

Effect of Perinatal Exposure to Low Dose Bisphenol A on Hepatic and Renal Tissues of Male Albino Rat Offspring: Histological, Immunohistochemical and Morphometric Studies

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ABSTRACT

Introduction: Bisphenol A (BPA) is one of the environmental endocrine disrupting chemicals with worldwide human oral exposure. Guideline studies established a no observed adverse effect level (NOAEL) at 5 mg/kg body weight /day.

Aim of The Work: To assess the hazards of oral maternal intake to low dose of BPA below NOAEL during pregnancy and lactation, on hepatic and renal tissues of male albino rat offspring through histological, immunohistochemical and morphometric studies.

Material and Methods: Thirty pregnant albino rats were equally divided into three groups; group A did not receive any treatment, group B received 1mL corn oil orally once daily by gastric tube, group C received 200 µg/kg bw /day of BPA (dissolved in 1mL corn oil) by gastric tube once daily from 6th day of gestation until weaning. Forty male rat pups from each group were subdivided into four subgroups according to the age of sacrifice (1st, 3rd, 6th and 9th) postnatal weeks (PW). After weaning, treatment was stopped. Livers and kidneys' specimens were collected and prepared for histological, immunohistochemical, morphometric studies and statistical analysis.

Results: Multiple histological degenerative changes in the hepatic and renal tissues of male albino rat offspring were observed after oral administration of low dose BPA to their mothers. These changes were obvious at 1st PW and progressed to become massive and extensive at 3rd and 6th PW then became less severe at 9th PW in spite of the cessation of BPA treatment at weaning. These results were confirmed by immunohistochemical and statistical studies.

Conclusion: Perinatal maternal intake to low dose of BPA below the dose of NOAEL, induced histopathological changes in livers and kidneys of male albino rat offspring. These changes may be considered as indicator for possibility of neoplastic liability.

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INTRODUCTION

Bisphenol A (BPA), an environmental chemical used in epoxy resins and polycarbonate plastics synthesis, is an organic plasticizer produced in large quantities all over the world (more than 6 billion pounds are produced per year)^[1]. It is used in food and drink packaging, food cans coating, bottle tops and water supply pipes synthesis^[2]. It is also present in many daily used products such as baby bottles, mobile phone casings and eyeglasses^[3]. Human exposure occurs mainly orally from food and beverage plastic containers and through saliva from dental sealants^[4]. Also, BPA exposure could occur via dermal contact with thermal-paper receipts^[5]. Polymerized BPA molecules are ester bonds linked and are subjected to hydrolysis and leach from plastics when they are subjected to heat, basic or acidic conditions^[6]. This clearly indicates that daily human exposure to BPA at different ages is unavoidable and making it a reason for concern.

Bisphenol A is a contaminant that applies both toxic and estrogenic effects on mammalian cells with increasing exposure to it. In adult human and animals, high BPA levels increase coronary heart disease incidence^[7], decrease thyroid hormone levels^[8], reduce fertility^[9], alter gene expression with subsequent neoplastic transformation^[10] and induce abnormal liver and kidney function^[11]. Being daily used, BPA exposure does not occur only in the adult period, but also occurs in the fetal and neonatal period. It has been found in a high concentration in the placental tissue, amniotic fluids, neonatal blood, umbilical cord and in blood of pregnant women, breast milk and fetal liver^[12-14]. Consequently, BPA exposure during pregnancy is of particular concern since key body organs are undergoing growth and differentiation during this critical time.

Several studies have investigated the potential harmful effect of prenatal BPA exposure on offspring such as abnormal glucose metabolism^[15], developing immune

system affection^[16], altered rodent behavior^[17], increased risk of wheezing and asthma^[18] and multiple organ developmental involvement such as brain^[19], heart^[20], mammary glands^[21] and uterus^[22].

In Animal research, U.S. Environment Protection Agency (U.S.EPA) determined a no observed adverse effect level (NOAEL) of BPA at 50 mg/kg bw /day while European Union set it at 5 mg/kg bw /day^[23]. However, the European Food Safety Authority (EFSA) and the U.S.EPA have established tolerable daily intake (TDI) or reference dose (50 µg/kg bw /day) for BPA as a 'safe dose. After careful evaluation, the ESFA down-regulated the TDI of BPA to 4 µg/kg/day in 2015, yet the U.S.EPA retained the old standard. However, the 'safe' dose of BPA remains controversial^[24].

Several studies have been conducted on a low dose BPA exposure (ranged from 2.5 to 5,000 µg/kg bw /day) with established adverse effects^[23,25,26]. However, to our knowledge, the effect of perinatal exposure to low dose of BPA on liver and kidney histopathology still needs more studies.

Keeping the previous literature in mind, the present study was undertaken to elucidate whether low dose of BPA given orally to mothers during gestational and lactational period could induce histopathological changes on liver and kidneys of male albino rat offspring. Light microscopy, Immunohistochemical and morphometric studies were used in this evaluation.

MATERIAL AND METHODS

Chemical

1. BPA powder was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). It was administered as 200 µg/kg bw /day dissolved in 1 ml corn oil.
2. Immunohistochemical kits for localization of proliferating cell nuclear antigen (PCNA) (the primary monoclonal antibody was anti PCNA IgG antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and for Caspase-3 (apoptosis marker) (the primary monoclonal antibody was the mouse monoclonal primary antibody to Caspase-3 (Ab-7, Mouse Mab. MS.).

Animals

Apparently healthy 30 female and 15 male adult albino rats (180-200 gm weight) were obtained from scientific medical research center (ZSMRC) at the Faculty of Medicine, Zagazig University. The experiment has been reviewed and approved by Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC) (approval number: ZU-IACUC/3/F/34/2019).

Experimental procedures: Throughout the experimental period, all animals were housed under controlled laboratory conditions (12-h light and 12-h dark cycle at 25°C and with appropriate humidity). The rats were kept in metal cages and

were fed a standard rodent pellet diet. Food and tap water were provided ad-libitum supply via glass bottles to avoid BPA exposure. After adaptation for 2 weeks, females were daily examined using vaginal smear technique to ensure that they were in regular estrous cycle. When proved that females were in their estrous phase, mating with mature male rats was allowed in separate cages at a ratio of 2:1. After mating, vaginal smears were obtained and presence of sperms indicated zero day of gestation^[6]. The process was repeated until all females became pregnant. Thirty pregnant rats were divided randomly into 3 equal groups:

Group A (negative control): 10 pregnant rats did not receive any treatment and were served as negative control group.

Group B (positive control): 10 pregnant rats were given 1ml corn oil only as vehicle orally by gastric tube once daily.

Group C (BPA treated): 10 pregnant rats were given BPA (at a dose of 200 µg/kg bw/day dissolved in 1ml corn oil) by a gastric tube once daily^[27].

The treatment to mothers (groups B and C) was from the 6th day of gestation until birth and during lactation until weaning (3rd week after birth).

After birth, forty male rat pups were selected from the total first generation of each group to be housed with their mothers (10 rats /group) in a ventilated room at a constant temperature (25°C) throughout the lactation period until weaning. After weaning, the male pups were separated from their mothers. They did not receive any treatment and they were fed only a standard diet. The female pups were excluded from this study.

The male rat pups of each group (A, B and C) were labeled and subdivided randomly into four subgroups (each contained 10 pups) according to the age of sacrifice^[28] as follow:

Subgroups (A1, B1 and C1): pups were sacrificed at the end of the first postnatal week (1st PW).

Subgroups (A2, B2 and C2): pups were sacrificed at the end of the third postnatal week (3rd PW).

Subgroups (A3, B3 and C3): pups were sacrificed at the end of the sixth postnatal week (6th PW).

Subgroups (A4, B4 and C4): pups were sacrificed at the end of the ninth postnatal week (9th PW).

By the end of the experiment, rat offspring of each subgroup injected intraperitoneally of 50 mg/kg bw pentobarbital^[29] to be anesthetized. The abdominal cavities were opened; livers and kidneys were rapidly dissected, delivered, rinsed with cold normal saline and well prepared to be examined by histological, immunohistochemical and morphometric studies.

A-Light Microscopy

Specimens for histological examination were fixed in 10% neutral formol saline and processed In ascending

grades of alcohol (50%, 70%, 90% and 95%), the samples were dehydrated each for one hour. Then, in absolute alcohol (100%), two changes one hour for each. After clearing in xylene, samples were embedded in soft paraffin wax at 55 °C for 2 hours and in hard paraffin at 60 °C for another 2 hours. Sections of 5 µm thick paraffin sections were prepared for Hematoxylin and eosin, staining^[30]. Examination and photography using light microscope were established at anatomy Department, Faculty of Medicine, and Zagazig University.

B-Immunohistochemistry

Liver and kidney paraffin sections were immunohistochemically stained for localization of proliferating cell nuclear antigen (PCNA) and for Caspase-3 (apoptosis marker).

Paraffin sections of 4-µm-thickness of hepatic and renal tissues of male albino rat offspring were passed through xylene and graded alcohol and rinsed in phosphate-buffered saline (PBS). Endogenous peroxidase was inactivated using 3% hydrogen peroxide (Sigma). Slides were microwaved in citrate buffer for 20 min, followed by an overnight incubation at 4°C with 1-2 drops of anti-PCNA (1:200) and with anti-Caspase-3 (1:50 dilution) antibodies in PBS. Following washes, the reaction product was detected with an avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, Calif., USA). The antigen antibody reaction was finally visualized with 0.05% diaminobenzidine (DAB) reagent at room temperature. The material was counterstained with Mayer's Hematoxylin then dehydrated and mounted. The PCNA immunoreactions were indicated by brown coloration in nuclei of hepatic and renal cells. The Caspase immunoreactions were expressed as brown yellow reaction product in cytoplasm of the cells, whereas the background was stained blue^[31].

C- Morphometric Study

Immunohistochemical- stained Sections were morphometrically analyzed using the image analyzer computer system (software Leica Quin 500) at Oral Pathology Department, Faculty of Dental Medicine, Cairo University. In hepatic and renal tissues of rats, the numbers of PCNA positive cells were counted and the area % of the Caspase-3 protein expression/ unit area (Unit area = microscopic field) was measured in a standard measuring frame using a magnification X400 by light microscopy transferred to the monitor's screen. The number of PCNA positive cells and the area % values of the Caspase-3 protein expression were assessed per field of different non-overlapped fields for the same slide of each animal. A mean of 10 readings was recorded from 5 serial sections from slides of each animal in each group. Values were assessed (mean ±standard deviation) and processed for statistical analysis.

D-Statistical Analysis

Continuous data were expressed as mean±SD as data were normally distributed (parametric). Normality was

checked by Shapiro-Wilk test. A one-way ANOVA was used to detect statistical differences between groups. Post hoc Tukey's test was performed for multiple comparisons between groups. The one-way repeated measure ANOVA was used to test for differences between groups when the dependent variable is normally distributed continuous variable. One group is measured on three or more different occasions. Adjusted Bonferroni posttest was used for multiple comparisons following one-way repeated measure ANOVA to detect which occasion in particular differ from other occasions. The differences were considered significant at $P<0.05$. All statistical comparisons were two-tailed. All statistical calculations were carried out using Graph Pad Prism, Version 5.0 Software (Graph Pad Software, San Diego, CA, USA).

RESULTS

A-Light Microscopy Using HandE Staining

Histological examination showed no difference between the negative control group (group A) and positive control groups (group B) in all subgroups and displayed normal hepatic parenchyma or normal renal cortex.

1-The Liver

-At 1st PW: (Figures 1a-1e)

The hepatic lobules of the two control subgroups (A1 and B1) contained normal thin walled central veins, blood sinusoids and some hemopoietic cells. The hepatocytes showed rounded central vesicular nuclei and acidophilic cytoplasm (Figure 1a). The portal area was seen containing normal portal vein with thin lining endothelium. The bile ductules were lined with one layer of simple cuboidal epithelium (Figure 1b). In subgroup (C1) treated with BPA, the parenchyma of the liver showed moderate degenerative changes in the form of moderate dilation of central vein and sinusoids, proliferation of kupffer cell lining, vacuolation of peripheral hepatocytes and swelling of pericentral hepatocytes with darkly stained nuclei (Figures 1c and 1d). Moreover, moderate dilatation of portal veins with thick wall and hyperplasia of wall of bile ductules were also demonstrated. Some inflammatory cell infiltration was seen in the periportal area (Figure 1e).

