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Histopathological Changes Produced by Bispenol A in the Renal Cortex of Adult Male Albino Rats

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Abstract

Background: Bisphenol A (BPA) is an important endocrine disrupting chemical that is widely used in plastic containers and has tendency to accumulate in many organs and affect their functions.

Aim of Study: The aim was to clarify the effect of BPA on the histological structure of the renal cortex in adult male albino rat and to assess the efficacy of its withdrawal in amelioration of this effect.

Material and Methods: This study was conducted on forty adult male albino rats which randomly divided into four groups (10 rats each). Group I served as negative control, Group II was vehicle group and received corn oil, Group III which received a daily oral dose of BPA 50mg/kg body of weight for eight weeks and finally Group IV which received the same dose of BPA as Group III then kept for another four weeks without treatment for spontaneous recovery (BPA recovery). Renal cortices were processed for light and electron microscope examinations. Blood samples were collected for biochemical parameters (urea and creatinine). The level of Malondialdehyde (MDA) and catalase activity were estimated in the kidney tissue. In addition, the diameter of renal glomeruli and Bowman's space surface area were morphometically and statistically analyzed.

Results: It was noticed that BPA led to degenerative changes in the renal cortex with partial improvement of such structural findings in the BPA recovery group. Compared with the control groups (negative control, corn oil), the BPA group showed no significant difference in body weights (p=.52) but there was a higher significant increase in kidney weights (p<.001). Comparing between the BPA-treated and BPA recovery groups, no statistically significant differences (p=.34) was reported. Tissue MDA, urea and creatinine levels showed significant increase (p<.001) while catalase activity was dramatically decreased (p<.001) in the in BPA-treated and BPA recovery groups with no statistically significant differences between BPA treated and BPA recovery groups. The glomerular histomorphometry showed a significant decrease in mean glomerular diameter but very statistically significantly higher mean Bowman's space surface area in the BPA-treated and BPA recovery groups than the control groups, with no significant difference between the BPA-treated and BPA recovery groups (p=.67, p=.61) respectively.

Conclusion: The previous results supported that BPA had toxic effect on the renal cortex structure and the recovery from such effect might need more than four weeks.

Key Words: *Bishpenol A – Recovery – Kidney and rats.*

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Introduction

BISPHENOL A (BPA) is one of the highly produced chemicals worldwide; its production is estimated to exceed 4.5 million tons per year [1]. BPA is an essential component in polycarbonate plastic and epoxy resin. All reusable food and drink containers, water pipes and mineral water bottles are made of polycarbonate plastic. Resins are used in the lining of metallic food containers, such as food and beverage cans, to prevent rusting and corrosion [2]. Dialysis patients are at high risk of exposure to BPA leaching from polycarbonate hemodialysis equipment with an increase in inflammatory markers [3]. In fact, BPA is present in many everyday used products, including mobile phone casings, eye glasses and dental sealant [4].

BPA molecules in polycarbonate plastic and resins undergo hydrolysis, which leads to the leaching of BPA monomers into water at room temperature. Moreover, this leaching greatly increases upon exposure to high temperatures and acidic or basic solutions [5].

This extensive use of BPA results in high human exposure [6]. Although the concentration of BPA in food products is low, large doses accumulate in the human body with the daily use of such products [7]. BPA exposure can occur through oral, inhalation or transdermal absorption [8], but in humans, the oral route is the main exposure route. According to previous studies, over 90% of tested individuals

had detectable levels of BPA, which is proof of the widespread, continuous exposure of BPA in the general population [9]. BPA can be detected in the urine of adults and children as well as in human serum, saliva and placenta [10].

Normally, BPA is rapidly metabolized after oral ingestion in the liver and intestine to a hormonally inactive form with oestrogenic properties [11]. Based on several studies, BPA, even at low concentrations, can stimulate cellular responses and bind to oestrogenic receptors with the same efficacy and potency as oestradiol [12].

The absorption of large amounts of BPA causes extensive damage to the liver, kidney and other vital organs in humans [13]. BPA also has adverse effects on the brain, reproductive system and metabolic processes [14].

Several studies showed an association of high urine BPA and low grade albuminuria in children and adults [15] also, high plasma levels of BPA have been recorded in patients with chronic kidney diseases [16]. These data raise the concern of the possible adverse effects of BPA on the kidney that may progress to serious renal injury.

BPA has adverse effects on renal tubules in the kidneys of rats and mice [17]. Several studies reported the occurrence of tissue oxidative stress and peroxidation after BPA exposure in rats and mice, that induce the formation of Reactive Oxygen Species (ROS) and cause tissue injury in the liver, kidney, brain and other organs [18,19]. Bindhumol et al., [20] proved that low doses of BPA can generate ROS by decreasing the activities of antioxidant enzymes and increasing lipid peroxidation, thereby causing oxidative stress.

Based on previous data, the purpose of the present study was to clarify the effect of BPA on the structure of the renal cortex of adult male albino rats and evaluate the influence of its withdrawal by using light and electron microscopy.

Material and Methods

Animals:

Forty adult male albino rats with an average weight of 180-200gm were used in the present study. Rats were obtained from the Zagazig Scientific and Medical Research center (ZSMR), Faculty of Medicine, Zagazig University during 2017. All animals were housed at room temperature and allowed free access to food and water throughout the experiment. All rats were handled in accordance with the guidelines of the Ethical Committee of Zagazig University.

Chemicals:

Bisphenol A (BPA) CAS No 80-05-7, was purchased from Sigma Chemical Company (St. Louis, Missouri, USA) in the form of crystalline white powder.

Study design:

After acclimatization for 2 weeks, the animals were divided into four groups, with 10 rats in each group:

- Group I (control): Rats were given no treatment.
- Group II (vehicle): Each rat received 1mL corn oil orally by gastric tube for 8 weeks.
- Group III (BPA): Rats received BPA powder dissolved in corn oil as a daily oral dose of 50 mg/kg body weight by a gastric tube once daily for 8 weeks [21].
- Group IV (BPA recovery): Rats received the same dose for the same duration as Group III and were maintained for another 4 weeks without treatment for spontaneous recovery.

After 24h of the last dose, rats received sodium thiopental (30mg/kg body weight), the body and kidney weights of rats in all groups were recorded to the nearest gram at the time of sacrifice.

1- *Histological study:* For light microscopy, immediately after sacrificing the animals, the kidneys were dissected and fixed in 10% formalin solution. After fixation, specimens were dehydrated using a series of ascending grades of alcohol (70, 90, 95 and 100%), cleared in xylol and finally impregnated and embedded in paraffin. Sections were cut at 5 μm and stained with Haematoxylin and Eosin to examine structural changes [22].

For electron microscopy, some specimens were cut into smaller pieces of 1mm^3 and fixed in buffered glutaraldehyde (2.5%) solution at 4ºC and post-fixed with 1% osmium tetroxide solution. After dehydration in ascending grades of ethanol, followed by clearing in propylene, the specimens were embedded in epoxy resin (Embed B12) and sectioned by an ultra-microtome to obtain semithin and ultrathin sections [23]. Semithin sections of 1μm thickness were cut and stained with toluidine blue for examination and photography by using a light microscope. Next, ultrathin sections (60nm) were cut and stained with uranyl acetate and lead citrate. Sections were examined using a JEOL-JEM 1010 electron microscope (Tokyo, Japan) at

the Mycology and Regional Biotechnology Center, Al-Azhar University, Cairo, Egypt.

