

The Assessment of Histopathological Impacts of Bisphenol-A on the Liver in Mice Model

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ABSTRACT

Background: Bisphenol-A (BPA) is one of the synthetic materials which is the chief part of polycarbonate plastics and it is the cheap alternate for metals available at time. It is considered as an ancient ecological contaminant which has harmful and severe effects on living beings all over the world. A worrisome effect of BPA is that there is incorporation in the living beings through the use of domestic appliances.

Objectives: The current study was carried out to investigate the lethal effects of BPA on the liver in a mice model.

Methodology: Following Complete Random Design-Model, forty (40) mice (*Mus musculus*) (24g ± 5g) were categorized into 4 groups (n=10) and administered with oral BPA as “Low Dose” (300 mg/kg/BW) and “High Dose” (600mg/kg/BW), respectively. The doses were planned by keeping in view the LD50 value of the drug. This experimental treatment was conducted for 28 days, consecutively. Numerical data were analyzed statistically through ANOVA by using software SPSS (Statistical Program for Social Science Version 20) followed by the Tukey’s test to observe the differences among the groups.

Results: A substantial difference was observed between the treated and control groups. There was a significant elevation in the biochemical analysis of serum. Microscopic and micrometric examination indicated that BPA has reduced the body and liver weight in treated groups as compared to control group. Histopathological (H & E stained sections) studies revealed that there were deleterious impacts found in hepatic cells which were symptoms of hepatotoxicity. Necrosis due to BPA disintegrates the normal composition of the liver, causing depression of body and liver weight when compared with control group.

Conclusion: The findings indicated BPA as a toxicant that is capable of acting on hepatocytic cells, resulting in histopathological alterations. BPA also show negative effects on Liver Function Tests (LFTs).

Keywords

Bisphenol A, Hepatotoxicity, LD50, *Mus musculus*, Liver Function Tests, Necrosis.

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INTRODUCTION

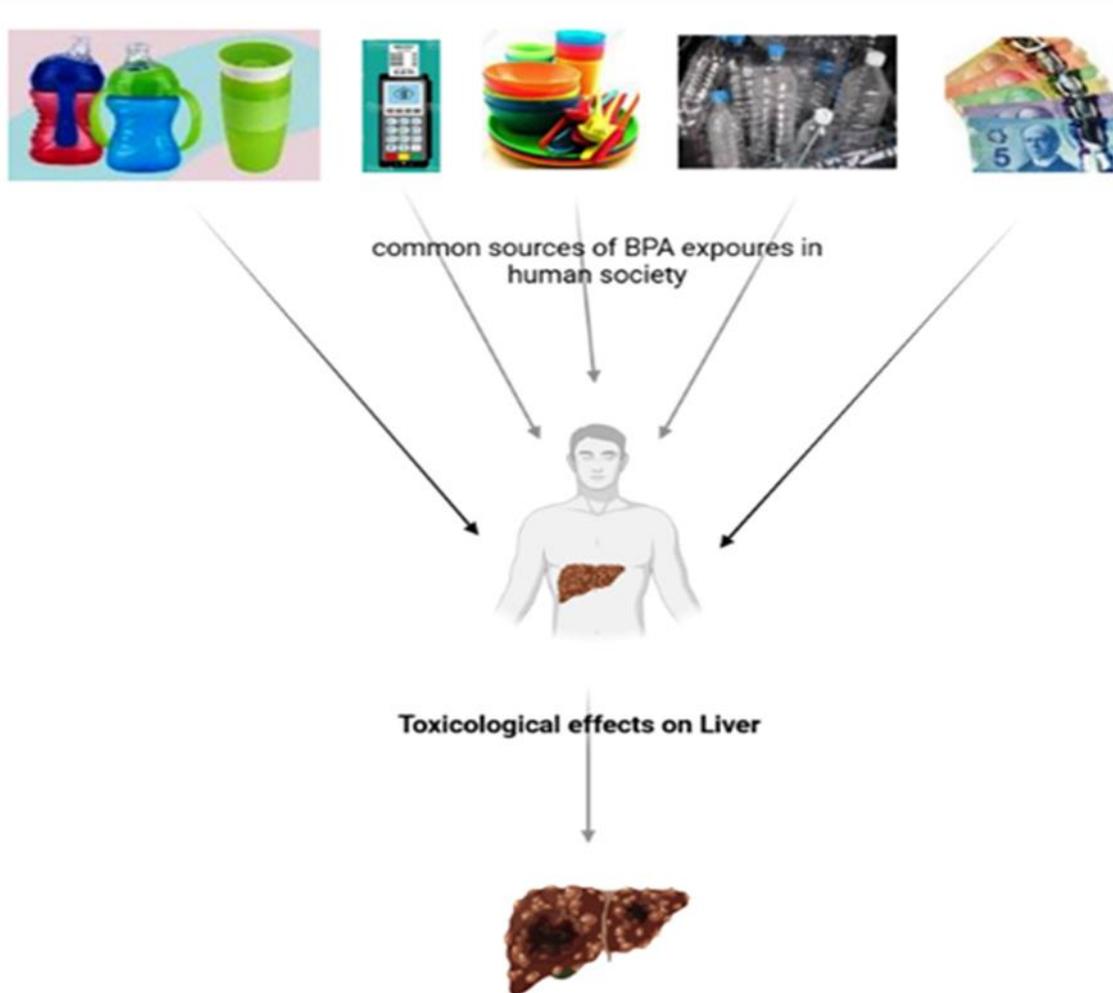
Back in 1891, Alexander Pavlovich Dianin, a Russian chemist was the one who first depicted the artificial capability of BPA polymers¹. For the first time in 1950, it was used as a component in the production of polycarbonate. It is a lightweight, transparent, heat resistant and almost indestructible chemical. BPA was considered as one of the highest capacities of the chemical

