EFFECT OF BISPHENOL A ON THYROID, LIVER AND TESTICULAR FUNCTIONS IN ADULT MALE RATS

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ABSTRACT

This study was carried out in Veterinary Medicine College / University of Basrah to investigate the effect of Bisphenol A on thyroid, liver and testicular functions. A total of 24 adult male rats were randomly divided into four equal groups, six animals in each group. Animal of group (1) served as control and received a daily oral administration of corn oil throughout the experimental protocol. Animals of group 2, 3 and 4 were administered orally 50,100 and 200 mg/kg body weight of BPA respectively dissolved in corn oil, the experiment extended for 30 days.   The results of the present study showed a significant decrease in serum thyroxin (T4) concentration and a significant increase in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentration in bisphenol A treated groups. A significant decrease in serum testosterone   and LH concentrations in all BPA-treated groups compared with control. On other hand non significant decrease in serum concentrations of FSH were observed in BPA-treated groups compared with control. A significant decrease in epididymal sperm count and sperms motility in all BPA treated groups. However no significant differences were noted in sperms viability between all BPA treatment groups and control. Histopathological changes were found in thyroid glands of male rats with different doses of BPA, also central vein dilation, enlarged nuclei, vacuolation of hepatocytes were observed in the liver of BPA treated groups and different degrees of histological changes include depression of spermatogenesis, decrease of leydig cells in dose dependent manner were found in testicular tissues of BPA treated groups.

INTRODUCTION

The endocrine disruptors are widespread in the environment and food chains and include some common environmental contaminants such as pesticides, plastic ingredients, dioxins,and biocides (١ & ٢).

Bisphenol A(BPA)(2,2-bis(hydroxyphenyl) propane is one of the environmental contaminants widely used in the manufacture of polycarbonate plastic such as baby bottles, epoxy resin (inside coating in metallic food cans),and as a non-polymer additive to other plastics(٣ & ٤). BPA is also found in polymers that are used in dental materials (5).

Although several authors have speculated that specific environmental chemicals might bind to thyroid receptors(TRs) and alter thyroid hormone signaling (6).In vitro studies have demonstrated that BPA binds weakly to the thyroid hormone receptor and suppresses transcriptional activity that is stimulated by T3(7).

Animal studies have shown that BPA affects the male reproductive system including androgen receptors ,male sex hormone levels, male reproductive organs including testes, epididymis, seminal vesicles, the prostate gland and sperm production(8). Lang *et al* (9) reported adverse effect of BPA on,reproductive system, metabolic processes including alterations in insulin homeostasis and liver enzymes.

BPA can be hydrolyzed under high temperature and acidic or basic conditions leading to leaching into food and drink containers (10).Human exposure to BPA occurs through multiple routes, however oral exposure is considered the major route of exposure. Oral exposure occurs due to the leaching of BPA from polycarbonate containers and from the plastic lining of cans containing food and beverage .BPA is also detected in indoor air primarily associated with dust ,which indicates exposure can occurs through inhalation. BPA is also found in streams and rivers, and leaches from landfills , suggesting that BPA is a common contaminant of water used for drinking and bathing (11).

The present study aimed to evaluate the effect of high doses of BPA on thyroid gland function , liver enzyme and male sexual functioning.

MATERIALS AND METHODS

Animals: Adult male rats (*Rattus Novergicus*) weighing (225±10) g were caged in a well-ventilated animal house with a 12h dark/light cycle and controlled temperature and all had free access to standard diet and drinking water *ad libitum,* and allowed 7 days for acclimatization prior to dosing.

Experimental design:

A total of 24 adult male rats were randomly divided into four equal groups, six animals in each group. The rats groups were administrated BPA (Sigma- Aldrich, USA) orally by gavage with pure corn oil once daily for 30 days as follows: Animal of group (1) served as control and received a daily oral administration of corn oil throughout the experimental protocol. Animals of group 2,3 and 4 were administered orally 50,100 and 200 mg/kg body weight of BPA respectively dissolved in corn oil, the experiment extended for 30 days. At the end of the experiment ,the animals were anaesthetized with ether and blood samples were obtained by heart puncture .Serum samples were separated for measurement of thyroid stimulating hormone(TSH), triiodothyronin (T3) , tetraiodothyronin (T4), testosterone (T), follicle stimulating hormone (FSH), and luteinizing hormone (LH) concentrations, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes level. Immediately after blood samples were collected, animals were sacrificed and their thyroid glands, liver and testes, were rapidly excised and part of them immediately maintained in 10% formalin for histopathological study.

Hormonal assay {Enzyme-Linked Immunosorbent Assay(ELISA)}:

Measurement of serum TSH, total T3 and total T4 concentration are generally regarded as a valuable tool in the diagnosis of thyroid dysfunction; Kits were used (Monobid Inc. lake forest CA 92630,USA).

Serum T, FSH, and LH were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits provided by (Monobid Inc. lake forest CA 92630,USA).

