

HISTOPATHOLOGICAL STUDY OF LUNG, KIDNEY, SPLEEN AND PROSTATE IN ADULT MALE RATS TREATED WITH BISPHENOL A

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ABSTRACT

The present study performed to evaluate the effect of Bisphenol A on some organs (lung, kidney, spleen and prostate) in male rats in veterinary medicine college/university of Basrah. A total of 24 adult male rats were randomly divided into four equal groups, six animals in each group. Animal of first group served as a control and received daily an 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 histopathological in lung of male rats treated with different dose of BPA revealed peribronchial inflammatory cells, necrosis in pulmonary cells, moderate to severe edema and emphysema, hyperplasia of bronchiolar epithelial cells. The histopathological examination of kidney demonstrated vacuolation of epithelial cells lining renal tubules, hemorrhagic area in renal tubules in male treated group with 50 and 100 mg/kg B.w. The histopathological section of spleen revealed moderate to severe atrophy in white pulp. Finally the result of prostate sections showed degeneration of epithelial cells and hyperplasia of lining epithelial of papillary projection.

INTRODUCTION

Bisphenol A (BPA), a key monomer in the production of polycarbonate plastics and epoxy resin, is widely used in a variety of products, including food and beverage packaging, compact disks, eye glass lenses, dental sealants, artificial teeth, cans, drums, reinforced pipes, adhesives, nail polish and carbonless papers used in receipts making BPA a

ubiquitous part of our daily life[1] and [2].BPA can be hydrolyzed under high temperature and acidic or basic condition leading to leaching into food dring container. [3].

BPA can contaminate the environment in significant amount by leaching from products(plastic food and water containers) and as by products of manufacturing[4].In rodent,BPA is associated with early sexual maturation ,altered behavior,and effects on prostate . There are several reports suggesting testicular toxicity of BPA in rats and mice [5] [6].Accumulation of BPA in male reproductive organs have some clinical implications since exposure to low doses of BPA during fetal life has been shown to decrease the efficiency of sperm production in the offspring of male mice[7]. [8]studied the effect BPA on lung of adult male albino rats by using histological and immunohistochemistry method.

The present study has been aimed at investigating the effects of BPA on kidney,lung,spleen and prostate, in male rats.

MATERIAL AND METHOD

3.2:Experimental Animals:

Atotal of (24) Adult Male rats (*Rattusnovergicus*) were used in this study, their weights were ranged from (225-10 grams). They were taken from the animals house / Veterinary Medicine College / University of Kufa ; and housed in laboratory animal house in College of Veterinary Medicine / University of Basrah, with (20±25°C) temperature. Lightness system was (10+ 14) hrs. darkness/lightness and mechanical ventilations were used to control the suitable humidity. They were kept for adaptation in these environments for 7 days before the study. These animals were divided into four equal group and put in specific plastic cages. The rats in each cage were only six to avoid crowding, the nutrient for rats was pellet, and water *ad libidum* until the end of the study. Throughtout the experiments, a plastic cage contained hard-wood chip as bedding. The bedding was changed continuously to ensure a clean environment .

3.5: Experimental design:

The experiment animals were divided into four groups each group contained six male rats as the following :

- **Group (1):** received a daily oral administration of corn oil throughout the experimental protocol doses daily for (30) days .
- **Group (2):** received (50mg of bisphenol A / per Kg) dissolved in corn oil daily by gavage for (30) days which served as low dose group.
- **Group (3):** received (100mg of bisphenolA per Kg) dissolved in corn oil daily by gavage for (30) days which served as intermediate dose group.
- **Group (4):** received (200mg of bisphenol per Kg)dissolved in corn oil daily by gavage for (30) days which served as high dose group.

: Histological study:

At the end of the experiment, (30) days. The rats were generally anaesthetized by inhalation of Chloroform and then sacrificed.

Then the following internal body organs (lung,kidney,spleen and prostate)were isolated and put in petri dish containing normal saline to remove the adipose tissues which were attached to it, then put in containers containing formalin (10%) as fixative solution for histological preparations .

According to [9] and [10], The preparation of histological sections include:

Washed the specimen with running water after fixed in formalin then that dehydrated in serial alcohol concentrations by upgrading the alcohols (70-80-90-100%) to absolute alcohol overnight. The specimen cleared in two stages of xylene, the tissue infiltrated with paraffin at (56-57°C). Then embedded in paraffin followed block making. Thin tissue section was cut at (6 µm) with rotary microtome.

