

Histological study of Bisphenol-A effect on the adrenal gland in the female rats

دراسة نسيجية لتأثير مادة البسفينول على الغدة الكظرية في إناث الجرذان

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Abstract

The present study found that the great effects of Bisphenol-A (BPS) on adrenal gland. There were two groups of pregnant gestation rats that administered bisphenol-A at dose 50mg/kg orally, one treatment group give Bisphenol- A day, days 6 of gestation to delivery. Control group was administered with the corn oil . The administration was continuous for the rats fetuses until weaning period subsequently, the adrenal gland was taken from the sixteen puberty rats fetuses ,eight control and eight treatment with Bisphenol-A and Ex amined under light microscope . The histological results revealed that the cells of treatment adrenal gland contain less granules in cytoplasm than adrenal of group control, this study also showed that the cortex in treatment group thinner than cortex of control group in this state give indicate the treatment adrenal gland has weakness in their secretion. The medulla has numerous of follicles which contain (7-10) chromaffin cells as well as that, the treatment group thinner than control group.

المستخلص

اجريت هذه الدراسة على مجموعتين من الجرذان الإناث المختبرية أحدهما مجموعة سيطرة والآخرى حيوانات المعاملة. تم إعطاء الأولى (زيت الذرة) أما المجموعة الثانية تم تجريعها 50 ملغ/كغم من وزن الجسم بمادة البسفينول-أ معلقة في 0.25 مل من زيت الذرة. جرعت المجاميع من اليوم السادس من الحمل حتى الولادة. وبعد ذلك استمر التجريع على المواليد الحديثة حتى مرحلة الفطام، ثم أخذت الغدة الكظرية لستة عشر من الأجنة البالغة ثمانية منها حيوانات السيطرة والآخرى حيوانات المعاملة. أجريت عليها الدراسة النسيجية تحت المجهر الضوئي. أظهرت هذه الدراسة إن هناك تأثير كبير لمادة البسفينول-أ على الغدة الكظرية حيث وجدت إن مناطق القشرة في الغدة المعاملة بمادة البسفينول-أ تكون أقل قطرا كذلك إن الحبيبات الإفرازية أقل تواجدا في الساتوبلازم وتوزيع الخلايا في المناطق الثلاث غير منتظما مقارنة بغدد السيطرة. قد لوحظ إن منطقة اللب في غدد المعاملة أسمك وكذلك عدد الخلايا المتواجدة داخل الحويصلات أقل عددا مقارنة مع حيوانات السيطرة. قد تبين من خلال هذه الدراسة إن منطقة القشرة في حيوانات التجربة ضعيفة الإفراز بينما منطقة اللب أكثر نشاطا.

Introduction

Adrenal gland: Paired glands, each located at the superior pole of a kidney; consist of two distinct subdivisions with different embryological origins subdivisions cortex, derived from mesoderm and constitutes the major steroid producing and medulla, derived from neural crest and is a major source of epinephrine and norepinephrine neurohormones surrounded by a dense capsule (1). The cortex is subdivided histologically into three zones. Cells in the outer zone, or zona glomerulosa, are arranged in clusters (glomeruli) and produce the hormone aldosterone. In the zona fasciculata, which comprises the bulk of the cortex, rows of lipid-laden cells are arranged radially in bundles of parallel cords (fascies). The innermost zone of the cortex, the zona reticularis, consists of a tangled network of cells. The fasciculata and reticularis, which produce both cortisol and the adrenal androgens (2). The two medullar born catecholamines epinephrine and norepinephrine are classical acute stress hormones and function as “fight or flight” hormones. They modulate the cardiovascular system (increased cardiac output, vasoconstriction in the skin and guts, vasodilation of the arterioles in leg muscles), increase oxygen uptake and supply the body with energy in the form of glucose. Norepinephrine has a further, non-hormonal function and can act as a neurotransmitter (3). Bisphenol-A (BPA) is an industrial chemical, used to manufacture

polycarbonate and numerous plastic products including compact disks, food can linings, thermal (fax) paper, safety helmet, adhesives, powder paints. Recent studies have shown that it can leach out of certain products, including the plastic lining of cans used for food, polycarbonate babies' bottles and tableware, and white dental fillings and sealants (4). Low levels of (BPS) have also been reported to cause biological effects and its mode of action appears to mimic that of the female hormone, estrogen and is therefore classified as an endocrine-disrupting chemical (5),(6),(7). Thyroid and liver weight were significantly increased in the bisphenol-A treated group. No significant differences were detected in the testis, epididymis and adrenal weight of the treatment animals when compared with the control. Several *in vivo* studies on rats have also shown that nonylphenol caused lower epididymal sperm count, lower percentage of motile sperm and disrupt the development of the male reproductive system (8),(9). (10) observed that bisphenol-A affected the male rats' reproductive system by causing seminiferous tubule degeneration and loss of elongated spermatids. Other study is definitive and indicates that Bisphenol-A does not have an effect on puberty. For this reason, he did not believe additional studies on puberty would be particularly helpful (11). Bisphenol-A (BPA) has been demonstrated in both *in vivo* and *in vitro* experiments to act as an endocrine disrupting chemical (EDC). There is extensive evidence that BPA is an estrogen-mimicking chemical, although recent findings have revealed that BPA is a selective estrogen receptor modulator (SERM), since BPA and the potent endogenous estrogen estradiol do not always show identical effects, and in some studies BPA has been shown to antagonize the activity of estradiol. There is evidence that, similar to other estrogens, BPA can bind to androgen receptors and inhibit the action of androgen (12). Treatment of Leydig cells (the cells in the testes that secrete testosterone) in culture with a 2.3 pg/ml (0.01 nM) dose of bisphenol A resulted in a 25% decrease in testosterone synthesis associated with a decrease in the androgen-synthesizing enzymes (13). The previous finding suggest that bisphenol A inhibits the function of the NE transporter by acting on a site different from that of 17beta-estradiol in the adrenal medulla and probably in the brain noradrenergic neurons (14).

