Bisphenol A Toxicity in Adult Male Rats: Hematological, Biochemical and Histopathological Approach

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Abstract: The purpose of this study was to investigate the hematological, biochemical and histological alterations induced by bisphenol A (BPA) which is an estrogenic environmental contaminant. Albino adult male rats were administrated orally BPA (150 mg/kg). After 70 consecutive days of exposure, blood, sera and tissues were collected for hematological, biochemical and histopathological examination. Lymphocytes were separated from the spleen for rosette formation test. BPA-treated rats showed a significant reduction in red cell count, hemoglobin concentration and packed cell volume. The serum activity of alanine amino transferase, aspartate amino transferase, alkaline phosphatase, total cholesterol, low-density lipoprotein, urea and creatinine levels showed increased in BPA-treated group concomitant with reduction in glucose and triglycerides levels. Leucogram, E-rosettes forming count, total protein, albumin, globulin and high-density lipoprotein values did not show significant differences. Histopathological alterations were detected in the liver, kidneys and spleen of BPA-treated rats. From the present study it could be concluded that exposure to BPA is accompanied with anemia, hepatic and renal injuries.

Key words: Bisphenol A • Hematology • Liver Enzymes • Kidney Function

INTRODUCTION

Bisphenol A (BPA) is a high molecular polymer organic compound widely used all over the world. It used as a component of many industrial products, such as plasticizers, thermal stabilizers, pesticides, paints and dental materials [1]. In addition, it used in the production of polycarbonate and epoxy resins. Because of the use of BPA in the production of materials used for food and potable water, it has been detected in food and water consumed by humans as well as animals [2,3]. It is possible that humans may gain exposure to BPA through the air and by absorption through the skin [4]. Additional studies have quantified BPA levels in various aqueous media, including fresh and marine surface waters, treatment plant influents and effluents and groundwater [4, 5]. BPA is absorbed from gastrointestinal tract into the blood and redistributed to other tissues [6]. It is highly conjugated in the liver to form bisphenol Aglucuronide, a major metabolite, which is excreted in urine [7]. BPA has been demonstrated in both in vivo and in vitro experiments to act as an endocrine disrupting chemical [8]. It also acts as a xenoestrogen modulating the endocrine pathways via a receptor-mediated process [9], exhibiting a mechanism of action similar to that of the sex hormone at the receptor. In addition, BPA can bind to androgen receptors and inhibit the action of androgen [10]. Therefore, numerous studies have investigated the effects of BPA in male and female reproductive systems. However, few studies have concern the toxic effect of BPA on other tissues and its potential to increase the risk of metabolic disorders [11,12]. Indeed, the endocrine disrupting chemicals not only act as hormone-mimics or antagonists that act via binding to receptors, but also can interfere with hormone synthesis and clearance, as well as other aspects of tissue metabolism [13].
This study investigated the possible effect of BPA in rat, following oral administration, by examining alterations in the hematological and biochemical parameters. In addition, we evaluated the histopathological alterations in the liver, spleen and kidney.

MATERIALS AND METHODS

Chemicals: Bisphenol A (Sigma-Aldrich Company, Germany) was dissolved in corn oil (vehicle) as stock before administration.

Reagent kits for measuring of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, glucose, urea and creatinine were purchased from Biodiagnostic Company, Egypt. Total cholesterol, triglyceride (TG) and high-density lipoprotein (HDL) was performed using reagent kits from Spin react (Spain). RPMI 1640 medium was purchased from Sigma, St. Louis, MO.

Animals and Experimental Design

Experimental Animals: Twenty-four adult male Albino rats weighing 100-120 g were kept under standard laboratory conditions, fed with a commercial diet and given water ad libitum. The animals were equally divided into two groups.

Experimental Design

Group 1: A vehicle control group was gavaged with corn oil

Group II: The rats were orally gavaged with 150 mg/kg of BPA daily for 70 successive days.