2- Kidney function parameters (urea & creatinie): Blood samples were collected and centrifuged at 3000r/min for 10min and the obtained serum then stored at 20ºC for determination of kidney functions (urea and creatinine) according to the colori-metric methods described by Fawcett and Scott **[24]**, Peters **[25]** respectively.

3- Biochemical analysis of lipid peroxidation and oxidative stress parameters (MDA & catalase): The renal tissue from each animal was rapidly removed and frozen until analyzed. The tissue was homogenized in 6ml ice cold phosphate buffer (50mM pH 7.4, 0.1% Triton X and 0.5mM EDTA). The homogenates were centrifuged for 15min, and the clear supernatants were separated for analysis. The level of Malondialdehyde (MDA) was assayed according to the method of Ruiz-Larrea et al., **[26]** and catalase activity was measured based on the spectrophotometric method described by Aebi **[27]**.

4- Morphometric study: Olympus Soft Imaging System software and a Leica 500MCO (Germany) were used for quantitative analysis of the kidney. Serial non-overlapping fields from five rat kidney sections were randomly selected and analyzed. The diameter of renal glomeruli and Bowman's space surface area were measured from photographs of sections stained with haematoxylin and eosin in a high-power field (400x).

5- Statistical analysis:

Continuous data are expressed as the mean ± SD for normally distributed (parametric) data. One-way ANOVA was used to detect significant differences between groups. Post hoc Tukey's test was performed for multiple comparisons between groups. The differences were considered significant at $p<.05$. All statistical comparisons were two-tailed. All statistical calculations were carried out using GraphPad Prism, Version 7.0 software.

Results

Body and kidney weights: There was no significant difference ($p=.52$) in final body weights of all tested groups. Regarding the kidney weights, the BPA group showed a significant increase ($p<.001$) compared with that in the control groups. However, no statistically significant difference ($p=.34$) was detected between the BPA and BPA recovery group as shown in (Table 1).

Biochemical results (urea and creatinine): BPA treated and BPA recovery groups had very highly statistically significantly higher mean blood urea and serum creatinine concentration compared to control groups (negative control and corn oil) ($p<.001$ for each), but no statistically significant differences between BPA treated and BPA recovery groups were found ($p=.12$ and $p=.23$, respectively). Histogram (1).

Tissue MDA and catalase: The oxidative effect of BPA was assessed by determining the concentration of lipid peroxides in rat kidneys. Compared with the control groups, the BPA group showed a significant ($p<.001$) increase in MDA and no significant difference ($p=.58$) was observed between the BPA and BPA recovery groups. On the other hand, catalase activity revealed significant decrease ($p<.001$) in BPA and BPA recovery groups compared with control group with no significance difference ($p=.07$) recorded between the BPA and BPA recovery groups. Histogram (2).

Glomerular histomorphometric results: BPA treated and BPA recovery groups had a very highly statistically significantly lower mean glomerular diameter compared to control groups (negative control, corn oil) ($p<.001$), but no statistically significant difference between BPA treated and BPA recovery groups were found ($p=.67$). BPA treated and BPA recovery groups had a very statistically significantly higher mean Bowman's space surface area compared to control groups (negative control, corn oil) ($p<.001$), but no statistically significant difference between BPA treated and BPA recovery groups was found ($p=.61$) as shown in (Table 2).

Histological results: Group I (negative control) and Group II (vehicle) exhibited similar results; thus, only the histological results of Group I are presented.

Histological results of H & E stained sections: The renal cortex of Group I showed normal renal corpuscles and renal tubules. Proximal Convoluted Tubules (PCTs) were lined with an eosinophilic simple cuboidal epithelium and had a narrow lumen. In contrast, Distal Convoluted Tubules (DCTs) had a less acidophilic cytoplasm with a wide lumen. The renal corpuscles comprised tufts of glomerular capillaries with Bowman's capsule formed of visceral and parietal layers surrounding it Fig. (1).

Examination of Group II (BPA group) showed that both renal tubules (PCTs and DCTs) appeared with damaged epithelial lining, widened lumen and had vacuoles within their cytoplasm with darkly stained nuclei. Some renal corpuscles appeared segmented, atrophied with widening of the renal space while others appeared enlarged with

hyper-cellularity and congestion Fig. (2). Intra-tubular hemorrhage was observed Fig. (3). Thickened blood vessels with inflammatory cellular infiltrations were also seen Fig. (4).

Bisphenol recovery group showed that some renal tubules regained their vesicular nuclei, while others remained darkly stained nuclei and less Intra-tubular hemorrhage could be noticed. Some renal corpuscles regained their normal appearance with a narrow renal space Figs. (5,6).

Histological results of semithin sections:

In the control group cuboidal cells with rounded vesicular nuclei were lining the PCTs together with brush border and well-defined basal striations. In contrast, the DCTs showed numerous basal striations and ill-defined brush border. Mesangial cells and normally shaped podocytes were also observed lining the inner visceral layer of Bowman's capsule with a regular basement membrane Figs. (7,8).

In BPA group both renal tubules (PCTs and DCTs) showed irregular darkly stained nuclei, had vacuoles within their cytoplasm and homogenous substances and exfoliated part of their lining cells in their lumens. Congested dilated glomerular capillaries with distorted mesangial cells and podocyte nuclei were also noted Figs. (9,10). While in BPA recovery group partial improvement in the form of preservation of some vesicular nuclei was noted in PCT and DCT structures with few cytoplasmic vacuoles. Less congested glomeruli were also seen Figs. (11,12).

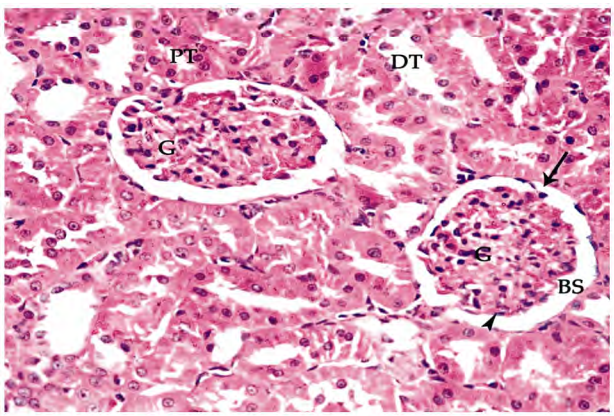


Fig. (1): A photomicrograph of a section a rat renal cortex from Group I (control group) showing normal architecture. The renal corpuscle is formed of parietal layer of Bowman's capsule of kidney formed of proximal (PT) and distal (DT) convoluted tubules, normal appearance of the glomerulus (G) surrounded with Bowman's capsule which is formed of parietal layer of (arrow) and visceral layer (arrow head). Note the preserved Bowman's space (BS). X400 H & E.

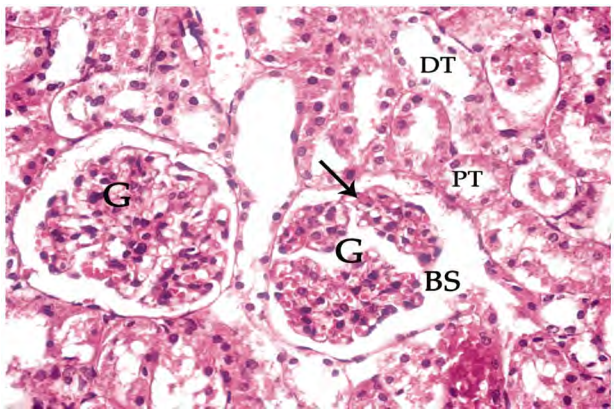


Fig. (2): A photomicrograph of a section of a rat renal cortex from Group III (BPA group) showing fragmented hyper-vascularized and hyper-cellular glomerulus (G) with widened Bowman's space. X400 H & E.

