Original Article

The Possible Protective Role of Vitamin C against Bisphenol-A-Induced Structural Changes on the Adrenal Cortex of Adult Male Albino Rat: Histological, Immunohistochemical and Morphometric Study

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ABSTRACT

Introduction: Bisphenol-A is a monomer in epoxy resins and polycarbonate plastics synthesis. However, it is an endocrine disruptor participating in several adverse health problems. Vitamin C is a potent antioxidant.
Aim of the Study: This study aimed to detect the effects of Bisphenol-A on the structure of the adrenal cortex and the possible impact of concomitant administration of vitamin C in protection against these changes in the adult male albino rat.
Material and Methods: 45 adult male albino rats weighing (200-250) gm were used. The rats were randomly divided into three equal groups. Group 1: control group was given distilled water for six weeks. Group 2: received 25 mg BPA /kg/day, once daily orally, for six weeks. Group 3: received 25 mg BPA /kg/day and 60 mg vitamin C /kg/ day, once daily orally for six weeks. At the end of the experiment, the rats were sacrificed. The left adrenals were removed, and processed for light and electron microscopic, immunohistochemical and morphometric studies.
Results: Bisphenol-A intake caused structural changes in the adrenal cortex, such as loss of normal arrangement of cells, congestion of blood capillaries, vacuolization of cytoplasm, nuclear pyknosis, shrinkage of the nuclei, thickening of the capsule, strong positive caspase-3 cytoplasmic reaction, marked significant reduction of the thickness and cell diameter of all three layers, and increase of the %Area of caspase-3 immunoreactivity. Vitamin C cancelled most of these light and electron histological, immunohistochemical, and morphometric effects.
Conclusion: Bisphenol-A administration has damaging effects on the adrenal cortex and vitamin C protects against these effects.

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Key Words: Adrenal cortex, albino rat, bisphenol-A, vitamin C.

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INTRODUCTION

Bisphenol-A (BPA) is a common ingredient in the manufacture of synthetic polymers like epoxy resins. The inner coat of water bottles and metal cans is made with epoxy resins[1]. BPA interferes with the normal human body's endocrine functions. Many studies have found a link between BPA exposure and a variety of health issues, including cancer, sterility, diabetes, and obesity[2]. BPA is known for its endocrine disruptor properties, which have been linked to metabolic abnormalities, brain dysfunction, and obesity[3].

According to recent studies, BPA exposure during pregnancy causes aberrant adrenal gland structure, development, and function with increased levels of plasma corticosterone in the descendants of adult mice[4,5].

The adrenal gland is located on the upper kidney pole. It is made up of two parts: cortex and medulla. The cortex, which is derived from mesoderm, is the body's primary steroid-producing organ. The medulla is the principal generator of catecholamines and is formed by neural crest cells[6]. The cortex is divided into three parts: zona glomerulosa (outer zone), zona fasciculata (intermediate zone), and zona reticularis (inner zone). The zona glomerulosa is important for the generation of the aldosterone hormone. The zona fasciculata makes up most of the cortex. The cells of this layer are laden with lipids and arranged in parallel rows of cords. The deepest area is the zone reticularis, which consists of an interlacing network of cells. Zona fasciculata is concerned with the production of cortisol and zona reticularis produces androgen[7,8].

Vitamin C is one of the most considered essential dietary antioxidants as it markedly declines the adverse reactions of reactive oxygen species (ROS) produced in the cell. It has a beneficial effect on some diseases such as cardiovascular disorders, malignancy, and ophthalmological disorders through its antioxidant effect[9].

Despite the previous studies on BPA have concentrated on its effect on the development of the adrenals, however, there is limited information about its effects on the histological, immunohistochemical and morphometric

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aspects of adult male adrenals. Therefore, this study aimed to detect the effects of BPA on the adrenal gland and to evaluate the co-administration of vitamin C in protection against the induced structural changes in the adult male albino rat.

MATERIAL AND METHODS

Chemicals

Bisphenol-A in the form of powder (97% purity) was purchased from Sigma Aldrich Company, USA (CAS Number 80-05-7). Vitamin C powder (100% purity) was purchased from EL-Gomhureia pharmaceutical and chemical company, Cairo, Egypt.

Experimental Animals

A total number of 45 adult male albino rats weighting (200-250) gm were used in this study. The rats were obtained and housed in cages of fine wood bedding at the animal house of Assiut University. The animals were kept under optimal appropriate conditions with (12/12) hours light/ dark cycle at optimal temperature (25 ± 5) ºC. Food and water ad libitum were given. The experiment was carried out according to the rules of the Scientific Research Ethics Committee of the Faculty of Medicine of Assiut University that follow the recommendations of the National Institute of Health Guides for Laboratory Animals Care and Use[10].

Experimental design

The albino rats were randomly separated into three groups (fifteen rats in each one).

Group 1 (Control group): was given distilled water only.

Group 2 (BPA-treated group): The rats of this group received bisphenol-A dissolved in olive oil orally via gastric tube in a dose of 25 mg/kg/day, once daily, for six weeks[11].

Group 3 (BPA+vitamin C-treated group): The rats of this group received bisphenol-A dissolved in olive oil orally via gastric tube in a dose of 25 mg/kg/day, once daily, for six weeks in concomitant with 60 mg vitamin C/ kg/day once daily, orally via gastric tube, for six weeks[11].

At the end of the six weeks, all rats were anesthetized by ether inhalation and their chest wall was opened. Intracardiac perfusion started in the left ventricle by 0.9% saline and 4% paraformaldehyde solution after ligation of the descending aorta. The perfusion continued until the right atrial venous return became clear. The abdominal cavity was opened to rapidly extract the left suprarenal glands[12].

Histological study

Light microscopic study

The randomly selected adrenals for light microscopic study were cut into small pieces then rapidly fixed in 10% formaldehyde solution for 48 hours. Dehydration was done by butting the specimens in ascending alcohol grades. Clearing was done by xylene. Then, the specimens were embedded in milted paraffin. Lastly, the paraffin blocks were cut into 5 mm thickness slices and stained by haematoxylin and eosin to demonstrate the general histologic structure and Masson's trichrome to assess collagen fibers[13]. The stained slides were examined and photographed by the optical microscope (OLYMPUS CX31-Japan) at the Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University.

Electron microscopic study

The randomly selected adrenals for electron microscopic study were dissected into very small pieces (≈ 1mm3) and rapidly fixed in 4% cold glutaraldehyde for twenty-four hours. Then, the fixed specimens were washed 3-4 times in phosphate buffer (PH 7.2) for 20 minutes each. Then, the specimens were put in1% Osmium tetroxide for 2h. After that, the specimens were washed again in the same buffer 4 times. Dehydration was done by butting the specimens in ascending grades of alcohol for 2 hours. Finally, embedding in Epon araldite mixture was performed and semithin sections (0.5 micron) and ultrathin sections (50 nm in thickness) were done by the ultra-microtome. The semithin sections were stained with Toluidine blue and the ultrathin sections were stained with lead citrate and uranyl acetate[14]. The ultrathin sections were photographed by the transmission electron microscope (Joel-JEM-100 CXII, Joel; Tokyo, Japan) at Assiut University Electron Microscopic Unit.

Immunohistochemistry

Immunohistochemical study for caspase-3 was done to evaluate the state of apoptosis. Suprarenal sections were washed by phosphate buffered saline for 5 minutes, incubated with antibody to caspase-3 at a dilution of 1:200 (In *vitro* gen; Sweden AB; Stockholm Sweden) overnight at 4°C, washed and incubated with (1:500) secondary goat -anti-rabbit antibody (In *vitro* gen; Molecular Probes; Eugene; Oregon; USA) for 1hour at room temperature. Finally, the slides were incubated in 3,3-diaminobenzidene for 15 minutes, counter-stained by Mayer's haematoxylin, dehydrated, and mounted with dibutyl phthalate in xylene (DPX)[15].