-At 3rd PW: (Figures 2a-2e)

The subgroups (A2 and B2) showed normal appearance of hepatic cords with a cell radial arrangement surrounding the thin walled central vein. The blood sinusoids appeared between the hepatic cords as vascular spaces lined by flat endothelial and Kupffer cells. The hepatocytes appeared polygonal in shape and contained acidophilic cytoplasm with rounded vesicular central nuclei (Figure 2a). The portal area contained the normal thin walled portal vein, hepatic artery and bile ductules lined with simple cuboidal epithelial cells (Figure 2b). However, subgroup (C2) showed extensive degenerative changes in the parenchyma of the liver in the form of marked dilatation of central vein with discontinuation and delamination of its

epithelial lining. Hydropic swelling, vacuolation, rupture and darkly staining nuclei of hepatocytes were also well seen (Figure 2c). Some hepatic lobules showed congested central vein with thickened wall, obliterated sinusoid with prominent Kupffer cells lining and abundant hepatocytes with darkly stained nuclei (Figure 2d). As regard to the portal area, there were marked dilatation and congestion of portal vein, hyperplasia of bile ductules, periportal inflammatory cell infiltration and thickening of the wall of both portal vein and hepatic artery (Figure 2e).

-At 6th PW: (Figures 3a-3g)

Light microscopic examination indicated normal architecture of liver tissue in the subgroups (A3 and B3), (Figures 3a and 3b). The subgroup (C3) still showed extensive degenerative changes despite of the cessation of BPA treatment to the offspring at age of weaning. The parenchyma of the liver displayed dilatation and congestion of central vein, extensive hydropic swelling and vacuolation of mono and binucleated hepatocytes. Different forms of nuclear degeneration were also seen as; pyknosis, karyolysis and Karyorrhexis (Figure 3c). Other degenerative changes were apparent in some hepatic lobules in the form of marked pericentral inflammatory cell infiltration around the central vein, marked dilation of some blood sinusoids and proliferation of kupffer cell lining (Figures 3d-3e). The portal vein was dilated with thickened wall and partly filled with blood. Bile ductules still exhibited hyperplastic epithelium or increased in their number (proliferation) as well. Hepatic artery still had thick wall. Excessive edema and marked inflammatory cell infiltration were also seen around the periportal area (Figures 3f-3g).

-At 9th PW: (Figures 4a-4d)

Subgroups A4 and B4 showed normal architecture of liver tissue (Figures 4a and 4b). In subgroup (C4), the hepatic parenchyma still showed less severe histopathology. Mild dilation of both the central vein and its draining sinusoids were observed. The peripheral hepatocytes still showed mild vacuolation. However, the pericentral hepatocytes appeared nearly normal and exhibited rounded vesicular central nuclei and acidophilic cytoplasm. No swelling of hepatocytes or nuclear degeneration was observed (Figure 4c). The portal vein was mildly dilated and still had thick wall. The bile ductules appeared with normal lining of one layer of cubical epithelium. Neither hyperplasia nor proliferation of bile ductules was shown. Little periportal edema and few inflammatory cell infiltrations were observed. Some Hepatic arteries appeared normal and others were still having thickened wall (Figure 4d).

2-The kidney

-At 1st PW: (Figures 5a-5f)

Light microscopic examination in the subgroups (A1 and B1), indicated normal renal architecture. The superficial renal cortex showed nephrogenic mesenchyme

contained immature renal developmental stages including ureteric bud (lined with simple cuboidal cells), renal vesicles (lined with columnar cells with central lumen) and different forms of immature glomeruli (Figure 5a). Deeper in the cortex, more mature glomeruli and differentiated proximal and distal convoluted tubules were well demonstrated (Figure 5b).

In subgroup (C1), the renal cortex showed some degenerative changes as regard to the glomeruli and tubules. Some glomeruli appeared with necrotic glomerular tuft and others had contracted tuft with wide urinary space. The cells of the ureteric bud had darkly stained nuclei. In certain cortical areas, the nephrogenic mesenchyme showed necrosis (Figure 5c, d, e). In the deep cortex, the proximal convoluted tubules had cytoplasmic vacuolization, darkly stained nuclei and cellular necrotic exfoliates into tubular lumen. Distorted glomeruli with partially obliterated Bowman's capsule were also observed (Figure 5f).

-At 3rd PW: (Figures 6a-6d)

Histological examination indicated normal architecture of kidney tissues in the subgroups (A2 and B2). The renal cortex contained proximal, distal convoluted tubules and mature glomeruli. The proximal convoluted tubules appeared lined with large pyramidal cells with apical brush borders, while the distal with simple cuboidal cells. The renal glomeruli consisted of glomerular tuft surrounded by Bowman's capsule formed of outer parietal layer (lined by simple squamous cells) and inner visceral layers (lined by podocytes and mesangial cells) with a urinary space in between the two layers (Figures 6a).

The subgroup (C2) showed extensive degenerative changes in renal cortex. Some glomeruli showed hypercellularity with obliterated urinary space. Others appeared atrophic with degenerated glomerular tuft and wide urinary space. Most proximal convoluted tubules were swollen with obliterated lumen. However, some tubules were severely degenerated and had dilated lumen with cytoplasmic vacuolization and sloughing of lining cells into tubular lumen (Figures 6b-6c). Intertubular congestion was also observed (Figure 6d).

-At 6th PW: (Figures. 7a-7e)

Normal architecture of the kidney tissue was shown in the subgroups (A3 and B3) (Figure 7a). The subgroup (C3) still showed extensive degenerative changes in spite of stoppage of BPA treatment to the offspring. Many glomeruli still displayed hypercellularity with obliterated urinary spaces. Adherent glomeruli (Figure 7b), atrophic glomeruli with necrotic glomerular tuft and wide urinary spaces (Figure 7c), lobulated (Figures 7d) and hypertrophied glomeruli (Figure 7e) were well recognized. Most proximal convoluted tubules were still swollen with narrow lumen and obvious intertubular congestion. Other tubules showed necrotic epithelium with darkly stained nuclei and wide lumen. In certain cortical areas,

some tubules appeared disrupted. Severely congested and dilated cortical renal blood vessels were well demonstrated (Figures 7b-7e).

-At 9th PW: (Figures 8a-8c)

The subgroups (A4 and B4) showed normal architecture of the kidney tissue (Figure 8a). However, the subgroup (C4) revealed less severe degenerative changes. Some glomeruli still showed slight lobulation. Some tubules appeared restoring normal architecture with patent lumen and normal cell lining. However, other tubules still showed cellular vacuolization and necrotic exfoliation into tubular lumen. In certain cortical areas, mild intertubular congestion was still observed (Figures 8b and 8c).

B- Immunohistochemistry (IHC)

1- IHC Staining of PCNA for Liver (Figures 9a-9h)

The numbers of PCNA-positive hepatic cells ranged from moderate at 1st PW (9a) to few at other successive PW (9b-9d) of control groups. However, the BPA treated groups showed apparent increase in number and intensity of PCNA staining in comparison to relevant control groups (Figures 9a-9h).

2- IHC Staining of PCNA for Kidney (Figures 10a-10h)

In the control groups, the numbers of PCNA-positive renal cells were ranged from moderate at 1st PW (10a) to few at other successive PW (10b-10d). However, the BPA treated groups showed apparent increase in number and intensity of PCNA staining in comparison to comparable control groups (Figures 10a-10h).

3-IHC Staining of Caspase-3 for Liver (Figures 11a-11h)

The Caspase-3 expressions (as brown yellow coloration) were weak in cytoplasm of hepatocytes of control groups at all postnatal weeks (Figures 11a-11d). However, expressions of Caspase-3 of BPA treated liver were ranged from moderate at 1st PW in pericentral hepatocytes (Figure 11e), to strong at 3rd, 6th PW in most hepatocytes (Figures 11f-11g) to mild at 9th PW (Figure 11h).

4-IHC Staining of Caspase-3 for Kidney (Figures 12a-12h)

There was weak expression of Caspase-3 (as brown

yellow coloration) in cytoplasm of renal glomeruli and tubules in all control groups (Figures 12a-12d). However, Caspase-3 expressions of the BPA treated kidney were ranged from moderate at 1st PW in cytoplasm of renal glomeruli and tubules (Figure 12e), to strong at 3rd, 6th PW (Figures 12f-12g) to mild at 9th PW (Figure 12h).

C-Statistical Analysis

1- The Mean Number of PCNA for Liver and Kidney

By using one-way ANOVA, in hepatic and renal tissues, the mean number of PCNA-positive cells showed high statistical significant differences ($P < 0.001$) among the studied groups of different ages. By Post hoc Tukey's test, BPA-treated groups showed high statistical significant increase in the mean number of PCNA-positive cells of different age groups when compared with their counterpart of control groups ($P < 0.001$). No significant differences were found between negative and positive control groups ($P > 0.05$) (Tables 1 and 2).

Moreover, Post hoc analysis with adjusted Bonferroni posttests revealed no statistical significant differences in mean number of PCNA-positive cells between 1st, 3rd, and 6th PW of BPA treated groups ($P > 0.05$) however, was significantly decreased in 9th PW ($P < 0.001$) (Figures 13, 14).

2- The Area % of Caspase-3 Expression For Liver and Kidney

By using one-way ANOVA, in renal and hepatic tissues, the mean values of area% of Caspase 3 expression showed high statistical significant differences among the studied groups at different ages ($P < 0.001$). Post hoc Tukey's test showed high statistical significant increase in area % of Caspase 3 expression of treated age groups when compared with their relevant control groups ($P < 0.001$). No significant differences were found between negative and positive control groups ($P > 0.05$) (Tables 3 and 4).

However, Post hoc analysis with adjusted Bonferroni posttests revealed significant increase of the area % of Caspase 3 expression from 1st to 3rd or from 1st to 6th PW of BPA treated groups ($P < 0.001$). No statistical significant difference were observed from 3rd to 6th postnatal weeks ($P > 0.05$), however, there was significant decrease from 3rd to 9th or from 6th to 9th postnatal weeks of BPA treated groups ($P < 0.001$) (Figures 15 and 16).