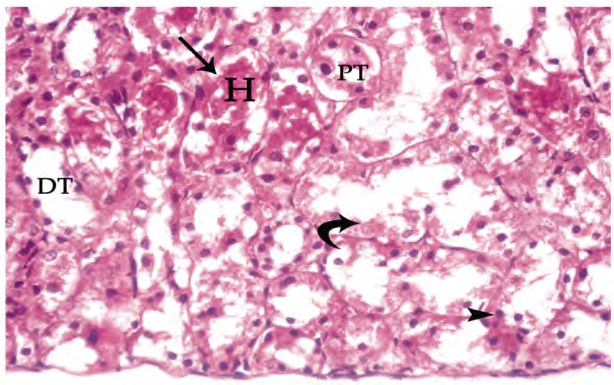


Fig. (3): A photomicrograph of a section of a rat renal cortex from Group III (BPA group) showing both PT & DT with wide lumen, distorted epithelial lining (curved arrow), vacuolated cytoplasm and darkly stained nuclei (arrow head). Hemorrhage (H) can be seen inside the renal tubules. X400 H & E.

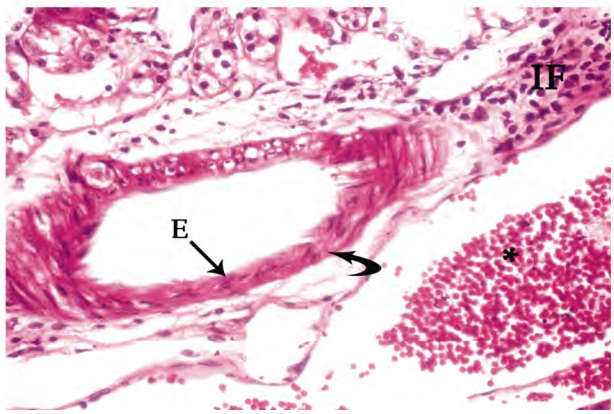


Fig. (4): A photomicrograph of a section of a rat renal cortex from Group III (BPA group) showing thickened wall blood vessel lined by endothelial cells (E) and show smooth muscle hyperplasia (curved arrow). Monocellular infiltration (IF) and extravasated blood can be seen (*). X400 H & E.

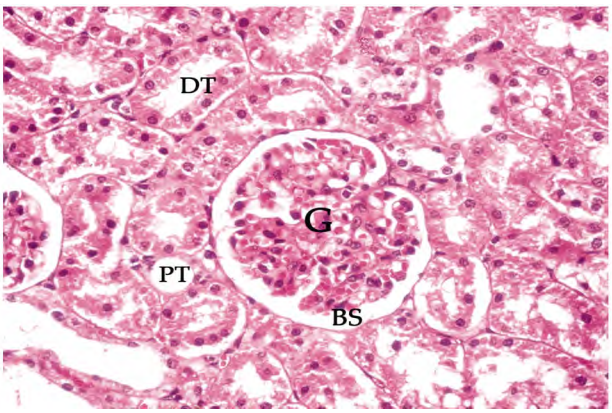


Fig. (5): A photomicrograph of a section of a rat renal cortex from Group IV (BPA recovery) showing that most of the renal tubules (PT & DT) regain their vesicular nuclei with less vacuolated cytoplasm. Glomerulus (G) appeared hypercellular with considerable widening of Bowman's Space (B S). X400 H & E.

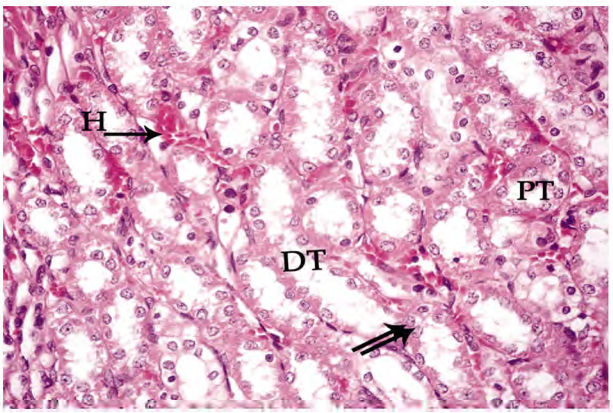


Fig. (6): A photomicrograph of a section of a rat renal cortex from Group IV (BPA recovery) showing both renal tubules (CT & DT) with vesicular nuclei (double arrow). Less hemorrhage (H) can be noticed (arrow). X400 H & E.

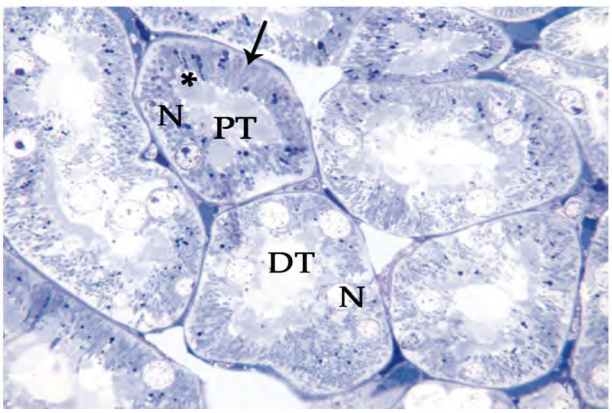


Fig. (7): A photomicrograph of a semithin section of a rat renal cortex from Group I (control group) showing PT with its high cuboidal epithelium, brush border (*) and well-formed basal striations (arrow). DT appeared with pale cytoplasm and good vesicular nuclei (N). Toluidine blue X 1000.

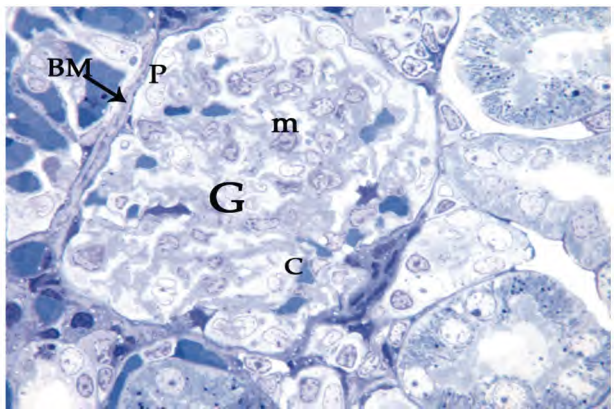


Fig. (8): A photomicrograph of a semithin section of a rat renal cortex from Group I (control group) showing part of the renal corpuscle which is formed of Bowmann's capsule with its parietal layer and the visceral layer which is formed of podocytes (P). Glomerulus (G) is formed of tuft of capillaries (C), mesangial cells (m) surrounded with mesangial matrix. Toluidine blue X 1000.