Serum enzymes assay:

AST and ALT enzymes were determined by reagent kits (SPECTRUM AST and ALT-kits, Egypt) (12).

Epididymal sperms analysis:

Epididymal sperms were collected by the method of (13). The epididymis were cut into small pieces in 5ml of normal saline at 32c. The sperms obtained were used

for determination of sperm viability, sperm motility, and sperm count. Sperm viability test was done by the method as described in the WHO laboratory manual (14). The proportion of live spermatozoa can be determined by using staining technique of 0.5% eosin solution. Epididymal sperm motility was evaluated by the method as described by (15). Epididymal sperm count were counted by method as described by (14), by using improved Neubauer hemocytometer.

Histological study:

Thyroid gland, liver, and testis were cleared from the adhering tissues and were fixed in 10% formalin for histological studies. After dehydration in alcoholic series and cleaning in xylol, the tissue were embedded in paraffin wax. Sections were stained with haematoxylin followed by eosin and examined under light microscope.

Statistical Analysis of Data:

Statistical analysis was performed by one- way ANOVA (analyses Variation) followed by LSD test. Data were expressed as mean ± SD.

Statistical significance was set at P≤ 0.05 (SPSS, 2001).

RESULTS

The results of the present study (Table,1) revealed that no significant differences were was observed in serum TSH and T3concentration between BPA-treated groups and control. On other hand a significant (P≤0.05) decrease in serum concentrations of T4 in (50, 100 and 200) mg/kg bw BPA- treated groups compared with control .

Finally in the same table a significant (P≤0.05) increase in serum AST and ALT levels were found in all BPA-treated groups compared with control.

Table(1); Effect of BPA treatment for 30 days on thyroid hormones concentration and liver functions (Mean ±SD) .   n=6

Parameters / Groups	TSH µÙ/ml	T3 ng/ml	T4 ng/ml	AST U/L	ALT U/L
Control	0.38±0,01	0.35±0.02	8.64±·.51 a	46,59±5.76 d	21.01±2.47 b
BPA (50mg/kgbw)	0.39±0.02	0.35±0,03	8.04±0.15 b	I63.10 ±3.29 c	36.20 ±2.03 ab
BPA(100mg/kg bw)	0.39±0.02	0.36±0.02	7.24±0.20 c	176.60±8.00 b	62.49±4.44 a
BPA (200mg/kgbw)	0.40±0.1	0.36±0.01	6.27±0.21 d	288.00±9.08 a	92.81±3 60 a
LSD	NS	NS	0.59	13.49	41.48

Small different letters referred to significant difference between groups (P≤0.05).

Table (2) showed a significant (P≤0.05) decrease in serum testosterone and LH concentrations in all BPA-treated groups compared with control. On other hand non significant decrease in serum concentrations of FSH were observed in BPA-treated groups compared with control.

Table (2).Effect of BPA treatment for 30 days on serum T, FSH and LH hormones concentrations in adult male rats (Mean ±SD). n=6

Parameters / Groups	Testosterone(ng/ml)	FSH(IU/L)	LH(IU/L)
Control	9,54 ± 0.54 a	1.41 ± 0.38	7.57 ± 0.24 a
BPA 50mg/kg bw	8.50 ± 0.52 b	1.34 ± 0.43	6.53 ± 0.32 b
BPA 100mg/kg bw	7.12 ± 0.03 b	1.22 ± 0.34	5.90 ± 0.20 c
BPA 200mg/kg bw	5.10 ± 0.05 c	0.94 ± 0.38	5.10 ± 0.09 d
LSD	1,04	NS	0.63

Small different letter reflect to significant difference (P≤0.05).

The results mentioned in the table (٣) indicated a significant (P≤0.05) decrease in epididymal sperm count and sperms motility in adult male rats administered orally (50, 100 and 200) mg /kg bw BPA daily for 30 days compared with that of control. However no significant differences were noted in sperms viability between all BPA treatment groups and control.

Table (3).Effect of BPA treatment for 30 days on epididymal sperms characteristics in adult male rats (Mean ± SD). n=6

| Parameters / Groups	Sperm count (x10^6/ml)	Sperm viability (%)	Sperm motility (%)
Control	70.83 ± 9.15 a	79.50 ± 2.66	88.00 ± 1,67 a
BPA 50mg/kg bw	61.33 ± 8,75 b	79.00 ± 2.19	79.00 ± 2.09 b
BPA 100mg/kg bw	53.00 ± 5.86 c	78.00 ± 2.28	66,17 ± 5.74 c
BPA 200mg/kg bw	46.50 ± 5.01 d	78.17 ± 4.40	56.33 ± 5.27 d
LSD	6.50	NS	7.00

Small different letters referred to significant difference between groups (P≤0.05).

Histopathological Study

A histological examination of thyroid gland of control group revealed normal architecture of thyroid follicles of different sizes lined by single layer of epithelial cells, filled with colloid and normal Para follicular cells figure (1), while the thyroid glands of adult male rats treated with 50,100,and 200mg/kgbw daily of BPA showed thyroid follicles of different sizes with vacuolated colloid and thickening of Para follicular cells, and some follicles with flattened thyrocytes figures (2, 3, 4).