manufacturers with a worldwide consumption. In the last 80 years, BPA has grown widespread, and it is now on the list of pollutants linked to estrogenic disruption. The impending disastrous impacts of BPA on human health hazards were documented². This artificial compound has its gigantic pervasiveness in our environment as an anthropogenic pollutant. Regardless of this, it is continuously used in chief

industries including food and brewing industry, medicals and safety appliances, water and milk bottles, and toys³. The underprivileged quality of plastic proliferates its leaking capacities. The toddlers having less body weight are more prone to its harmful effects⁴. A worrisome effect of BPA is that there is incorporation in the living beings through the use of domestic appliances⁵ (Schematic 1). There is also a reported strong association between upraise of BPA and diabetes. Many republics banned the use of BPA in edibles and their allied equipment. Unfortunately, Asian countries realized the hazardous impact of BPA in recent decades on living beings and injuries of organs⁶. Hines *et al.*, 2018 documented that employees of the BPA industries have more chances to become the victim of it⁷. A unique observation from the piles of the experiment revealed the bio-magnifications of the BPA in the organism⁸. The most

recent observation evaluated long-term exposure causing organ catastrophe and having a casual role in making behavior anomalies⁹. Nowadays, hormones disruptor mechanism of BPA is the leading cause to declare BPA as carcinogenic. Lots of investigations were done to study the intrusions of BPA toxicity, signposted by a preliminary survey of the literature¹⁰.

The political and economic interference has crafted a misconception regarding BPA fatal effects in layman's mind. Although, there is a wide-range use of BPA products however, the statistics about liver toxicity via BPA is still inconclusive, and unluckily in Pakistan there is little awareness about BPA. Therefore, this study was carried out to investigate the lethal effects of BPA on the liver.



Schematic 1. Representation of Bisphenol-A (BPA) toxicity on Liver.

MATERIALS AND METHODS

Animals:

In this investigation, 40 male albino mice (Swiss strain) were used. The mice were hosted in an animal house at the Department of Zoology, University of the Punjab, Quaid-Azam campus, Lahore, Pakistan. The animals were familiarized with the native circumstances of the animal house a week before. Bioethical protocol permitted by the committee of the University of Punjab, Lahore was applied in this research.

Husbandry:

To diminish the peripheral interference, stainless steel cages were used under control conditions. Up to 12hrs of light and dark cycle were upheld during investigational work. Chick feed No. 14 (National) was fed to the animals along with water ad libitum.

Chemicals:

All chemicals used in the study were 99.9% pure, by Duskan USA.

Dosing:

Two dosages were used in this research. These doses were decided based on the LD50 value. Bisphenol-A was administered orally *via* gavages (introduction of material into the stomach by a tube). Initial body weight was noted before dosing. The dose was administered daily for twenty-eight days. Soya bean oil was used as vehicle control.