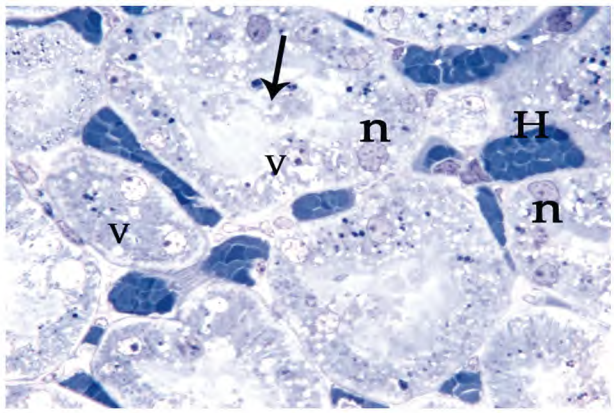


Fig. (9): A photomicrograph of a semithin section in a rat renal cortex from Group III (BPA group) showing both renal tubules with irregular dark nuclei (n), cytoplasmic vacuoles (V) and exfoliated parts can be noticed (arrow). Hemorrhage (H) in between tubules is also appeared. Toluidine blue X 1000.

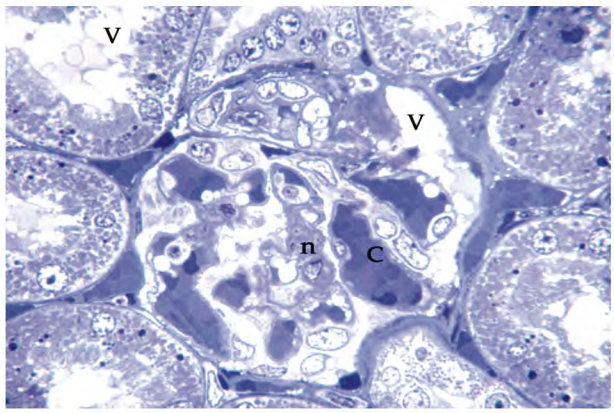


Fig. (10): A photomicrograph of a semithin section in a rat renal cortex from Group III (BPA group) showing part of the renal corpuscle with dilated congested glomerular capillaries (C) and some of nuclei of podocytes and mesangial appear distorted (n) with many cytoplasmic vacuoles (V). Toluidine blue X 1000.

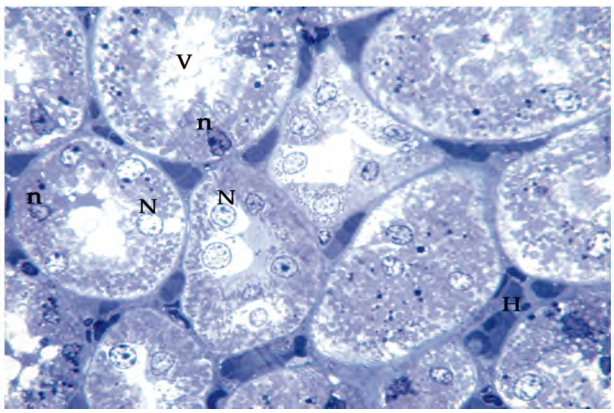


Fig. (11): A photomicrograph of a semithin section in a rat renal cortex from Group IV (BPA recovery) showing some renal tubules restores their normal architecture with vesicular nuclei (N) while others still show irregular nuclei (n) and less hemorrhage (H) still be noticed. Toluidine blue X 1000.

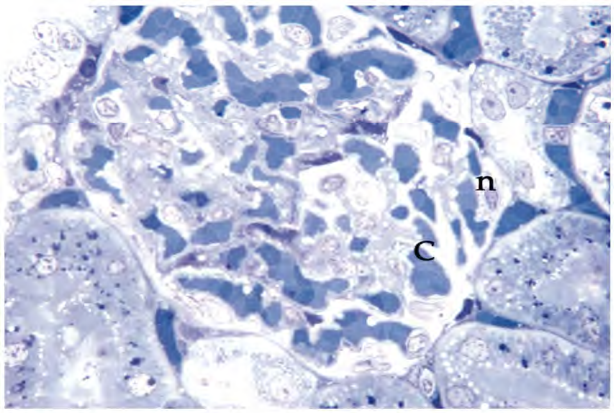


Fig. (12): A photomicrograph of a semithin section in a rat renal cortex from Group IV (BPA recovery) showing less congested renal corpuscle (C) with few irregular nuclei (n). Toluidine blue X 1000.

Ultrastructure results:

In Group I, examination of the renal cortex revealed PCTs with well-developed basal enfolding containing elongated mitochondria, euchromatic nuclei, long apical microvilli and thin regular basement membrane Fig. (13). DCTs showed substantial basal enfolding with regularly arranged mitochondria, few apical microvilli and euchromatic nuclei and regular basement membrane Fig. (14). The renal corpuscles showed regular podocytes with folded heterochromatic nucleus, an electron dense cytoplasm and long primary processes, which in turn branched to give raise to secondary processes and numerous thin foot processes. The tri-laminar fenestrated basement membrane was also observed Fig. (15).

In BPA group, PCTs showed shrunken irregular nuclei, few apical microvilli, reduced basal enfolding with disorganized mitochondria and many lysosomes of variable size Fig. (16). In DCTs, rarified cytoplasm with irregular heterochromatic nuclei appeared, dispersed mitochondria with ruptured cristae and thickened basement membrane were noticed Fig. (17). The renal corpuscles showed focal thickening of the glomerular Basement Membrane (BM) and fusion of foot processes of podocytes Fig. (18). While in BPA recovery group PCTs showed partial restoration of apical microvilli but nuclei still appeared irregular and some cells retained a vacuolated cytoplasm. Some mitochondria appeared swollen Fig. (19). DCTs had irregular nuclei and preserved basal enfolding containing some disorganized mitochondria, thickening of basement membrane was still seen Fig. (20). Regarding the renal corpuscle, thickening of the basement membrane with fusion of the foot processes was observed to a lesser extent with restoration of some podocytes's foot processes Fig. (21).

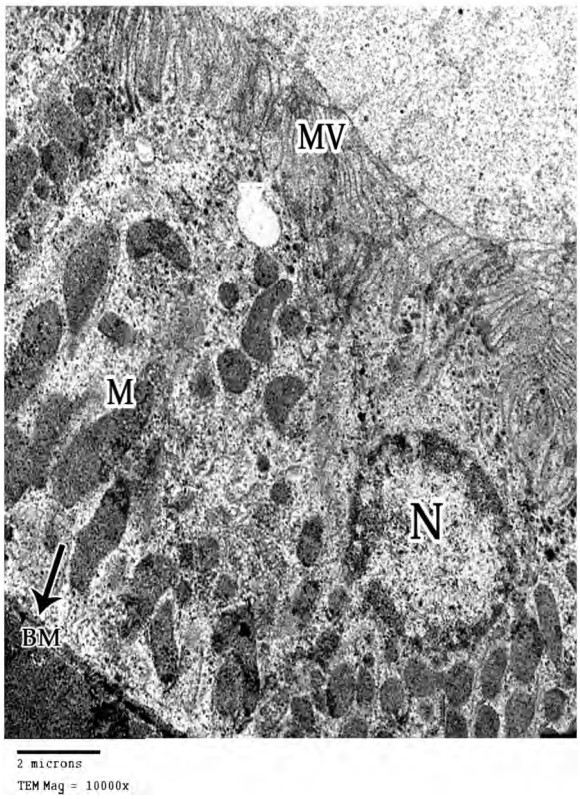


Fig. (13): An electron micrograph of PCT from Group I (control group) showing numerous apical long microvilli (MV), well-developed basal enfolding containing regularly arranged mitochondria (M), and euchromatic nucleus (N). X10000.