The aim of this study for explain the main of effect for Bisphenol-A on the histological characteristics in adrenal gland and study arrangement of adrenal cells in the layers.

Materials & Methods

Sixteen adrenal glands were used for histological study, eight from control group and eight from treatment group . The experimental animal was administrated of either 50 mg/kg.B.W of Bisphenol –A dissolved with (0.25)ml per animal of corn oil from gestation day 6 until weaning via their mother then F1 rat administration till day 90 of age. while, the control group was administered with the corn oil only. Histopathological evaluations were performed on the adrenal gland all experimental animal . The samples were immediately removed and fixed in 10 % buffered neutral formalin. Routine histological techniques were done on the samples. (Hematoxylin , eosin and Periodic-acid shiff (PAS) reagent were used for staining) was adapted from (15). Ocular micrometer was used for measurements. Analysis of data was done according to (16).

Results

Adrenal gland

The present study showed the adrenal gland consist of an outer cortex and an inner medulla, surrounded by a thin connective tissue capsule, the diameter of control group (16.8) μm while in treatment group (11.8) μm , The current results revealed that the septa of connective tissue and blood supply was weak in treatment group (Fig.1,2).

Control adrenal cortex

1-Zona glomerulosa

Is formed of columnar or rather pyramidal cells arranged in glomeruli-like structure, which are separated by delicate trabeculae extending from the capsule (Fig, 2). Its cells contain eosinophilic cytoplasm with fairly large rounded to oval having distinct nucleoli. The diameter of this zone (33.7) μm and cell diameter (7.8) μm .(Fig, 3).

2-Zona fasciculata

Is composed of polyhedral or columnar cells arranged in one or two cell thick in long radial cords or fasciculae and they are separated by narrowed blood capillaries. The cells have granulated eosinophilic cytoplasm embodying spherical basophilic nuclei showing distinct nucleoli. Binucleate cells are seen frequently. The diameter of this zone (151.2) μm and cell diameter (14.3) μm .(Fig, 3).

3-Zona reticularis

Is characterized by an irregular anastomosing network of intermingled cords .The cells of these cords are columnar cells having moderately eosinophilic cytoplasm, containing certain discrete granules and have rounded basophilic nuclei possessing centrally located. The diameter of this zone (496.2) μm and cell diameter (20.9) μm .(Fig, 5).

4-Medulla

The medulla in control group consist from numerous of follicles and chromaffin cells ,while the follicles in control group smaller in size. The blood supply less than treatment group. The diameter of medulla (215) μm while, the diameter of follicles (28) μm . (Fig,7)

Treatment adrenal gland

1-zona glomerulosa

This layer contain numerous of the cells arranged in glomeruli-like structure appearance as chromophopic cells and granules poorly, has basophilic nuclei rounded in shape. The diameter of zona glomerulosa (23) μm and cells diameter(3.1) μm , (fig,4).

2- Zona fasciculata

The cells in this layer appear as oval in shape are not arranged as a vertical column in adrenal treatment, but the cells spread and contain amount of lipid droplets while, the zona fasciculate cells in control group appearance was arranged as vertical column. The diameter Zona fasciculata (192.2) μm and cell diameter (16.8) μm (Fig, 4).

3-Zona reticularis

The cells in this layer characterized by small, irregular in shape and speared randomly. Each cell has basophilic cytoplasm granules and rounded nucleus, located in center of the cell, the diameter of zona reticularis (326) μm and cell diameter (9) μm (Fig, 6).

4-Medulla

Composed of numerous of follicles structure each follicles contains (7-10) cells called (chromaffin cells). The cellular limits was unobvious which form the syncitium contain basophilic granules, has nucleus oval in shape. The medulla in treatment group has highly vascularized. The diameter of follicles (43.7) μm and thicker of medulla(768.7) μm (Fig,6,8).