Morphometric Study and Statistical Analysis

Morphometric study was performed on the haematoxylin and eosin-stained sections using image J program. Ten non-overlapping fields were randomly chosen from 10 sections of each group for measuring the thickness of each of the 3 layers of the adrenal cortex and the cell diameter of each layer. The magnification used was X400 for the thickness of the layers of the adrenal cortex and X1000 for the cell diameter. Morphometric study was also performed on the caspase-3 immunohistochemically stained sections to measure the %Area of the caspase-3 immunoreactivity. The magnification used was X400 for the %Area of the caspase-3 immunoreactivity. SPSS (Inc., Chicago, Illinois, USA) version 21.0 was used for the statistical analysis and measuring mean, standard deviation (SD) and *p-value*. One-way analysis of variance (ANOVA) test followed by post-hoc Tukey test were performed. The results were expressed in the form of mean ± SD. The *p-value* < 0.05 was considered statistically significant.

RESULTS

Histological Results

light microscopic results

Haematoxylin and eosin-stained results

Group 1 (Control group): On examination of the haematoxylin and eosin-stained sections of the adrenal cortex of the control animals, it was observed that the adrenal cortex was consisted of three layers. The zona glomerulosa was situated superficially. The zona fasciculata was localized inner to the zona glomerulosa and represented most of the thickness of the adrenal cortex. Finally, the zona reticularis was immediately inner to the zona fasciculata (Figure 1A). Regarding the zona glomerulosa, its cells appeared rounded to oval and arranged in clusters with darkly stained nuclei and acidophilic vacuolated cytoplasm. Blood capillaries were noted among the cells (Figures 2,3 A). On examination of the zona fasciculata, its cells with rounded vesicular nuclei and prominent nucleoli were arranged in cords. The cytoplasm showed multiple vacuoles giving it foamy appearance. Blood capillaries in between the cords of cells were observed (Figures 2,4 A). The zona reticularis was formed of anastomosing cords of vacuolated cells with blood capillaries in between (Figure 5A).

Group 2 (BPA-treated group): On examination of the haematoxylin and eosin-stained sections of the bisphenol- treated group, it was observed that, the adrenal cortex was consisted of the three layers; zona glomerulosa, zona fasciculata and zona reticularis (Figure 1B). The zona glomerulosa cells showed marked disorganization and widely separated with empty spaces (Figures 1,2 B). Some cells revealed deeply acidophilic cytoplasm and pyknotic nuclei (Figure 3B). Dilated and congested blood capillaries were seen (Figure 3B). Zona fasciculata cells with marked vacuolization of their cytoplasm lost their normal arrangement and marked congestion of their blood capillaries were noticed. Some cells revealed pyknosis (Figure 4B). Some zona reticularis cells showed features of apoptosis in the form of acidophilic cytoplasm and darkly stained pyknotic nuclei (Figure 5B). Marked congestion of blood capillaries was noticed among the cells of the zona reticularis (Figure5B).

Group 3 (BPA+vitamin C-treated group): On examination of the haematoxylin and eosin-stained sections of the bisphenol and vitamin C-treated group, it was observed that apart from some residual pyknotic cells, most of the studied sections exhibited marked improvement of the adrenal cortex. The improvement involved the cells and blood capillaries of the three layers of the adrenal cortex (Figures 1,2,3,4,5 C). The zona reticularis revealed some binucleated cells (Figure 5C).

Masson's trichrome stained results

Group 1 (Control group): It showed fine collagen fibers throughout the suprarenal cortex (Figure 6A).

Group 2 (BPA-treated group): It showed an apparent increase of collagen fibers of the cortical capsule (Figure 6B).

Group 3 (BPA+vitamin C-treated group): Masson's trichrome result of this group was nearly like the control group (Figure 6C).

Toluidine blue-stained results

Group 1 (Control group): Examination of the Toluidine blue-stained semithin sections of the control group showed that the zona glomerulosa was adjacent to the capsule. The cells of the zona glomerulosa were arranged in clusters. Cytoplasmic vacuoles, round vesicular nuclei and prominent nucleoli were revealed (Figure 7A). The zona fasciculata cells had well-distinct cell boundaries and rounded nuclei with prominent nucleoli. The cytoplasm had characteristic foamy vacuolated appearance. The zona reticularis cells were seen with well-distinct cell boundaries and rounded nuclei with prominent nucleoli. The cytoplasm had vacuolated foamy appearance (Figure 8A).

Group 2 (BPA-treated group): In BPA-treated group the zona glomerulosa was seen adjacent to the capsule and showed numerous irregularly arranged cells separated by intercellular spaces. The cells revealed highly vacuolated cytoplasm and pyknotic distorted nucleoli (Figure 7B). In zona fasciculata, the cells were disorganized with ill-detected cell boundaries, darkly stained (pyknotic) and distorted nuclei. The cytoplasm appeared rarified with marked vacuoles. The zona reticularis cells revealed pyknosis and marked vacuolation (Figure 8B).

Group 3 (BPA+vitamin C-treated group): In BPA and vitamin C-treated group, there was marked improvement and restoration of the normal arrangement of cells of zona glomerulosa adjacent to the capsule. Most of the nuclei appeared normally vesicular rounded with prominent nucleoli. Residual cytoplasmic vacuoles, pyknotic nuclei and dilated blood capillaries were noticed (Figure 7C). In the zonae fasciculata and reticularis the cells revealed well detected boundaries and rounded nuclei with prominent nucleoli. The cytoplasm showed some vacuoles but less than those noticed in the bisphenol-treated group (Figure 8C).

Electron Microscopic Results

Group 1 (Control group): On ultrathin sections examination of the control animals, it was observed that the cells of the zona glomerulosa had rounded nuclei with intact nuclear membrane. The cytoplasm showed multiple rounded mitochondria and lipid droplets (Figure 9A). The cells of the zona fasciculata exhibited rounded nuclei with peripheral dense heterochromatin and intact double walled nuclear membrane. The cytoplasm exhibited numerous mitochondria and foamy appearance due to lipid droplets (Figure 10A). The zona reticularis cells of the control group showed accumulation of lipid droplets of variable sizes. Round to oval nucleus with regular nuclear envelope and prominent nucleolus were noticed. Mitochondria were apparent (Figure 11A).

Group 2 (BPA-treated group): The zona glomerulosa of the BPA-treated group revealed distorted nuclei with irregular nuclear membrane. The cytoplasm looked rarified with large lytic area (area of cytoplasm containing few remnants of destroyed organelles). Mitochondria showed marked degeneration (Figure 9B). The cells of the zona fasciculata of the BPA-treated group revealed degenerated shrunken nuclei with indistinct nuclear membrane. The cytoplasm exhibited large lytic area and numerous lipid droplets (Figure 10B). The zona reticularis of the BPA-treated group revealed small sized nucleus with irregular nuclear envelope and numerous lipid droplets (Figure 11B).

Group 3 (BPA+vitamin C-treated group): The cells of the zona glomerulosa of the BPA and vitamin C-treated group appeared nearly normal as control. The cells retained vesicular rounded nuclei, prominent nucleoli and intact nuclear membranes. The cytoplasm showed multiple mitochondria and lipid droplets (Figure 9C). The zona fasciculata of the BPA and vitamin C-treated group restored rounded nuclei with an intact double walled nuclear membranes. The cytoplasm declared numerous lipid droplets. An adjacent blood capillary containing RBCs was noticed (Figure 10C). The cells of the zona reticularis of the BPA and vitamin C-treated group restored the normal appearance. The cells revealed rounded nuclei with intact nuclear membranes. The cytoplasm showed mitochondria and multiple lipid droplets. Adjacent blood capillaries containing RBCs were observed (Figure 11C).

