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BPA; An Endocrine Disruptor Induced Biochemical Changes and Histopathological Damage in the Kidneys of Rats (*Rattus norvegicus)*

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Competing interests
The authors have declared that no competing interests exist.

Abstract

Background: *Xenoestrogens are chemical compounds that are similar to estrogen in effect but not in structure. Bisphenol A is an endocrine disruptor, that mimics the action of endogenous estrogen and activates the estrogen receptor. It is produced in large volumes and incorporated in many plastic industries worldwide. BPA is extensively used in food and beverages. The ubiquitous and extensive use of BPA containing products results in high human exposure, and its effects on the human body are of great concern. The aim of the present study was to evaluate the effects of BPA on biochemical and histopathological parameters of the kidney.*

Material and Methods: *Forty adult male rats were assigned into five groups of eight rats each. One group was served as a control and other groups were treated with BPA. Rats were administered orally with different doses of BPA 10mg/Kg and 25mg/Kg for 6 and 12 weeks, respectively. All doses of BPA were dissolved in corn oil and orally administered to rats. After 6 and 12 weeks, blood and kidney samples were collected for evaluation of biochemical parameters and histopathological analyses.*

Results: *Serum levels of urea and creatinine were significantly increased, and uric acid levels in serum were increased but not significantly. The biochemical parameters variations were confirmed by histopathological investigations. BPA induced toxicity may lead to harmful health effects.*

Conclusion: *Results showed that the oral administration of BPA significantly affects biochemical parameters and renal tissue architecture.*

Key words: *Bisphenol A, Histopathology, Kidney, Toxicity, Xenoestrogen*

Introduction

In recent years, endocrine-disrupting chemicals have been introduced into the environment on a large scale as a result of rapid advances in human lifestyle, and living organisms are directly or indirectly exposed to dangerous compounds like Bisphenol A (BPA) (Bosch et al., 2016; Khan., et al., 2021). In environmental and toxicological research, BPA; which is a xenoestrogen has received much attention due to its ubiquitous effects in biological system (Ola-Davies et al., 2018). BPA was first synthesized in late the 19th century (Hassan et al., 2013).

BPA is a xenoestrogen, which is a common constituent found in food and beverage containers as well as plastic baby bottles (Bindhumol et al., 2003; Rubin, 2011). The xenoestrogen BPA has been shown to mimic estrogen and showed estrogenic activity both *in vivo* and *in vitro* (Zahra et al., 2022). BPA is utilized in manufacturing of polycarbonate plastic and epoxy resins (Ribeiro et al., 2017). Plastic containers are made of polycarbonate, which is frequently used at home and in food industry, and epoxy resins are utilized as coatings for beverages and food cans (Dekant and Volkel, 2008). BPA adds inflexibility, light weight, clarity, and temperature resistance to plastic products (Rahimi et al., 2015). Exposure occurs because BPA monomers are linked together by ester bonds that are subject to hydrolysis as a response to contact with acidic/basic

conditions and high temperatures (Kang et al., 2003; Krishnan et al., 1993). The main routes of BPA exposure are oral, dermal, and inhalation (Bosch et al., 2016). Human exposure to BPA is increasing at risk level as it is leaching out of polymers and entering sources of water and food (Koch and Calafat, 2009). In another study, extensive accumulation of BPA in the human cord blood, placenta, amniotic fluid, and breast milk has been reported (Pal et al., 2017).

Endocrine disruptors, as being exogenous agents are capable of interfering with the synthesis, metabolism, and function of endogenous hormones in humans and animals (Bindhumol et al., 2003; Nakagawa and Tayama, 2000). The function of natural estrogen mimicked by BPA, which is an endocrine disrupter and has adverse health effects. Apart from the estrogenic activity of BPA, it has been revealed that BPA causes dysregulation of cytokines and induces oxidative stress in the liver, kidney, and brain (Morgan et al., 2014).

The generation of multinucleated giant cells in hepatocytes of the rats, reactive oxygen species formation by DNA oxidation of liver, biochemical profile changes in the liver and kidney, as well as renal tubule degeneration in mice and rats, are the BPA adverse effects on the liver and kidney (Bindhumol et al., 2003; Nakagawa and Tayama, 2000). Cellular macromolecules such as nucleic acids, proteins, and lipids are directly damaged by the oxidative stress of reactive oxygen species (Canbek et al., 2011). Reactive oxygen species cause injury to the kidney, brain, liver, and other organs because reactive oxygen species are cytotoxic agents that cause oxidative damage by attacking DNA and cell membranes, which could lead to diseases (Kabuto et al., 2003).