Investigational Scheme:

This investigational scheme consists of four groups, having 10 mice in each group (n=10).

Group (C): This group act as a negative control, (was not given any dose except water and feed for 28 days).

Group (LD): This group was treated with a low dose of BPA 300mg/kg body weight for 28 days.

Group (HD): This group was treated with a high dose of BPA 600mg/kg body weight for 28 days.

Group (VC): This group acts as a positive control and is given oil *via* gavages for 28 days.

Blood Samples:

The mice were kept fastened overnight, weighed, and anesthetized. Blood samples were collected 24hrs after the last treatment before dissection, by cardiac puncture, for biochemical analysis. After this, mice were dissected and liver tissues were removed carefully. The liver was instantaneously preserved in preservatives after trimming extra fats.

Histology:

Liver samples were tranquilized in 10% formalin for histopathological analysis. To observe the liver morphology, several laboratory phases were performed for slide preparation. Liver samples were dehydrated by series of ethanol, cleared with xylene, embedded in paraffin wax, and sliced into 5 μ m sections by a rotatory microtome. Tissues were processed and embedded in paraffin wax. Slides were examined using microscope model No.M4000-D Swift, Japan supported by portable camera "Ease-Imageur universal" with H & E staining.

Statistical Analysis:

All the data were expressed as mean \pm / standard error of the mean. The data were statistically analyzed by SPSS (Statistical Program for Social Science Version 20) software using One-Way Analysis of Variances (ANOVA), followed by Tukey test for multi-comparison at significance level $p < 0.05$.

RESULTS

On the initial day of the treatment, the body weight (g) of mice have been recorded. The average final body weight (g) before dissection of mice recorded in distinct groups has been summarized in Table 1.

Table 1. The Table shows the Effects of BPA on the Body Weight and Liver Weight of Mice.

Groups	Body Weight (Initial) (g)	Body Weight (Final) (g)	Liver Weight (mg)
Control (C)	20.075 \pm 1.33	23.785 \pm 1.44	1506.8 \pm 41.87
High Dose (HD)	23.815 \pm 1.91	23.125 \pm 1.54	1256.1 \pm 29.11
Low Dose (LD)	24.080 \pm 1.70	23.397 \pm 1.73	1322.2 \pm 61.87
Vehicle Control (VC)	23.400 \pm 1.86	23.732 \pm 1.64	1545.6 \pm 5.98

BPA treatment caused a significant reduction in the body as well as the liver weight of both treated groups (HD & LD) as compared to the control group (C). However, the intensity of weight reduction is associated with exposure to BPA dose and *vice versa*. Besides, no discrepancy differences ($p < 0.05$) were noted between control and vehicle control. The damage due to BPA that occurred in (LD) group was less unlike in the (HD) group. The occurrence of alterations in the fundamental structure of the liver are revealed in Fig 1. Histological sections were observed at three different magnifications i.e., (10×04) (10×10) (10×40). Sections of liver tissues were evident in

massive destructions and dysfunctions in the BPA exposed groups. There was the finding of cytoplasmic vacuolization and blocking (congestion) of the blood vessel within the liver cells, and inflammatory cellular and infiltration occurred in the bile duct. The destructive symptoms of necrosis were prominent in treated groups. The whole architecture of the liver cells was deformed. Centrally arranged leucocytes were scattered from their original position. Clumps of leucocytes with the impact of microsteatosis as well as macrosteatosis were prominent in the high dose (Table 2; Fig 1).