Caspase-3 immunohistochemically stained results

On examination of the caspase-3 immunohistochemically stained sections of the control group, weak cytoplasmic reaction of the cells of the adrenal cortex was detected in the form of very light brown coloration especially in the zona glomerulosa and to a lesser extent in zonae fasciculata and reticularis (Figure 12A). In Bisphenol-treated group, strong positive cytoplasmic reaction in the form of dark brown coloration of cells of the zona glomerulosa and to a lesser extent in zonae fasciculata and reticularis was observed (Figure 12B). In Bisphenol and vitamin C- treated group, moderate cytoplasmic reaction in the form of light brown coloration was noticed in the cells of the zona glomerulosa mainly and to a lesser extent in zonae fasciculata and reticularis (Figure 12C).

Morphometric results

Layers thickness

A statistically significant marked reduction in thickness of all the layers was observed in BPA-treated group in comparison with the control. There was no statistically significant difference in the layers thickness of BPA+vitamin C treated group in comparison with the control (Tables 1,2,3, Histograms 1,2,3).

Cells diameter

The cells diameter of all layers exhibited a highly statistically significant decrease in the BPA-treated group in comparison with the control. There was no statistically significant difference in the cells diameter in all layers of the BPA+vitamin C-treated group in comparison with the control (Tables 1,2,3, Histograms 1,2,3).

%Area of the caspase-3 immunoreactivity

The %Area of the caspase-3 immunoreactivity exhibited a highly statistically significant increase in the BPA-treated group in comparison with the control. There was no statistically significant difference in the %Area of the caspase-3 immunoreactivity of the BPA+vitamin C-treated group in comparison with the control (Table 4, Histogram 4).

Fig.1

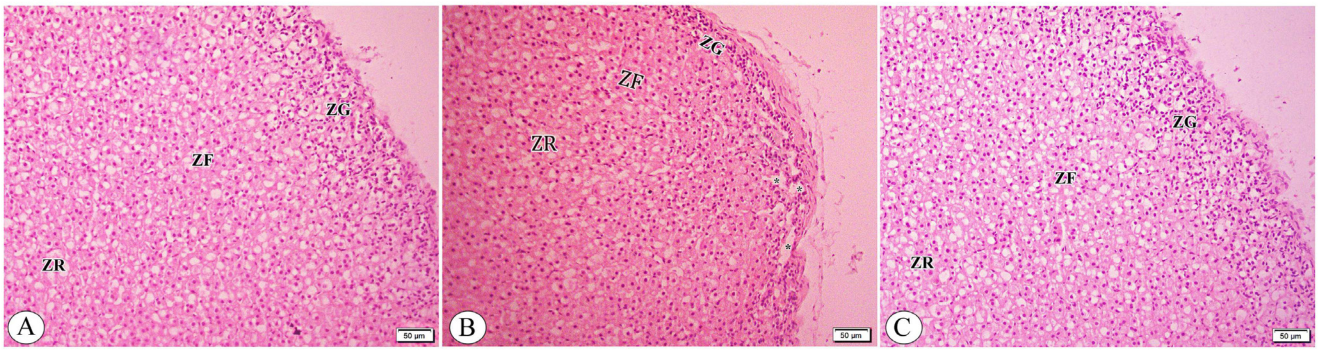


Fig. 1: Photomicrographs of sections of the adrenal cortex of adult male albino rats. (A) control group showing; well organized zona glomerulosa (ZG). Zona fasciculata (ZF) occupies most of the cortex. Zona reticularis (ZR) is the deepest cortical layer. (B) BPA-treated group showing the adrenal cortical three layers; zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). The zona glomerulosa cells (ZG) shows marked disorganization. (C) BPA+ vitamin C-treated group showing well organized zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). (Haematoxylin and eosin, X200)

Fig.2

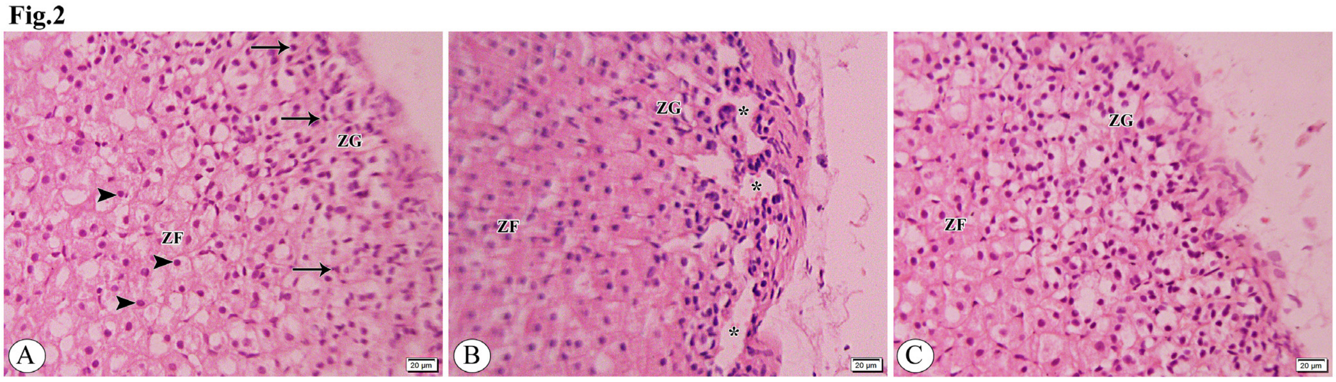


Fig. 2: Photomicrographs of sections of the adrenal cortex of adult male albino rats. (A) control group showing, the cells of the zona glomerulosa (ZG) are well organized in groups containing darkly stained nuclei (arrows), zona fasciculata (ZF) in which the cells are arranged in cords with rounded vesicular nuclei (arrowheads). (B) BPA-treated group showing, the cells of zona glomerulosa (ZG) appear markedly disorganized and widely separated with empty spaces (*). Notice the adjacent cells of the zona fasciculata (ZF). (C) BPA+ vitamin C-treated group showing restoration of the normal arrangement of the cells of the zona glomerulosa (ZG) and adjacent zona fasciculata (ZF). (Haematoxylin and eosin, X400)

Fig.3

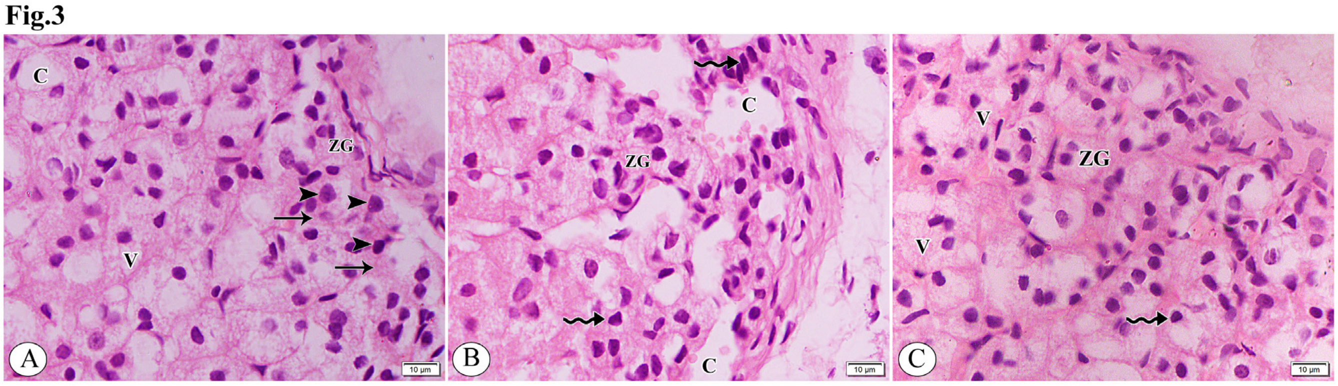


Fig. 3: Photomicrographs of sections of the adrenal cortex of adult male albino rats. (A) control group showing, the cells of the zona glomerulosa (ZG) are rounded to oval and arranged in clusters with darkly stained nuclei (arrowheads) and acidophilic (arrows) vacuolated cytoplasm (V). Blood capillaries among the cells are noticed (C). (B) BPA-treated group showing, some cells of the zona glomerulosa (ZG) reveal deeply acidophilic cytoplasm and pyknotic nuclei (wavy arrow). Dilated and congested blood capillaries are seen (C). (C) BPA+ vitamin C-treated group showing restoration of the normally arranged vacuolated cells (V) of the zona glomerulosa (ZG). Residual pyknotic nuclei (wavy arrow) are noticed. (Haematoxylin and eosin, X1000)