The liver sections of the male rats treated with 50,100 and 200 mg/kgbw daily BPA for 30 days demonstrated homogenization of the hepatocytes content with no clear demarcation between hepatocytes in 50 mg/kgbw treated group figure (6) compared with control figure   (5). On other hand clear flattening of hepatocytes with vacuolation, enlarged nuclei and disappearance of radiation structure of hepatocytes in 100 mg/kgbw BPA treated group was observed(7), while necrotic foci, disappearance wall of some hepatocytes, cytoplasmic vacuolation of hepatocytes with mild lymphocytic infiltration around central vein of male rats treated with 200 mg/kgbw BPA compared with control figure(8).

Finally the histopathological examination of the testes revealed normal appearance of seminiferous tubules with sperms in the lumen and normal inter seminiferous tubules Leydig cells figure (9). On other hand some seminiferous tubules have enlarged lumen with few spermatogonia and spermatids, some tubules have enlarged nuclei of sertoli cells in 50 mg/kgbw BPA treated group figure (10). Also disturbances in the structure of some seminiferous tubules including destruction in the epithelial lining of some seminiferous tubules and decrease of interstitial tissue in both 100 mg and 200 mg/kgbw BPA treated groups(11 , 12   ) compared with control.
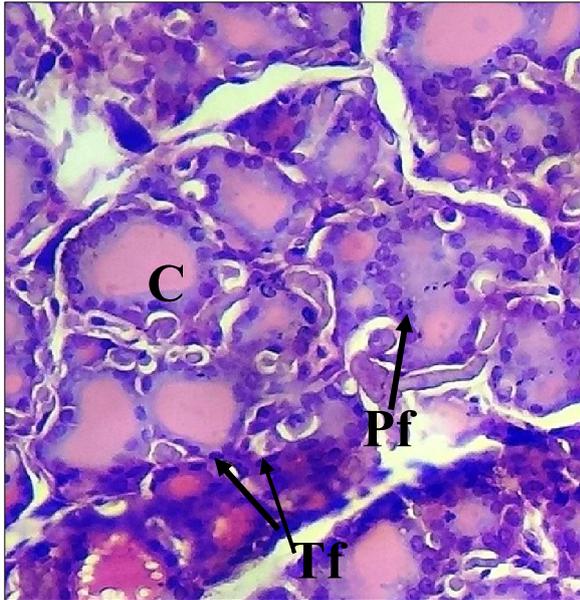
Figure(1).Thyroid gland of control group, showing normal thyroid follicles (Tf) filled with colloid(c) & parafollicular cells(Pf).(H&E).200X.
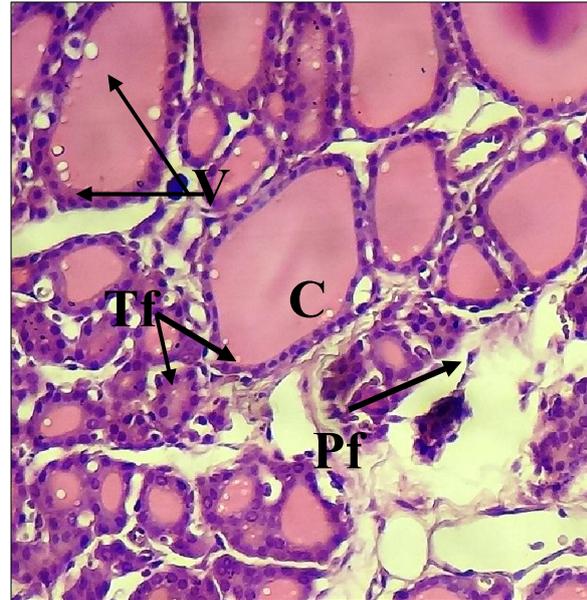
Figure(2).Thyroid gland of male rats group treated with 50mg/kg bw BPA, showing different sizes of thyroid follicles(Tf), filled with vacuolated colloid(v).(H&E).200X.
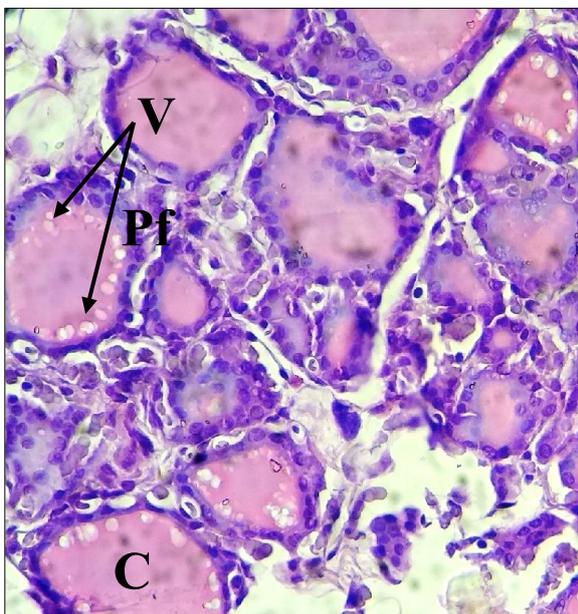
Figure(3). Thyroid gland of control male rats treated with 100mg/kg BPA, shows different sizes of thyroid follicles with vacuolated colloid (v).(H&E).200X.
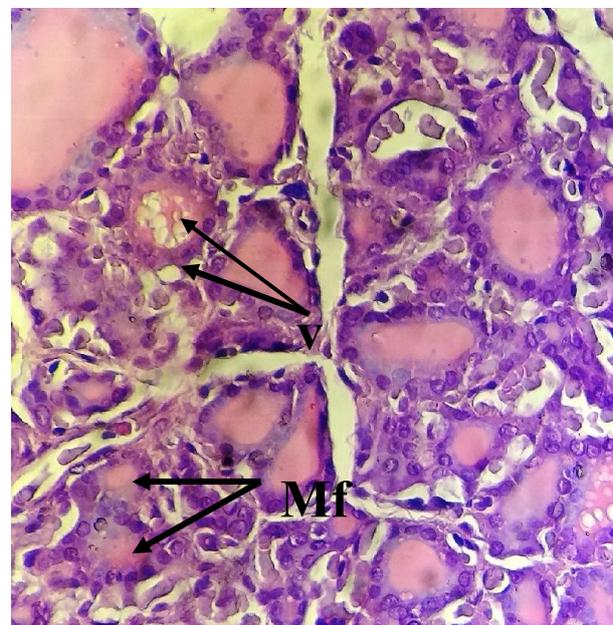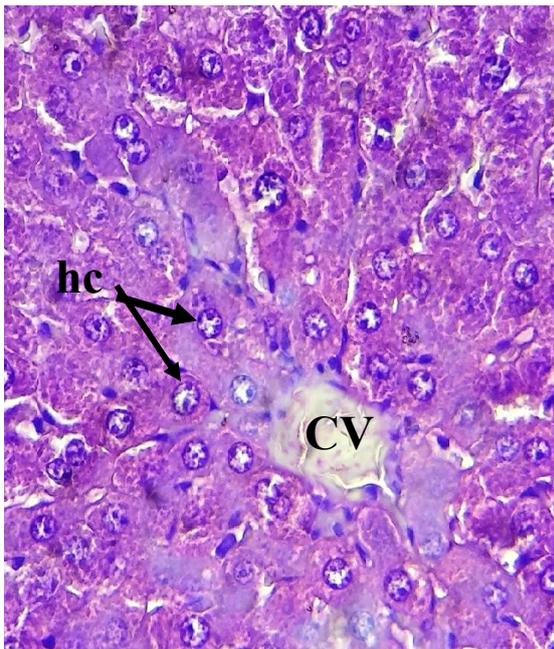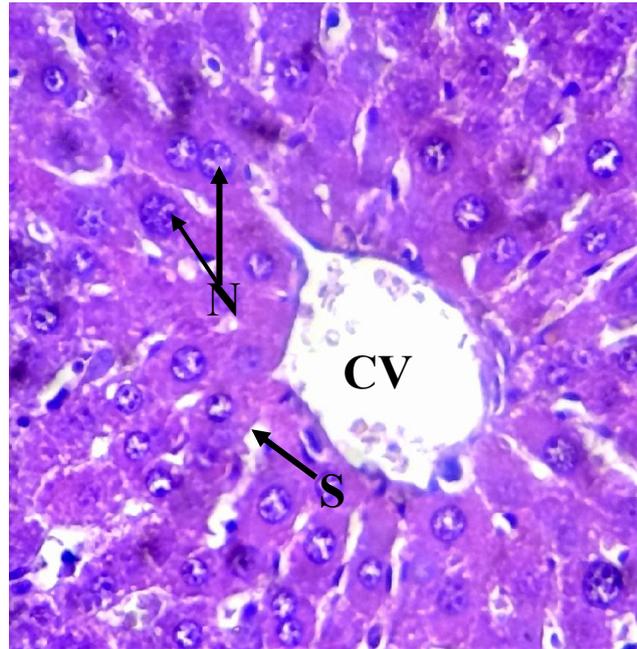

Figure (4). Thyroid gland of male rats group treated with 200mg/kgbw BPA, showing vacuolated colloid (v) and micro follicles (Mf). H&E.200X

Figure(5). Liver tissue of control group, showing normal architectures of central vein (cv) & intact hepatocytes (hc). H&E 400X