The section was mounted on the glass slides, attached to the slide its surface smeared by small drops of Mayer's albumin then put on hot plate at (40C⁰) for (24) hours. The specimens were stained with hematoxylin-eosin. The slides were then examined by light microscope to detect any changes.

RESULT

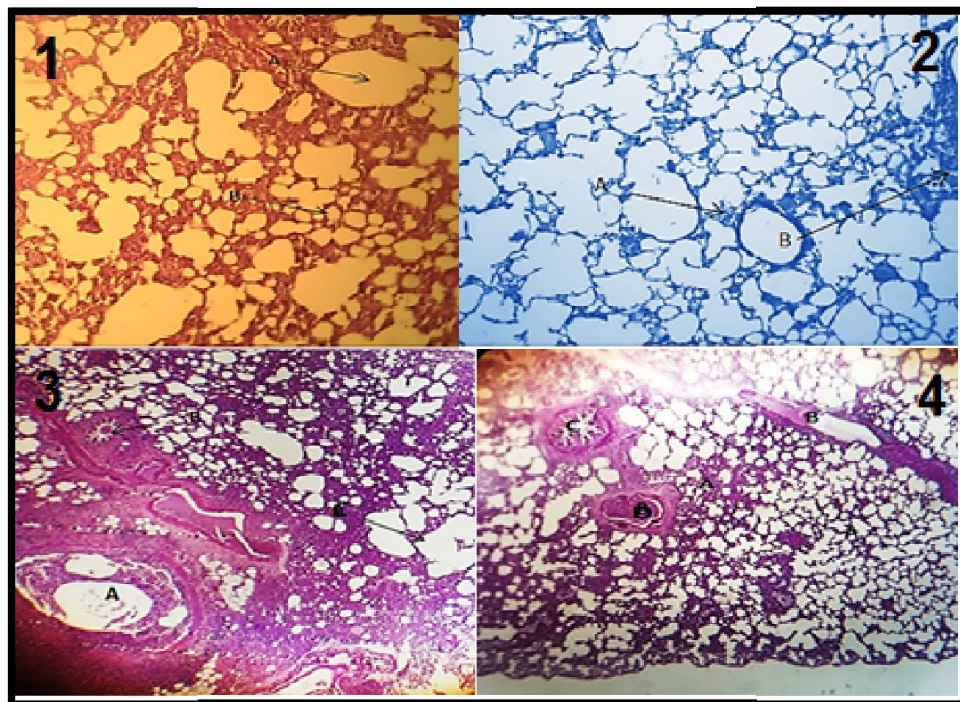
The histological examination in lung of the control group revealed normal structure of alveoli ,bronchioles ,bronchi and other tissue and blood vessels as shown in figure(1). While the histological changes in lung of adult male rats treated with 50,100 and 200mg/kg B.w daily of BPA,shown peribronchial inflammatory cells aggregation ,necrosis in alveoli,moderate to sever edema and emphysema, congestion of blood vessels, infiltration of eosinophil and hyperplasia of bronchiolar epithelium cells figure(2,3,4).The histological examination of kidney of the control group revealed normal structures which contain normal glomeruli, proximal and distal convoluted tubules as in figure (5).Histopathological examination of kidney tissue of the male rats treated with 50mg/k.g.b.w.daily BPA for 30 days demonstrated vacuolation of epithelial cells lining renal tubulesfigure(6),narrowing lumen sections,While male rats treated group with 100 mg/k.g BPA revealed vacoulation in glomeruls cell,massive hemorrhagic area and infiltration of the inflammtary tuftt surrounding blood vessels. Figure(7). On the other hand sever vacuolation in glomerular capillary tuft,necrosis in the epithelial cells lining glomeruli and renal tubuli and atrophy in glomerular capillary tuft also showed sever vacuolation in renal tubular and necrosis in the epithelial cells lining renal tubuli and atrophy in renal tubular in male rats treated with 200mg/k.g.b.w.figure(8).

The histopathological examination of spleen of the control group showed normal structure which composed of normal white and red pulp, normal capsule and blood vessels as in figure (9).

The histological section of spleen of male rats treated with 50 mg/k.g /. b.w showed atrophy in white pulp and odematous in red pulp, ,congestion around blood vessels with fibrosis figure. (10) .On the other hand the histological examination of spleen of the treated group with 100mg/k.gb.w. showed atrophy of lymphoid tissue of white pulp, while the red pulp showed foamy vacuolated macrophages as in figure. (11) . Also the spleen of high dose group200 mg/k.g. showed severe atrophy of lymphoid tissue of white pulp while the red pulp showed foamy vacuolated macrophages, congestion of sinusoids and hemosiderin laden macrophages as shown in figure (12).