Fig.4

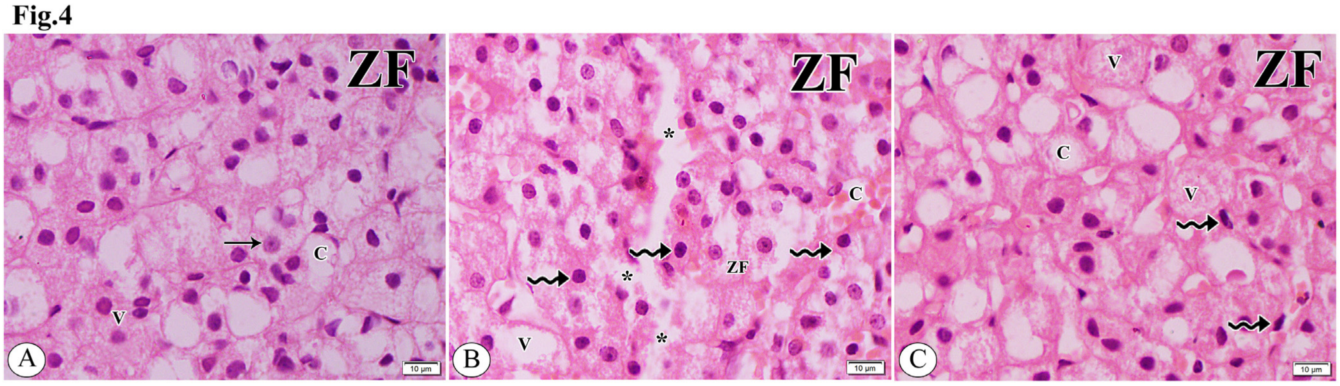


Fig. 4: Photomicrographs of sections of the adrenal cortex of adult male albino rats. (A) control group showing, the cells of the zona fasciculata (ZF) are aligned in cords with blood capillaries (C) in between. The nuclei are rounded vesicular with prominent nucleolus (arrow). The cytoplasm contains multiple vacuoles (V). (B) BPA-treated group showing, the cells of the zona fasciculata (ZF) reveal marked cytoplasmic vacuolization (V), disorganization and widely separated (*), pyknosis (wavy arrows) and marked congestion of the blood capillaries (C). (C) BPA+ vitamin C-treated group showing restoration of the normal arrangement of the vacuolated cells (V) of the zona fasciculata (ZF) with normal blood capillaries (C) in between. some pyknotic cells (wavy arrows) are seen. (Haematoxylin and eosin, X1000)

Fig.5

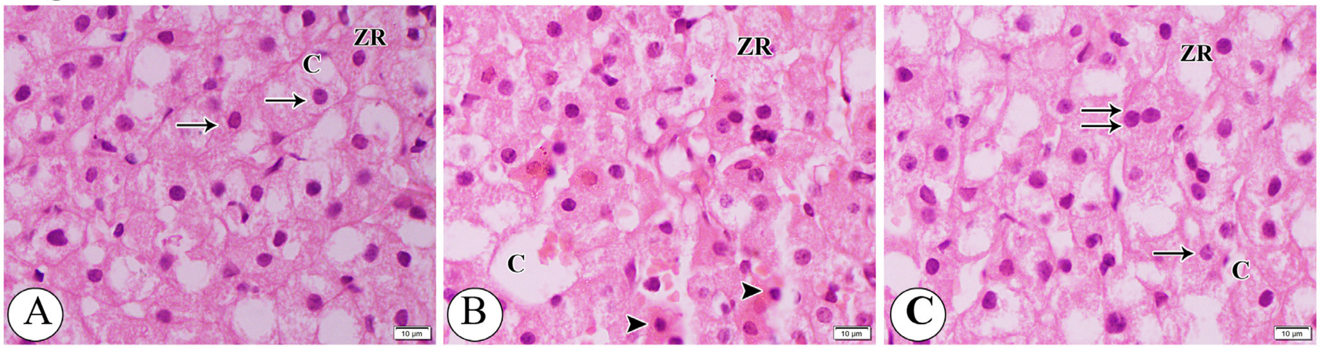


Fig.5: Photomicrographs of sections of the adrenal cortex of adult male albino rats. (A) control group showing, the cells of the zona reticulata (ZR) are formed of anastomosing cords of vacuolated cells with blood capillaries (C) in between. The nuclei are rounded vesicular with prominent nucleolus (arrows). (B) BPA-treated group showing, cells of the zona reticularis (ZR) show features of apoptosis in the form of acidophilic cytoplasm and darkly stained pyknotic nuclei (arrowheads). Marked congestion of blood capillaries (C) are noticed among the cells of the zona reticularis (C) BPA+ vitamin C-treated group showing restoration of the normal arrangement of the vacuolated cells (arrow) of the zona fasciculata (ZR) with blood capillaries (C) in between. Binucleated cells (double arrow) are observed. (Haematoxylin and eosin, X1000)

Fig.6

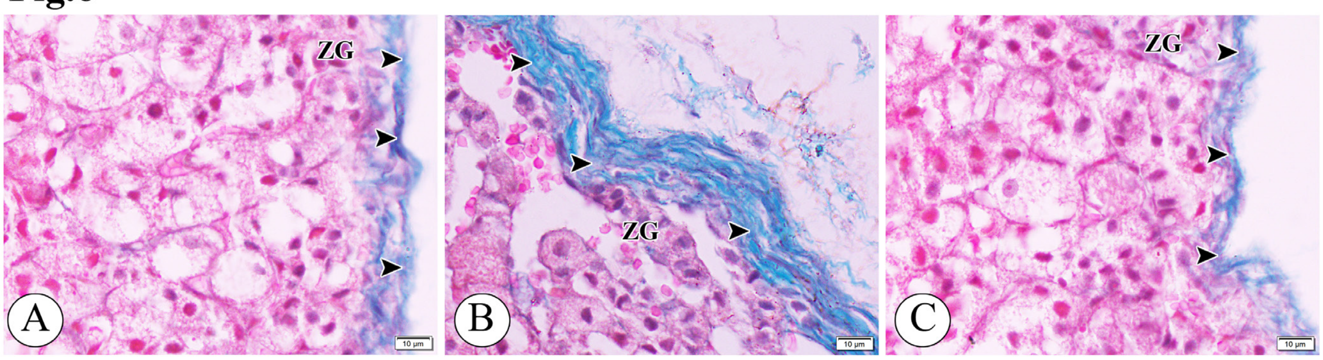


Fig. 6: Photomicrographs of sections of the adrenal cortex of adult male albino rats. (A) control group showing, fine capsular collagen fibers (arrowheads) adjacent to the zona glomerulosa (ZG). (B) BPA-treated group showing, dense capsular collagen fibers (arrowheads) adjacent to the zona glomerulosa (ZG). (C) BPA+ vitamin C-treated group showing fine capsular collagen fibers (arrowheads) adjacent to the zona glomerulosa (ZG). (Masson's trichrome, X 400)