At the end of the experiment, rats from treated as well as control groups were fasted overnight, weighed and anaesthetized with ether. Blood samples were collected with EDTA anticoagulant for hematological examination and without anticoagulant to obtain serum. Liver, spleen and kidney were dissected and weighted in order to calculate the organ/body weight ratios for each animal. Portions of liver, spleen and kidneys of each rat were kept in 10% formalin for the histopathological examination. Spleen was used for lymphocyte separation to evaluate rosette formation test. All experimental procedures and animal use in the current study were approved by the Beni-Suef University Local Committee on Animal Research Ethics.

Hematological Assays: Total erythrocyte and leukocyte counts were determined using an improved Neubauer hemocytometer. Packed cell volume (PCV) was estimated by microhematocrit technique. Hemoglobin concentration (Hb) was estimated using Drabkin’s method [14]. Differential leukocytic count was done on Giemsa stained blood smears [15]. Mean corpuscular volume (MCV) and Mean corpuscular hemoglobin concentration (MCHC) were calculated according to standard formulas.

Biochemical Analysis of Serum

Liver Function: Serum activities of ALT and AST [16] as well as ALP [17] were evaluated in both control and BPA-treated groups.

Serum total proteins [18] and albumin [19] were measured in both groups while the total globulins were determined by subtracting serum albumin value from the value of serum total protein to obtain the albumin-globulin (A/G) ratio.

Estimation of Blood Glucose: Glucose level was estimated according to Trinder [20]

Serum Lipid Profile: The serum total cholesterol [21], triglyceride levels [22], HDL [23] were estimated by enzymatic colourimetric reaction. Low-density lipoprotein cholesterol (LDL) was estimated by computation, according to the methods described by Friedewald et al. [24]. LDL = Total cholesterol - HDL - (TG/5).

Kidney Function: Urea and creatinine were measured according to Tabacco et al. [25] and Fabiny and Eringhausen [26], respectively.

Erythrocyte Rosette Test

Preparation of Spleen Mononuclear Cell: Spleen of rats obtained on the end of experiment and washed in 0.15 M NaCl. Cells from spleen were obtained by grinding the tissue in cold PBS, pH 7.2 and filtering the cells through a mesh. After washing with RPMI 1640 medium, the cells were harvested. Single cell suspensions of the spleen of each rat were maintained in RPMI 1640 medium with 10% fetal calf serum. Two volumes of the cell suspension were layered onto one volume of Ficoll-Hypaque and centrifuged for 25 min at 1500 xg. The mononuclear cells were collected, washed by centrifugation three times with RPMI 1640 containing 10% fetal calf serum and then adjusted to a concentration of 10^7 lymphocytes/ml in the same medium for rosette-formation test [27].
Rosette Formation: Sheep erythrocytes (SRBC) were collected, washed with PBS by centrifugation at 300 xg for 5 min and adjusted to a 1% suspension. The E-rosette test was performed as follows: 500 µl of the purified spleen cells was added to 250 µl of the well mixed 1% SRBC suspension. The mixture was centrifuged immediately at 250 xg for 2 min and left to stand overnight at 4°C. The supernatant was then removed and the pellet gently resuspended with 50 µl of 1% toluidine blue in PBS. Under light microscope, the number of rosettes counted out of 200 lymphocytes counted up to 200 nucleated cells were scored as a rosette.

RESULTS

Body Weight and Organ Weights: From Table 1, it appears that there was no change in the body weights, relative liver, kidney and spleen weights of BPA-treated rats compared with the control values.

Hematological Assays: As shown in Table 1, there was a significant reduction in red cell count, Hb concentration and PCV in BPA-treated group compared to control. There were no significant differences regarding MCV and MCHC between the control group and the BPA-treated group. Leucogram values showed no significant changes between the control group and BPA-treated group.

Clinical Biochemical Assays: In Table 2, the activities of serum ALT, AST and ALP exhibited a significant increase in BPA group compared to control group. There were no significant differences in total proteins, albumin, globulin levels of BPA group compared to the control group.