Figure(6). Liver of male rats group treated with 50 mg/kgbw BPA, showing dilated central vein (cv), enlarged nuclei (N) & widening of sinusoid (s). H&E. 400X.
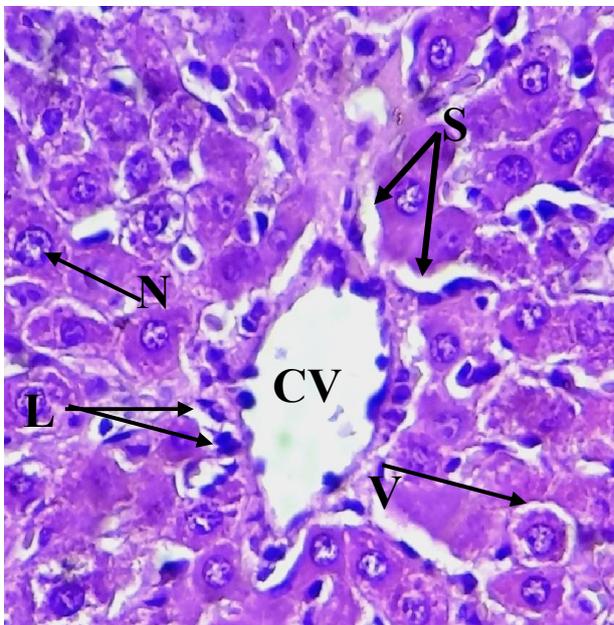
Figure (7). Liver of male rats group treated with 100 mg/kgbw BPA, showing dilated central vein (cv), dilated sinusoid (s), enlarged nuclei (N) & per central vein lymphocytic infiltration (L). H&E. 400X
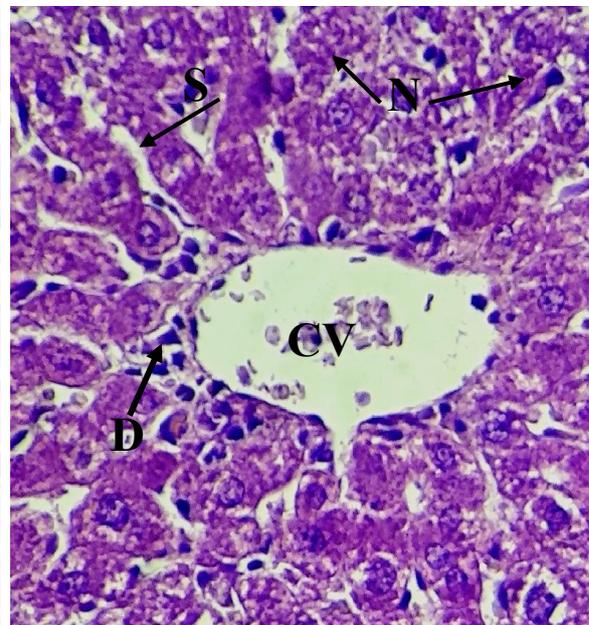
Figure (8). Liver of male rats trated with 200 mg/kgbw BPA, showing dilated central vein (cv), dilated sinusoid (S), per central vein degeneration(D) & necrotic area (N). H&E.400X.
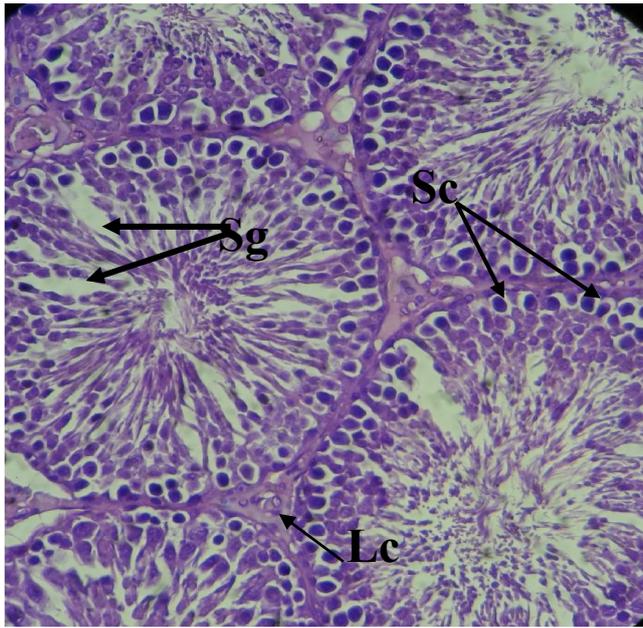
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Figure (9). Testis tissue of control male revealed normal appearance of seminiferous tubules with sperms in the lumen, spermatogonia (Sg), Sertoi cells (Sc) and leydig cells (Lc). H&E.200X.
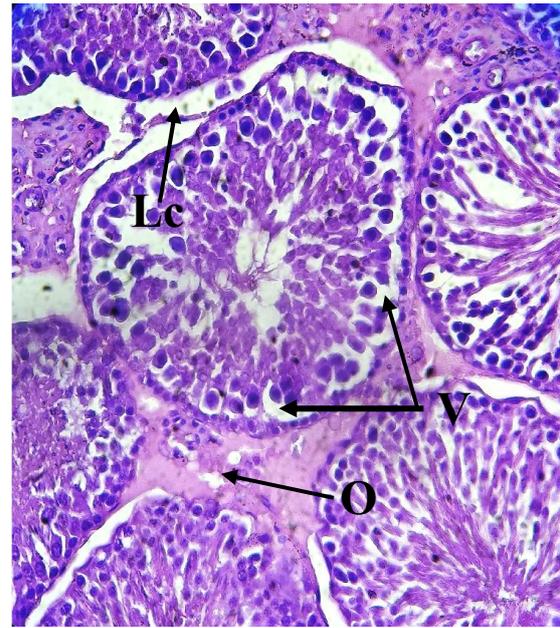
Figure (10). Testis tissue of male rats treated with 50 mg/kg BPA, showing decrease in leydig cells (Lc), vacuolation of spermatogonia and sertoli cells (v), interstitial oedema (O). H`&E. 200X