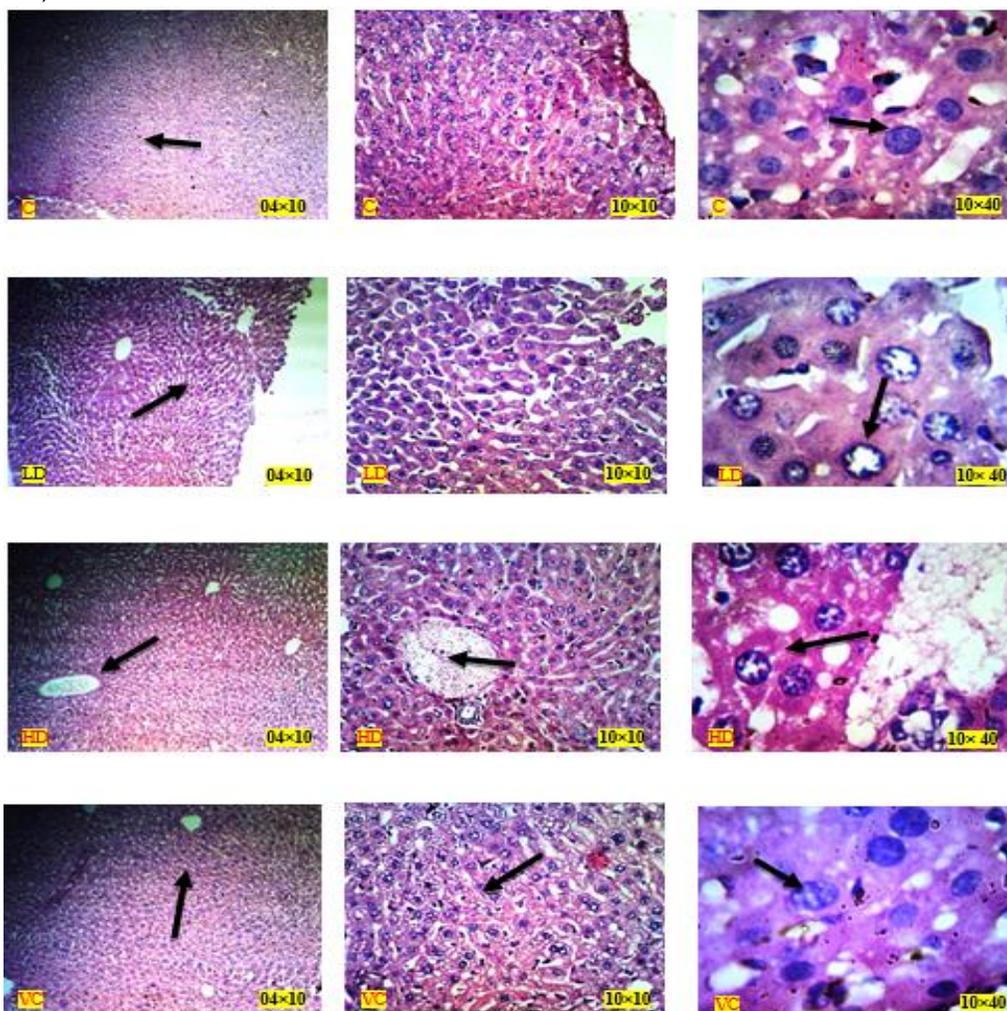


Figure 1. Hematoxylin and Eosin (H & E) staining. Histopathological analysis of the hepatic cells. C and VC are the images for liver slides of the control group illustrating normal architecture. HD and LD sections show effected liver sections by BPA doses. Both shows distended and choked main vital veins and hepatic sinusoids. Liver sections of the high dose of BPA experimental group show hepatocytes with marked vacuolar degeneration, H & E stain shows constant changes. 10x04 (1st column), 10x10 (2nd column).

Serum analysis shows that there was a significant decrease in ALP and AST, however, ALT was normal and does not show any change in the treated groups (Fig. 2a-i).

Table 2. Histopathological Impacts of BPA on Mice Liver Serum Assay.

S. No.	Liver Lesions	Control (C)	High Dose (HD) 600mg/Kg/B.wt	Low Dose (LD) 300mg/Kg/B.wt	Vehicle Control (VC)
1	Hepatic necrosis and peri-portal congestion	No	Yes	Yes but less than HD	Negligible
2	Cellular infiltration of lymphocytes in the portal area	Negligible	Yes	Yes	No
3	Disarrangement of hepatic plates with swollen	No	Yes	Yes	No
4	Pyknotic nucleus	No	Yes	Yes but less than HD	No
5	Proliferative bile ducts	Negligible	Yes	Yes	No
6	Cytoplasmic vacuolization	No	Yes	Yes	No