Table (1) Shows the thickness of structures & cells dimensions in adrenal gland

Structure	Thickness cont\group\ μm	Thickness\ treat\group\ μm	Diameter cell&fol\cont\ μm	Diameter cell\treat\ μm
Capsule	16.8 $\pm$	11.8 $\pm$	-----	-----
Zona glom	33.7 $\pm$	23 $\pm$	7.8 $\pm$	3.1 $\pm$
Zona fasc	192.2 $\pm$	151.2 $\pm$	14.3 $\pm$	16.8 $\pm$
Zona retec	496.2 $\pm$	326 $\pm$	20.9 $\pm$	9 $\pm$
Medulla	215 $\pm$	768.7 $\pm$	28 $\pm$	43.7 $\pm$

The number represent the mean $\pm$

Cont = control group

Treat = treatment group

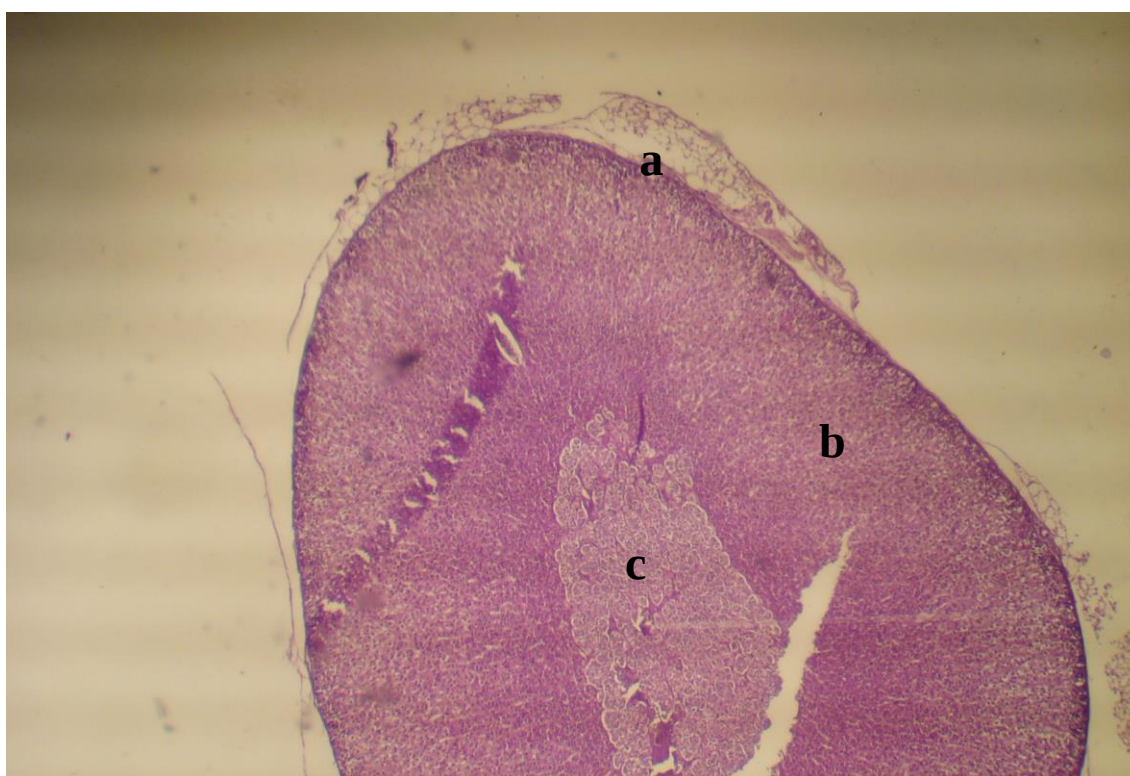
fol = follicles

μm = Micron

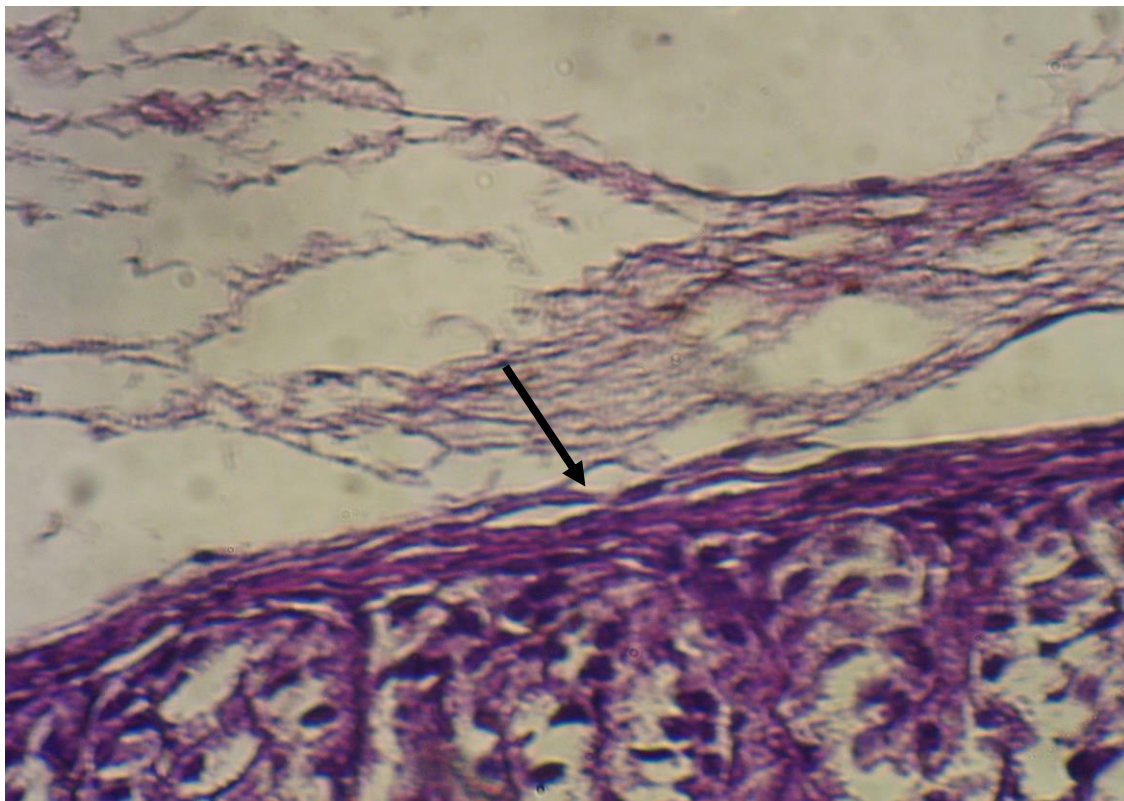
glom = Glomerulosa

fasc = fasciculata