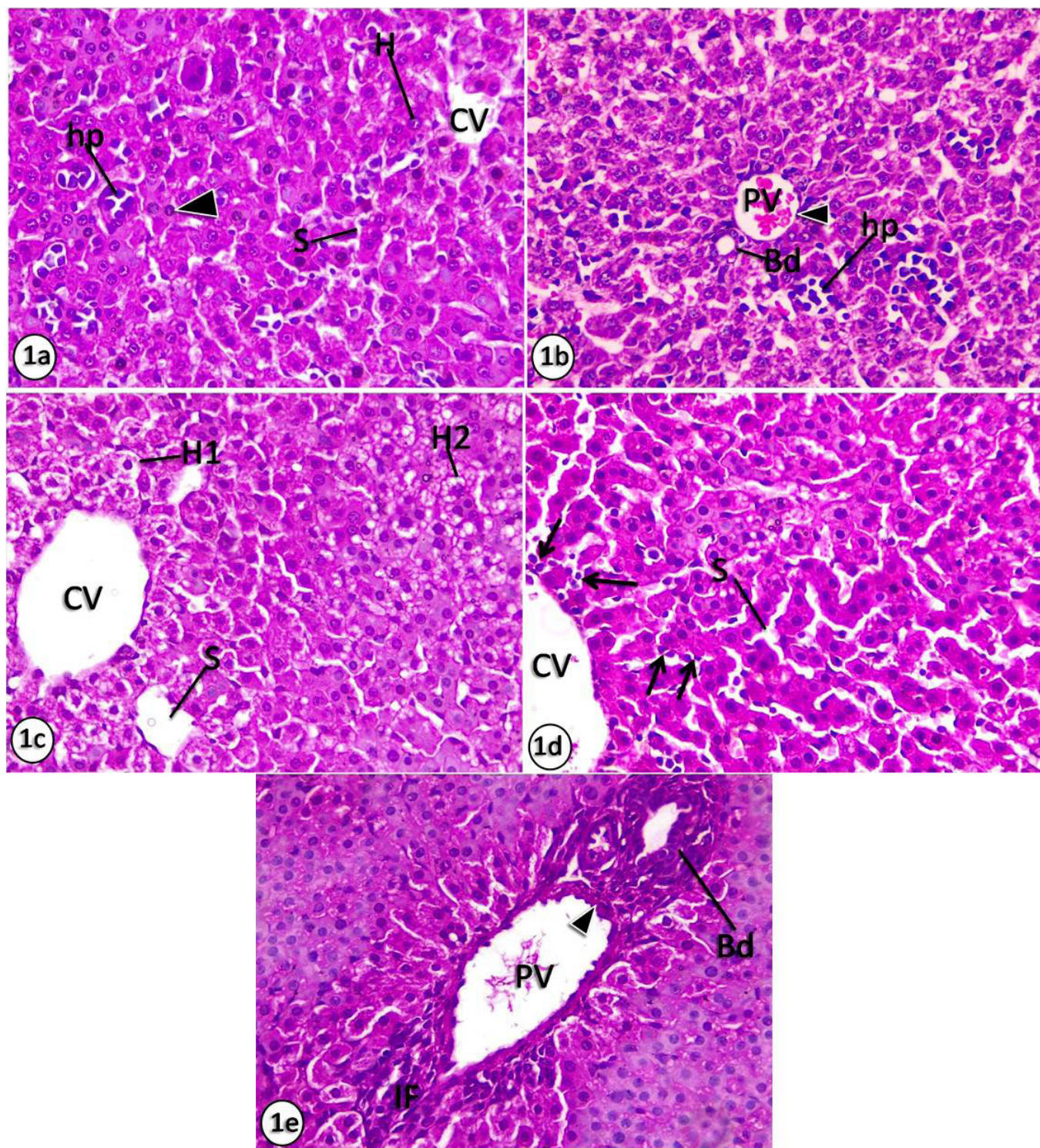


Fig. (1.a-e): Photomicrograph of liver sections of control and treated groups of 1st PW male albino rat pup. (a-b): Sections of a control rat liver (a): showing normal central vein (CV), sinusoids (S), hepatocytes (H) with its central nuclei (arrowhead), and hemopoietic (hp) cells. (b): showing normal portal vein (PV) with thin lining endothelium (arrowhead) and bile ductules (Bd). Notice the Hemopoietic (hp) cells. (c-e): Sections of BPA treated liver. (c): showing moderate dilation of central vein (CV) and sinusoids (S). The pericentral hepatocytes (H1) show moderate swelling with darkly stained nuclei. The peripheral hepatocytes (H2) contain accumulated vacuoles. (d): showing moderately dilated sinusoids (S) with proliferation of kupffer cell lining (arrows). Notice the central vein (CV). (e): showing moderately dilated portal vein (PV) with thickened wall (arrowhead). The wall of bile ductule (Bd) shows hyperplastic epithelium. Mild periportal inflammatory cell infiltration (IF) is seen. (HandE X 400)

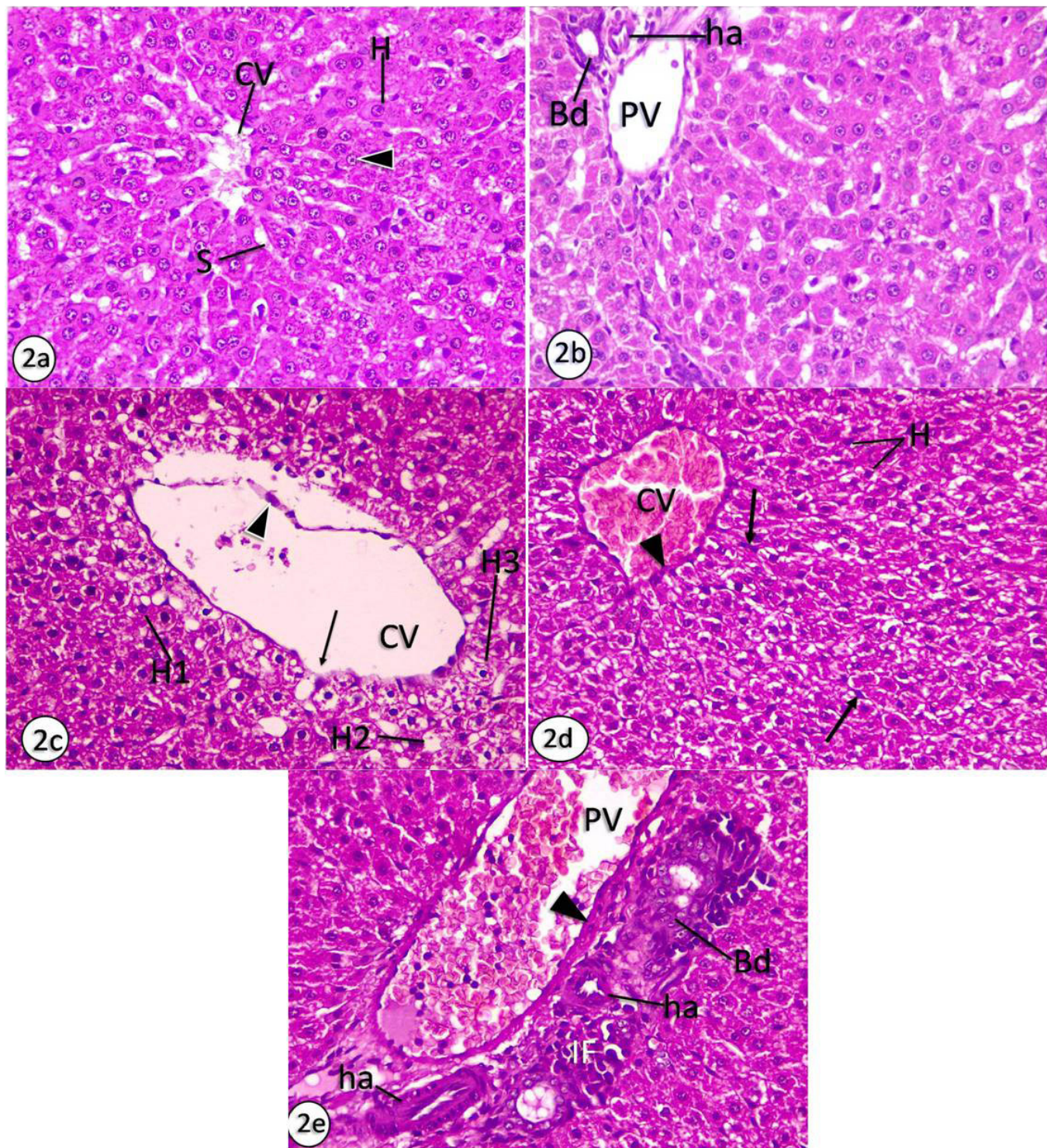


Fig. (2.a-e): Photomicrograph of liver sections of control and treated groups of 3rd PW male albino rat pups. (a-b): Sections of a control rat liver (a): showing normal central vein (CV), sinusoids (S) and hepatocytes (H) with central vesicular nuclei (arrowhead). (b): showing normal portal vein (PV) with thin lining endothelium (arrowhead), hepatic artery (ha) and bile ductule (Bd) lined by one layer of cubical epithelium. (c-e): Sections of BPA treated liver.(c): showing markedly dilated central vein (CV) with discontinuation (arrow) and delamination (arrowhead) of its epithelial lining. Vacuolated (H1), ruptured (H2) and swollen hepatocytes with pyknotic nuclei (H3) are well seen.(d):showing dilated and congested central vein (CV) with thick wall (arrowhead), obliterated sinusoid with prominent Kupffer cells (arrow) and abundant hepatocytes (H) with darkly stained nuclei. (e): showing markedly dilated and congested portal vein (PV) with thick wall (arrowhead). Hyperplasia of bile ductule (Bd) wall is well seen. An inflammatory cell infiltration (IF) is observed in periportal area. Thick walled hepatic artery (ha) is also shown. (HandE X 400)

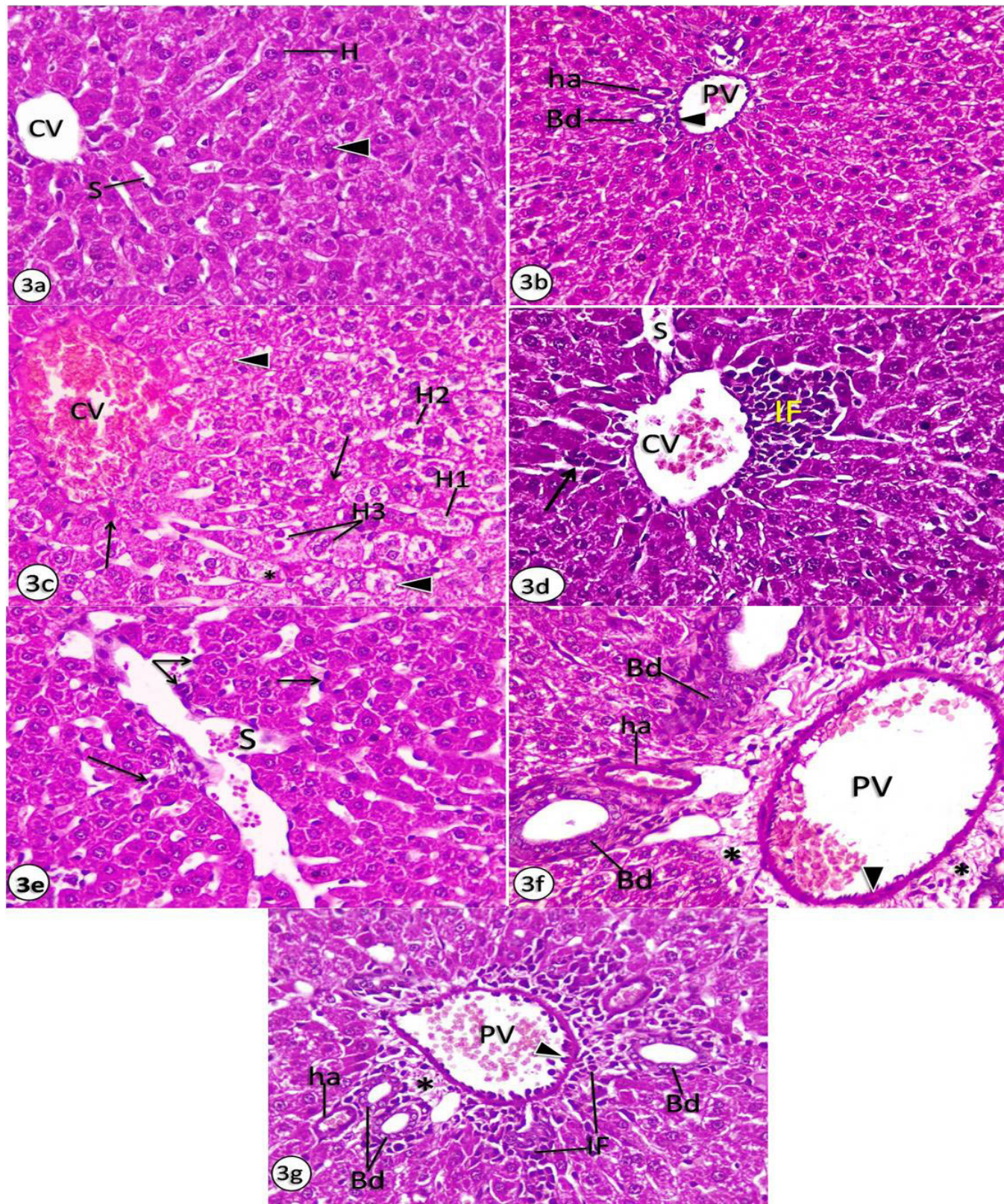


Fig. (3.a-g): Photomicrograph of liver sections of control and treated groups of 6th PW male albino rat pups. (a-b): Sections of a control rat liver (a): showing normal central vein (CV), sinusoids (S) and hepatocytes (H) with central nuclei (arrowhead). (b): showing normal portal vein (PV) with thin lining endothelium (arrowhead), hepatic artery (ha) and bile ductule (Bd). (c-g): Sections of BPA treated liver. (c): showing dilated and congested central vein (CV). Extensive hydropic swelling (H1), vacuolation of the mono (H2) and binucleated (H3) hepatocytes are clearly seen. Different nuclear deformities of hepatocytes appear as; pyknosis (arrows) karyolysis (*) Karyorrhexis (arrowheads). (d-e): showing marked inflammatory cell infiltration (IF) around the central vein (CV), marked dilatation of sinusoids (S) and proliferation of kupffer cell lining (arrows). (f-g): showing dilated portal vein (PV) with thickened wall and partly filled with blood (arrowhead). Bile ductule (Bd) is lined by hyperplastic epithelium or increased in number. Hepatic artery (ha) have thick wall. Excessive edema (*) and marked inflammatory cell infiltration (IF) are well seen in all around the periportal area. (HandE X 400)