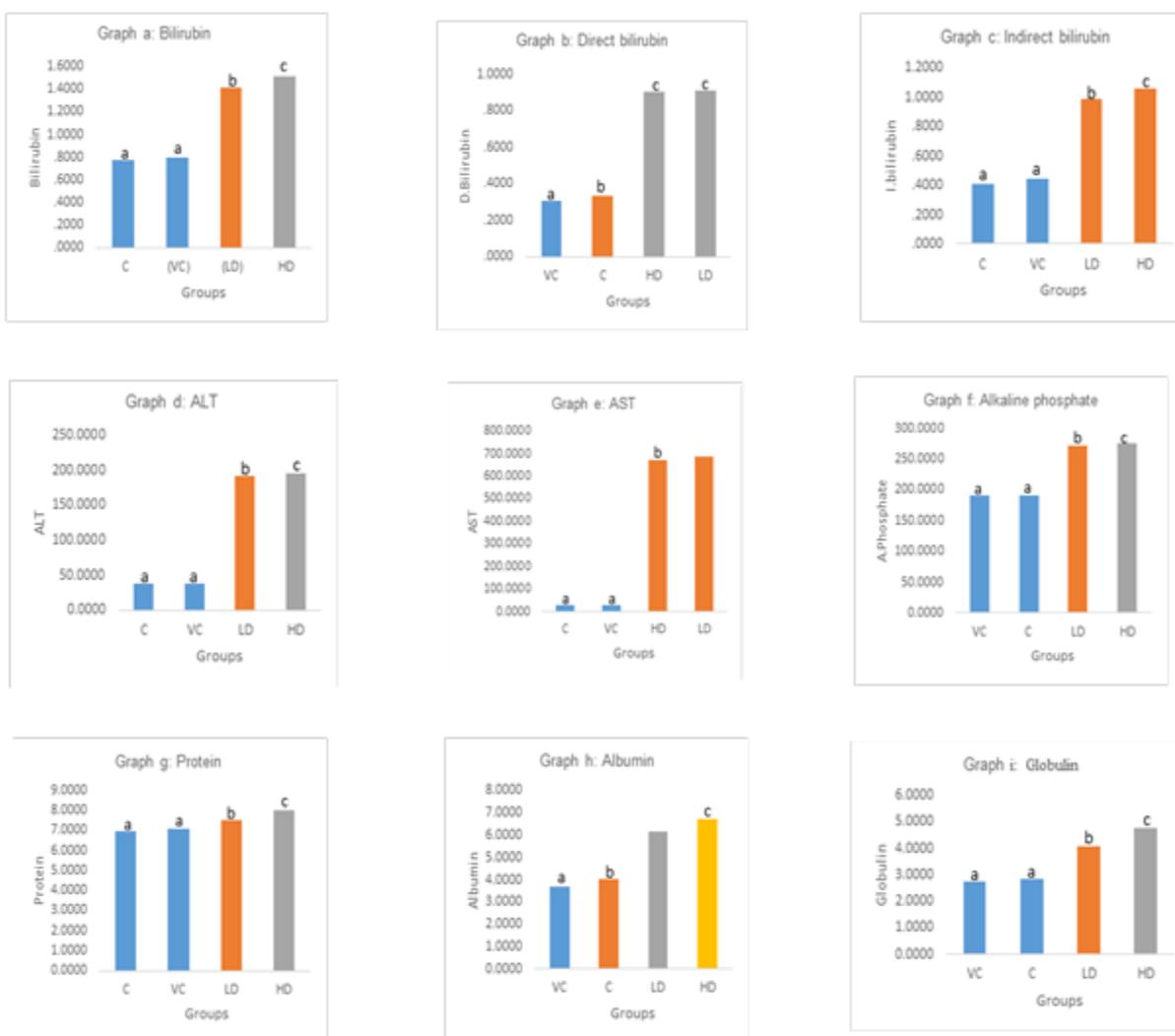


Fig 2 (a-i). Fluctuations in different parameters of serum analysis in BPA exposure.