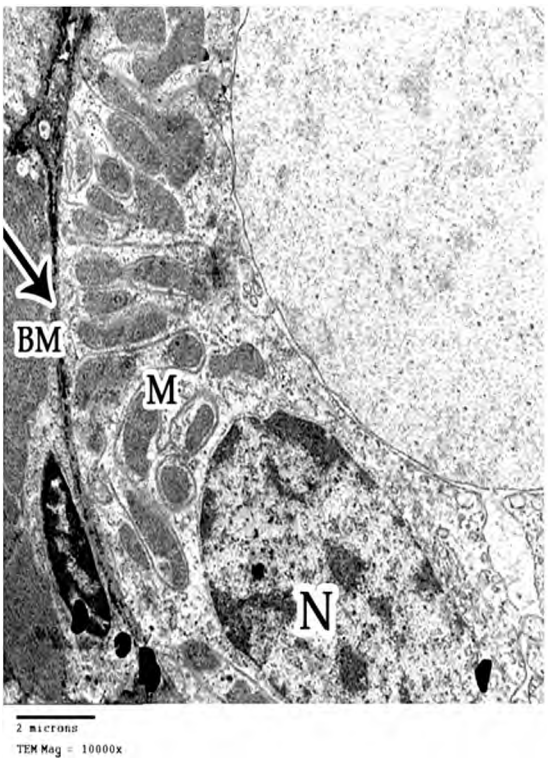


Fig. (14): An electron micrograph of DCT from Group I (control group) showing the basal enfolding with normally appeared mitochondria (M), euchromatic nucleus (N) and regular basement membrane (BM). X10000.

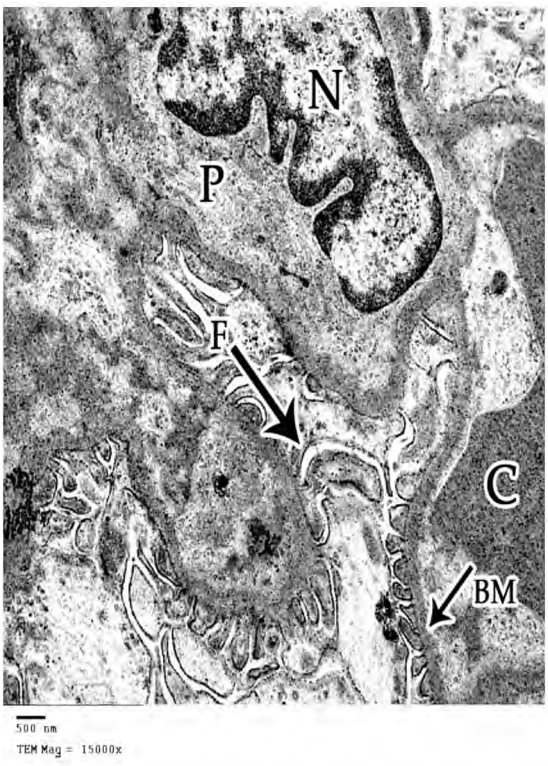


Fig. (15): An electron micrograph of the renal corpuscle of Group I (control group) showing podocyte (P) with folded euchromatic nucleus (N) and secondary foot processes (F), fenestrated trilaminar glomerular basement membrane (BM) and glomerular capillary (C). X15000.

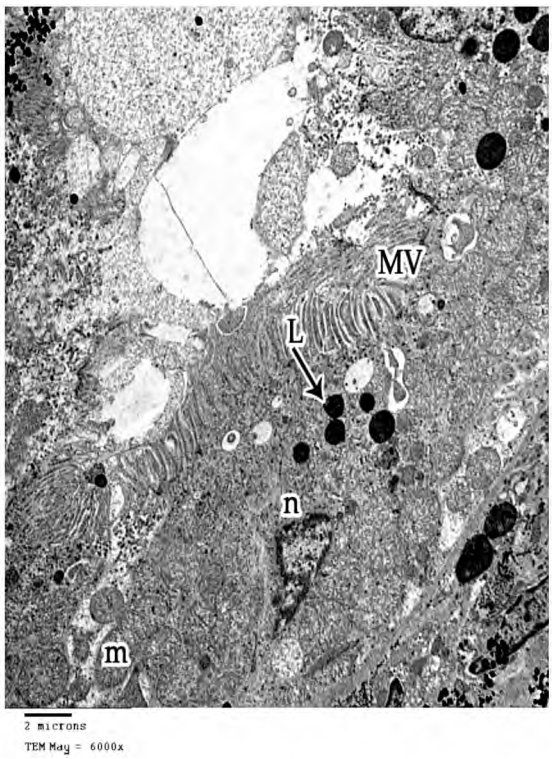


Fig. (16): An electron micrograph of PCT from the Group III (BPA group) showing apical microvilii (MV), reduced basal enfolding with disarranged mitochondria (m), shrunken irregular electron dense nucleus (n) and many lysosomes of variable size (L). X6000.

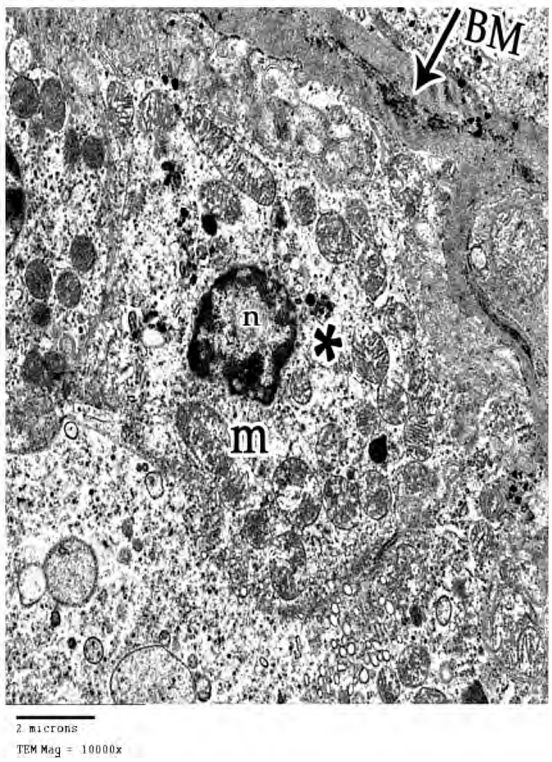


Fig. (17): An electron micrograph of DCT from the Group III (BPA group) showing dispersed mitochondria with rupture crista (m), heterochromatic irregular nucleus (n) rarified cytoplasm (*) and cell rest on thick basement membrane (BM). X10000.