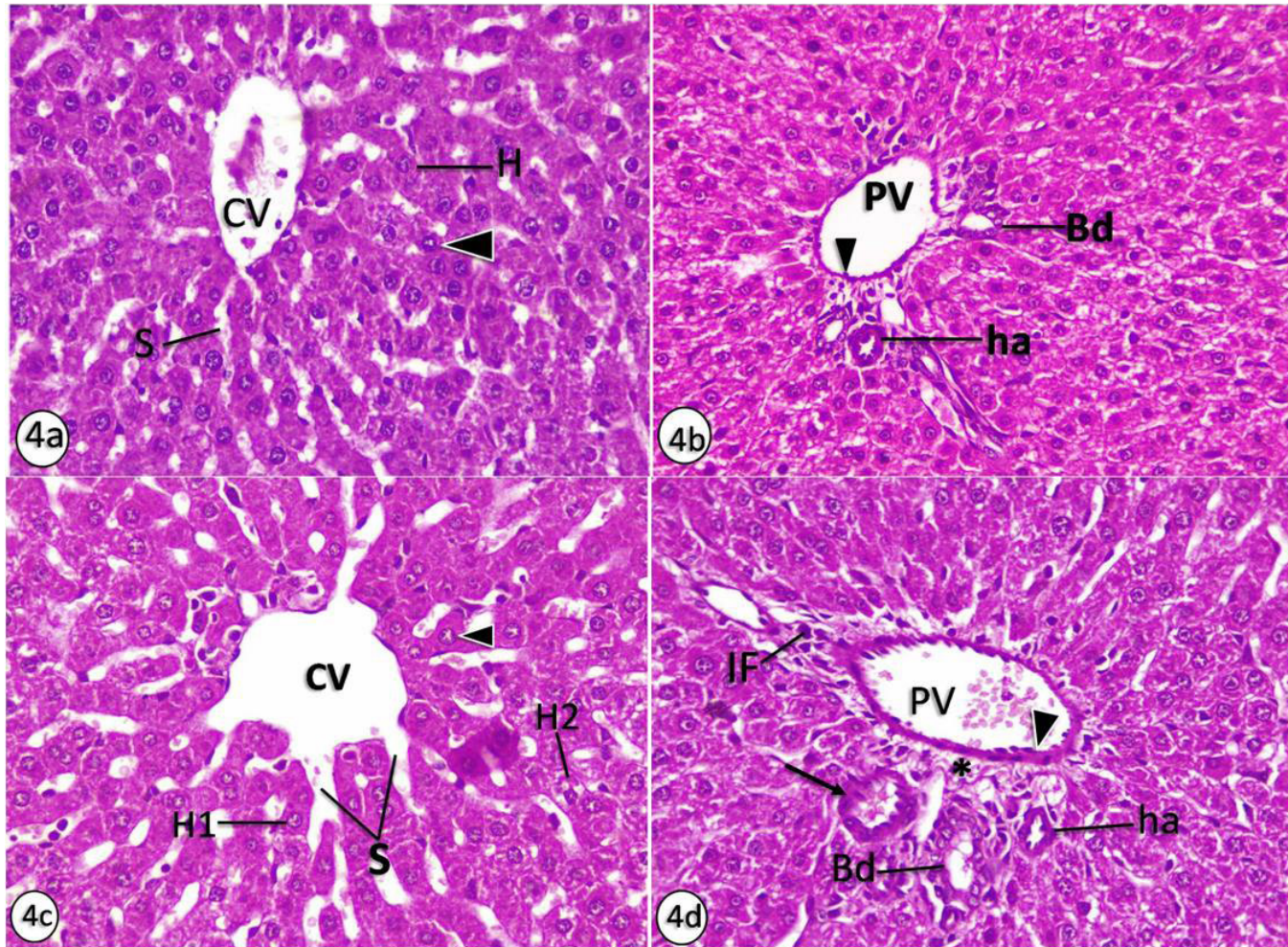


Fig. (4.a-d): Photomicrograph of liver sections of control and treated groups of 9th PW male albino rat pups. (a-b): Sections of a control rat liver (a): showing normal central vein (CV), sinusoids (S) and hepatocytes (H) with central nuclei (arrowhead). (b): showing normal portal vein (PV) with thin lining endothelium (arrowhead), hepatic artery (ha) and bile ductules (Bd). (c-d): Sections of BPA treated liver. (c): showing mild dilation of both the central vein (CV) and its draining sinusoids (S). The pericentral hepatocytes (H1) appear nearly normal and exhibit rounded vesicular central nuclei (arrowhead). The peripheral hepatocytes (H2) show mild vacuolation (d): showing mildly dilated portal vein (PV) with thick wall (arrowhead). Little edema (*) and few inflammatory cell infiltration (IF) are still present in the periportal area. Bile ductule (Bd) appears normal with one layer of cubical epithelial lining. Hepatic artery appears normal (ha) and other is still having thickened wall (arrow). (HandE X 400)

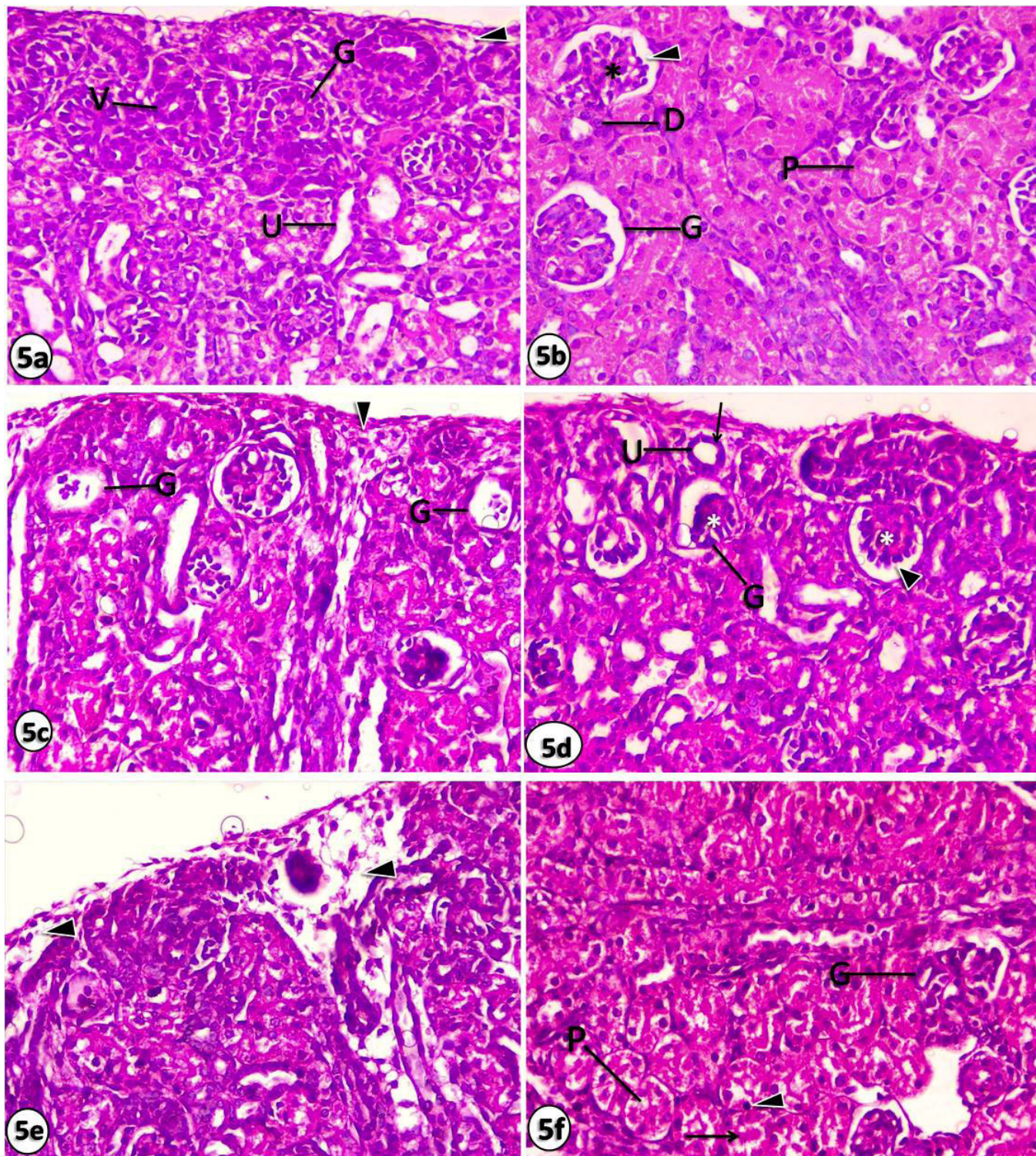


Fig. (5.a-f): Photomicrograph of kidney sections of control and treated groups of 1st PW male albino rat pups. (a-b): Sections of a control rat kidney. (a): showing the superficial cortex composed of subcapsular nephrogenic mesenchyme (arrowhead) containing ureteric buds (U), renal vesicles (V) and immature glomeruli (G). (b): showing the deep cortex containing mature glomeruli (G) with glomerular tuft (*) and urinary space (arrowhead). Proximal (P) and distal (D) convoluted tubules are also seen. (c-f): Sections of BPA treated rat kidney. (c): showing the nephrogenic mesenchyme (arrowhead) containing glomeruli (G) with necrotic glomerular tuft. (d): showing glomeruli (G) with contracted tuft (*) and wide urinary space (arrowhead). The cells of the ureteric bud (U) have darkly stained nuclei (arrow). (e): Showing some areas of necrosis of nephrogenic mesenchyme (arrowhead). (f): showing proximal convoluted tubules (P) with cytoplasmic vacuolization, darkly stained nuclei (arrowhead) and necrotic exfoliates into tubular lumen (arrow). Distorted glomerulus with partially obliterated Bowman's capsule (G) is observed. (HandE X 400)

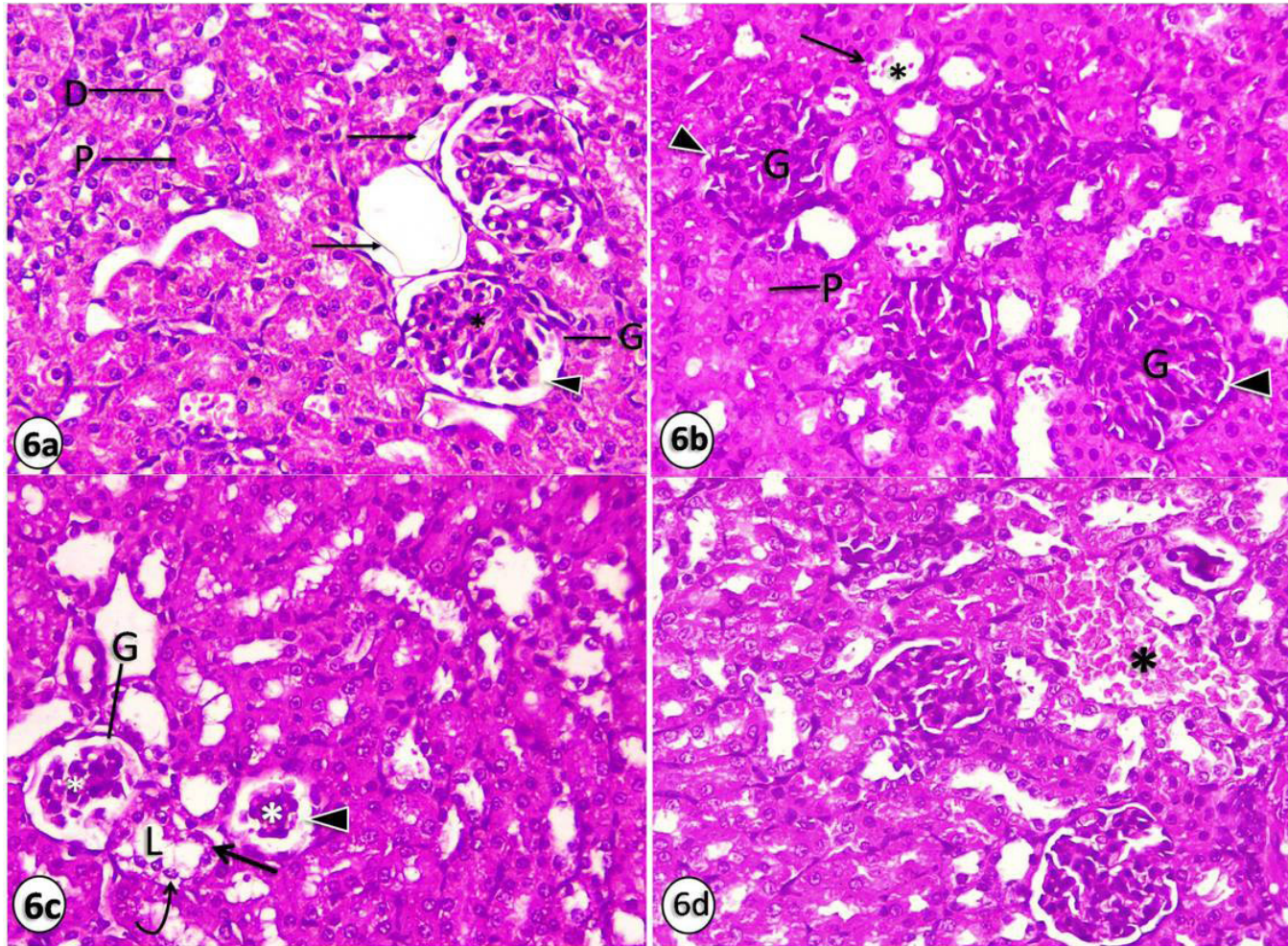


Fig. (6.a-d): Photomicrograph of kidney sections of control and treated groups of 3rd PW male albino rat pups. (a): Section of a control rat kidney showing normal structure of the renal cortex containing proximal (P), distal (D) convoluted tubules and mature glomeruli (G) with urinary space (arrowhead) and glomerular tuft (*). Thin walled blood vessels (arrow) are observed. (b-d): Sections of BPA treated rat kidney. (b): showing hypercellular glomeruli (G) with obliterated urinary space (arrowhead) and swollen proximal convoluted tubules (P) with obliterated lumen. Other tubules (arrow) show sloughing of lining cells into tubular lumen (*). (c): Showing atrophic glomeruli (G) with degenerated glomerular tuft (*) and wide urinary space (arrowhead). Severe degeneration of renal tubular epithelium (arrow), dilated lumen (L) and cytoplasmic vacuolization (curved arrow) are seen. (d): showing intertubular congestion (*). (HandE X 400)