Cholesterol and LDL values significantly increased in BPA-treated group while no significant difference in HDL value was noticed in BPA-treated group compared with the control. On the other hand, significant decrease in glucose and triglyceride levels were observed in the BPA group compared to the control group.

Urea and creatinine levels in BPA group significantly increased when compared to the corresponding control values.

Table 1: Body weight (g) and relative organs weights (mg/g), hematological analysis and E-rosette count of rats in the control and BPA groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Bisphenol A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>Initial</td>
<td>Terminal</td>
</tr>
<tr>
<td>120±0.45</td>
<td>261.3±11.93</td>
<td>122±4.78</td>
</tr>
<tr>
<td>Liver Absolute (g)</td>
<td>7.68±0.49</td>
<td>30.22±0.74</td>
</tr>
<tr>
<td>Relative (mg)</td>
<td>30.22±0.74</td>
<td>6.59±0.26</td>
</tr>
<tr>
<td>Kidney Absolute (g)</td>
<td>1.89±0.15</td>
<td>7.45±0.33</td>
</tr>
<tr>
<td>Relative (mg)</td>
<td>7.45±0.33</td>
<td>1.59±0.07</td>
</tr>
<tr>
<td>Spleen Absolute (g)</td>
<td>0.97±0.06</td>
<td>0.71±0.04</td>
</tr>
<tr>
<td>Relative (mg)</td>
<td>3.86±0.32</td>
<td>0.71±0.04</td>
</tr>
<tr>
<td>Red blood cell (x 106/µl)</td>
<td>7.73±0.11</td>
<td>6.62±0.27*</td>
</tr>
<tr>
<td>Packed cell volume %</td>
<td>47.67±1.45</td>
<td>42.67±0.33*</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>15.60±0.21</td>
<td>14.13±0.20**</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>66.64±1.70</td>
<td>64.46±2.19</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.77±0.83</td>
<td>33.13±0.52</td>
</tr>
<tr>
<td>White blood cell (x 103/µl)</td>
<td>10.53±0.49</td>
<td>9.39±0.54</td>
</tr>
<tr>
<td>Lymphocyte (x 103/µl)</td>
<td>7.46±0.32</td>
<td>7.08±0.06</td>
</tr>
<tr>
<td>Neutrophil (x 103/µl)</td>
<td>2.8±0.21</td>
<td>2.2±0.17</td>
</tr>
<tr>
<td>Monocyte (x 103/µl)</td>
<td>0.26±0.03</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>E-rosette count</td>
<td>14.20±3.2</td>
<td>13.00±2.7</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE (Standard error of means). * differ significantly (P<0.05) from control group
Abbreviations: MCV, mean corpuscular volume (MCV); MCHC, mean corpuscular hemoglobin concentration
Table 2: Biochemical analysis of serum samples of rats in control and BPA groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Bisphenol A</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>13.53±0.74</td>
<td>37.48±1.34***</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>54.43±2.25</td>
<td>69.73±4.3*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>77.92±3.6</td>
<td>257±11.54***</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.16±0.16</td>
<td>6.30±0.11</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.16±0.08</td>
<td>2.25±0.09</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>3.99±0.22</td>
<td>4.17±0.09</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.62±0.06</td>
<td>0.54±0.02</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>121.7±6.4</td>
<td>82.07±1.4***</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>130.49±3.9</td>
<td>161.71±4.5**</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>41.98±1.8</td>
<td>22.93±2.89***</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>62.67±2.33</td>
<td>71.00±5.5</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>59.46±2.8</td>
<td>86.25±4.9*</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>45.37±0.79</td>
<td>70.24±3.3*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.65±0.02</td>
<td>0.83±0.06*</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE. * significantly different from control group.

Histopathological and Histochemical Results.