Researchers have also found BPA in the human hairs of head (Tzatzarakis et al., 2015). In the liver and intestine, BPA is conjugated with glucuronic acid and excreted as BPA glucuronide in urine (Dekant, & olkel, 2008). BPA has renal excretion and accumulates if the glomerular filtration rate is reduced. Furthermore, the plasma level of BPA is increased in patients with chronic kidney disease (Gonzalez-Parra et al., 2013).

The aim of the present study was to investigate the effect of oral administration of different doses of BPA for two different time periods on kidney histopathology and biochemical parameters.

Materials and Methods

Animals

Forty adult male Wistar rats weighing 125-130g were used in the present study. The rats used for the present study were raised at the Institute of Zoology, University of the Punjab, Lahore. The animals were caged in a well-aerated and clean environment at 23 ± 2 °C. They were all kept in controlled 12 hours light/dark cycles in stainless steel wire cages. They were fed with normal rat chow and fresh water *ad libitum* during the study. All the experiments were performed in accordance with the institutional guidelines for the use and care of animals for research purposes.

Dose preparation and administration

To investigate the toxicity of BPA, rats were randomly divided into five groups of eight rats each. One group served as a control and the other four groups were the experimental groups. Group I was given BPA dose of 10mg/kg/day for 6 weeks. Group II received BPA dose of 25mg/kg/day for 6 weeks. Group III was given 10mg/kg/day of BPA for 12 weeks and group IV received 25mg/kg/day BPA for 12 weeks. Doses of BPA were freshly prepared by dissolving it in corn oil and orally administered to experimental animals with the help of oral gavage. Animals were sacrificed after 6 and 12 weeks of BPA exposure.

Blood sampling

After receiving the last dose, animals were anesthetized with intraperitoneal injection of equal ratio of ketamine and pyrogen free water. The blood samples from the heart of rats were collected by using sterilized disposable syringes. 4ml of blood from each animal was collected in gel vacutainer for serum separation. Blood samples were incubated at room temperature for 2-3 hours and centrifuged for 20 minutes at 4000 rpm. Then serum was collected in new eppendorf cups and stored at -20 °C for biochemical analysis.

Biochemical analysis

Biochemical analysis was performed using ready to use kits (Chemohouse). Samples from the control and experimental groups were processed according to the instructions provided by the manufacturer.

Histopathological Analysis

Immediately after blood samples were collected, kidneys were excised quickly, and part of them was put/kept/preserved in 10% formalin. Tissue sections of the kidney were stained with hematoxylin and eosin for histopathological analysis.

Statistical analysis

All collected data were analyzed by Graph Pad Prism 5 software and presented as mean ± standard error of the mean (SEM). Statistical significance was calculated by one way ANOVA test followed by Turkey's *post hoc* analysis. A p value of ≤ 0.05 was considered statistically significant.

Results

Assessment of biochemical parameters

Urea levels were significantly increased ($P \leq 0.05$) in BPA treated groups as compared to control group. The level of urea in group IV was significantly higher as compared to group I of low dose for 6 weeks. Level of creatinine in BPA administered groups was increased in dose dependent manner. BPA treated group II showed significant, while groups III and IV showed highly significant increase as compared to control group. Uric acid level in the BPA treated groups was increased compared to the control group, but not significantly (Table 1, Figure 1).

Table 1: Mean ± S.E.M of serum urea, creatinine, and uric acid levels in rats treated with BPA.

Groups/Parameter	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Con	21.50 ± 0.50	0.55 ± 0.02	1.43 ± 0.17
I	29.33 ± 3.38	0.64 ± 0.02	1.60 ± 0.10
II	36.50 ± 1.32	0.70 ± 0.03	1.75 ± 0.05
II	35.80 ± 2.87	0.72 ± 0.01	1.70 ± 0.20
IV	43.00 ± 2.48	0.75 ± 0.02	2.00 ±0.11

Histopathological findings:

The histopathological findings of the kidneys of the control group revealed that normal glomerular tufts with normal convoluted and distal tubules were present. Podocytes of normal