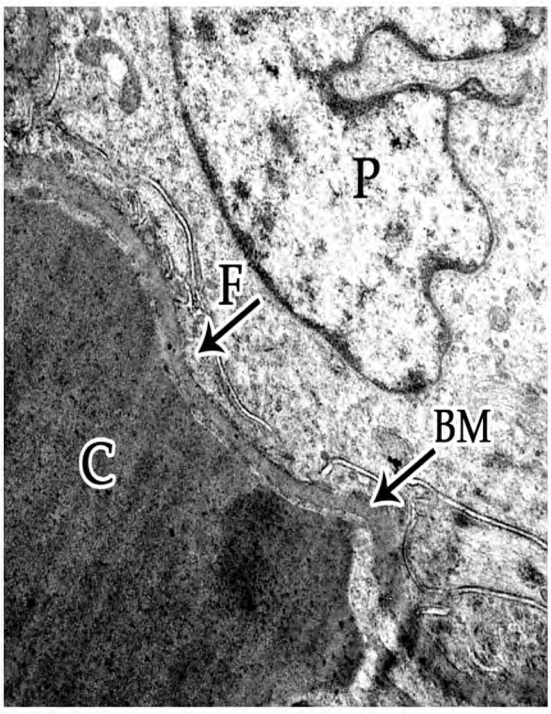


Fig. (18): An electron micrograph of the renal corpuscle of Group III (BPA group) showing focal thickening with disrupted appearance of basement membrane (BM) and broadening and effacement of foot processes (F) of podocytes (P). X15000.

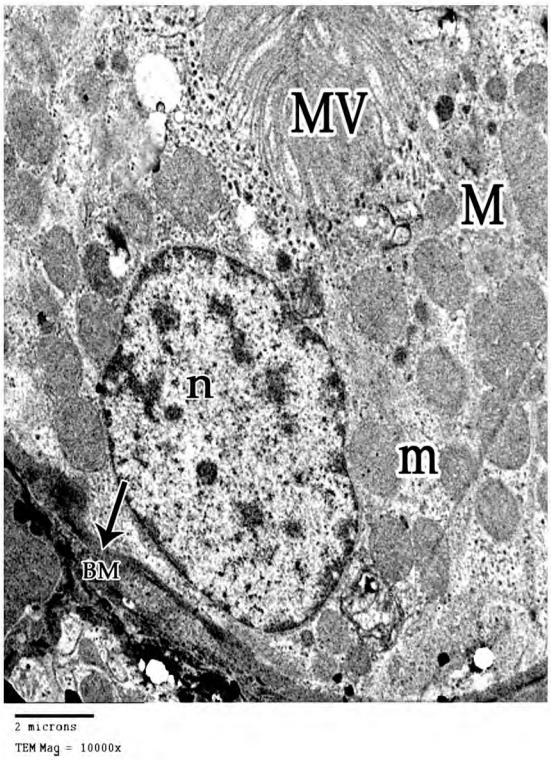


Fig. (19): An electron micrograph of DCT from the Group IV (BPA recovery) showing long apical microvilii (MV), basal enfolding containing multiple mitochondria some are normal (M) others are swollen (m) with irregular nucleus (n). Thickening of basement membrane (BM) can be seen. X10000.

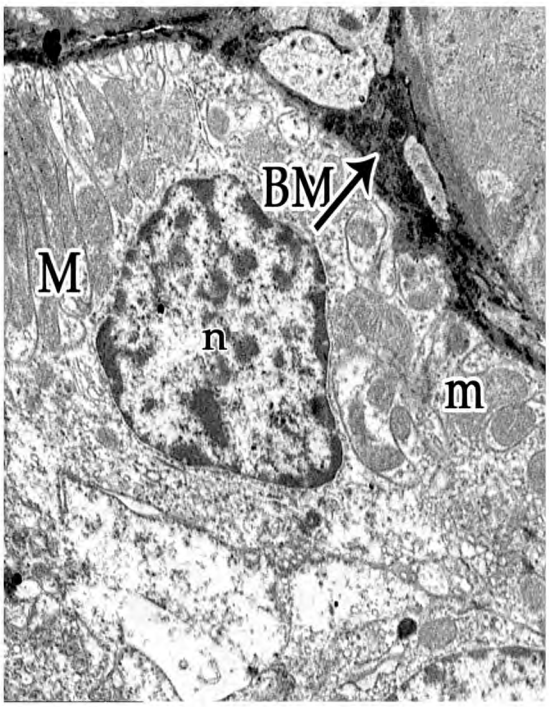


Fig. (20): An electron micrograph of DCT from the Group IV (BPA recovery) showing basal enfolding containing multiple mitochondria; some of them regularly organized (M) and others appear disorganized and swollen (m), with irregular nucleus (n). Thickening of basement membrane (BM) is still seen. X10000.

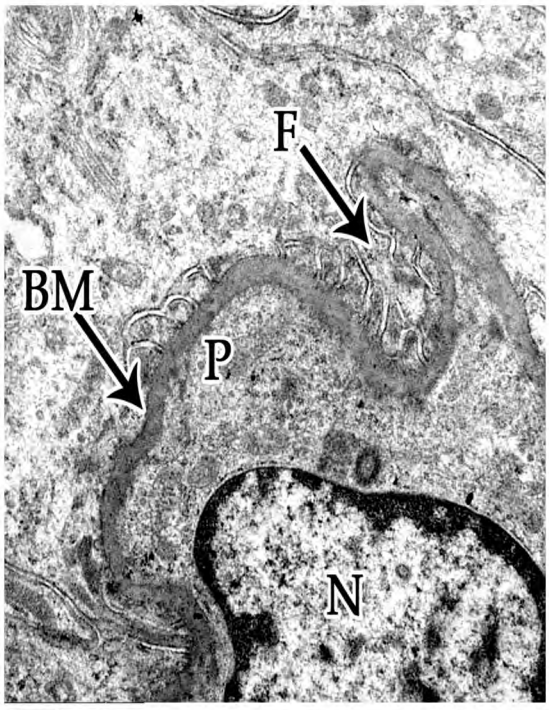
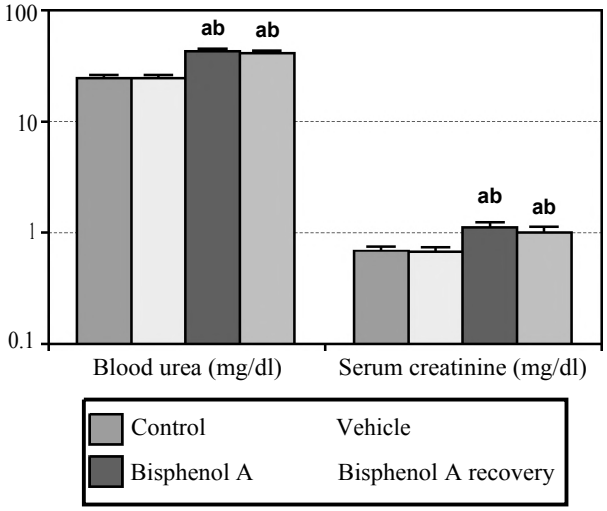


Fig. (21): An electron micrograph of the renal corpuscle of Group IV (BPA recovery) showing thickening of basement membrane (BM) and restoration of some podocyte's (P) foot processes (F) but still fewer than normal. N X20000.

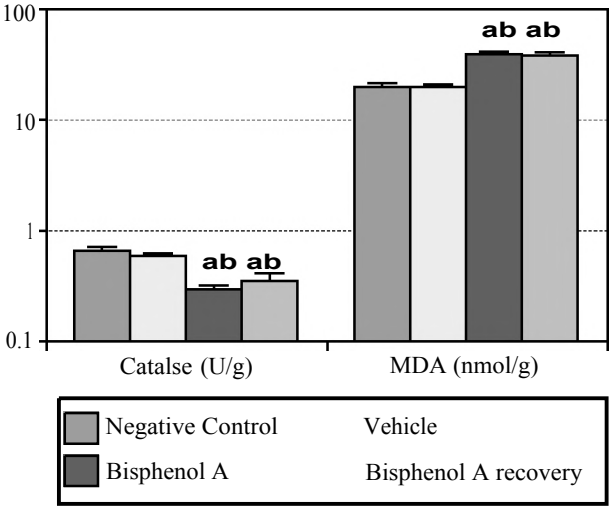
Table (1): Effect of Bisphenol A (BPA) on body and relative kidney weights in adult male albino rats.