Microscopic examination of prostate of control male rats male shows normal prostatic gland and lobules containing *mucous secretory units (acini)* figure.(13) . The

prostate section of the male rat treated with 50,100 and 200mg/kg B.w. daily BpA for 30 days demonstrated degeneration of epithelial cell and sloughing of some epithelia and hyperplasia of lining epithelia and of papillary projections toward alveolar lumen , sever hyperplasia found in most of acini ,the mass of hyperplasia obstructed the lumen of some alveoli in addition to the presence of papillary projections in other alveoli figure(14,15,16).

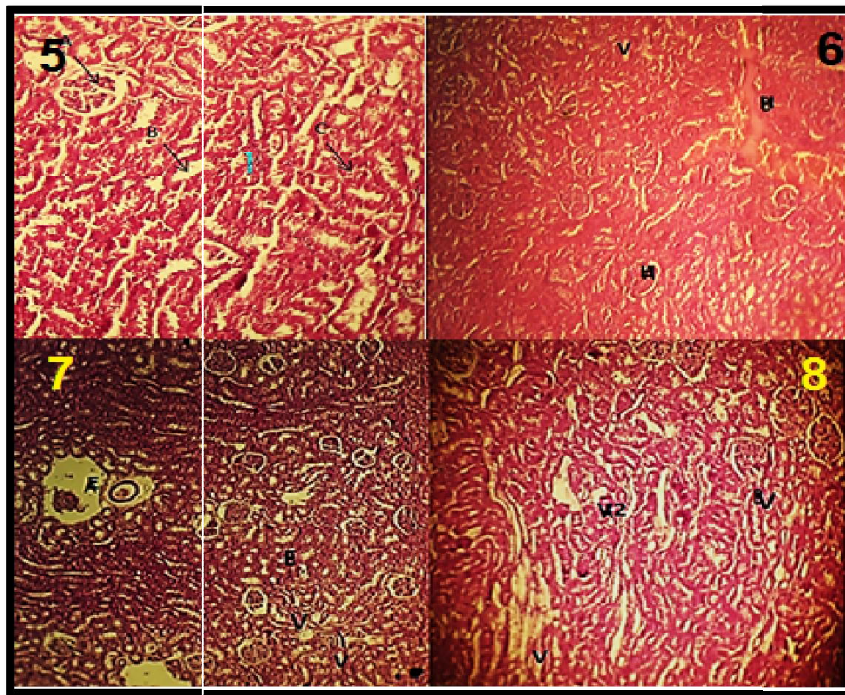


Figure(1):Transverse section through the lung of control group showing A.alvoli.B.Bronchiolei (H&E stain 100X).

Figure(2):Transeverse section through the lung of male rats group treated with 50mg/kg.B.W.BPA showing A.peribronchial inflammatory cells B.,congestion of blood vessels(H&Estain 100X)

Figure(3):Transeverse section through the lung of male rats group treated with 100mg/k.g.B.W.BPA showing A.necrosis inflammatory tissue.B.edema C.hyperplasia in bronchiole.D.congestion in blood vessel(H&E stain 100X).

Figure(4):Tranverse section through the lung of male rats treated with 200mg/k.g.B. W.showing.A.emphysema.B.hyperplasia of bronchiolar.C.sever edema(H&E stain 100X).