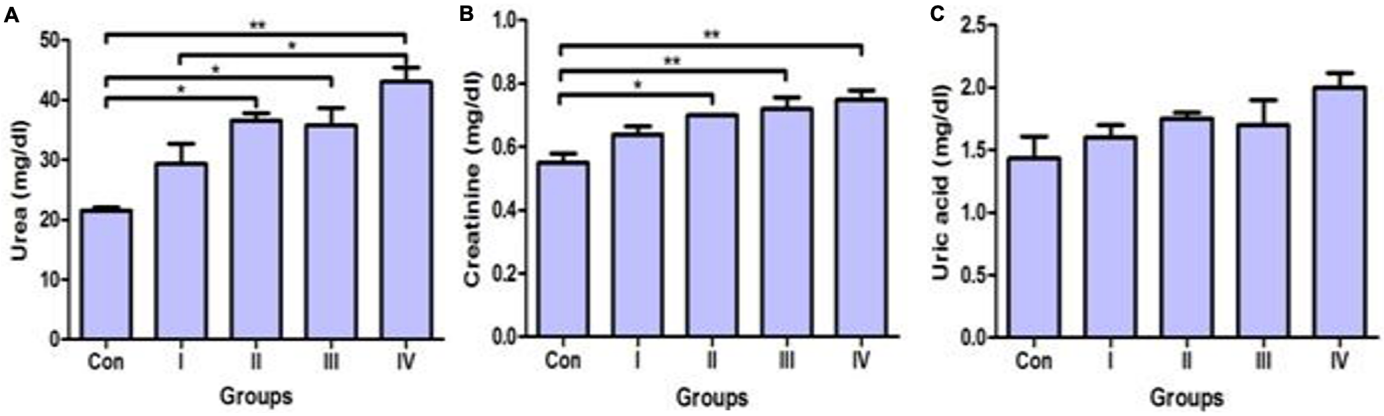

*Figure 1: Change in serum urea (A), creatinine (B), and uric acid (C) levels in control and BPA treated groups. Values are mean ± SEM, error bar shows the standard error of the mean. **P≤0.01, *P≤0.05.*