Figure (11). Testis of male rats treated with 100 mg/kg bw BPA , showing widdening of inter seminiferous tubules with decrease of Leydige cells (Lc), degenerated sperms in some tubules (Sp).H&E.200X

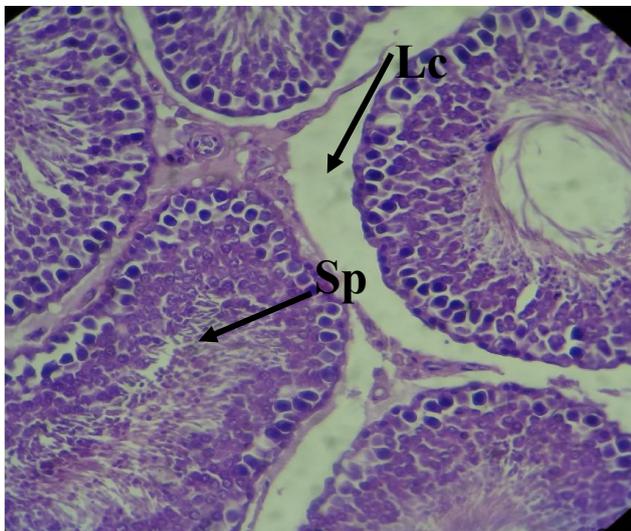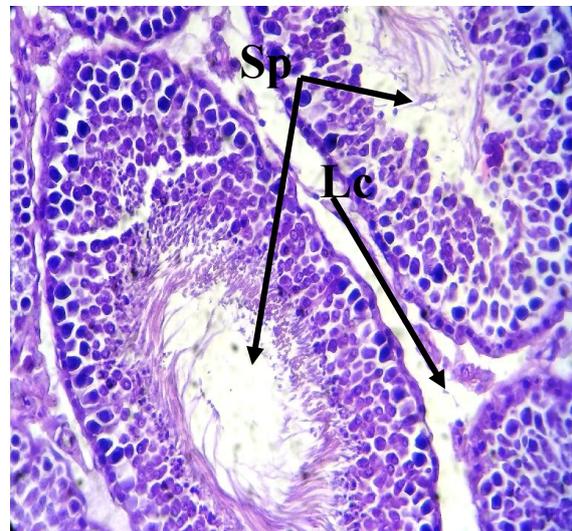Figure (12). Testis of male rats treated with 200 mg/kg bw BPA, showing arrested of spermatogenesis (Sp), decrease of interstitial leydig cells(Lc). H&E. 200X

DISCUSSION

The obtained results revealed that there was a significant decrease in serum T4 level in male rats treated with 50, 100, and 200 mg/kg bw/day BPA for 30 days compared with control. While no significant differences were observed in TSH and T3between all treated groups and control. BPA act as an agonist or antagonist of thyroid hormone receptor because of its structural similarity to thyroid hormone. Hence given that thyroid hormone receptor are expressed ubiquitously and abundantly in various organs, BPA may perturb thyroid hormone action throughout the body tissue (16. In contrast to the results of the present study either no effect or no consistent effects were found on thyroid hormones levels in adult male rats after BPA exposure (17).

On other hand prenatally exposured pups showed a significant increase in T4 levels (18). In vitro studies have demonstrated that BPA binds weakly to the thyroid hormone receptor and suppress transcriptional activity that is stimulated by T3 (7).Mechanistic studies indicate several mechanisms by which BPA may interfere with thyroid function. BPA inhibits human thyroperoxidase activity (19), and accordingly block T3-induced metamorphism of tadpoles (20). At receptor level BPA bind to thyroid hormone receptor as a weak ligand and act as antagonist to T3 thus inhibiting TR-mediated transcriptional activity (21 & 22).

Thyroxin binding proteins include thyroxin-binding globulin(TBG), transthyretin (TTR), and albumin, because T4 is more avidly bound to these proteins, it has a much longer half-life than T3. Adult male rats do not appear to produce TBG (23).,but pregnant females and pups produce high levels of TBG (24).There are chemicals that are well known to displace T4 from serum binding proteins such as polychlorinated diphenyl ether (25).Chemicals that can displace TH from these binding proteins may cause a very rapid decline in serum hormone levels. Xenobiotic chemical causes a decrease in circulating levels of T4, then serum TSH should increase. However there are a number of chemicals that cause decrease in serum total and free T4 without causing concomitant increase in serum TSH (26).The mechanism by which a toxicant can cause a decrease in the circulating level of T4 without affecting serum TSH level is not clear (27).