Variables	Groups			
	Negative control	Corn oil (vehicle)	BPA	BPA recovery
• Body weight (g).	220.4±5.9	220.9±4.1	219.4±5.3	223.2±7.3
• Relative kidney weight (g kidney weight/100g BW)	0.40±0.013	0.40±0.017	0.58±0.1ab	0.62±0.02ab

All values are expressed as mean ± SD, n=10.
a: Significant vs negative control group.
b: Significant vs corn oil group (p<.05).
A one-way ANOVA followed by post hoc Tukey test multiple comparisons between groups.



Histogram (1): Effect of Bisphenol A (BPA) on kidney function test in adult male albino rats. Asignificant Vs. negative control group, b significant Vs. corn oil group (p<.05). All values are expressed as mean ± SD.



Histogram (2): Effect of Bisphenol A (BPA) on renal oxidative stress biomarkers in adult male albino rats. asignificant Vs. negative control group, b significant Vs. corn oil group within a row (p<.05). All values are expressed as mean ± SD.

Table (2): Effect of Bisphenol A (BPA) on glomerular diameter and Bowman's space surface area in adult male albino rats.

Variables	Groups			
	Negative control	Corn oil (vehicle)	BPA	BPA recovery
• Glomerular diameter (μm)	88.57± 2.0	88.45± 2.5	58.9± 8.2ab	61.8± 7.2ab
• Bowman's space surface area (μm^2)	2331 ± 198.7	2160± 329.6	3636± 573.6ab	3458± 528.8ab

Discussion

The renal cortex is the most part of the kidney affected by Bisphenol A (BPA), because it receives most of the nutrient blood flow to the organ and may potentially recover after BPA withdrawal. In this study, the chosen dose of BPA; (50mg/kg/day); is the Lowest Observed Effect Level (LOAEL) below which no adverse effects could be observed [28].

Leaching from plastic products is the main mechanism by which the population is exposed to BPA; thus, oral gavage was chosen as the route of administration in our study [5]. For easy administration and absorption of BPA, corn oil was used as a vehicle.

In the current study, rats treated with BPA showed no significant difference in their body weights compared with that of control groups. Similar results were observed by Bindhumol et al., [20] and Poormoosavi et al., [29] who stated that oral exposure to BPA revealed no significant change in the body weight of adult male rats.

In contrast, Simerly, [30] reported a statistically significant reduction in the body weights after BPA administration which can be explained by the effect of BPA on the brain; BPA may affect some brain centers involved in food intake and metabolism. Such differences may be attributed to different doses and route of administration.

In addition, in the BPA group, there was a significant increase in the absolute weights of kidneys. These findings were consistent with Tyl et al., [31] who stated that this increase may be due to increased lipid and cholesterol content within the kidney. In contrast, Mahmoudi, et al., [32] showed that treatment with BPA causes a significant reduction in the absolute kidney weights of mothers and young rats. However, compared with the BPA group, the BPA recovery group showed non-significant differences in both body weights and kidney weights.

Oxidative stress is an imbalance between the production of ROS and antioxidant defenses, which finally results in oxidative damage [33]. This study reported that treatment with BPA resulted in a significant increase in MDA, which is one of the end products of cell death, together with significant decrease in enzymatic antioxidant; catalase. These results were in agreement with Hassan et al., [34] who reported a significant decrease in catalase activity after BPA administration. This finding was confirmed by the study of Gong et al., [18] who explained that catalase activity reduction after BPA exposure may be due to the inability of liver mitochondria to eliminate hydrogen peroxide resulting from BPA exposure. Pigeolet et al., [35] explained that such reduction in catalase activity may be attributed to exhaustion of the enzyme to get rid of hydrogen peroxide produced after BPA exposure or due to excess ROS that result in enzyme inactivation.

In contrast Mourad and Khadrawy [36] showed a non-significant change in oxidative parameters in the kidney following 6 weeks of BPA exposure.

In the current study, light microscopy examination showed that rats treated with BPA had multiple degenerative changes in renal tubules, cytoplasmic vacuolization, darkly stained pyknotic nuclei and loss of normal architecture. Archana et al., [37] explained that darkly stained nuclei indicate apoptotic changes in the cell. The results of the present study revealed cellular vacuolation in proximal and distal tubules Robbins et al., [38] stated such cellular vacuolation could be a cellular defense mechanism against injurious substances. These substances were segregated in vacuoles and were prevented from interfering with cellular metabolism. In addition, Sakr et al., [39] claimed that this change is one of the primary responses to all types of cell injury in which cell membrane permeability increases, leading to water accumulation inside the cells.

Concerning the glomeruli, the present study showed shrunken glomeruli with widening of Bowman's space, in addition to hypercellular, fragmented and congested glomeruli. Mayer [40] mentioned that tubular injury can slow the glomerular filtration rate, which may lead to subsequent glomerular shrinkage. However, the hypercellularity and congestion of glomerular capillaries presented in this study can be explained by Stoev et al., [41] who demonstrated that glomerular endothelial proliferation leads to an increase in glomerular size. Such degenerative changes in the renal corpuscle proven by glomerular histomorphometry which revealed a significant decrease in the mean glomerular diameter in the BPA-treated and BPA recovery groups compared to that in the control groups (control, corn oil) (p<.001). Additionally, a higher mean Bowman's space surface area was detected in the BPA-treated and BPA recovery groups compared to that in the control groups (p<.001). In contrast with our results, Yıldız and Barlas [42] reported no significant differences in glomerular histomorphometry between the control and BPA treated groups.

The appearance of inflammatory cells may be related to oxidative stress induced by BPA with increased ROS that have the ability to induce an inflammatory response and result in the generation of some mediators, such as IL-8 and cytokine-induced neutrophils, which attract inflammatory cells into the microcirculation. The thickening of the blood vessel wall demonstrated in the present study can be traced back to He et al., [43] who stated that in stress conditions, many vasoactive substances can be released, such as nitric oxide and Endothelin (ET-1), which promotes the proliferation of endothelial cells and vascular Smooth Muscle Cells (SMCs).

All previous changes were consistent with those of Neha et al., [44] who reported that these changes could be attributed to an increase in lipid peroxidation with a reduction in antioxidant capacity in the rat kidney. Similarly, our results were consistent with those of Singh et al., [45] who showed that administration of BPA leads to renal dysfunction. Fernandez et al., [46] proved that chlorinated derivatives of BPA had the ability to accumulate in different tissues due to poor excretion by urine. These derivatives are more toxic than BPA; thus, their accumulation plays a major role in kidney damage [47].

All previous results were confirmed by transmission electron microscopy examination, which revealed loss of integrity of the apical brush border of PCTs, partial loss of basal striations and increased lysosomes. Lysosomal accumulation reportedly indicates cell injury [48].