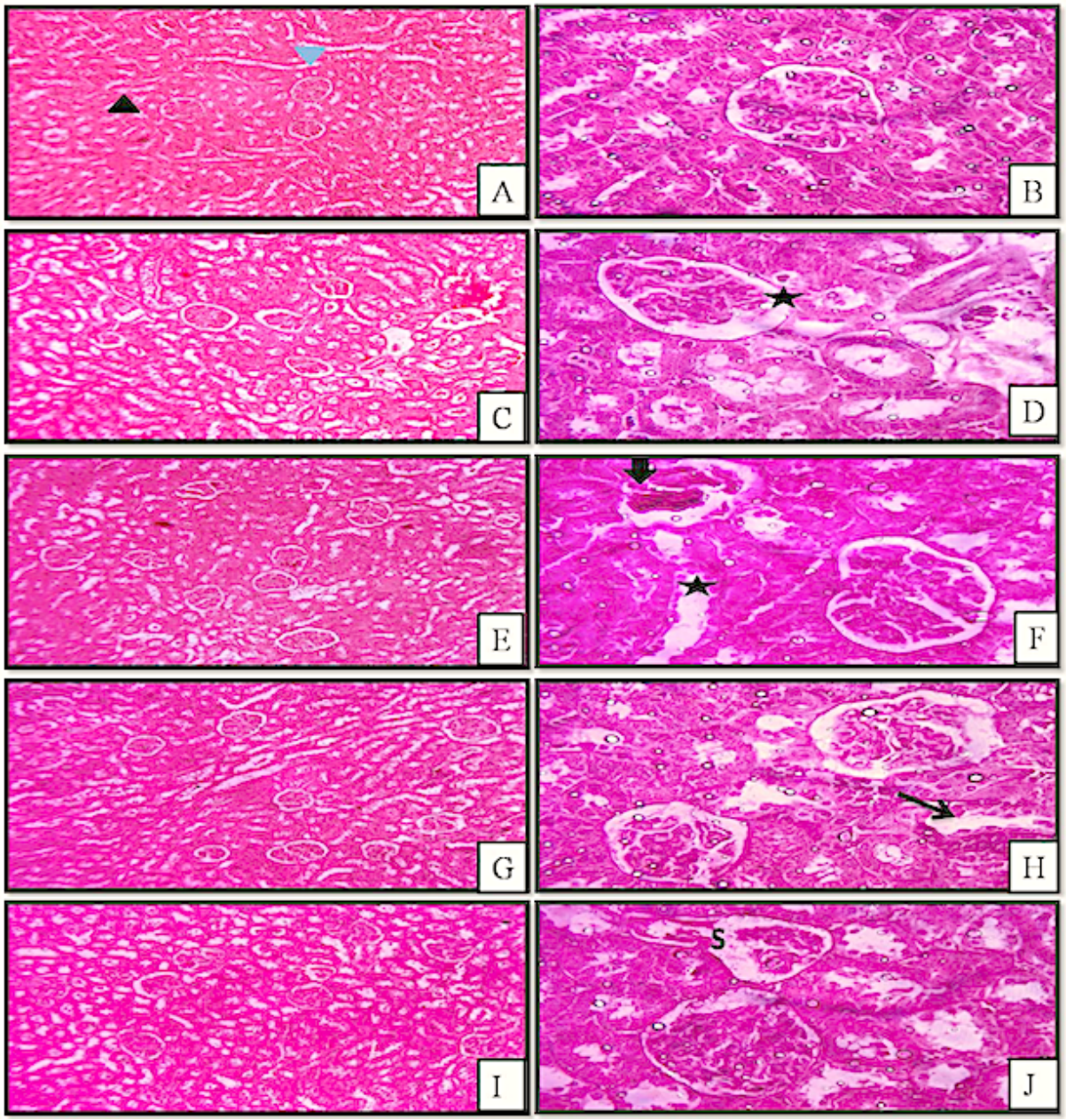

Figure 2: Hematoxylin & eosin staining of kidney sections of BPA induced toxicity in groups I & III (10mg/Kg for 6 and 12 weeks respectively) on 10× and 40× (C, D, G, and H). Groups II & IV (25mg/Kg for 6 and 12 weeks respectively) on 10× and 40× (E, F, I, and J). Control sections (A, B) on 10× & 40×. Arrow heads show normal renal tubules and glomeruli; black thick and thin arrows indicate infiltration and necrotic cells; S shows the shrinkage of the glomerulus and stars indicate the dilation of the glomerulus and renal tubules.

shape with an intact glomerular basement membrane were also seen in the control group (Figure 2A & B). Shrinkage of glomeruli was observed in all BPA treated groups, and shrinkage was more prominent in high dose groups as compared to low dose. Destruction of the glomerular membrane and congestion were observed in all treated groups. Severe chronic tubular necrosis was observed in groups II, III and IV (Figure 2E, F, G, & H). More prominent renal tubule distortion and infiltration were observed in high dose exposure for 12 weeks (Figure 2I & J). Mild to severe degeneration and dilation of renal tubules were seen in low as well as in high dose groups. Dilation of Bowman's space was observed in all BPA treated groups. Bowman's space dilation became more prominent as the dose of BPA was increased.

Discussion

Some chemical substances exhibit hormone like action, which is known as an endocrine disruptor, has been associated with reproductive abnormalities, immune dysfunction, and an increased rate of cancer. Currently, the effect of endocrine disruptors in the human body has become a social issue. BPA is one of the endocrine disrupting chemicals, that is produced in the highest volume worldwide. Its effects on human body are of great concern.

It has earlier been reported that estrogenic compounds have endocrine disrupting effects on male and female reproductive systems, but there are very few studies concerning the estrogenic effects of these compounds on the spleen, liver, and kidneys (Yildiz and Barlas, 2013).

The current findings revealed that BPA caused damage in the renal tissue, as indicated by increased serum urea, creatinine, and uric acid levels as well as altered histopathological findings. Creatinine is a breakdown product of muscle metabolism, in which creatine is converted non-enzymatically to creatinine. The glomerular filtration rate was measured by it. The level of creatinine excreted in the urine is equal to the creatinine filtered by glomerulus per minute time. Creatinine is built up in the blood when GFR decreases because of renal dysfunction. These results were in congruence with (Edres et al., 2018) who found that oral administration of BPA induced a significant increase in the levels of serum urea, creatinine, and uric acid when compared to control group due to nephrotoxicity variations.

It was also suggested that BPA caused nephrotoxicity due to the accumulation of toxic BPA metabolic substances and the inability of the kidneys to eliminate these toxic substances in rats, which resulted in nephrotoxicity. While another study has indicated that BPA insignificantly increased the creatinine level after 15, 30, and 60 days as compared to control (Murmu and Shrivastava, 2014). The BPA effects on serum kidney markers were positively correlated with previous studies (Ola-Davies et al., 2018). Levels of these biomarkers was increased in the present chronic study of BPA exposure, which was also confirmed by changes in renal tissue architecture.

Histopathological investigation of renal tissue showed shrinkage of the glomerulus and destruction of glomerular membrane, as well as congestion, in the BPA treated groups. Renal tubules degeneration, distortion and severe chronic necrosis were also found in treated groups. Bowman's space and renal tubules dilation was seen in all BPA treated groups. These results are correlated with (Ahmed et al., 2015) who revealed that renal tissue section of an orally treated rat showed severe dilation as well as congestion in the renal blood vessels. The kidney tissue of rat exposed to BPA showed large hemorrhagic areas, congestion, necrotic lesions, and infiltration (Edres et al., 2018).

Conclusion

It can be concluded from the present study that oral administration of different concentrations of BPA for different time periods significantly affects renal function as well as renal tissue architecture.

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