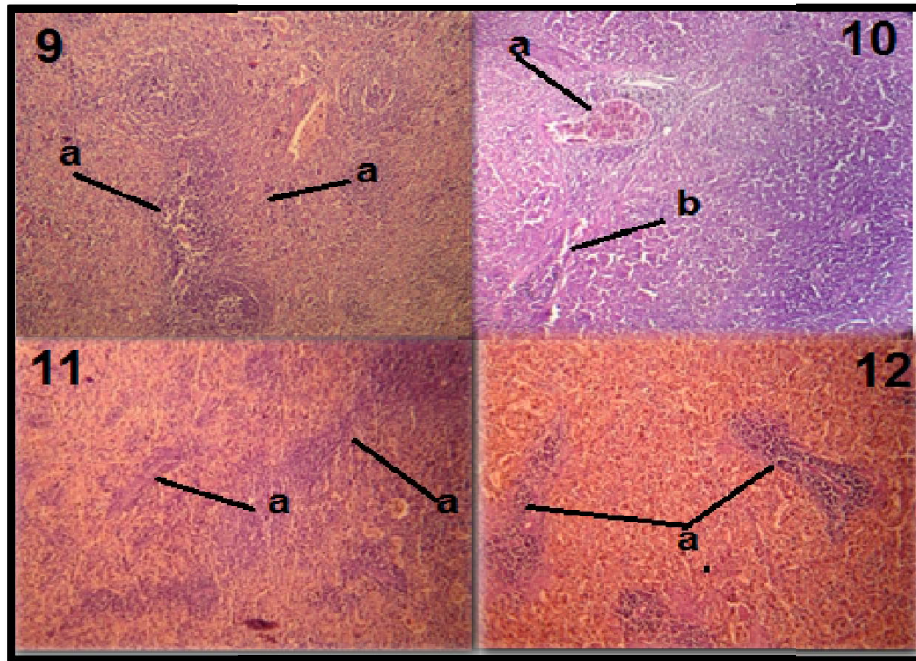


Figure(5):Tranverse section through the kidney of control group showing A.glomeruli.B.proximal convoluted tubule C.Distal convoluted tubule (H&E stain 100X).

Figure(6):Tranverse section through kidney of mals rats treatedgroupwith50mg/kg.B. W.BPAshowing V.vacuolation of epithelial cells.H.hemorrhage blood vessl.C.degenration of epithelial cell (H&E stain 100X).

Figure(7):Tranverse section through kidney of mals rats treatedgroup with with100mg/kg.B. W.BPAshowing.v.vacuolation.E.edema.C.congestion of blood vessel. (H&E stain 100X).

Figure(8):Tranverse section through kidney of mals rats treatedgroup with with200mg/kg.B. W.BPA showing.V1.sever vacuolation of mesengeal cell.V2 sever vacuolation in renal tubule.B.atrophy of glomeruli.(H&E stain 100X).

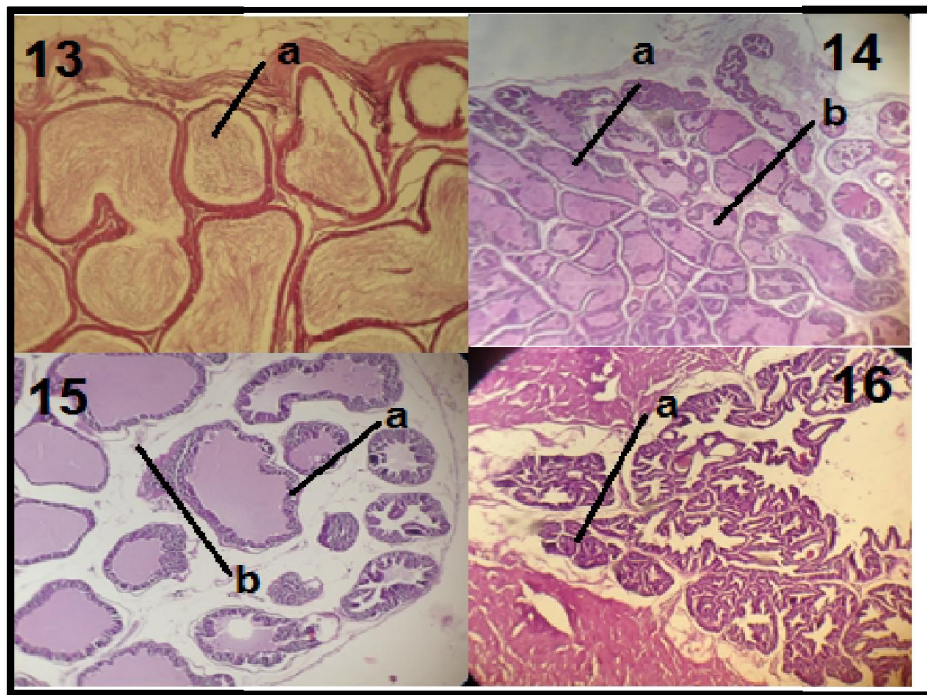


Figure(9):Tranverse section through the spleen of mals rats control group showing normal structure.A.Redpulp.B.white pulp (H&E stain 100X).

Figure(10):Tranverse section through spleen of mals rats treated groupwith50mg/kg.B.W.BPA showing A.congestion of blood vessel.B.mild atrophy of white pulp (H&E stain 100X).

Figure(11):Tranverse section through spleen of mals rats treated group with with100mg/kg.B.W.BPA showing A..moderate atrophy of white pulp (H&E stain 100X).

Figure(12):Tranverse section through spleen of mals rats treated group with with200mg/kg.B.W.BPA showing A..sever atrophy of white pulp (H&E stain 100X).



Figure(13):Tranverse section through prostate of mals rats control group showing normal prostate gland (H&E stain 100X).