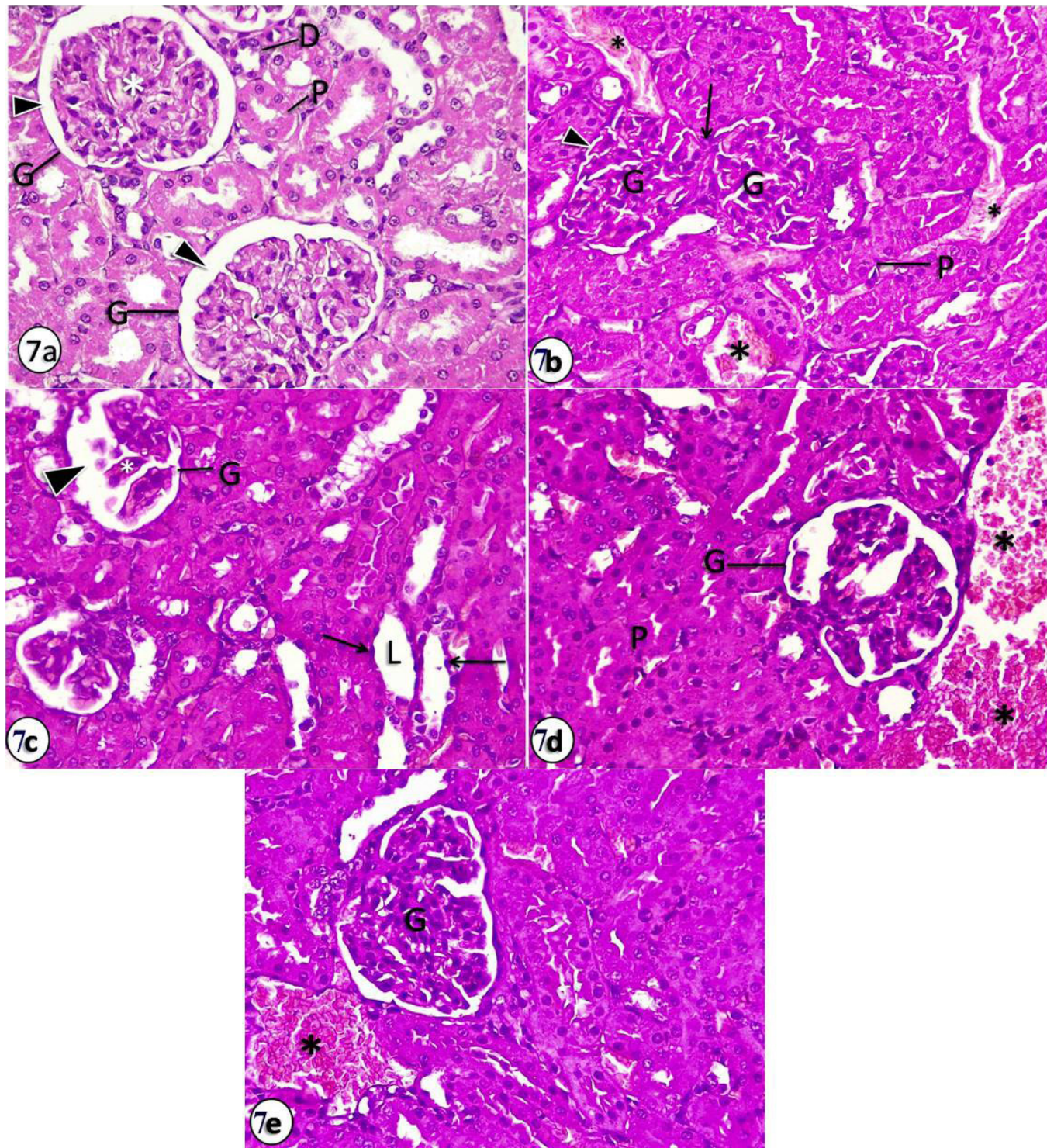


Fig. (7.a-e): Photomicrograph of kidney sections of control and treated groups of 6th PW male albino rat pups. (a): Section of a control rat kidney showing normal glomeruli (G), urinary space (arrowhead), glomerular tuft (*), proximal (P) and distal (D) convoluted tubules. (b-e): Sections of BPA treated rat kidney. (b): showing glomeruli (G) with hypercellularity, obliterated urinary spaces (arrowhead) and adhesion (arrow). Swelling of proximal convoluted tubules (P) and intertubular congestion (*) are observed. (c): showing atrophic glomerulus (G) with necrotic glomerular tuft (*) and wide urinary space (arrowhead). Some tubules show necrotic cell lining with darkly stained nuclei (arrow) and wide lumen (L). (d): showing severe dilatation and congestion in the cortical renal blood vessel (*), lobulated glomerulus (G) and markedly distorted proximal convoluted tubules (P). (e): showing hypertrophied glomerulus (G). (HandE X 400)

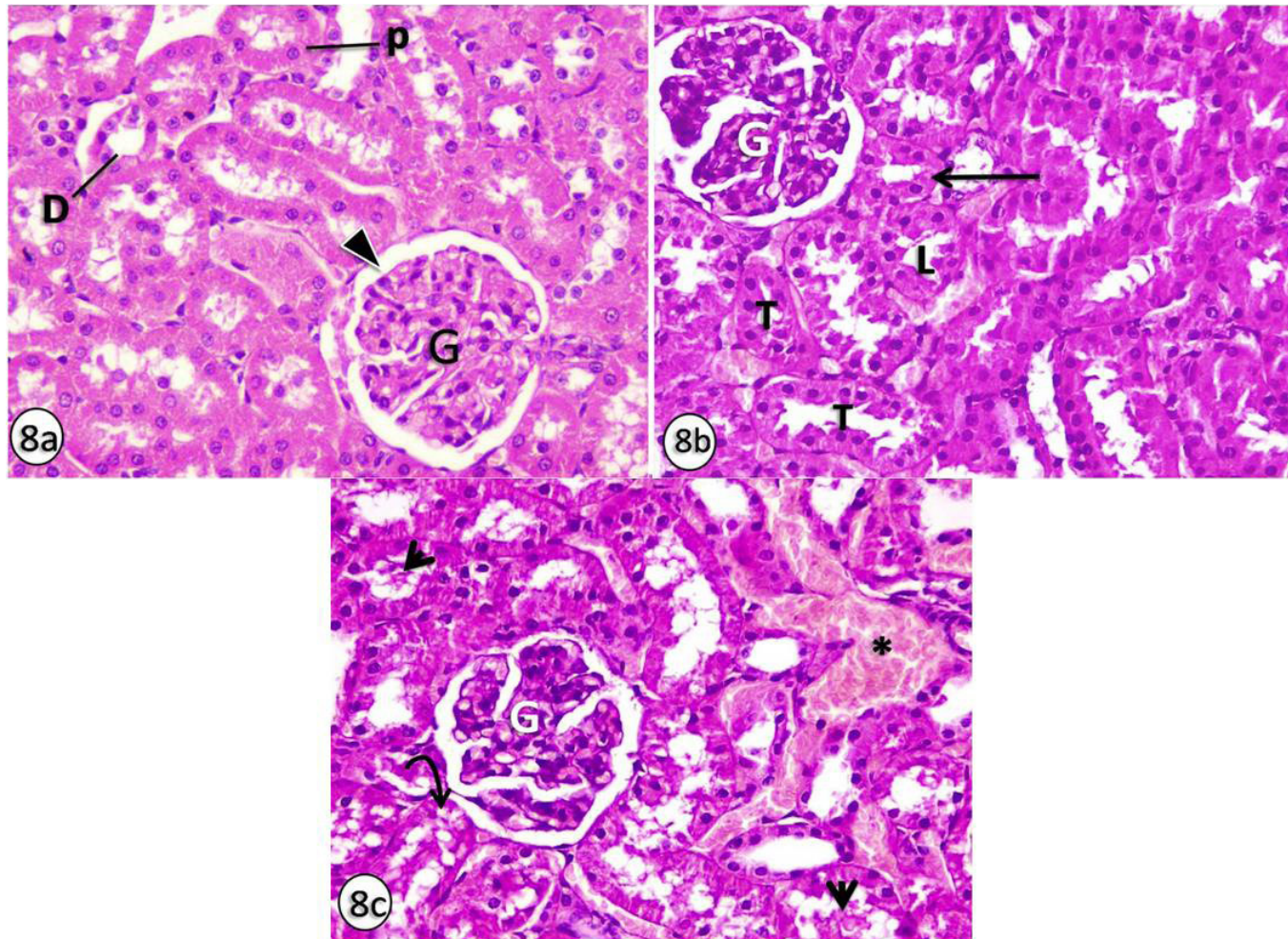


Fig. (8.a-c): Photomicrograph of kidney sections of control and treated groups of 9th PW male albino rat pups. (a): Section of a control rat kidney showing normal glomeruli (G), urinary space (arrowhead), proximal (P) and distal (D) convoluted tubules. (b-c): Sections of BPA treated kidney showing some tubules (T) nearly restoring normal epithelial cell lining (arrow) and patent lumen (L). However, few tubules still show slight vacuolization (curved arrow) and necrotic exfoliation (arrowheads) into tubular lumen. Some glomeruli (G) show mild lobulation. Mild intertubular congestion (*) is still observed. (HandE X 400)