The reduction in serum T4 level may be related to the estrogenic action of BPA as that found by (28) who reported that estrogenic treatment in rats decreases serum

T4.Thus a given of endocrine disrupter can interfere with thyroid hormone synthesis, altering serum transport proteins or increasing catabolism of thyroid hormones (7).

The results as mentioned in the table (1) also revealed a significant increase in serum AST and ALT levels in male rats treated with 50,100 and 200 mg/kg bw/day BPA compared with control. These results were matched with the results obtained by (29) who reported an increase in the activity of AST in male rats treated with ≥ 200mg /kg /day. Similarly (30)demonstrated that oral administration of BPA(0.1, 10, and 50 mg /kg /day) to rats for four weeks causes significant increase in ALT and ALP levels. In agreement with the present study (31) found that serum AST and ALT levels increased in BPA group compared with control. It was suggested that PBA caused tissue injury in the liver, kidney, brain and other organs by formation of reactive oxygen species (ROS) (32).The significant increase in serum AST and ALT levels indicates to the damage in the cytosol and also in mitochondria(33).The dose-dependent elevation in serum AST and ALT levels indicated to the toxic effect of BPA witch confirmed by histopathological changes as seen in figures (6, 7, 8).

In the present study serum levels of T, and LH decreased significantly (P≤0.05) after 30 days of BPA   treatment compared with control. while no significant differences in serum FSH concentrations were observed between all treated groups and control Table (2) . These results are in agreement with (34) who demonstrated that exposure to environmentally relevant BPA levels has adverse effects on testicular function by decreasing pituitary LH secretion and reducing leydig cell steroidogenesis. Similar to the results of the present study serum testosterone concentration was decreased in the rats treated with high dose of BPA for four weeks (35). Although BPA suppress T production via decreased LH secretion ,there is also evidence that BPA interfere with LH receptor ligand binding (36). In the same line(37) found that plasma free testosterone levels were dramatically decreased following 8 weeks of BPA treatment compared with control. Mendiola et al (38) reported that in fertile men exposure to low level of BPA causes modest decrease in T levels.BPA has been shown to act as an androgen antagonist that interrupts normal androgen receptor binding activity and therefore the interaction between androgen receptor and endogenous androgen (39).In addition BPA has been reported to interfere with the function of leydig cells resulting in a reduction of testosterone

biosynthesis (34).The serum levels of T and FSH as well as the level of GnRH mRNA in BPA-rats were lower than those of control (40).

Unlike the present results no significant difference in plasma LH hormone levels were seen between BPA and control groups (41 & 37).Also in contrast to the present results (42) reported that plasma concentrations of LH were increased significantly in adult male rats treated with BPA (1mg/rat/day) subcutaneously for two weeks, while FSH levels in the plasma were not changed by BPA treatment. However the same researcher found that serum T concentrations were decreased significantly in BPA-treated group compared with control group similar to that found in the present study. The differences in the response of FSH and LH to BPA could be due to differential sensivity of the system regulatory FSH and LH secretion to BPA at the level of the pituitary or the hypothalamus (43).

The effect of BPA on epididymal sperm count, motility and viability revealed a significant decrease in sperm count and motility in BPA-treated groups compared with control group, while no significant differences in sperm viability were observed between BPA-treated groups and control Table (3). These results are in consistent with previous studies (44 & 45) who found that administration of BPA caused a reduction in the epididymal sperm motility and sperm count in dose dependent manner and the sperm viability remained unchanged. The results suggested that graded doses of BPA elicit depletion of antioxidant defence system and induce oxidative stress in epididymal sperm of rats. Similarly (46) reported that subcutaneous administration of BPA at dose of 200 mg/kg/day for four weeks significantly decrease the testis, epididymis, prostate and seminal vesicle weights and daily sperm production in wistar rats. In male mice injected with 25 and 100 microgram/kg bw BPA showed a significant reduction testicular sperm count and in the efficiency of sperm production. Epididymal sperm count were also significantly reduced in males that had observed in the offspring of the females exposed to BPA (47). Accordinance with present results (40) showed that oral administration of BPA 2 microgram /kg bw for 14 consecutive days in adult rats significantly reduced the sperm count and the number of germ cells compared with control. A significant increase in apoptosis of spermatocytes and spermatid was recorded by (48). In contrast to the present study Tyl et al (49) found no change in sperm motility of percent of normal sperm in mice exposed to as much as 600 mg/kg/day of BPA. Spermatogenesis is regulated by FSH and T released from the Leydig cells in

response to LH, in adult animals androgen acting on sertoli cells can promote and maintain the development of germ cells (50). FSH stimulate spermatogenesis through increasing the number of spermatogonia and enhancing subsequent entry of these cells into meiosis (50). Animal studies have shown that BPA affects the male reproductive system including androgen receptors, male sex hormones, male reproductive organs including testes, epididymis, seminal vesicles the prostate and sperm production (7 and 4). BPA has been show n to cause atrophy of seminal vesicles, prostate, and degeneration of testicular spermatogenesis in sprague-dawely rats (29).The decrease epididymal sperm motility and sperm count may be due to increased lipid peroxidation (51). A decrease in serum T also compromises the epididymal functions of synthesis and release of protein thereby decrease epididymal sperm motility (52). Anahara et al (53) demonstrated a decrease in actin binding necessary for sperm motility, via a decrease in cortactin expression in the testes.