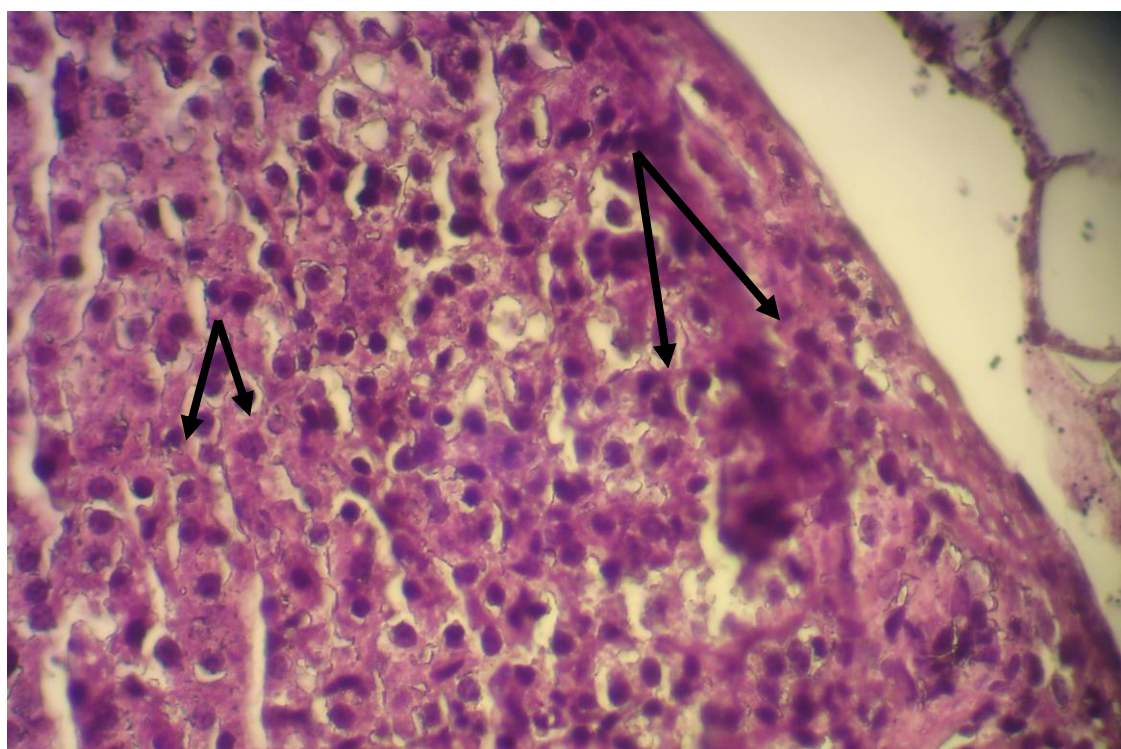
retec = reticularis



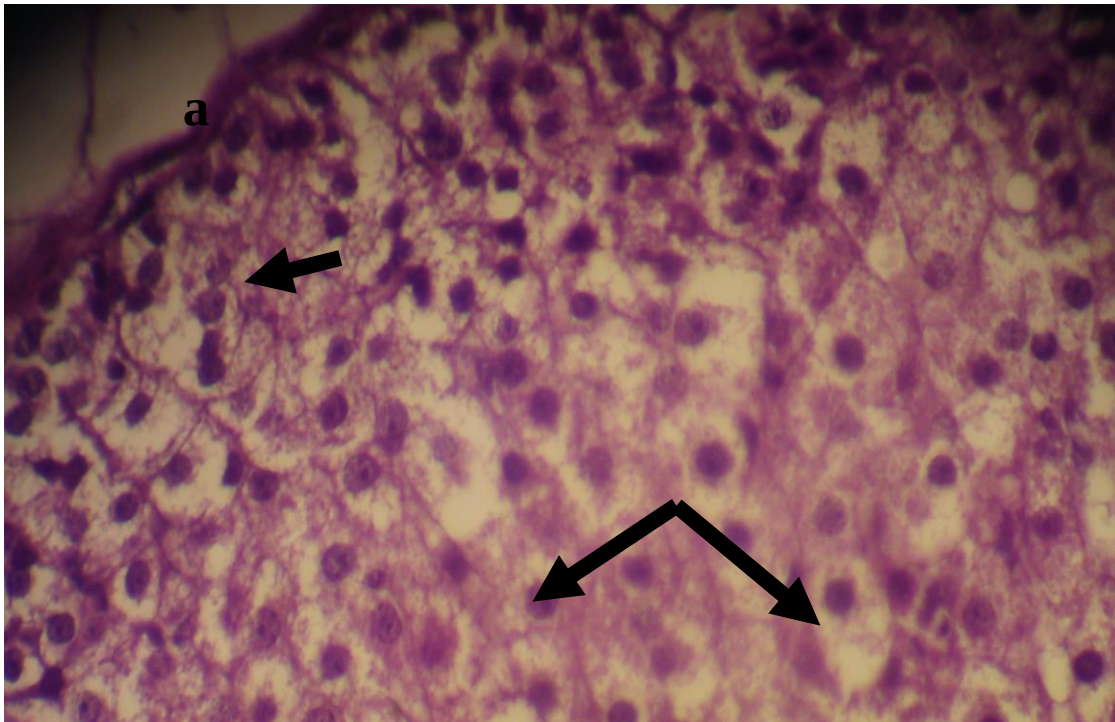
(Fig.1) Adrenal gland in treatment group showing capsule (a) cortex (b) and medulla (c) .H&E stain 4X



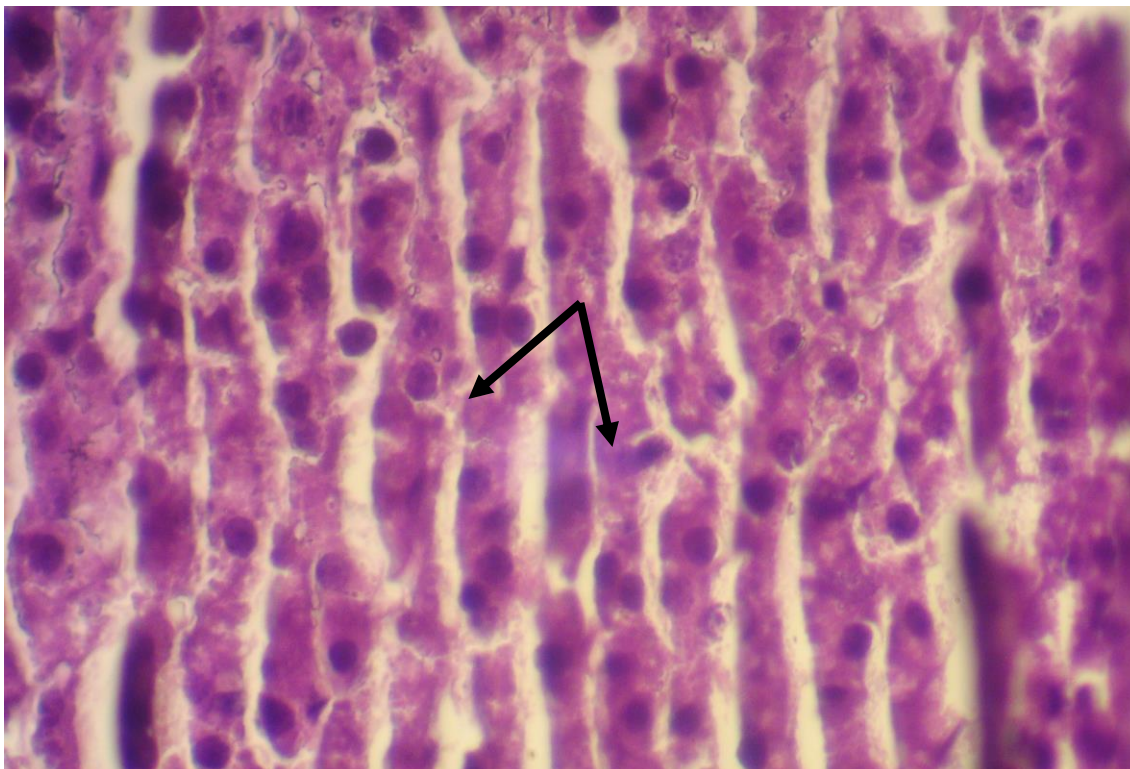
(Fig.2) Adrenal gland in control group showing the capsule (arrow). PAS stain 40X.