The Liver Tissue: In control group, the liver sections revealed normal lobular architecture with ill-distinct interlobular connective tissue septa. Each hepatic lobule consisted of hepatocyte plates radiating from the thin-walled central vein. Hepatocytes were large, polygonal cells with acidophilic cytoplasm containing rounded vesicular nuclei and some of them were binucleated. The blood sinusoids separating the hepatocyte plates lined with endothelial cells and von kupffer cells (Fig. 1.1). By using PAS technique, the general mucopolysaccarides appeared as strong magenta coloration in the hepatocytes cytoplasm (Fig. 1.2). The total proteins were seen as intensely dark blue coloration in the cytoplasm by using bromophenol blue stain (Fig. 1.3). The bisphenol treated rats showed severe degenerative changes in the renal corpuscles and the renal tubules. Atrophied renal corpuscles with contracted glomerular tufts and widening of the urinary space were detected (Fig. 1.4). Progressive damage of the renal tubular epithelium appeared as loss of their apical brush borders, cytoplasmic vacuolation, nuclear pyknosis and exfoliation of the degenerated cells (Fig. 1.5). Hyaline casts and desquamated cellular debris accumulated in the dilated renal tubular lumina (Fig. 1.6). Severe congestion and dilatation of the cortical renal blood vessels were noticed (Fig. 1.7). Kidney sections showed thickening of the basement membranes of Bowman’s capsule and the renal tubules which manifested by strong PAS positive reaction while the degenerated renal tubular epithelium showed negative reaction (Fig. 1.8). Great depletion of the total protein as indicated by faint blue coloration in renal tubular epithelium after application of bromophenol blue stain was seen (Fig. 1.9).

The Spleen Tissue: The spleen of control rats showed normal histological picture with distinct white pulp and red pulp that covered by a dense fibrous connective tissue capsule emerging trabeculae carrying blood vessels (Fig. 1.1 and 1.2). The white pulp composed of separated small sized splenic lymphoid follicles containing one or two eccentric central arteries which surrounded by a periarterial lymphoid sheath (PALS). Some of these follicles contain a central lightly stained area called a germinal center which capped by densely stained area called corona or mantle zone (Fig. 1.3). The red pulp consisted of splenic cords and splenic sinuses (Fig. 1.4). The examined spleen sections of BPA treated rats showed histological alterations as, increasing in the white pulp area with progressive lymphocytic hyperplasia and
Fig. 1.1: A liver section of control rat showing normal hepatic plates radiating from a thin walled central vein (cv) separated by blood sinusoids(S) lined by endothelial cells and von kupffer cells, polyhedral hepatocytes contained rounded vesicular nuclei (arrows). Some hepatocytes were binucleated (arrowheads). H&E, X400. 2. A section of control rat liver showing presence of general mucopolysaccarides indicated by strong magenta red coloration in the cytoplasm of hepatocytes, PAS X200. 3. A photomicrograph in a section of control rat liver showing presence of total protein the hepatocytes indicated by intensely blue coloration. Bromophenol blue staining method, X200. 4. A photomicrograph in a section of BPA treated rat liver showing severe swollen hepatocytes, congested blood sinusoids (S) with numerous Von Kupffer cells (wavy arrows), different signs of nuclear degenerations karyorhexsis(an arrow) and karyolysis (arrowheads) H&E X400. 5. A photomicrograph in a section of treated rat liver with bisphenol showing diffuse inflammatory cellular infiltration in the portal area (F). H&E, X400. 6. A photomicrograph in a section of treated rat liver with bisphenol showing dilated and congested portal vein surrounded by a highly fibrous tissue. Crossman's trichrome stain, X 200. 7. A photomicrograph in a section of treated rat liver with bisphenol showing marked depletion of general mucopolysaccharides as indicated by weak magenta red colour. PAS staining method, X400. 8. A photomicrograph in a section of treated rat liver with bisphenol showing marked depletion of total protein content as indicated by faint blue colour. Bromophenol blue staining method, X400.
Fig. 2: A photomicrograph in kidney section of control rat showing normal histological structure; renal corpuscles contained glomerular tuft (G) lined by podocytes and mesangial cells surrounded by partial layer lined by simple squamous cells (an arrow), proximal convoluted tubules (P), distal convoluted tubules (D). H&E, X400. 2. A photomicrograph in kidney section of control rat showing normal mucopolysaccharides as indicated by strong magenta red coloration of renal tubular brush borders (arrows) and glomerular tuft surrounded by thin glomerular and tubular basement membranes (arrow heads). PAS staining method, X400. 3. Control kidney showing normal total protein content as indicated by intense blue stainability. Bromophenol blue staining method, X400. 4. A photomicrograph in kidney of treated rat with bisphenol showing atrophied renal corpuscles (arrows) with contracted glomerular tuft (G) and wide urinary space (U). H&E, x200. 5. Kidney section of treated rat with BPA showing severe degeneration of renal tubular epithelium; loss the apical brush borders, cytoplasmic vacuolations (arrows) H&E, X400. 6. A photomicrograph in kidney section of BPA treated rat showing hyaline casts, exfoliated cells and cellular debris accumulated in the renal tubular lumina (arrows). H&E, x400. 7. Kidney section of BPA treated rat showing severe dilatation and congestion in the cortical renal blood vessels (c). Crossman’s trichrome stain, x200. 8. A photomicrograph in kidney section of treated rat with bisphenol showing marked thickening of the glomerular and the renal tubular basement membranes (arrows), pronounced reduction of mucopolysaccharides in renal tubular epithelium as indicated by weak magenta red color. PAS staining method, X400. 9. A photomicrograph in kidney section of treated rat with bisphenol showing marked reduction in the total protein content as indicated by faint blue stainability. Bromophenol blue staining method, X 400.
hypertrophy of the splenic lymphoid follicles with ill-distinct germinal centers (Fig. 3.5). In addition, pronounced dilatation and congestion of trabecular splenic blood vessels and splenic sinuses were recorded (Fig. 3.6). Hemosiderosis was increased and clearly distributed throughout the red pulp area (Fig. 3.7).
DISCUSSION