Histopathological changes:

The thyroid gland of BPA treated rats showed dose-dependent histological changes as seen in figures(2, 3, 4).It has been suggested that BPA could act on thyroid glands through different mechanism such as interference with the binding of T3 to trasthyretin or antagonism to T3 binding to thyroid receptors (7).

On other hand liver rats treated with BPA in different doses showed widening of central vein, multinuclear giant cell in the liver, disappearance of radiation structure of hepatocytes and periportal , pericentral lymphocytic infiltration Similarly (54) found high incidence of multinucleated giant cells in the liver after administration of BPA in male and female mouse. AST and ALT are two enzymes of the most reliable markers of hepatocellular injury or necrosis which are recorded in the present study table (1). The liver of rats treated with BPA in different doses and periods revealed dilated and congested central vein, dilated sinusoid together with increased proliferation of von kupffer cells and lymphocytic infiltration, hyalinization of some portal veins with haemolysed blood cells and mild inflammatory areas in periportal zoon (55). Finally the histological changes of the rats testes exposed to the different doses of BPA demonstrated different degrees of changes in dose dependent manner included enlarged seminiferous tubules lumen with few spermatogonia and spermatids, some spermatogonia and sertoli cells have large nuclei and decrease the number of leydig cells. In agreement with the present results (46) showed that seminiferous tubules degeneration and loss of elongated spermatids were observed,

the severity being related to BPA dose. Hypoplasia of leydig cells was noted by (56). Salian et al (57) proposed that this effect is caused by disturbance to blood-testes barrier. Germ cells were detached from basement membrane of seminiferous tubules and sertoli cells in BPA treated groups and the administration of high doses of BPA produce both pyknotic nucleus and vacuolated nucleus in germ cells and germ cells (37).

Conclusion: The results of the present study demonstrated that exposure of adult male rats to high doses of BPA(50, 100, and 200) mg/kg bw orally has adverse effects on pituitary-thyroid axis, liver function and testicular function.

تأثير البسفينول أ على وظائف الغدد الدرقية والكبد والخصيتين في ذكور الجرذان البالغة

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الخلاصة

اجريت هذه الدراسة في كلية الطب ألبيطري/ جامعة البصرة لتقصي تأثير البسفينول أ على وظائف الغدد الدرقية والكبد والخصيتين في ذكور الجرذان البالغة. أستخدم في هذه الدراسة ٢٤ من ذكور الجرذان البالغة قسمت عشوائيا الى اربع مجاميع متساوية. المجموعة الاولى (السيطرة) جرعت زيت الذرة يوميا خلال فترة التجربة اما المجموعة الثانية والثالثة والرابعة فجرعت ب ٥٠ و ١٠٠ و ٢٠٠ / ملغم /كغم من وزن الجسم بسفينول أ مذابا في زيت الذرة على التوالي. استمرت التجربة لمدة ٣٠ يوما. أظهرت النتائج انخفاض معنوي بتركيز هرمون الدرقيين (T4) وارتفاع معنوي بتركيز بعض الانزيمات AST و ALT في مصل جميع المجاميع المعاملة بالبسفينول أ مقارنة بمجموعة السيطرة. في حين لوحظ انخفاض معنوي بتركيز هرمون الشحمون الخصوي (Testosterone) والهرمون اللوتيني (LH) في مصل جميع المعاملات مقارنة بمجموعة السيطرة. ومن ناحية اخرى لوحظ انخفاض غير معنوي بتركيز الهرمون المحفز للجريبات (FSH) في مصل جميع المعاملات مقارنة بمجموعة السيطرة. في حين اظهرت جميع المجاميع المعاملة بالبسفينول أنخفاض معنوي في كل من اعداد النطف البربخية وحركة النطف مقارنة بمجموعة السيطرة مع ذلك لم يلاحظ فرق معنوي في حيوية النطف بين جميع مجاميع المعاملات ومجموعة السيطرة. كما اظهرت الدراسة النسجية تغيرات في نسيج الغدة الدرقية اعتمادا ألجرعة ، كذلك لوحظ توسع الوريد المركزي ، خلايا كبدية عملاقة وتفجي الخلايا الكبدية في المجاميع المعاملة بالبسفينول ، كما يلاحظ تغيرات نسجية مختلفة في خصى الحيوانات المعاملة بالبسفينول بدرجات مختلفة اعتمادا على الجرعة.

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