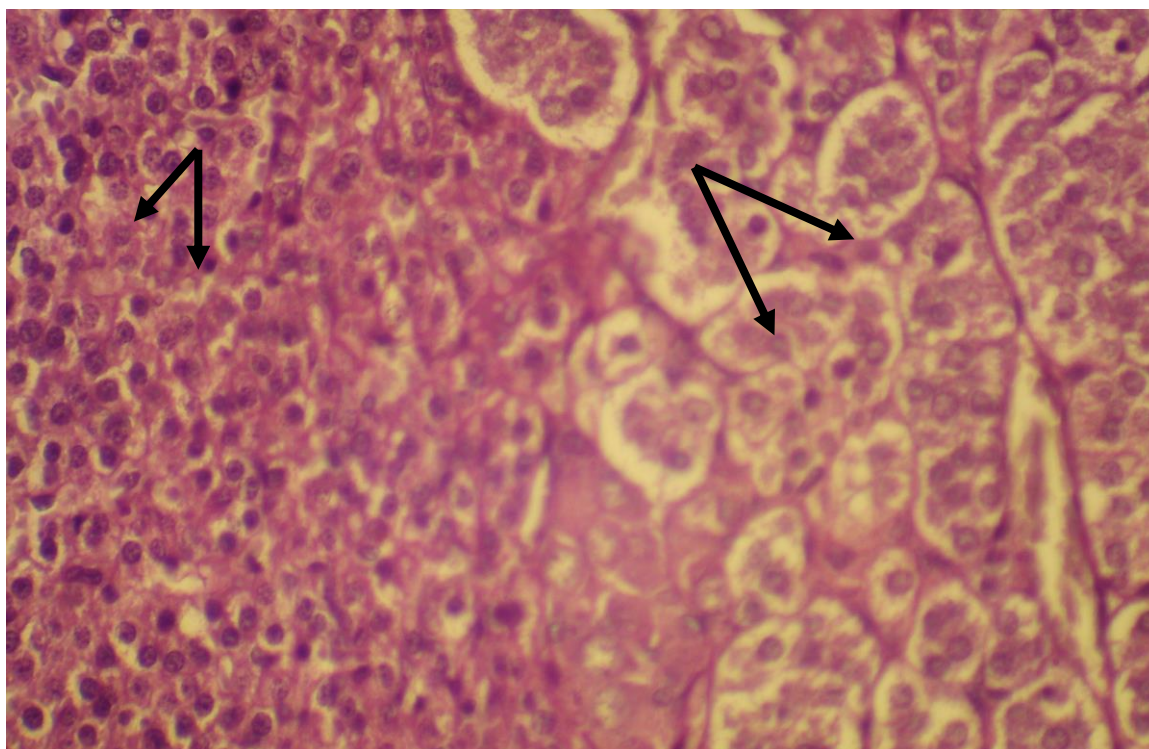
(Fig.3) Adrenal gland of control group, showing the cells in zona glomerulosa (large arrows) and the cells of zona fasciculata (small arrows) .H&E stain 40x.



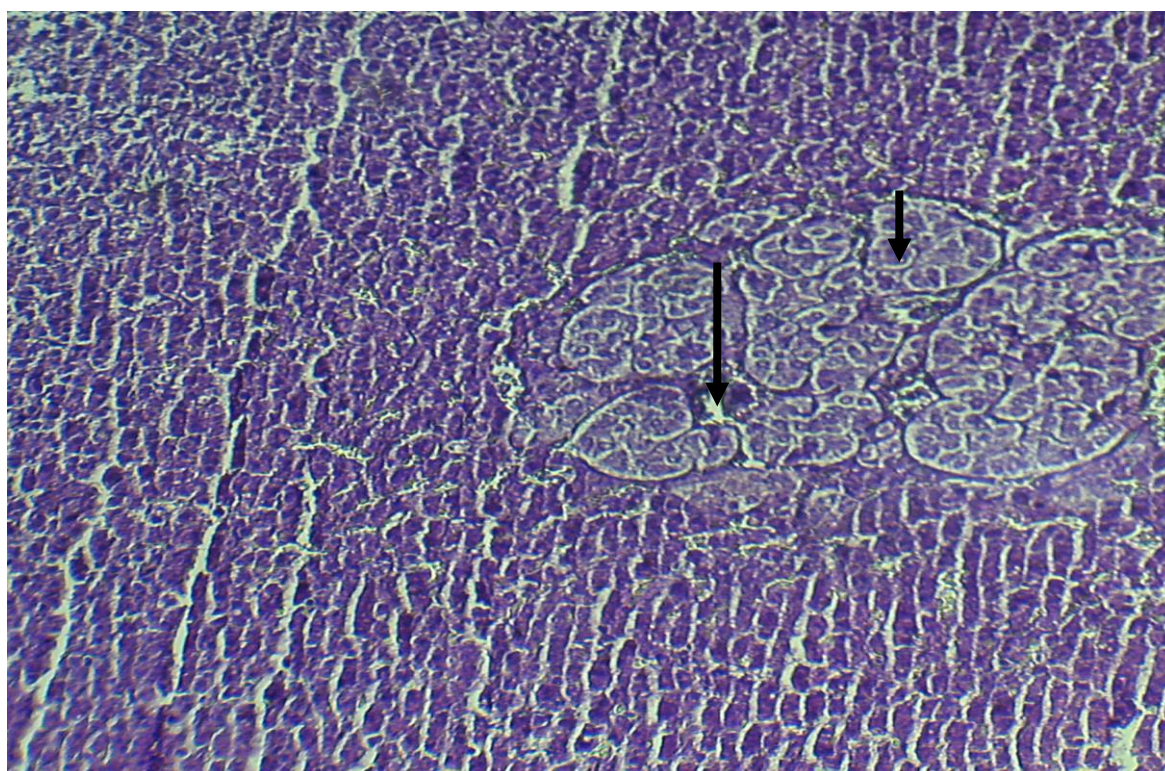
(Fig.4)Adrenal gland, treatment group showing capsule(a), the cells in zona glomerulosa,(small arrow) and cell of zona fasciculata lipid droplet (large arrow) H&E stain 40x .