Bisphenol A is a chemical that ubiquitously infiltrates our environment because of continuous release [29]. Its release can occur via effluent discharge from municipal wastewater treatment plants, leaching from landfills, combustion of domestic waste and the natural breakdown of plastics in the environment [5].

Current results showed that oral exposure of adult male rat to BPA does significantly reduce the erythrocytes number, Hb concentration and PCV compared to control values. Exposure to BPA resulted in normocytic normochromic anemia. The present findings come in accordance with Ulutağ et al. [30] and Yamasaki and Okuda [31] who evaluated the BPA hematotoxicity in rat at dose of 125 mg/kg and 100 mg/kg, respectively. They found that BPA induced a significant decrease in red cell count, Hb concentration and PCV. The decrease in the red blood cells may indicate a disruption of erythropoiesis. The administration of estrogens has been known to reduce erythropoiesis in male rats [30]. The present data of leukogram revealed that BPA did not induce any change in leucocytic count or differential count when compared with the control. Ulutağ et al. [30] reported that BPA at doses of 125 and 250 mg/kg in rats induce no effect on leucogram.

In the present study, the number of rosettes forming lymphocytes was unchanged in the BPA treated group as compared to the control group suggesting that BPA did not affect the activity of splenic T-lymphocytes. Yamashita et al. [32] reported no change in T-cell subpopulations with BPA treatment.