Fig.7

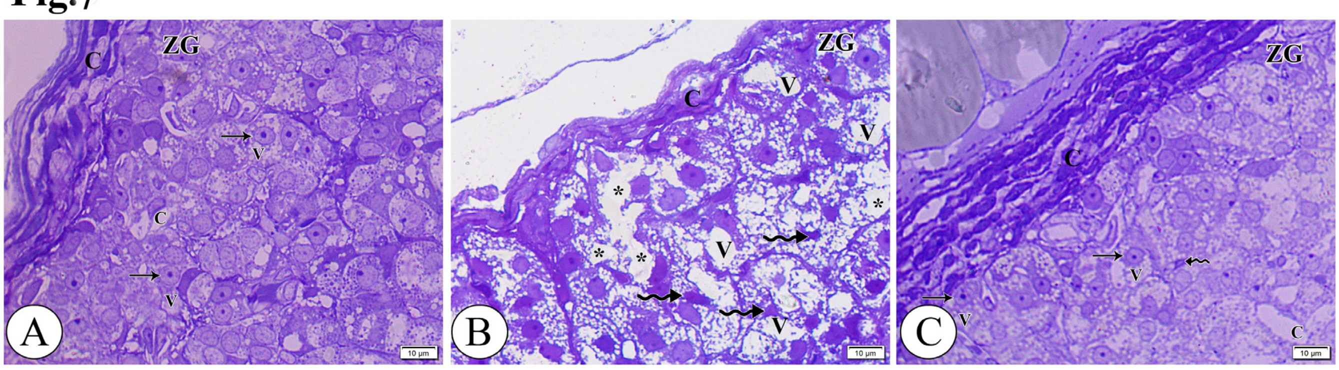


Fig. 7: Photomicrographs of semithin sections of the adrenal cortex of adult male albino rats. (A) control group showing, the capsule (C), the cells of the zona glomerulosa (ZG) are arranged in clusters with rounded vesicular nuclei and prominent nucleolus (arrows). Cytoplasmic vacuoles are noticed (V). (B) BPA-treated group showing, the capsule (C), the cells of the zona glomerulosa (ZG) are irregularly arranged with marked intercellular spaces (*), the nuclei are distorted and pyknotic (wavy arrows). Numerous cytoplasmic vacuoles are noticed (V). (C) BPA+ vitamin C-treated group showing, the capsule (C) and normal arrangement of the cells of the zona glomerulosa (ZG) with vesicular rounded nuclei and prominent nucleoli (arrows). Residual cytoplasmic vacuoles (V), pyknotic nuclei (wavy arrow) and dilated blood capillaries are noticed (C). (Toluidine blue, X1000)

Fig.8

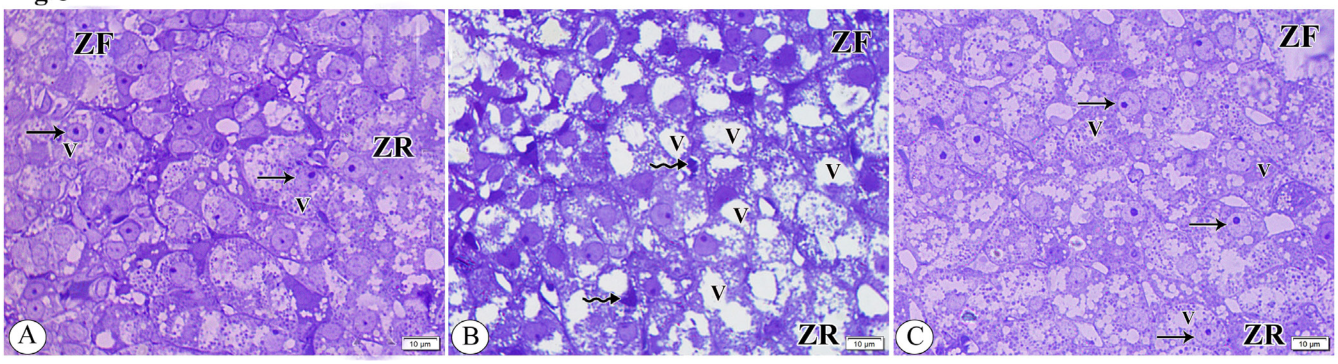


Fig. 8: Photomicrographs of semithin sections of the adrenal cortex (ZF) of adult male albino rats. (A) control group showing, the cells of the zona fasciculata (ZF) and zona reticularis (ZR) have well distinct cell boundaries. The nuclei (arrow) are round with prominent nucleoli. The cytoplasm reveals characteristic foamy vacuolated appearance (V). (B) BPA-treated group showing, the cells of the zonae fasciculata (ZF) and reticularis (ZR) are disorganized with ill detected cell boundaries and distorted pyknotic nuclei (arrows). The cytoplasm appears rarified with multiple vacuoles (V). (C) BPA+ vitamin C-treated group showing, the zonae fasciculata (ZF) and reticularis (ZR) retain well detected cell boundaries and rounded nuclei with prominent nucleoli(arrows). The cytoplasm reveals some vacuoles (V) but less than those noticed in the bisphenol-treated group. (Toluidine blue, X1000)

Fig.9

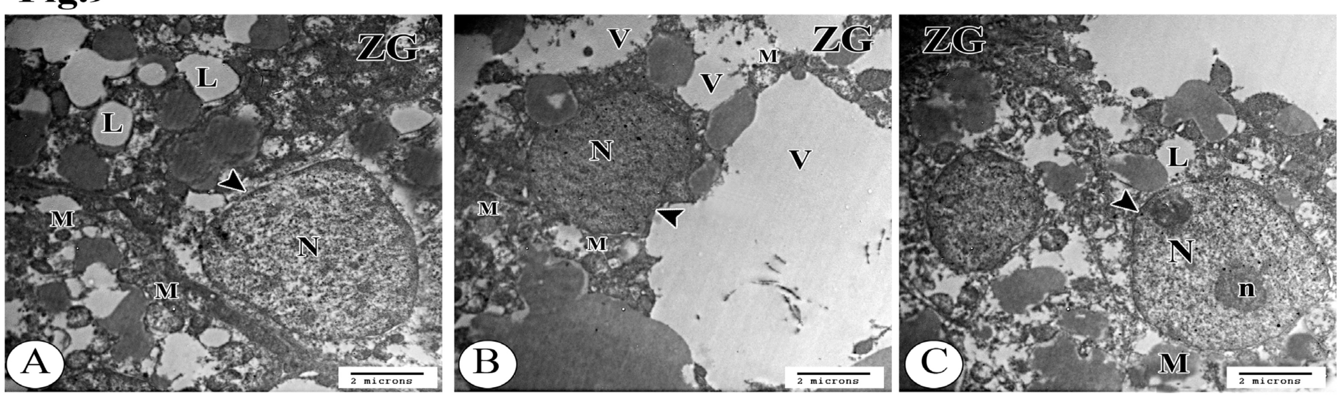


Fig. 9: Electron photomicrographs of the zona glomerulosa (ZG) of the adrenal cortex of adult male albino rats. (A) control group showing, a cell of the zona glomerulosa (ZG) has a rounded nucleus (N) with intact nuclear membrane (arrowhead). The cytoplasm shows multiple mitochondria (M) and lipid droplets (L). (B) BPA-treated group showing, a cell of the zona glomerulosa (ZG) has a distorted deeply stained nucleus (N) with irregular nuclear membrane (arrowhead). The cytoplasm is rarified with large lytic area (V) and degenerated mitochondria (M). (C) BPA+ vitamin C-treated group showing, the cells of the zona glomerulosa (ZG) have vesicular rounded nucleus (N), prominent nucleolus (n) and intact nuclear membrane (arrowhead). The cytoplasm shows multiple mitochondria (M) and lipid droplets (L). (TEM, X7200)