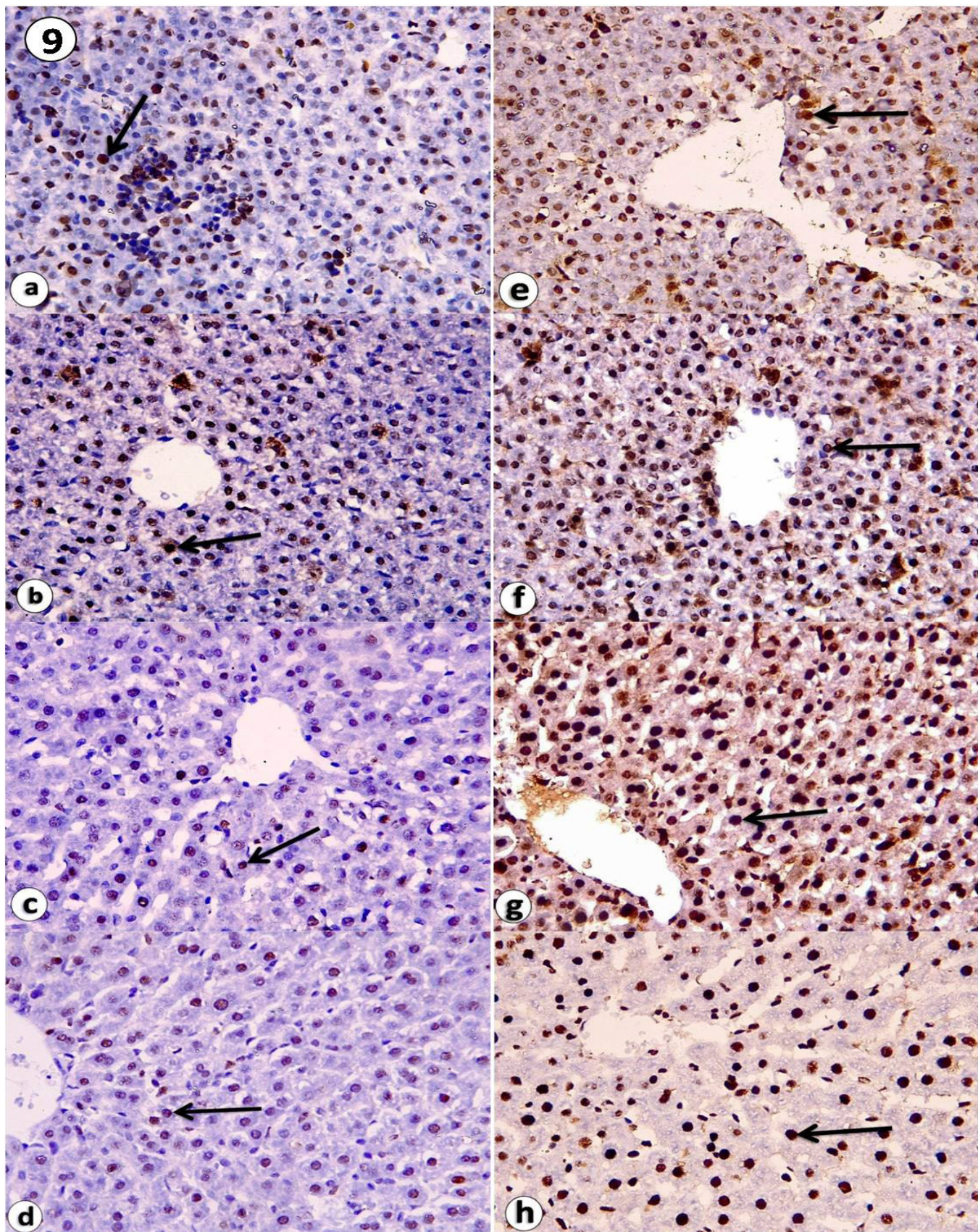


Fig. (9.a-h): Photomicrograph of liver sections of control and BPA treated male albino rat pups at different age group showing nuclear immunoreactions (arrows) of PCNA in the hepatocytes. (a-d): Sections of a control rat liver showing moderate (in Fig. 9a) to few numbers (in Figs 9b-9d) of PCNA-positive hepatic cells. (e-h): Sections of BPA treated liver showing apparent increase in the number of PCNA-positive hepatic cells in comparison to the relevant control group. (PCNA IHC X400)

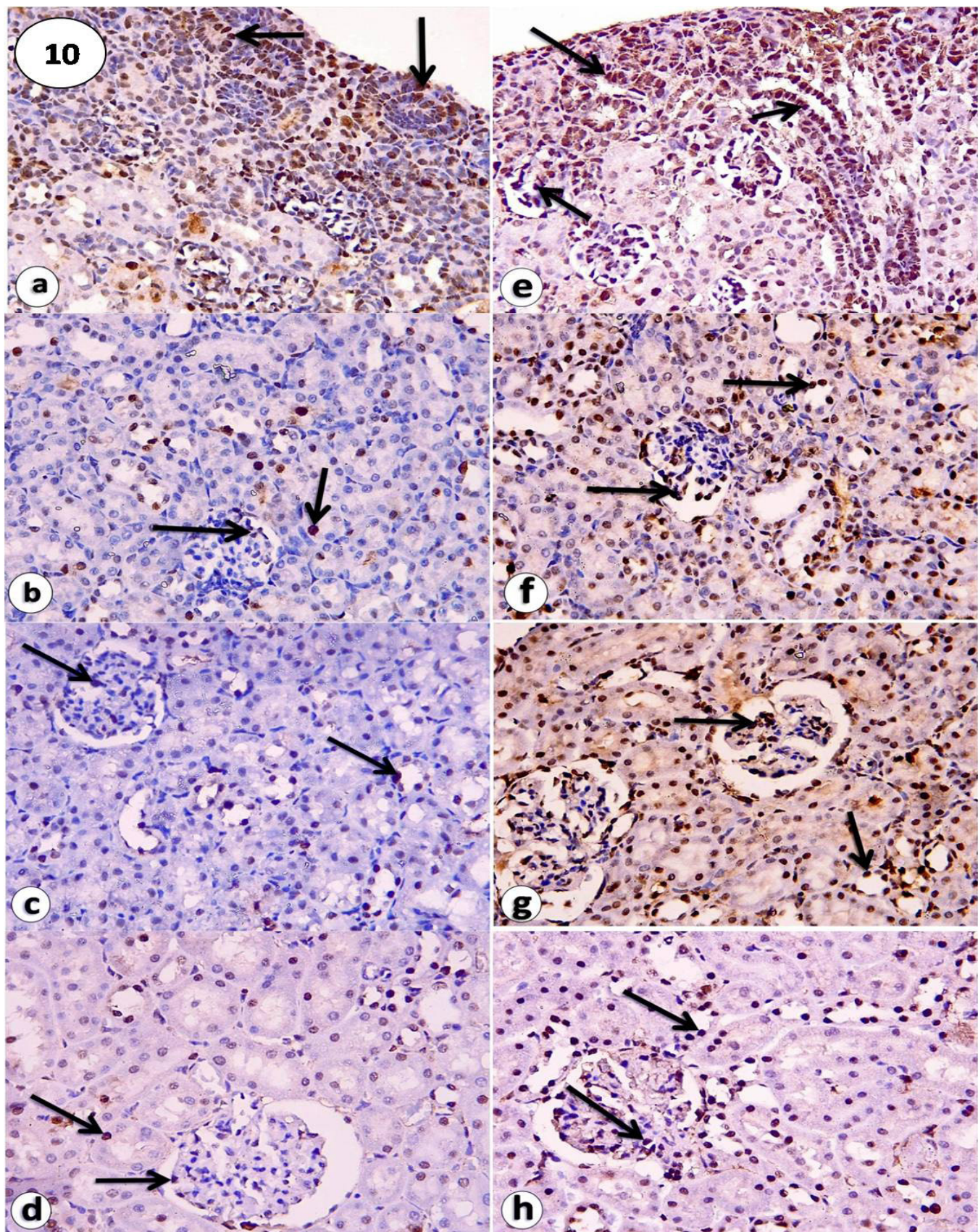


Fig. (10.a-h): Photomicrograph of kidney sections of control and BPA treated male albino rat pups at different age group showing nuclear immunoreactions (arrows) of PCNA-positive renal cells. (a-d): Sections of a control rat kidney showing moderate (in Fig. 10a) to few (in Figs 10b-10d) numbers of PCNA-positive renal cells. (e-h): Sections of BPA treated kidney showing apparent increase in the number of PCNA-positive glomerular and tubular renal cells in comparison to the relevant control group. (PCNA IHC X400)

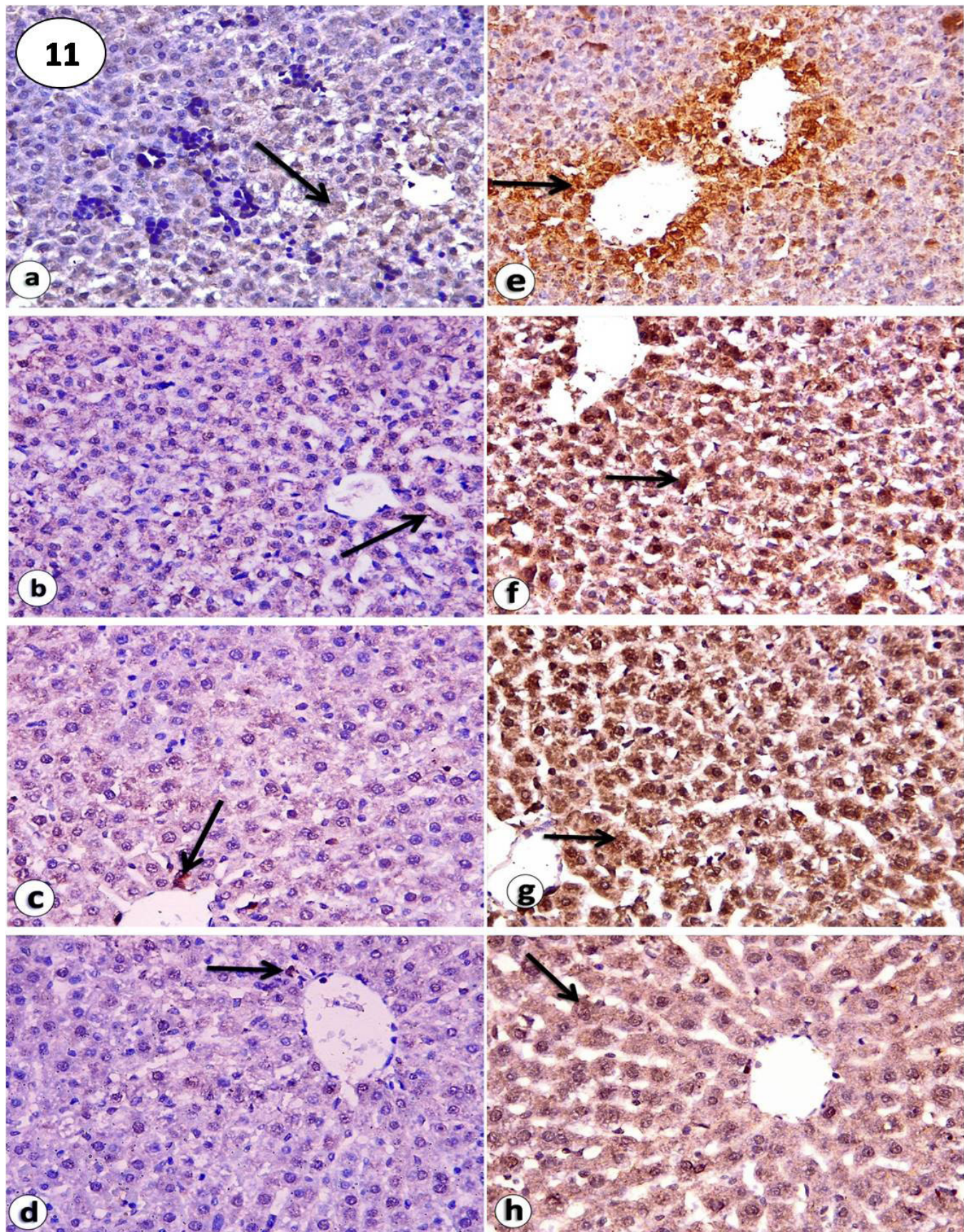


Fig. (11.a-h): Photomicrograph of liver sections of control and BPA treated male albino rat pups at different age group showing expressions of Caspase-3 (arrows) in cytoplasm of hepatocytes. (a-d): Sections of a control rat liver showing weak Caspase-3 expressions at all ages. (e-h): Sections of BPA treated liver exhibiting moderate Caspase-3 expression in the pericentral hepatic cells at 1st PW age (e), to strong expression in most hepatocytes at 3rd, 6th PW ages (f-g), while, mild expression is seen at 9th PW (h). (Caspase-3IHC X400)