DISCUSSION

A significant reduction in body and liver weight was found in this study, similar to the previous results by Verstraete *et al.*, (2018) where they found that BPA exposure caused significant decrease in the body as well as liver weight. They also concluded dysfunctions and abnormal variation in the level of serum. ALP and AST decreased significantly whereas ALT remained undisturbed via BPA exposure¹¹. The preliminary study of Elswefy (2016) on BPA's toxic effect on the histology of the liver clearly shows symptoms of necrosis. This likelihood of BPA includes chronic damage and fibrosis creation within the liver. The indication of conclusive necrosis correlated with this study outputs¹². There are dysfunctions and necrosis in the physiological and structural properties of the liver respectively. BPA disjunctions the regular gap between the liver cells radiating from the central vein of blood vessels. It was revealed that the potency of dose determines the extent of the deformation in the internal anatomy of the liver¹³. The finding of Kamal *et al.*, (2018) supported the current outputs as similar consequences are also part of his studied results. Our results also suggest the noticeable physical change in normal liver architecture with dilated sinusoids in treated groups. BPA was definite, intense and concrete in dose-related dysfunctions and injurious to the liver. The hostile consequence of BPA was studied by Kamal *et al.*, (2018)¹⁴. The current results coincided with the findings of Wang *et al.*, (2019) which revealed a massive degree of apoptosis is the common mean of damage in the liver. They also found an association between hepatotoxicity and the destruction¹⁵. The output correlated with the reports of Faheem *et al.*, (2016) as they concluded that BPA has a toxic impact on the liver. Its prolonged exposure caused significant serious degenerative changes in the liver^{16, 17}. The researchers revealed that hepatocyte cells have a unique sensitivity. The BPA toxicity is not only destructive and eccentric in hepatocytes but prominent. Such type of damage to the liver and its parameters was observed frequently in both treated groups of this study. Manna *et al.*, (2018) classified BPA as an endocrine-disrupting chemical, after finding changes in hepatocytes of *Oreochromis mossambicus* in his study. They validated the results as they matched

concerning serum variations in mice¹⁸. Serum concentration transformations after oral disclosure can be directly endorsed to the fluctuations in hepatic and gastric glucuronidation rates among the correspondents. Significant changes were found in serum analysis in treated groups when compared with control groups. A sheer elevation of ALT and AST were noted. However, the total bilirubin and its various components were also elevated in the treated groups. It was also revealed that variation in serum analysis in the exposed organism was more likely to be affected by molecular disturbance, leads to metabolic sickness¹⁹. They also verified that BPA is well associated with developmental mechanisms, as in these research outputs. These results correlated with our studies. Tassinari *et al.*, (2020) ascertained via an investigation that the liver, besides the thyroid gland, is the most sensitive organ to BPA exposure during the early stage of rodents²⁰. BPA is liable for inducing the basic mechanism disorders significantly involved in lipid metabolism creating irregularity in it; they also found that this abnormality is associated with the intensity of disorder in metabolism²¹. Elwakeel *et al.*, (2018) revealed and proved BPA a toxicant for the liver. He observed histopathological alternation in mice liver exposed to BPA²². BPA-induced genes that played a contributory role in liver steatosis. Results are supported by the work of Lin *et al.*, (2017)²³. Histological studies revealed BPAs' negative impacts include hepatocellular degeneration, edema formation, and necrosis of liver cells. These were finding of Khan *et al.*, (2016) which matched with the present study results. They also proposed during their study that with BPA hepatotoxicity, there was an elevation in the serum level of aminotransferase aspartate aminotransferase, alkaline phosphate, and dehydrogenase, which are the symptoms of BPA deleterious²⁴. Serum analysis found and proved BPA responsible for necrosis via his studies. The present results also have similar findings²⁵. Mahmoudi *et al.*, (2018) demonstrated that daily oral administration of BPA caused a significant abnormal increase of lipids, which lead to its dysfunctions²⁶. Eid *et al.*, (2015) and Faheem *et al.*, (2019) worked separately and noted significant degeneration in the liver due to BPA exposure^{27, 28}.

CONCLUSION

The findings of this study confirm that ingesting BPA has major deadly consequences, including serious malfunction as well as degenerations in the liver's normal architecture (hepatic toxicity). It also harms the liver cells' compatible activities. The BPA is dangerous to use, especially for the liver and it was discovered that it was linked to blood congestion. Severe haemorrhages and congestion in BPA-affected rats result in hormonal changes.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Ethical Board of the University of Punjab, Lahore, Pakistan.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ALP	Alkaline phosphatase
AST	Aspartate Aminotransferase
ALT	Alanine Transaminase
BPA	Bisphenol A
H & E	Hematoxylin and Eosin
LD50	Lethal Dose 50
SPSS	Statistical Package for the Social Sciences

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