 400x

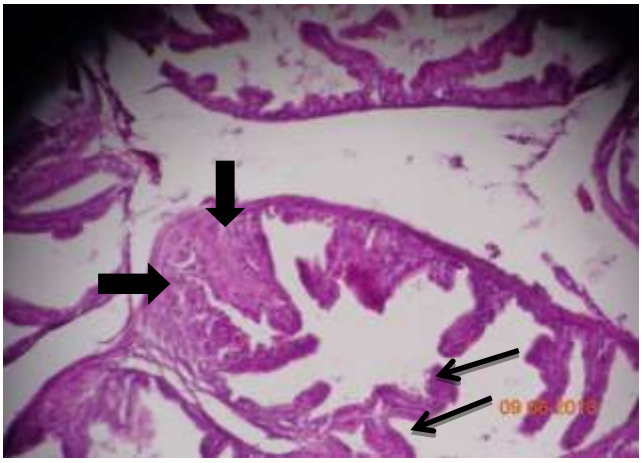

Figure -15: Light micrograph of prostate rat pre and postnatal exposed to BPA 50 mg/kg B. W. shows epithelial papillary projections toward lumen (light arrows)and hyperplasia of epithelial cells(thick arrows). H&E, 400x

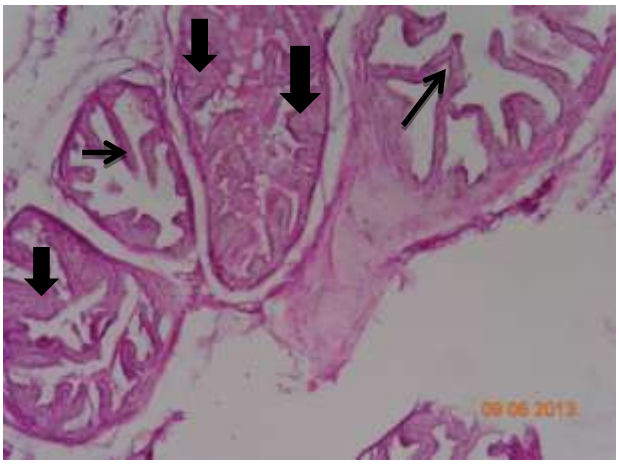

Figure 16 Light micrograph of prostate rat pre and postnatal exposed to BPA250 mg/kg B. W. shows more of epithelial papillary projections toward alveolar lumen (light arrows) and sever hyperplasia(thick arrows).. H&E, 400x

Discussion:

Our results are in agreement with (**17**and **18**). The liver is the major organ for the metabolism and detoxification of xenobiotics, including BPA (**19**). Therefore, the liver could be largely exposed to BPA, and could be susceptible to lower doses, than other organs (**20**). Liver enlargement is due to hepatocyte proliferation with the increase in cytochrome P-450 but not fat deposition (**21**), therefore, increase of relative weight of liver in the present study may be partly related to liver enzyme induction by BPA.

The increase in kidney weight was in agreement with results of (**22** and **23**).increase relative weight of kidney might be caused by defects and disorders in the kidney functions due to the long exposure of kidney cells to the toxic metabolites of BPA for 90 days which resulted in kidney dysfunctions and these findings and after BPA intoxication might be lead to reduced ability of the kidney to eliminate the toxic metabolic substances(**24**)

With regard to effect of BPA on relative testis weight, our results agree with that observed by Nakamura et al. (**25**) in rat; Wang et al. (**26**) in mice; Akingbemi et al., (**27**) in rats Chitra et al. (**28**) in rat and Kawai et al. (**29**) in mice and Takahashi and Oishi,(**30**) in rat and mice, but these results disagree with that obtained by Kato et al. (**31**) who found that BPA treatment had no effect on reproductive organs weight of male rats. This decrease in testis weight may be attributed to one of or all the four following reasons: first, during the perinatal period the remethylation process in rat testis mainly occurs during GD 11–15, indicating it may also be affected by BPA exposure (**32**), therefore, the perinatal exposure to BPA has the potential to modify epigenetic marks in various steroid responsive organs including testis. Second, suppression of normal increase in germ and sertoli cells per testis specially before puberty (**33**) as the sertoli cells never proliferate after puberty (**34**).Third, this decrease also may be due to decreased steroidogenic enzyme activity (**35** and **36**) resulting inhibition of spermatogenesis, which is parallel with histopathological findings observed in our study that revealed defective spermatogenesis and testicular degeneration in testis of mature F1 male rat offsprings exposed to all three doses of BPA, Figs (10 ,11 and 12)

.Fourth, it is well known that testicular growth is highly promoted by testosterone and inhibited by estrogen as proved by Balthazart and Hendrick (**37**) and Zeller (**38**) and this in coordinate with decrease of testosterone levels in the present study (table2).

From table (1) , the prostate corrected weights (g/100g B.W.) of male rats treated with BPA were significantly increased . These findings are consistent with studies by **(39**; **40** and **41**)Maternal exposure toDES , an increase in free serum E2 and BPA all caused a permanent increase in prostatic

androgen receptorsin mice in addition to an increase in adult prosate weight, relativeto negative controls(**40**;**41**. , and**42**).

The decreased serum testosterone level (table 2) could be primarilypostulated to the decreased expression of the steroidogeneicenzymes and cholesterol carrier protein "StAR" involving thetestosterone synthesis as mentioned by Xi et al. (**43**) andNakamura et al. (**25**). Also the reduced serum level of LH (table 2) mightdeteriorate testosterone biosynthesis by adversely affecting theexpression of cholesterol carrier protein or steriodogenic enzymesas mentioned by Nakamura et al. (**25**). Furthermore BPA isreported to act as antiandrogenic agent blocking the action ofdihydrotestosterone (**44**).