Fig.10

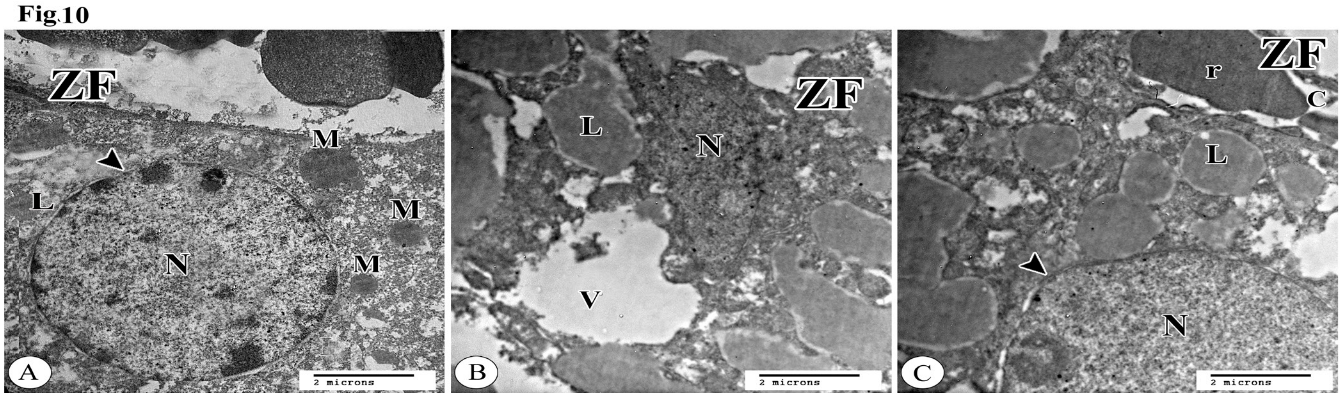


Fig. 10: Electron photomicrographs of the zona fasciculata (ZF) of the adrenal cortex of adult male albino rats. (A) control group showing, a cell of the zona fasciculata (ZF) has a rounded nucleus (N) with intact double walled nuclear membrane (arrowhead). The cytoplasm exhibits numerous mitochondria (M) and foamy appearance due to lipid droplets (L). (B) BPA-treated group showing, a cell of the zona fasciculata (ZF) has a degenerated shrunken nucleus with indistinct nuclear membrane (N). The cytoplasm shows large lytic area (V) and lipid droplets (L). (C) BPA+ vitamin C-treated group showing, a cell of the zona fasciculata (ZF) has a rounded nucleus (N) with an intact double walled nuclear membrane (arrowhead). The cytoplasm declares numerous lipid droplets (L). An adjacent blood capillary (C) containing RBCs (r) is noticed. (TEM, X10000)

Fig.11

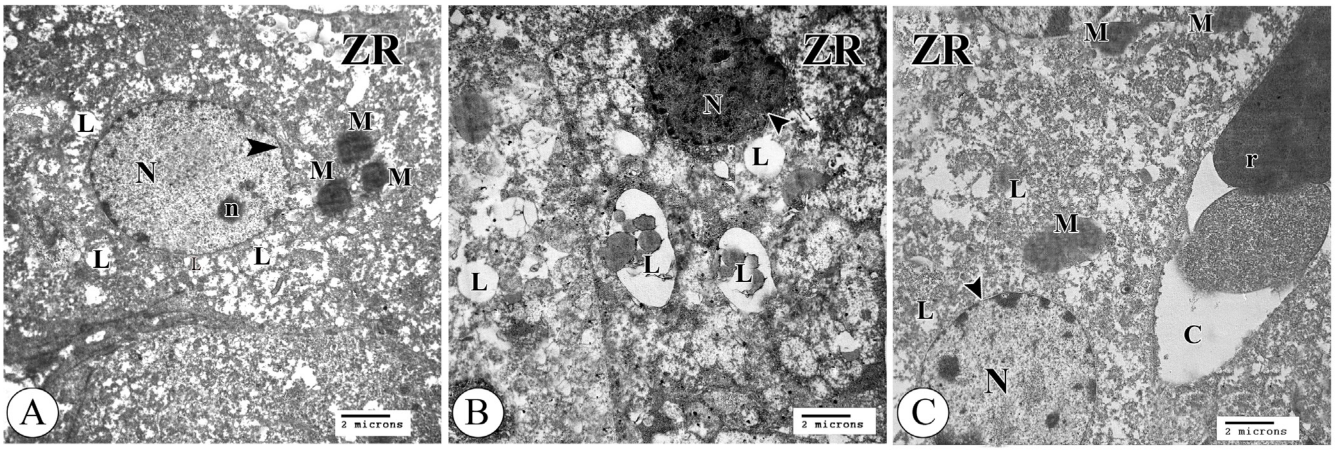


Fig. 11: Electron photomicrographs of cells of the zona reticularis (ZR) of the adrenal cortex of adult male albino rats. (A) control group showing, a rounded to oval nucleus (N) with intact nuclear membrane (arrowhead) and prominent nucleolus (n). The cytoplasm shows mitochondria (M) and multiple lipid droplets (L). (B) BPA-treated group showing, small sized shrunken nucleus (N) with irregular nuclear membrane (arrowhead). The cytoplasm showed numerous lipid droplets (L). (C) BPA+ vitamin C-treated group showing, rounded nucleus (N) with intact nuclear membrane (arrowhead). The cytoplasm shows mitochondria (M) and multiple lipid droplets (L). An adjacent blood capillary (C) containing RBCs (r) is noticed. (TEM, X7200)

Fig.12

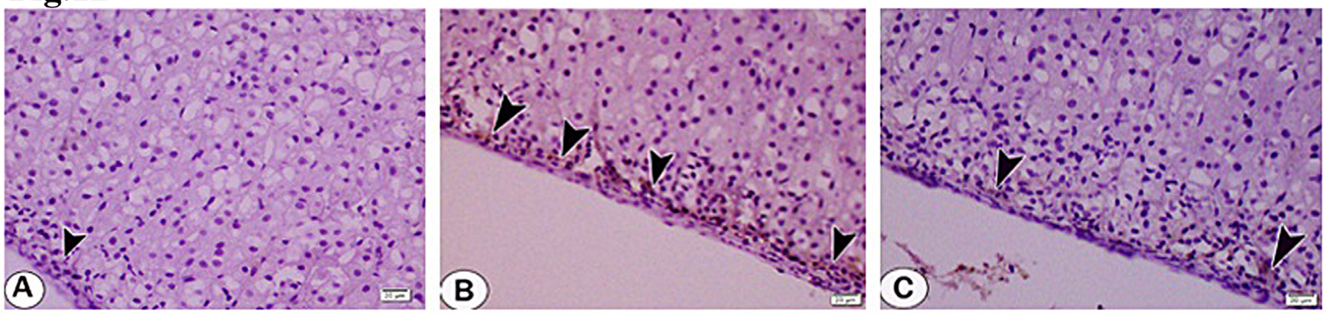


Fig. 12: Caspase-3 immune-reactivity photomicrographs of the adrenal cortex of adult male albino rats. (A) control group showing, weak immune reaction to caspase-3 in the form of very light brown coloration in zona glomerulosa mainly (arrowheads). (B) BPA-treated group showing, strong positive immune reaction to caspase-3 in the form of dark brown coloration mainly in the zona glomerulosa (arrowheads). (C) BPA+vitamin C-treated group showing, moderate positive reaction to caspase-3 in the form of light brown color mainly in the zona glomerulosa (arrowheads). (Caspase-3 immunostaining, X400)