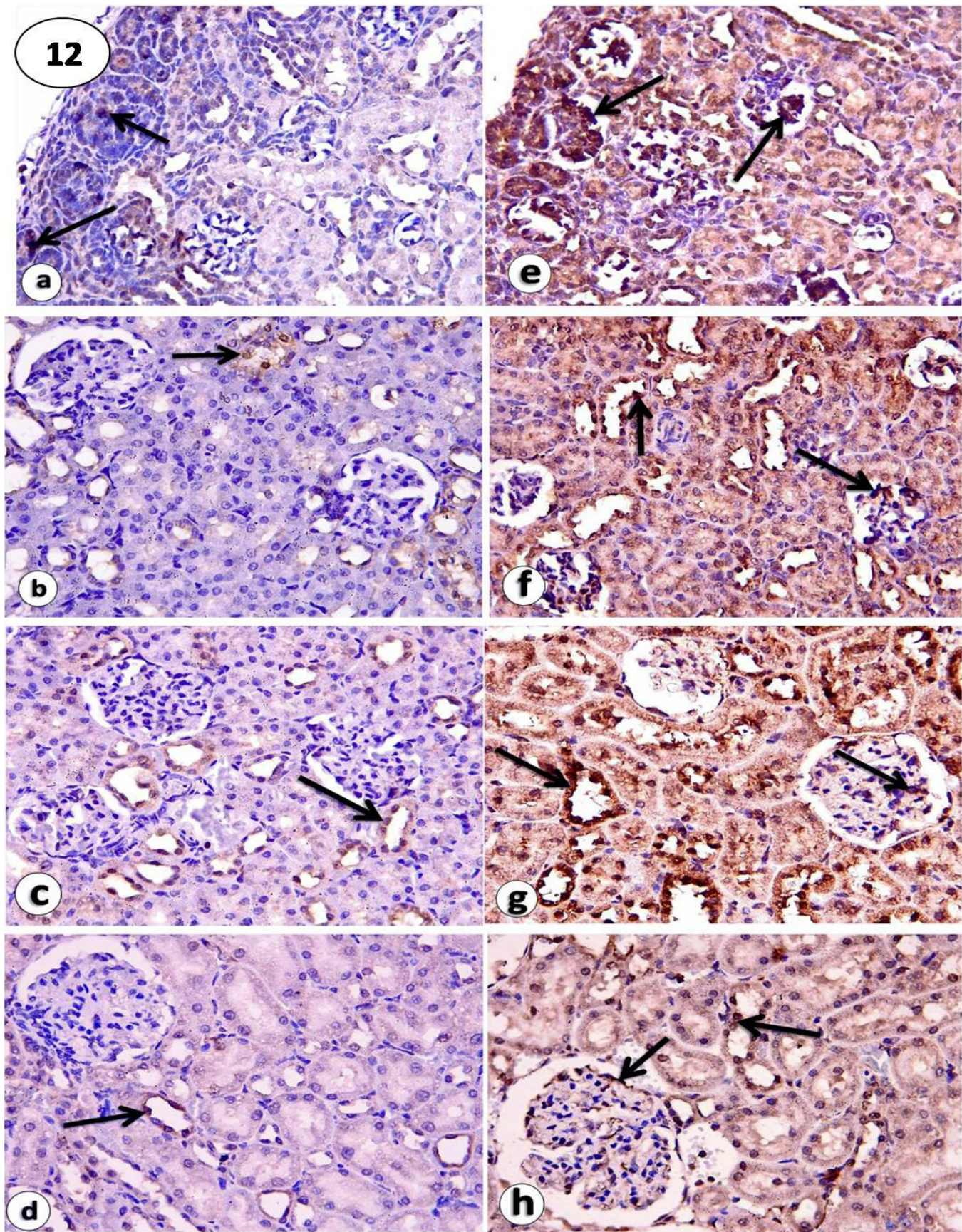


Fig. (12.a-h): Photomicrograph of kidney sections of control and BPA treated male albino rat pups at different age group showing expressions of Caspase-3 (arrows) in cytoplasm of renal glomeruli and tubules. (a-d): Sections of a control rat kidney showing weak expressions of Caspase-3 at all ages. (e-h): Sections of BPA treated kidney showing moderate expression of Caspase-3 at 1st PW (e), strong expression at 3rd, 6th PW (f-g) and mild expression at 9th PW (h). (Caspase-3 IHC X400)

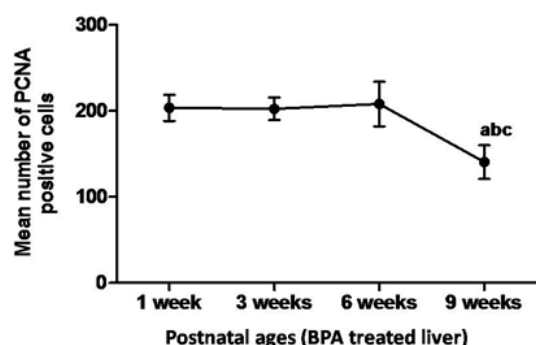


Fig. 13: The effect of BPA on liver cell proliferation. One way repeated measure ANOVA followed by adjusted Bonferroni posttests. Data are mean±SD, n=10 in each group, significant if $P \leq 0.05$, a significant vs 1st week, b significant vs 3rd weeks, c significant vs 6th weeks.

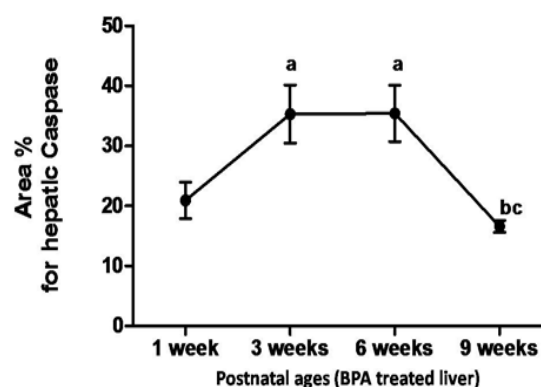


Fig. 15: The effect of BPA on area% of Caspase 3 expression in hepatic tissue. One way repeated measure ANOVA followed by adjusted Bonferroni posttests. Data are mean±SD, n=10 in each group, significant if $P \leq 0.05$, a significant vs. 1st week, b significant vs. 3rd weeks, c significant vs. 6th weeks.

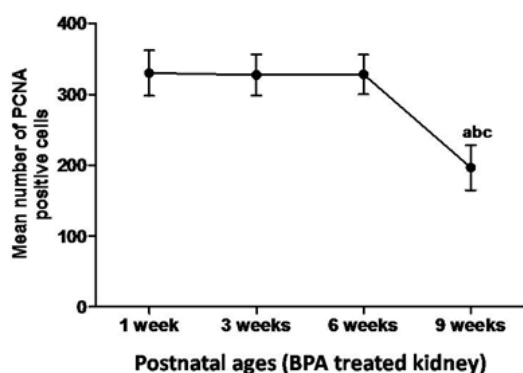


Fig. 14: The effect of BPA on kidney cell proliferation. One way repeated measure ANOVA followed by adjusted Bonferroni posttests. Data are mean±SD, n=10 in each group, significant if $P \leq 0.05$, a significant vs 1st week, b significant vs 3rd weeks, c significant vs 6th weeks.

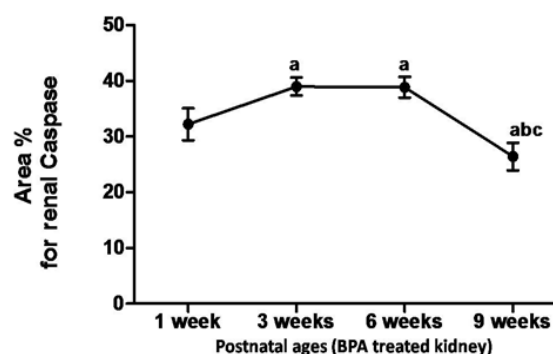


Fig. 16: The effect of BPA on area% of Caspase 3 expression in renal tissue. One way repeated measure ANOVA followed by adjusted Bonferroni posttests. Data are mean±SD, n=10 in each group, significant if $P \leq 0.05$, a significant vs. 1st week, b significant vs. 3rd weeks, c significant vs. 6th weeks.

Table 1: Statistical comparison between negative control, positive control and BPA treated groups regarding PCNA-positive cells in hepatic tissue in male albino rats by using A one-way ANOVA and post hoc Tukey's test.

Postnatal Date	PCNA-positive cells	Mean±SD			ANOVA	P-value
		Negative control group	Positive control group	BPA treated group		
1 week		95.4±19.2	96.4±18.5	203.5±15.0 ^{a,b}	F=123.7	< 0.001
3 weeks		93 ±9.2	92.6 ±8.7	202.4 ±13.1 ^{a,b}	F=361.5	< 0.001
6 weeks		47.20±6.6	46.30±5.6	208±25.9 ^{a,b}	F=350	< 0.001
9 weeks		40.6±7.0	39.8±6.3	140.5±19.6 ^{a,b}	F=213.3	< 0.001

n=10 in each group, significant if $P \leq 0.05$. a significant vs negative control group, b significant vs positive control group

Table 2: Statistical comparison between negative control, positive control and BPA treated groups regarding PCNA-positive cells in renal tissue in male albino rats by using A one-way ANOVA and post hoc Tukey's test.

Postnatal Date	PCNA-positive cells	Negative control group	Positive control group	BPA treated group	ANOVA	P-value
		Mean±SD				
1week		149.3±10.2	151.6±9.5	330±32.1 ^{a,b}	F=263.7	< 0.001
3 weeks		30.30±7.1	30.10±6.9	327.4±28.9 ^{a,b}	F=947.1	< 0.001
6 weeks		53.8±7.3	54.2±7.1	328±28 ^{a,b}	F=849	< 0.001
9 weeks		40±7.3	39±7.2	196.4±31.9 ^{a,b}	F=220	< 0.001

n=10 in each group, significant if $P \leq 0.05$. a significant vs negative control group, b significant vs positive control group

Table 3: Statistical comparison between negative control, positive control and BPA treated groups regarding area% of Caspase 3 expression in hepatic tissue in male albino rats by using A one-way ANOVA and post hoc Tukey's test.

Postnatal Date	Area% Caspase	Negative control group	Positive control group	BPA treated group	ANOVA	P-value
		Mean±SD				
1week		3.6 ±0.61	3.7 ±0.60	21.0±3.0 ^{a,b}	F=300.8	< 0.001
3 weeks		4.2±0.72	4.4±0.78	35.3±4.8 ^{a,b}	F=392.6	< 0.001
6 weeks		4.5±0.57	4.7±0.46	35.4±4.7 ^{a,b}	F=422.3	< 0.001
9 weeks		3.2±0.9	3.4±1.1	16.6±1.0 ^{a,b}	F=559.6	< 0.001

n=10 in each group, significant if $P \leq 0.05$
a significant vs negative control group, b significant vs positive control group

Table 4: Statistical comparison between negative control, positive control and BPA treated groups regarding area% of Caspase 3 expression in renal tissue in male albino rats by using A one-way ANOVA and post hoc Tukey's test.

Postnatal Date	Area% Caspase	Negative control group	Positive control group	BPA treated group	ANOVA	P-value
		Mean±SD				
1week		5.8 ±1.2	5.9 ±1.1	32.2 ±2.9 ^{a,b}	F=648.7	< 0.001
3 weeks		4.1 ±0.7	4.1±0.9	39 ±1.6 ^{a,b}	F=3165	< 0.001
6 weeks		3.6±1.3	3.8±1.3	38.8±1.9 ^{a,b}	F=1784	< 0.001
9 weeks		4.4±0.8	4.6±0.8	26.4±2.5 ^{a,b}	F=633.5	< 0.001

n=10 in each group, significant if $P \leq 0.05$
a significant vs. negative control group, b significant vs. positive control group

DISCUSSION

Bisphenol A (BPA) is metabolized in the liver and eliminated through the kidney, so, the histopathological study of liver and kidney appears to be an accurate indicator of any pollutant toxicity^[32]. In addition, the histological changes in more than one organ can attract attention to determine the biological effect of the toxicant and permit diagnosis of the observed changes^[33]. In the present study, there was experimental evidence that perinatal low dose (200 µg/kg bw/day) of BPA intake can induce multiple histopathological degenerative changes in hepatic and renal tissues in male albino rat offspring. The effect was approved in the four examined postnatal subgroups when compared to the control groups. These degenerative changes were obvious at 1st PW age and progressed to

become massive and extensive at 3rd and 6th PW ages then were less severe at 9th PW age in spite of the cessation of BPA treatment to the offspring at age of weaning.

Regarding the liver, in this work, vascular dilatation and congestion, kupffer cell proliferation, inflammatory cell infiltration and bile duct hyperplasia were well demonstrated. The hepatocytes showed multiple degenerative changes (especially pronounced in the centrilobular area) including vacuolization, hypertrophy, hydropic degeneration and nuclear degenerative changes (pyknosis, karyorrhexis and karyolysis). These findings were in consistence with the previous literature^[11,34-37].

However, most of the above scientists have focused on high dose BPA effect on adult animals without considerable attention to perinatal low dose exposure. The studies on

early developmental BPA exposure toxicity have been shown that BPA causes liver dysfunction and altered human fetal liver metabolism^[38,39].

In the present work, most hepatocytic degenerative changes were observed in pericentral vein area. This finding coincides with previous literature^[40]. They mentioned that the pericentral hepatocytes are supplied by lower levels of essential nutrients and O₂ making them more liable to injury in comparison with hepatocytes nearest to the portal area. The pericentral cellular degeneration may result from central vein congestion which makes flow of blood difficult, as blood flows from hepatic portal vein and hepatic artery into the central vein^[33].

The vascular congestion, inflammation and degenerative pericentrilobular changes, are reactions naturally occurring with cell damage since acute hepatotoxicity can induce inflammatory effects and apoptosis or necrosis of hepatocytes^[41,42]. The hepatocytic vacuolization might be due to an imbalance between the rate of synthesis of substances in parenchyma cells and rate of their release into the circulation^[33].