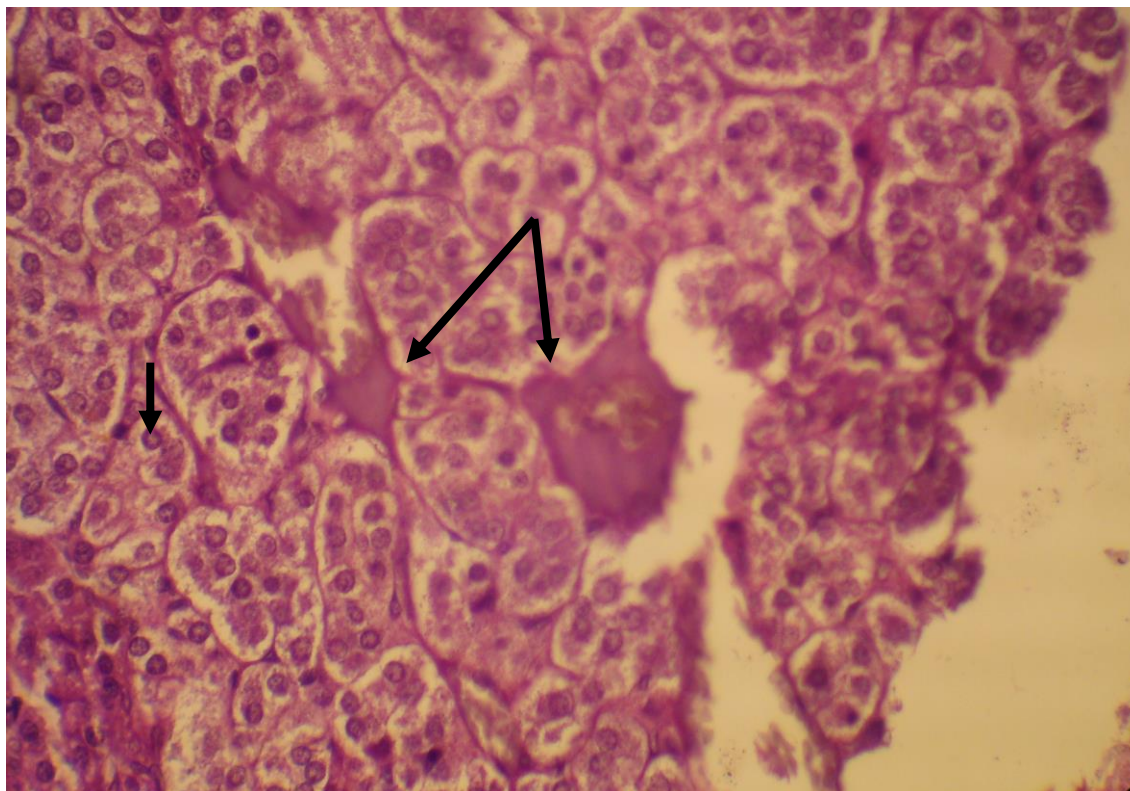
(Fig.5) Adrenal gland, control group showing the cells of zona reticularis (arrows) H&E stain.40x



(Fig.6) Adrenal gland, treatment group showing the cells in zona reticularis Spread as randomly (small arrows) and medulla cells (large arrows). H&E stain.40x



(Fig.7) Adrenal gland, control group showing the showing the chromaffin (small arrow) and blood supply (large arrows) cells in medulla region. PAS stain.10X



(Fig,8) Adrenal gland, treatment group showing the chromaffin (small arrow) and blood supply (large arrows) cells in medulla region. H&E stain.40X

Discussion

The cells of zona glomerulosa, zona fasciculata in cortex of adrenal gland of treatment a group was appear as chromophopic cells and poor for granules when compare with control group, give it a evidence for effect of Bisphenol-A on the adrenal gland that may indicate a special metabolic and hormonal activity. The present result different with the finding of (2) who described the cells in the outer zone, or zona glomerulosa, were arranged in clusters (glomeruli) and produce the hormone aldosterone. In the zona fasciculata, which comprises the bulk of the cortex, rows of lipid-laden cells are arranged radially in bundles of parallel cords (fascies). The innermost zone of the cortex, the zona reticularis, consists of a tangled network of cells. The cells spread as randomly in cortex of adrenal treatment this spread indicate to the hormonal disturbance. In this study I am agree with (5),(6)and (7) whom give an importance role to the low levels of bisphenol - A have also been reported to cause biological effects and its mode of action appears to mimic that of the hormone, estrogen and is therefore classified as an endocrine-disrupting chemical. The current result explained that the three zones in treatment group thicker than of zones in control group , gives evidence that the control group is more active from the treatment group, In this concern our results in agreement with (17) reported reduced adrenal weights in their bisphenol- A treated rats at doses of 50 mg/kg per day in a three generation reproductive toxicity study of dietary Bisphenol- A. In this study showed the accumulation of lipid droplets throughout the cytoplasm of the three zones cells of treatment adrenal, give evidence is more active. (18) who explained that the most striking change is the enlargement and extensive accumulation of lipid droplets throughout the cytoplasm of the three zones cells, this alteration was also reported an inhibitor secretion. The current study revealed the presence of large amount of connective tissue septa which carry high blood supply in medulla treatment and larger in diameter from control medulla, give an evidence of the activity of medulla in treatment group. The present result was in variance with (14) who found that the suggest