Fusion and flattening of podocyte foot processes (effacement) reportedly occur with BPA exposure. Ricardo et al., [49] explained that this effect may result from direct injury to the podocyte skeleton after exposure to ROS or may develop secondary to the thickened Glomerular Basement Membrane (GBM) to compensate for increased glomerular permeability and proteinuria. These results were consistent with those of Olea-Herrero et al., [50]

who demonstrated that BPA has a damaging effect on renal tubules as well as glomerular epithelial cells (podocytes), leading to tubular and glomerular dysfunction.

The present work elucidated some degenerative changes in the renal corpuscles in the form of irregularity and thickening of the GBM in addition to fusion and flattening of secondary podocyte processes. Mostafa **[51]** stated that a thickened GBM was a common finding of many pathological and experimental conditions that lead to increased permeability of glomerular capillaries, resulting in proteinuria. Devuyst and Guggino **[52]** clarified that GBM thickening could be attributed to increased glycoprotein deposition which might indicate the toxic insult to cells.

Reactive Oxygen Species (ROS), a consequence of BPA exposure, may result in lipid peroxidation that subsequently reacts with DNA, leading to DNA damage, which is expressed as nuclear changes in the form of pyknosis and irregularity. Additionally, ROS production associated with BPA exposure is responsible for mitochondrial swelling and cristae destruction. These changes were attributed to mitochondrial metabolism, which is responsible for the generation of energy as well as free radical production. Thus, ROS production is highly involved in mitochondrial swelling and apoptosis by opening the mitochondrial permeability transition pore **[53]**. These findings were parallel with those Kobroob, et al., **[54]** who suggested that BPA can worsen mitochondrial function.

Bisphenol A adverse effects are further supported by increased urea and creatinine levels in the tested animals. Increased levels of urea and creatinine have been mentioned in several studies; Singh et al., **[45]** reported an increase in plasma levels of urea and creatinine in rats treated with BPA also Poormoosavi et al., **[29]** recorded high levels of urea and creatinine following BPA exposure. These findings were in congruence with the results of the current research. However, compared with the BPA group, the BPA recovery group showed non-significant differences in the plasma levels of urea and creatinine.

In the current study, withdrawal of BPA exposure in the follow-up (recovery) group resulted in partial improvement in renal tubules, which partially regained their normal vesicular nuclei with less haemorrhage and a less vacuolated cytoplasm. The renal glomeruli nearly retained their normal structure. Ultrastructurally, PCTs showed irregular nuclei, and multiple mitochondria with fewer cytoplasmic vacuoles. DCTs exhibited some mitochondria that regained their normal appearance, but some nuclei retained irregularities. Restoration of some podocyte foot processes was observed, but these normal processes were fewer in number than normal. However, the basement membrane remained thick, indicating potentially incomplete recovery after stopping BPA administration.

Disagreed with our findings Helal, et al., **[55]** elucidated that during the BPA recovery period the histological architecture of the kidney was regained and kidney parameters were came back to normal. These differences may be relayed on the different dose of BPA which was only 20mg/kg of body weight and also different duration of exposure.

Conclusion:

In light of the current study, we concluded that BPA exposure resulted in alterations in the histological structure of the renal cortex in albino rats, and the withdrawal of BPA resulted in incomplete recovery that may be attributed to an insufficient recovery period.

Recommendation:

Minimizing the use of plastic containers and observing the use of BPA in different industries are recommended to minimize the risks of this substance on human health, especially in developing countries. In addition, follow-up of animals for a longer period may be needed.

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التغيرات الهستوباثولوجية المحدثة بواسطة البسفينول (أ) فى البنية النسيجية للقشرة الكلوية لذكور الجرذان البيضاء البالغة: دراسة بإستخدام المجهر الضوئى والإلكترونى

البسفينول (أ) هو مادة كيميائية مهمة لتعطيل الغدد الصماء ويستخدم على نطاق واسع فى الحاويات البلاستيكية ويميل إلى التراكم فى العديد من الأعضاء والتأثير على وظائفهم.

كان الهدف هو توضيح تأثير إعطاء البسفيول (أ) عن طريق الفم لمدة ثمانية أسابيع على التركيب النسيجى للقشرة الكلوية لدى ذكور الجرذان البيضاء البالغة وتقييم فعالية إنسحابها فى تحسين هذا التأثير. أجريت هذه الدراسة على أربعين من ذكور الجرذان البيضاء البالغة مقسمة بشكل عشوائى إلى أربع مجموعات (١٠ جرذان لكل منهما) وتم إعطاء الجرعات بواسطة أنبوب معدى مرة واحدة يومياً لمدة ٨ أسابيع. المجموعة الضابطة سلبية: لم تتلقى أى عقار، مجموعة السائل المذيب للبيسفينول (أ): تلقى كل جرذ جرعة يومية ١مج/كغ من وزن والجسم من زيت الذرة، مجموعة البيسفينول (أ): تم معالجتها بمسحوق البيسفينول (أ) المذاب فى زيت الذرة كجرعة يومية تبلغ ٥٠مغ/كغ من وزن الجسم، أما مجموعة الإنسحاب فقد تم معالجتها بجرعة مماثلة من البيسفينول (أ) للمجموعة السابقة ولنفس المدة الزمنية ثم تم الإحتفاظ بها لمدة أربع أسابيع أخرى من أجل الإسترداد التلقائى. تم أخذ عينات الدم ثم تم بعدها ذبح الجرذان وإزالة الكلى ومعالجته لفحصها بالمجهر الضوئى والمجهر الإلكترونى وجمعت عينات من الدم لقياس مستوى اليوريا والكرياتينين وتم تقدير مستوى Malondialdehyde (MDA) ونشاط الكاتلاز فى الأنسجة. بالإضافة إلى قياس قطر الكبيبات الكلوية ومساحة سطح بومان. وقد أظهرت النتائج أن البسفينول (أ) أدى إلى تغييرات تنكسية فى القشرة الكلوية مع تحسن جزئى لهذه النتائج الهيكلية فى مجموعة الإنسحاب. مقارنة مع مجموعات التحكم لم تظهر إى إختلاف كبير فى أوزان الجسم (p=.52) ولكن كانت هناك زيادة كبيرة فى أوزان الكلى (p<.001) عند مقارنتها مع مجموعات الإنسحاب ومجموعة البسفينول (أ)، وأظهرت الأنسجة MDA، مستويات اليوريا والكرياتينين فى الدم زيادة كبيرة (p<.001) فى حين إنخفض نشاط الكاتلاز بشكل كبير (p<.001) فى مجموعة الإنسحاب ومجموعة البسفينول (أ) وأظهر قياس التشريح الكبيبى إنخفاضاً كبيراً فى متوسط قطر الكبيبات مع زيادة فى مساحة سطح مساحة بومان فى مجموعات الإنسحاب ومجموعة البسفيول (أ) بالمقارنة مع مجموعات التحكم. أيدت النتائج السابقة أن البسفيول (أ) كان له تأثير سام على بنية القشرة الكلوية وأن الشفاء من هذا التأثير قد يحتاج إلى أكثر من أربعة أسابيع. لذلك يوصى بالإقلال من إستعمال الفوارغ البلاستيكية فى حفظ المشروبات والأغذية وكذلك الإقلال من إستعمال مادة البسفينول (أ) فى هذه المنتجات مع التوصية بعمل أبحاث تشمل مدة أطول من أربع أسابيع بعد إيقاف التعرض للبسفينول (أ).