In the present work, the BPA-treated groups revealed hyperplasia of bile duct (evidenced by histology) together with increase cellular proliferations of hepatic and renal tissues (indicated statistically). There was significant increase in the expression of PCNA at all postnatal ages of BPA-treated groups if compared to control groups. This may give a suspicion risk of neoplastic alteration induced by low dose BPA exposure.

A Similar finding (bile duct hyperplasia) was recorded previously^[23], while increased expression of PCNA in hepatic tissue was demonstrated by some investigators^[43] following BPA exposure. It has been investigated that, as an endocrine disruptor, BPA could disturb biological functions and organ structures in extremely low concentrations. As previously reported, 250 µg/kg bw BPA increased incidence of intraductal hyperplasia in mammary gland of female rats at pup day 400^[44]. Also, BPA lower than 1000 µg/kg bw could induce benign and malignant hyperplastic lesions in the ovary and uterus of mice^[45]. Moreover, BPA induced prostatic^[46], testicular^[47] and hepatic^[48] cancers were confirmed.

It has been suggested that, though the liver and kidney are non-reproductive organs, they possess the ability to express estrogen receptors and response to steroid hormone signaling^[49]. Consequently, the proliferative effect of BPA has been suspected to emerge from its endocrine disrupting activity as it could interact with many hormone receptors. The non-genotoxic and genotoxic pathways may be also involved^[23].

In addition, BPA directed DNA injury cannot be excluded since BPA has been shown to induce genetic mutations, cellular transformation and DNA adducts formation^[50]. Also, increased reactive oxygen species (ROS) production may have led to a concomitantly elevated

cellular proliferation in BPA exposed mice through ROS signaling^[35].

Moreover, in this work, the expression of moderate number of PCNA observed by IHC study during 1st and 3rd PW ages of control liver and during 1st PW age of control kidney indicated the cellular proliferation that occurred during normal postnatal development of liver and kidney, and also explained why there was no statistical significant difference in the mean number of PCNA-positive cells between 1st, 3rd PW ages ($P>0.05$) of BPA treated liver and kidney. This finding was in agreement with some literature^[51]. They mentioned that, in postnatal liver development, the increase in cell proliferation indicated by large numbers of PCNA-positive resident hepatocytes were evident from PD 5 to 20, with no to a few PCNA-positive cells observed at PD 0 and 25 respectively. Moreover, some authors postulated that the number of PCNA-positive cells per glomerulus was higher in 1- and 7-day-old control animals than in 30-day-old animals^[52].

As regards the kidney, in the current work, multiple degenerative changes in the renal cortex were apparently progressive through the successive postnatal weeks and became less severe at 9th PW. Glomerular necrosis, adhesion, lobulation and hypercellularity with either wide or obliterated urinary spaces were well demonstrated. In addition, proximal tubular cellular swelling with obliterated lumen, cytoplasmic vacuolization, necrosis, pyknosis, tubular dilation and intertubular congestion were observed.

Similar renal histopathological changes were reported following BPA administration in fish and rat [33, 53] and in other investigations^[11,54,55].

In BPA treated rats, accumulated BPA metabolites and in ability of renal excretion might affect renal tissue with subsequent tubular epithelial necrosis, degeneration and marked congestion^[6].

However, most of the above studies have given a lot of attention to high dose BPA exposure in adult rat kidney and to the best of our knowledge, there was little information about perinatal low dose kidney exposure.

To date, there is a controversy about dose of BPA toxicity on the kidney.

There was non-significant changes in oxidative stress parameters in the kidney due to BPA (10, 25 mg/kg) treatment. However, serum uric acid increased significantly after 6 weeks of the daily oral administration of BPA at low and high dose levels^[56].

However, other author revealed that BPA induced tissue oxidative stress and peroxidation in the brain, liver, kidney, and testes of mouse. This effect was achieved after administration of BPA (5 or 10µg/milliliter of drinking water) throughout the embryonic/fetal life and during infancy by feeding their pregnant/lactating mothers^[57].

Regarding renal physiologic capacity, the newborn kidney is functionally immature reaching full functional

level at weaning. Also, drug administration depends largely on maintaining the fine balance between metabolism and drug clearance mechanisms through the kidneys^[58].

Furthermore, levels of organic anion transporters needed to help with drug and toxin elimination, are also reduced in the juvenile kidney. Therefore, functional immaturity exposes the juvenile kidney to more toxic agents by decreasing drug clearance or inability to detoxify drugs or their metabolites^[59,60].

The range of toxicity or lack of toxicity in the juvenile animal is due to a number of factors including the competency of renal tubular function or the activity of enzymatic pathways in the immature animal^[61].

The present study showed long lasting effect of BPA exposure at early development stages evidenced by continued degenerative changes at 6th and 9th weeks postnatal in spite of stopping BPA treatment to the offspring at the age of weaning. This coincides with many studies on the long-term effects of early BPA exposure at environmentally relevant dose on the reproductive tract, neural behaviors, metabolic function and liver tissues^[62].

Previous studies examined by radioluminography the distribution of radioactivity in pregnant and lactating rats that orally dosed with 0.5 mg/kg bw 14C- BPA on gestational days (GD) 12, 15, or 18. At 30 min following maternal exposure, the concentration of BPA radioactivity was in liver (219-317µg eq/kg) and in kidney (138-270 µg eq/kg) of dams. Fetuses, fetal membranes, and yolk sacs did not contain quantifiable levels of radioactivity at 30 min, however, after 24 hr, the radioactivity was only detected in fetuses and fetal tissues from dams dosed on GD 18^[63].

The metabolism of BPA conjugates was examined in the liver using a perfusion method. In male rats, the infused BPA was conjugated primarily to mono-glucuronide and approximately 1/4 of the infused BPA was eliminated as a glucuronide/sulfate diconjugate during passage through the liver. The resultant metabolites were preferentially excreted into the bile. From bile, the excreted conjugates flow into the intestinal tract, where the metabolites are reabsorbed into the body^[64].

Moreover, *in vivo*, other study added that once BPA-glucuronide is excreted into bile and enters the intestine, it could be reabsorbed; otherwise, intestinal microflora hydrolyzes BPA-glucuronide, making free BPA sufficiently lipophilic for reabsorption. Repeated enterohepatic recirculation leads to long half-life of BPA in rats at a low dose of BPA (0.10 mg/kg)^[65].

In addition, the BPA conjugates pass through internal organs, such as the heart, lung, kidney and brain before being eliminated from the body^[64]. Moreover, BPA-glucuronide and diconjugate are absorbed from the

maternal blood stream and actively transferred into the fetus through the placenta^[66].

In this current study, BPA-treated group showed high statistical significant increase in the area percentage (%) of Caspase-3 expression in hepatic and renal tissues at different age groups when compared with control groups ($P<0.001$). This percentage showed significant increase from 1st to 3rd postnatal weeks ($P<0.001$), and significant decrease from 6th to 9th postnatal weeks ($P<0.001$). However, no statistical significant change from 3rd to 6th postnatal weeks ($P>0.05$) was observed.

These results were in consistence with some authors who demonstrated a significant increase in Caspase-3 with a significant depression in the antiapoptotic protein Bcl2 in liver rats treated by BPA^[67]. Moreover, BPA dose over 100 micromole (µM) can cause apoptosis in mouse hippocampal cells by elevating reactive oxygen species (ROS) and intracellular calcium and activating Caspase-3^[68]. Recently, BPA induced liver fibrosis as evidenced by caspase-3 increase and BCL2- immunopositive hepatocytes number decrease^[69].

It has been previously documented that, the cytotoxic effect of BPA is strongly linked with the production of ROS with consequent oxidant/antioxidant imbalance and cellular apoptosis in hepatic and renal tissues^[32,53,54,70,71]. Also, hepatic macrophages could generate ROS with increased proinflammatory cytokines which themselves can induce oxidative stress and decreased the antioxidant catalase enzyme activity^[35,72].

CONCLUSION

Maternal oral intake of BPA at low doses NOAEL was proved to have hazardous histological consequences on hepatic and renal tissues in male albino rat offspring. These changes may be indicator for possibility of neoplastic liability. This liability must be under focus of clinical practitioners. Hence the environmental exposure to BPA during early stage of life is important for more searches with particular attention during gestation, lactation and childhood.

RECOMMENDATIONS

Further measures should be needed to minimize environmental exposure to BPA during early stage of life. More researches are needed to evaluate the toxic effects of BPA for longer duration of exposure. If the period of the experiment is extended more than 9 weeks, will complete recovery occur?.

COFLICTS OF INTERESTS

There are no Coflicts of Interests.

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المخلص العربي

تأثير التعرض لجرعات منخفضة من البيسفنول أ في فترة ما قبل وبعد الولادة على أنسجة الكبد والكلية للذكور من نسل الجرذان البيضاء: دراسة نسيجية وهستوكيميائية مناعية ومورفومترية

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المقدمة: البيسفنول أ هي مادة من المواد الكيميائية البيئية المسببة لإختلال الغدد الصماء وذلك عند تعرض الإنسان لها عن طريق الفم في جميع أنحاء العالم. وقد وضعت الدراسات العالمية المختصة بذلك حدا لمقدار الجرعة المسببة تأثيرا ضارا وهي ٥ ملجم / كجم من وزن الجسم / يوم.

الهدف: لتقييم مخاطر تناول الأمهات لجرعة منخفضة من البيسفنول أ عن طريق الفم والتي هي أقل من الجرعة المصرح بها عالميا أثناء الحمل والرضاعة على أنسجة الكبد والكلية للذكور من نسل الجرذان البيضاء من خلال الدراسة الهستولوجية والهستوكيميائية المناعية والدراسة المورفومترية.

مواد وطرق البحث: قسم ثلاثون من إناث الجرذان البيضاء الحوامل إلى ثلاثة مجموعات متساوية. المجموعة (أ): (المجموعة الضابطة السلبية): لم تعط أي عقار والمجموعة (ب) (المجموعة الضابطة الموجبة): أعطيت زيت ذرة (١مل) عن طريق الفم مرة واحدة يوميا باستخدام الأنبوب المعدى، أما المجموعة (ج) (المعالجة بالبيسفنول أ) أعطيت البيسفنول أ (جرعة ٢٠٠ ميكروجرام/ كيلوجرام من وزن الجسم/ يوم) المذاب في (١مل) من زيت الذرة مرة واحدة يوميا باستخدام الأنبوب المعدى من اليوم السادس من الحمل حتى تمام الفطام (الإسبوع الثالث بعد الولادة)، ثم بعد الولادة أختير أربعون من الذكور من النسل الأول لكل مجموعة لتعيش مع أمهاتهم أثناء فترة الرضاعة حتى الفطام، وقسموا إلى أربع مجموعات فرعية متساوية وفقا لميعاد يوم التضحية بهم (الإسبوع الأول والثالث والسادس والتاسع بعد الولادة على الترتيب) وبعد الفطام لم يتلق الصغار أي علاج، وبعد التضحية بهم، جمعت عينات الكبد والكلية منهم وإعدت للفحص الهستولوجي والدراسة الهستوكيميائية المناعية والدراسة المورفومترية والتحليل الإحصائي.

النتائج: تبين إن إعطاء إناث الجرذان البيضاء الحوامل لجرعة منخفضة من البيسفنول أ (٢٠٠ ميكروجرام / كيلوجرام من وزن الجسم/ يوم) في فترة ما قبل وبعد الولادة أدى إلى ظهور تغييرات تنكسية متعددة في أنسجة الكبد والكلية للذكور من نسلها بعد الولادة. وأظهرت نتائج الدراسة الهستولوجية أن هذه التغيرات التنكسية كانت واضحة في عمر الإسبوع الأول بعد الولادة ثم تقدمت لتصبح ضخمة وواسعة النطاق في عمر الإسبوع الثالث والسادس بعد الولادة ثم أصبحت أقل حدة في عمر الإسبوع التاسع بعد الولادة على الرغم من توقف العلاج بالبيسفنول أ عند سن الفطام. وقد تم تأكيد هذه النتائج الهستوباثولوجية عن طريق الدراسة الهستوكيميائية المناعية والتحليل الإحصائي للدراسة المورفومترية.

الخلاصة: إن إعطاء أمهات الجرذان البيضاء في فترة ما قبل وبعد الولادة لجرعة منخفضة من البيسفنول أ عن مقدار الجرعة المصرح بها عالمياً أدى إلى إحداث تغييرات هيستوباثولوجية لأنسجة الكبد والكلية للذكور من نسلها بعد الولادة. ويمكن اعتبار هذه التغييرات بمثابة مؤشر لإحتمال تحولها لأورام.