Moreover the testicular response to hCG for progesterone andtestosterone release was found to be decreased or suppressed inBPA treated rats. So these finding , taken all together, suggests thatBPA affect testicular function in term of leydig and sertoli cellfunction leading to inhibition of testosterone secretion "primarygonadal failure" (**45**).On contrary the decreased serum testosterone level disagreewith results obtained by Kato et al. (**31**) in rats and Kawai et al.(**29**) in mice, they found that there was non-significant change intestosterone level following BPA exposure when compared withcontrol, but Ramos et al. (**46**) found that prenatal BPA exposure(25 and 250 µg/kg b.wt) resulted in significant increase in serumtestosterone level at PND 15 when compared with control.This variation may be due to differences in animal species,dose of BPA and time of exposure.

The decreased serum LH level (table 2) is similar to the resultsobtained by Nakamura et al. (**25**); Gharravi et al. (**47**) andAkingbemi et al. (**28**). This could be explained by ability of BPAto interfere with LH receptor ligand binding resulting in uncouplingLH from LH receptor that potentially contributes to diminished LHstimulation of steroidogenesis as reported by (**48**),or due to increased prolactin release after BPA exposure asmentioned by (**49**), where hyperprolactinemia hasbeen shown to cause reproductive dysfunction as confirmed byKoike et al.(**50**) and Hamada et al.(**51**), this dysfunction isnot mediated via direct action on testis but due to its effects at thelevel of hypothalamus-pituitary to inhibit LH-RH and LH secretionas confirmed by (**50**) and.(**52**).Controversially these results disagreed with results obtained by(**50**), who found an increase in serum LH level aftersubcutaneous administration of male rat with 0.3 mg BPA /kg b.wt/day for 2 weeks. This variation in LH level may be attributed tovariation in the doses as clarified by (**53**) and(**54)**who mentioned that responses of organs weights and serum hormone levels to estrogen vary in proportion tothe doses, where higher doses decrease LH and very small dosesonly decreases testosterone hormone without response to other.FSH level was unaffected by BPA.These findings are consistent with studies by(**55**)and (**56**).

Regarding the effects of BPA on epididymal spermcharacters, Theseresults agree with that obtained by (**57**) in mice;**(28**) in rat; (**58**) in rat; (**33**) in rat and (**59**)in mice and these resultsdisagrees with that obtained by (**31**); who found thatBPA administration has no effect on semen picture. This may beattributed to depletion of antioxidant defense system and inductionof oxidative stress as well as increased lipid peroxidation in ratspermatozoa by BPA (**28**). Lipid peroxidation ishigh toxic to spermatozoa and cause irreversible arrest of spermmotility, decrease sperm count and damage sperm integrity andincreased sperm cell abnormalities which confirmed by (**60**). Suppressed aromatase gene expression by neonatalexposure to BPA as reported by (**27**) couldresult in incomplete differentiation of spermatids in seminiferousepithelium and subsequently resulted in defective spermatogenesisand lowered sperm production as reported by (**51**).

Regarding histopathological finding of liver we observed histopathologicalchanges in liver indicating variable damage after BPAadministration as showed in (Figs.2,3 and 4). Microscopic examinationrevealed that liver could be susceptible to low dosesthis result was reported by several authors, (**20**and **62**). In presentstudy; it has been observed that BPA showeddegenerative changes in hepatic cells this also wasreported by (**63** and**64**).

Moreover, lightmicroscopic examination revealed signs ofvacuolatedhepatocytes, dilated sinusoids, and congested bloodvessels, increased in number of. It has been reported by previous findingsthat BPA causes cell infiltration and necrosis (**62**and **63**),vacuolated hepatocytes (**64**), liverdamage (**65**). The accumulation of metabolites of BPA and inability of kidney to excreted them might affect the kidney tissues of treated rats had proved necrosis, degeneration in tubular epithelium (figs.6,7and 8) Regarding histopathological findings of different malereproductive organs; focal and diffused testicular degeneration inseminiferous tubules with defective spermatogenesis were seen intestis of mature F1 male albino rat offsprings pre- and postnatallyexposed to (250 mg/kg b.wt /day) , (50 mg/kg b.wt/day) and (50 µg/kg b.wt/day) of PBA.(figs.10,11 and 12) These histopathological findings are similar to thatobtained by (**30**),(**31**),(**71**),(**35**),(**41**) and (**54**).

TheseTesticular pathological alterations may be due to xenoestrogenicproperties of BPA that can inhibit testicular growth as mentioned byBalthazart and Hendrick (**37**) and Zeller (**38**). Where BPAmay act as selective toxicant for the male reproductive organs anddirectly inhibit testicular function as reported by (**45**).The author attributedthese histopathological changes to decreased testosterone level ordue to reduction of 5- reductase activity in the epididymis and thisparallel with the decreased serum testosterone level in our studyTable (2).

Moderate to severe papillary hyperplasia seen in the liningepithelia with decrease prostatic secresion were seen inprostate gland of mature F1 male offsprings which pre-and postanatallyexposed to all doses of BPA(figs.14,15 and 16). These findings were similar to thatobtained by (**46** and **67**). The hyperplastic changesseen in prostatic acini may be due to an increase in the proliferation ofbasal epithelial cells as mentioned by (**40**).

From the present study it has been concluded that thexenoestrogen BPA adversely affect animal reproduction thereby itsaction on gonadal steroidogenesis, and subsequently the anomalous release of endogenous steroidhormones.

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