that bisphenol-A inhibits the function of the NE transporter by acting on a site different from that of 17 β -estradiol in the adrenal medulla and probably in the brain noradrenergic neurons (14).

References

- (1) Bakurer, A. S. and Bigbee, J. W. (2004). Digital histology an interactive CD atlas with Review text. Johan Wiley simultaneously in Canada . PP :156-162.
- (2) Maurice, H, (2003).Basic medical endocrinology. 3rd ed Mc.
- (3) Engelhardt WV & Breves G (2000). Nebennierenhormone. In Physiologie der Haustiere, Stuttgart: Enke im Hippokrates Verlag.
- (4) Lyons, G. (2000) Bisphenol A. A Known Endocrine Disruptor.A WWF European Toxic Programme Report. WWF, UK. Matthews, J.B., Twomey, K., Zacharewski, T.R., 2001. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. Chem. Res. Toxicol. 14 (2), 149_/157.
- (5) Nagel, S.C., vom Saal, F.S., Thayer, K.A., Dhar, M.G.Boechler, M., Welshons, W.V.(1997) Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the Xenoestrogens bisphenol A and octylphenol. Environ. Health Persp. 105 (1), 70_/76.
- (6) Vom Saal, F.S., Cooke, P.S., Buchanan, D.L., Palanza, P., Thayer, K.A.,Nagel, S.C., Parmigiani, S., Welshons, W.V.(1998) A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. Toxicol. Ind. Health 14 (1_/2), 239_/260.
- (7) Kloas, W., Lutz, I., Einspanier, R.(1999) Amphibians as a model to Study endocrine disruptors: II Estrogenic activity of environmental chemicals in vitro and in vivo. Sci. Total Environ. 225, 59_/68.
- (8) Lee, P.C., Arndt, P., Nickels, K.C.(1999) Testicular abnormalities in male rats after lactational exposure to nonylphenols. Endocrine 11 (1), 61_/68.
- (9) de Jager, C., Borman, M.S., Oosthuizen, J.M.,(1999) The effect of p -nonylphenol on the fertility potential of male rats after gestational, lactational and direct exposure. Andrologia 31 (2), 107_/113.
- (10) Takahashi,O., Oishi, S., 2001. Testicular toxicity of dietary 2,2-bis(4- hydroxyphenyl)propane (bisphenol A) in F344 rats. Arch. Toxicol. 75(1), 42_/51.
- (11) Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, et al.(2008) Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1(R) (Swiss) mice. Toxicol Sci 104(2):362-384.
- (12) Wilson NK, Chuang JC, Lyu C, Menton R, Morgan MK.(2003) Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home. J Expo Anal Environ Epidemiol 13(3):187-202.
- (13) Frederick S.(2011) Report of Very Low Real World Exposure to Bisphenol A is Unwarranted Based on a Lack of Data and Flawed Assumptions. Division of Biological Sciences, University of Missouri, Columbia, Missouri65211; †Department of Urology, University of Illinoisat Chicago, Chicago, Illinois60612; and ‡Department of Biomedical Sciences,UniversityofMissouri,Columbia,Missouri65211.
- (14) Toyohira, Y., K. Utsunomiya, S. Ueno, K. Minami, Y. Uezono, R. Yoshimura, M. Tsutsui, F. Izumi and N. Yanagihara (2003). Inhibition of the norepinephrine transporter function in cultured bovine adrenal medullary cells by bisphenol A. Biochem. Pharmacol. 65:2049-54.
- (15) Luna , G . (1968) . Manual of histological staining method of the armed forced institute of pathology . 3rd ed Mc . Graw hill book Co . New York.
- (16) Al- Mohammad , N. T. ; AL- Rawie, K. M; Younis, M. A & AL-Marany, W. K. (1986). Standard of statically. Dar alktib linasher. Musel.
- (17) Tyl, R.W., Myers, C.B., Marr, M.C., et al.(2002) Three generation reproductive toxicity study of dietary bisphenol A in CD Sprague_/Dawley rats. Toxicol. Sci. 68 (1), 121_/146.
- (18) Szabo D, Toth IE . and Szalay KS.,1996. Viscosity of rat adrenocortical lipids in different functional states: morphological characteristics. J. SteroidBiochem Mol Biol., 58: 329-35.