Figure(14):Tranverse section through prostate of mals rats treated group withwith50mg/kg.B.W.BPAshowing A.edema.B.hyperplasia (H&E stain 100X).

Figure(15):Tranverse section through prostate of mals rats treated group withwith100mg/kg.B.W.BPAshowing A..hyperplasia in most acini.B.edema (H&E stain 100X).

Figure(16):Tranverse section through prostate of mals rats treated group withwith200mg/kg.B.W.BPAshowing A.sever.hyperplasia (H&E stain 100X).

DISCUSSION

The present histopathological study of lung of BPA treated group revealed congestion, focal perivascular and bronchiolar lymphoid aggregation and mononuclear cell infiltration between alveoli, in majority of animal, Alveolar congestion mild to moderate emphysema, these results were similar to those observed in group II&III [11][12]. Also these result of present study similar by (8) showed disrupted lung architecture, congested blood vessels in male rats treated with BPA. The accumulation of metabolites of BPA and inability of kidney to excrete them might affect the kidney tissues of treated rats. or this kidney's histological changes might be caused by increase formation of ROS and increase the oxidative stress induced by BPA [13]. The previous histopathological alteration was reported by [14]. BPA is metabolized in liver and eliminated through the kidney. So the congenerated tissue of kidney were noticed [15]. Also, [16] demonstrated that histopathological examined of kidney of rats in BPA group revealed necrotic lesions, congestion and mononuclear cell infiltration, these results are in accordance with present study congestion in blood vessels, hyaline in the lumen of epithelial cell which lining convoluted tubular were noticed by [17].

The histopathological changes in the present study are considered as specific monitor for the toxicity of bisphenol A showed by histopathological changes of the rats spleen exposed to the different doses manner included multiple necrosis and atrophy of red pulp these changes of the rats showed by the histopathological finding of spleen tissue [18], also agreement with [19] who mentioned in mice fed bisphenol A for 4 weeks, the spleen tissue showed lymphocytic depletion and multiple focal necrosis [20] suggested that BPA induced the migration of the neutrophils into peritoneal cavity but reduced their phagocytic activity, also BPA reduced population of lymphocytic and macrophage in the spleen and its accumulation in the infected foci.-

Finally the histopathological examination revealed moderate to severe papillary hyperplasia seen in the lining epithelia with focally thickened were seen in prostate gland of treated males rats exposed to different doses of BPA. These findings were similar to that obtained by [21]; [22] and Imam [23].

Explain[24] The hyperplastic changes seen in prostatic acini may be due to an increase in the proliferation of basal epithelial cells as mentioned by[25] who found a 6-fold increase in prostatic androgen receptors, associated with a doubling of androgen receptors per cell

دراسة أمراضية نسجية للرئة والكلية والطحال وغدة البروستات في ذكور الجرذان البالغة

المعاملة بالبسفينول أ

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الخلاصة

صممت هذه الدراسة لمعرفة التأثيرات المرضية النسجية للبسفينول على الرئة والكلية والطحال وغدة البروستات في ذكور الجرذان البالغه. اجريت الدراسة في كلية الطب البيطري جامعه البصرة، استخدمت في الدراسه اربع وعشرون جرذ ذكر قسمت عشوائيا وبالتساوي الى اربع مجاميع متساويه احتوت كل مجموعه على ست جرذان أعطيت المجموعة الاولى زيت الذره يوميا خلال التجربه (مجموعه السيطرة) اما المجموعه الثانيه والثالثه والرابعه فجرعت ب ٠.٥ و ١.٠ و ٢.٠ ملغم/كغم من وزن الجسم بسفينول أ مذابا في زيت الذره على التوالي استمرت التجربه لمده ٣٠ يوما .

أظهر الفحص النسجي المرضي للرئة في ذكور الجرذان المعامله وجود خلايا التهابيه حول القصيبات الهوائيه، تنخر في الخلايا الرئويه،استسقاء معتدل الى شديد،انخماص في جدران الاسناخ وكذلك فرط تنسج في الخلايا الطلائيه للقصيبات الهوائيه . بينت الدراسات الحاليه للفحص النسجىللكلية . وجود تفجى في الخلايا المبطنه للنبيبات الكلويه ،مساحات نزفيه في النسيج الكلوي ناتج من تاثيرالبسفينول أ،اما في الطحال فكان التأثير واضح من خلال ظهور ضمور معتدل الى شديد في اللب الابيض .أخيرا اوضحت النتائج وجود تنكس وفرط تنسج في الخلايا الطلائيه المبطنه للبروزات الحليميه في البروستات ناتج من تاثيرالبسفينول أ .

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