Table 1: The glomerular layer thickness and the glomerular cell diameter of the three groups

	Group 1 Mean±SD n=10	Group 2 Mean±SD n=10	Group 3 Mean±SD n=10
Glomerular layer thickness (um)	97.27±1.61	43.44±1.95***	96.02±1.40
Glomerular cell diameter (um)	14.472±0.27	8.51±0.34***	14.31±0.27

*Differences at $p < 0.05$ were considered significant

Table 2: The fascicular layer thickness and the fascicular cell diameter of the three groups

	Group 1 Mean±SD n=10	Group 2 Mean±SD n=10	Group 3 Mean±SD n=10
Fascicular layer thickness (um)	405.09±2.36	360.57±1.90***	406.00±2.21
Fascicular cell diameter (um)	14.24±0.03	8.23±0.17***	14.18±0.03

*Differences at $p < 0.05$ were considered significant

Table 3: The reticular layer thickness and the reticular cell diameter of the three groups

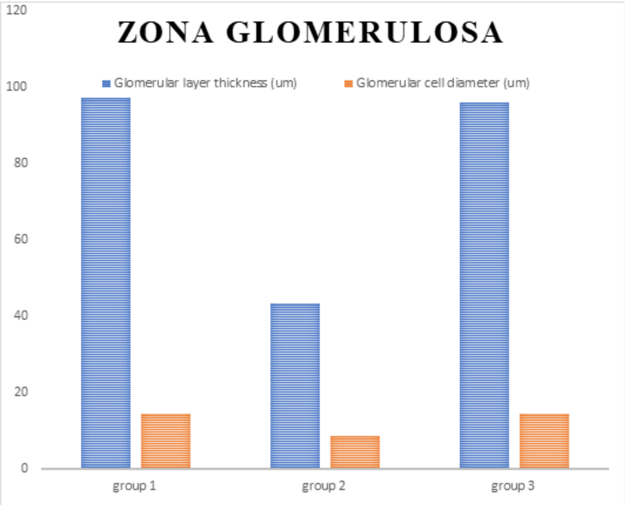
	Group 1 Mean±SD n=10	Group 2 Mean±SD n=10	Group 3 Mean±SD n=10
Fascicular layer thickness (um)	62.25±1.51	33.59±1.00***	61.59±1.92
Fascicular cell diameter (um)	18.39±0.25	10.98±0.54***	18.17±0.32

*Differences at $p < 0.05$ were considered significant

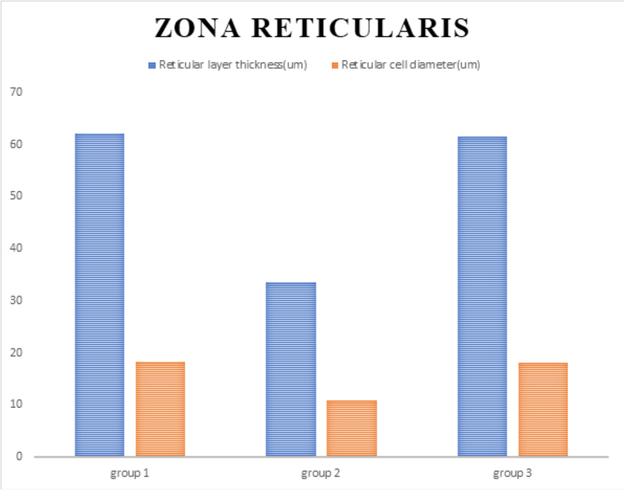
Table 4: The % Area of the caspase-3 immunoreactivity of the three groups

	Group 1 Mean±SD n=10	Group 2 Mean±SD n=10	Group 3 Mean±SD n=10
% Area of the caspase-3 immunoreactivity	0.043±0.002	1.542±0.001***	0.044±0.002

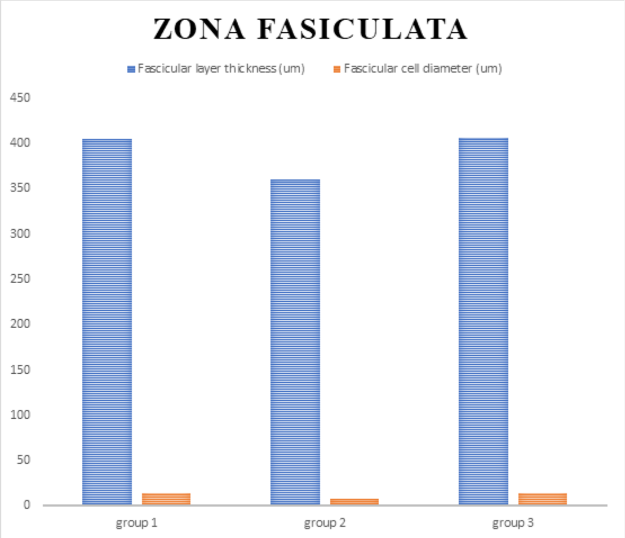
*Differences at $p < 0.05$ were considered significant



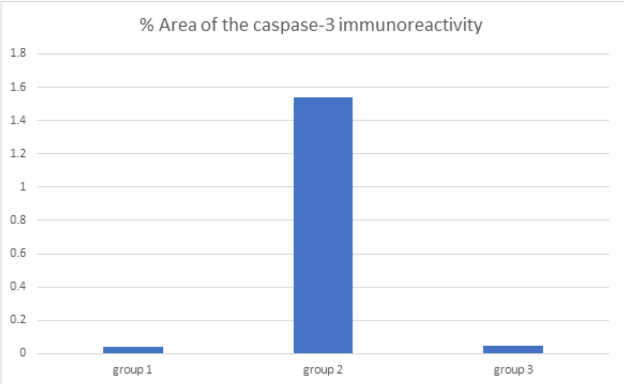
Histogram 1: The glomerular layer thickness and the glomerular cell diameter of the three groups



Histogram 3: The reticular layer thickness and the Reticular cell diameter of the three groups



Histogram 2: The fascicular layer thickness and the fascicular cell diameter of the three groups



Histogram 4: The % Area of the caspase-3 immunoreactivity of the three groups

DISCUSSION

Although bisphenol A is widely used in manufacture to produce synthetic polymers, its usage is associated with hazardous effects on the human body organs[1,3]. The animal of choice for this study was the albino rat because of the similarity in the anatomy and physiology of these animals to human. In addition, several studies carried out on albino rats often produced similar results with those conducted on humans[16].

The current work revealed that BPA induced histopathological and immunohistochemical changes that confirmed morphometrically on the adrenal cortex of the male albino rat. The administration of 25 mg bisphenol-A /kg/day once daily, for six weeks resulted in capillary congestion, cellular disorganization, cellular acidophilia, nuclear pyknosis, wide intercellular spaces and vacuolization of the cytoplasm. These results coincide with the findings of the study of Samuel and others[17]. They reported that the observed capillary congestion and pyknotic nuclei of the zona glomerulosa showed that bisphenol-A is injurious to the adrenal cortex and complements the elevated circulating levels of corticosterone and ACTH observed through their hormonal assay. They added that within the adrenal cortex, the zonae glomerulosa and fasciculata were described as regions responsible for adrenocortical cytogenesis where most of the intense mitotic activity of the adrenal cortex takes place.