The liver is a target tissue for endocrine-disrupting chemicals. Specific estrogen receptors exist in the liver and cellular responses involving estrogen interactions have been identified [33]. The current study demonstrates that BPA has adverse effects on the liver, as indicated by increased activities of ALT, AST and ALP enzymes. They are released in the blood stream when the liver is damaged [34]. Similar elevated levels of serum hepatic enzymes activities were previously observed that resulted from BPA at doses of 5 mg/kg [8] and 50 mg/kg [11] in rats. Hepatic damage induced by BPA may be due to generation of reactive oxygen species in the liver [8]. The elevated activities of liver enzymes in BPA group confirm our histopathological findings. In this study, severe alterations were recorded in the liver of BPA treated rats, which manifested by swollen and vacuolated hepatocytes with nuclear degenerations, dilatation and congestion in the blood sinusoids with increased number of Von Kupffer. Similar observations were recorded by previous studies [11, 35, 36]. In addition, dilatation and congestion of the portal vein with periportal infiltration of inflammatory cells and mild fibrosis were detected. These findings agreed with the results recorded by Daniela-Saveta et al. [37]. Bisphenol reported to increase the hepatic oxidative stress and mitochondrial dysfunction leading to structural changes of rat liver [38].

In the current study, hypoglycemia was recorded in BPA group compared to control. Alonso-Magdalena et al. [39] found that treatment with BPA at a dose of 10 µg/kg produces an increase in the pancreatic insulin content, a rapid increase in plasma insulin and a decrease in blood glucose.

In the present study, hypercholesterolemia was observed in BPA-treated group. Exposure to low doses of BPA increases the insulin expression and production by the pancreas [40, 41]. Insulin is known to increase lipogenesis by both post-translational protein modifications and transcriptional mechanisms [42]. Kotzka et al. [43] and Xie et al. [44] found that activity and expression of sterol regulatory element binding protein 1c (SREBP-1c), which regulates cholesterol metabolism, were activated by increased insulin levels. Thus, insulin is likely to contribute to hypercholesterolemia observed following BPA exposure. However, Marmugi et al. [41] did not rule out the contribution of other mechanisms, independent of insulin and possibly involving direct effects of BPA on the liver, to the hepatic transcriptional impacts detected in the BPA-treated mice.

A significant decrease level of triglyceride was observed after treatment with BPA. The obtained results go in parallel with those reported by Yamasaki and Okuda [31] who detected a decrease of triglyceride values using bisphenol A related compound at dose of 100 and 300 mg/kg.

The elevated levels of urea and creatinine recorded in this study resulted from BPA administration were confirmed by the renal histopathological alterations. There are similarities between the renal alteration detected in this study and those described by previous authors [11, 12, 37]. Bisphenol has a nephrotoxic effect due to accumulation of BPA toxic metabolites and inability of the kidney to eliminate them Sangai et al. [45].

According to the histochemical study, our results indicated marked depletion in the general mucopolysaccarides and the total protein in hepatic and renal tissue. Asahi et al. [46] reported that bisphenol induces mitochondria dysfunction and rough endoplasmic damage which in turn important for protein
pathway. In addition, BPA treatment impairs glycogen content by decreasing the glycogen phosphorylation and increase glycolysis [47]. The present study showed thickening of the basement membrane Bowman’s capsule and renal tubules which confirmed by strong PAS stainability as recorded by Manikkam et al. [48]. Several toxic substances induced nephrotoxicity may result in alteration of the glomerular basement membrane and affecting the glomerular filtration [48].

Regards to the spleen, in this study we observed diffuse lymphocytic hyperplasia and hypertrophy of lymphoid follicles as well as congested red pulp with hemosidrosis. Yoshino et al. [49] reported that male mice treated with BPA at 3000 µg/kg bw/day had an enhanced lymphoid cell proliferation in response to hen egg lysozyme HEL. Also, Youn et al. [50] observed significant splenocytic proliferation in male mice treated with BPA for 4 weeks at drinking water at concentrations of 0.015, 1.5 or 30 mg/ml. On other hand, BPAreported to induce a decline in the number of T cells (Total, CD4+ and CD8+), B cells and macrophages in the spleen [51].

CONCLUSION

It can concluded that oral administration of bisphenol A for 70 days in adult male rats resulted anemia, in addition to alteration in several biochemical parameters that indicate liver and kidney injuries which confirmed with histopathological alteration those organs.

REFERECNES