Thickening of the adrenal capsule of BPA-treated group when compared to the control group was observed in the Masson-stained sections of the present study. Previous several studies showed that adrenal viability depends mainly on conversion of the zona glomerulosa cells into the zona fasciculata cells and the signaling center that controls the cortical cells renewal is present in the adrenal capsule. So that, the adrenal capsule is considered an important factor maintaining adrenal zonation and continuous replacement of the destroyed cells throughout life[18].

In the present work, Toluidine blue-stained semithin sections of BPA-treated group showed an increase in the cytoplasmic vacuolization. Zonae fasciculata and reticularis cells also declared ill-defined cell boundaries and pyknotic darkly stained nuclei and these results agreed with the study of Ghassan[19]. The main site of lipid storages essential for steroidogenesis is the cells of adrenal cortex[20]. Disturbance in steroidogenesis results from oxidative damage. Thus, cholesterol synthesis would be reduced with marked accumulation of lipid droplets in the cytoplasm indicating the adrenal hypo-functional state[5].

In addition, the Caspase-3 immunohistochemically results of the BPA-treated group of the present work declared strong positive cytoplasmic reaction of the cells of the adrenal cortex especially the zona glomerulosa. This finding is an indicator of increased cellular apoptosis. Two known apoptotic pathways for activation of Caspase 3; the intrinsic (mitochondrial) pathway and the extrinsic (receptor) one which is activated by oxidative stress[21].

The marked reduction in the thickness and cell diameter of all layers of the adrenal cortex in the present work on administration of bisphenol-A comes in agreement with Samuel *et al*.[17]. The obvious reduction of the thickness of the different layers of the adrenal cortex comes parallel to the histologic results in the form of pyknotic nuclei of the three zonae of the adrenal cortex of the BPA-treated group of the present work and are also concomitant with Panagiotidou *et al*.[22]. They indicated that BPA is injurious to the cells of adrenal cortex and complements the elevated circulating serum levels of corticosterone and ACTH detected through hormone assay. They added that the ZG and ZF are regions where adrenocortical synthesis occurs and most of the mitotic activity of the adrenal cortex takes place.

Moreover, the studies of[23-30], attributed such results to the ability of BPA to induce peroxidation of lipid and decreased activities of antioxidant enzyme in many tissues such as the adrenal gland, testis, epididymis, prostate gland, central nervous system, liver, and lungs. They noted that the exposure to BPA increased the levels of H_2O_2 and malondialdehyde (MDA) while glutathione (GSH) activities were significantly decreased in the albino rats and this correlated with our result as regard destructive changes induced by bisphenol in adrenal cortex as loss of normal alignment, distorted nuclei, increased vacuolation and cellular degeneration.

According to Anjum *et al*.[31], the mice exposed to standardized dose of BPA resulted in lipid peroxidation and reduction in mitochondrial glutathione. BPA also reduces the antioxidant enzyme activity such as glutathione reductase, glutathione peroxidase, and superoxide dismutase. They reported that the decreased GSH activity and increased MDA levels in the adult rats are indicators of increased adrenal gland reactive oxygen species (ROS) production that led to lipid peroxidation, and this coincide with our results as mitochondrial degeneration and large lytic areas in cytoplasm.

Lan *et al*.[32] reported that the noticed reduction of the antioxidant enzymes activities and the ROS increase indicate that the BPA ameliorates the adrenal antioxidant pathway and induces ROS generation. Therefore, the increased circulating corticosterone and ACTH levels noticed in many studies are indicators of the adrenal gland exposed to the oxidative stress and this correlated with our findings as regard increased the apoptotic activity detected by strong positive reactivity to caspase 3 in bisphenol treated group.

Vitamin C's antioxidant function is mediated by the fact that it neutralizes ROS by providing one of its own electrons, effectively ending the "stealing" reaction and preventing cell and tissue damage[33,34].

The concomitant administration of vitamin C with BPA in the present work resulted in maintained adrenal cortical structure to a great extent. One of the important organs rich in vitamin C in the body is the adrenal gland. Vitamin C is stored in adrenal glands, and it is essential for synthesis of catecholamine and steroidogenesis[35]. Many Studies have proved the essential role of vitamin C on the cells of the adrenal cortex. Moreover, vitamin C affects the structure of adrenal cortex as its deficiency leads to decrease in the level of corticosterone and disturb the morphology of mitochondria of adrenal cortical cells[36].

Furthermore, the current immunohistochemical results of the animal group given BPA plus vitamin C showed reduced apoptotic cells in the adrenal cortex. Adikwu and Deo[37] reported that, vitamin C is considered important physiological antioxidant. They added that, it exerts its action through inhibition of DNA damage inside the mitochondria and decrease the lipid peroxidation directly or indirect through regeneration of vitamin E which is an antioxidant, too.

CONCLUSION

Bisphenol-A exposure resulted in marked histological, immunohistochemical and morphometric changes of the adrenal cortex. Concomitant administration of vitamin C greatly protects against these effects.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور الوقائي المحتمل لفيتامين ج ضد التغيرات التركيبية المستحدثة بمادة البيسفينول-أ على قشرة الغدة الكظرية في ذكر الجرذ الأبيض البالغ: دراسة نسيجية ومناعية ومورفومترية

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الخلفية: يعتبر بيسفينول-أ مونومرًا في تصنيع لدائن البولي كربونات وتركيب راتنجات الإيبوكسي. ومع ذلك، فهو يسبب اضطراب في وظائف الغدد الصماء ويساهم في العديد من المشاكل الصحية. فيتامين ج هو أحد مضادات الأكسدة القوية.

هدف الدراسة: هدفت هذه الدراسة إلى الكشف عن تأثيرات مادة بيسفينول-أ على بنية قشرة الغدة الكظرية والدور الوقائي المحتمل للتناول المتزامن لفيتامين ج ضد هذه التغيرات في ذكر الجرذ الأبيض البالغ.

المواد وطرق البحث: تم استخدام خمسة وأربعين من ذكور الجرذان البيضاء البالغ وزنها (٢٠٠-٢٥٠) جرام. تم تقسيم الفئران عشوائيًا إلى ثلاث مجموعات متساوية.

المجموعة الأولي: المجموعة الضابطة أعطيت الماء المقطر لمدة ستة أسابيع.

المجموعة الثانية: تناولت ٢٥ مجم بيسفينول-أ / كجم / يوم مذابًا في زيت الزيتون مرة واحدة يومياً عن طريق الفم لمدة ستة أسابيع.

المجموعة الثالثة: تناولت ٢٥ مجم بيسفينول-أ / كجم / يوم مذابًا في زيت الزيتون و٦٠ مجم فيتامين ج / كجم / يوم مرة واحدة يومياً عن طريق الفم لمدة ستة أسابيع. في نهاية التجربة، تمت التضحية بالفئران. مع إزالة الغدة الكظرية اليسرى، ومعالجتها للدراسة بالميكروسكوب الضوئي والمجهر الإلكتروني، والدراسة المناعية والمورفومترية.

النتائج: تسبب تناول بيسفينول-أ في حدوث تغيرات في قشرة الغدة الكظرية مثل فقدان الترتيب الطبيعي للخلايا، احتقان الشعيرات الدموية، تفريغ السيتوبلازم، تقلص النوى، زيادة سمك الكبسولة، تفاعل السيتوبلازم الإيجابي القوي لماده caspase-٣، انخفاض كبيرملحوظ في سمك وقطر الخلية في جميع الطبقات الثلاثة، وزيادة النسبة المئوية لمساحة النشاط المناعي لـ caspase-٣. ولقد وقى فيتامين ج من حدوث معظم هذه التأثيرات النسيجيّة الضوئية والإلكترونية والمناعيّة والمورفومتريّة.

الخلاصة: إن تناول مادة بيسفينول-أ له تأثيرات ضارة على قشرة الغدة الكظرية ويقي فيتامين ج من هذه التأثيرات.