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HISTOLOGICAL COMPARISON OF SPLENIC HEMOSIDEROSIS TOXIC EFFECT OF BISPHENOL A (BPA) AND BISPHENOL S (BPS) IN MUS MUSCULUS

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ABSTRACT

Bisphenol A (BPA) and Bisphenol S (BPS) are phenolic organic compounds. These compounds are commonly used in the manufacturing of plastic containers, epoxy resins, food and drink cans, water pipes, electronic equipment, thermal paper, kitchen utensils, toys, and dental sealants. The general population exposed to bisphenol S, both directly (through oral and/or topical routes) and indirectly (through environmental pollution and/or food chain).? The main objective of the study was to evaluate the effect of Bipshenol A and Bisphenol S on the spleen of mice. Mice were equally divided into three groups, i.e., Control group (given water only), and dose group II (orally administrated 40mg/kg body weight, Bisphenol A for the period of 21 days) and dose group III (orally administrated 40mg/kg body weight, Bipshenol S for the period of 21 days) after dissection the spleen of the all mice were extracted and further processed for morphological, morphometric and histological analysis. The Bisphenol A treated group of mice shown that an evident ($p<0.001$) increased in the body weight and the spleen weight of the Bipshenol A treated dose II treated group as compared to the control group, where significant ($p<0.001$) decreased in the body and spleen weight were observed in the dose group III bisphenol S in comparison of with the mice of dose group II Bisphenol A. Histological analysis of dose group II Bisphenol A showed some abnormalities in the Spleen of mice like parenchymatic cells with an increased number of megakaryocytes, vacuolated cytoplasm, and irregular nuclei, thickening in the capsule, destruction of marginal zone red pulp and destruction in the white pulp. It concluded that the excessive and long-term use of Bisphenol A and Bisphenol S leads to impaired vital organs of albino mice, causing the impairment in blood parameters and splenic toxicity. This plastic product must be used with care, particularly for products underuse by children.

1. INTRODUCTION

According to estimates, toxicant exposures account for about 24% of human anomalies and disorders, with the possibility that these conditions could be passed on to subsequent generations even in the absence of direct exposure (Hou et al., 2012).

The mature phenotype and susceptibility to abnormalities/diseases in later life significantly influenced by environmental toxicant exposure during intrauterine life, postnatal life, early life, and/or germ cell, according to previous studies (Skinner et al., 2013). Analyzing the chemical toxicity of compounds like bisphenol A (BPA), cadmium, mercury, dioxin, and tri chloro ethane reveals that some of these toxins have an immediate impact on the ecosystem.

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Hemosiderosis is a general term used to describe the overload of iron in tissues and other organs. Increase in iron concentration results the epidermis tissue that turn bronze colored skin. Hemosiderin is a protein that store iron in the tissue (Chin et al., 2019). Iron excess occurs when iron intake increases indefinitely due to regular transfusions of whole blood and red blood cells or increased iron absorption through the gastrointestinal tract.

Bisphenol A also called endocrine disrupting agent because their ability to disrupt endocrine organs. Bisphenol A is component epoxy resins, which is use worldwide to produce plastic products such as drinking pipeline lining, paints, dental sealants, in paper industry production of paper, adhesives, food containers (Qiu et al., 2015). Bisphenol A was quickly removed from consumer products due to their harmful effects on environment and human health. Chemicals that are used as alternatives to bispheno. A have been reported toxic and with parallel physiological effects in living organisms (Rochesteri & Bolden, 2015).

Bisphenol S is used as an industrial product for production of thermal paper in which it is used as a cash receipt and also in cleaning agents (Siracusa et al., 2018). Bisphenol S used in chemical as well as food industry for different kind consumer product production its used as in a variety of application such as dental sealants, dental composite filling materials, varnishes, paints, coating flooring, pipe and tank lining (Cabaton et al., 2009). Exposure of Biphenol S through different route like dermal, inhalation, oral causes damaging effects on human health and cause environmental pollution (Yang, Guan, Yin, Shao & Li., 2014).

2. MATERIALS AND METHODS

Animal model Development

This research is based on the qualitative research method. The workplace was Animal House, School of Zoology, Minhaj University Lahore. Thirty mice aged 5-6 weeks with a body weight of 28-30 grams purchased from the University of Veterinary and Animal Sciences.

These mice were placed under well managed and controlled conditions, such as a 12-hour day/night cycle, temperatures of 27 ± 2°C, and humidity of 40-50. These were housed in iron cages 24 inches long and 12 inches wide. The animals received commercially prepared grain pills. (Chick Feed No.14 National Feeds, Lahore). The mice were fed a feed that was high in minerals, proteins, and multivitamins.

Experimental design

Theexperimentation was performed to contemplate the harmful impact of Bisphenol S and Bisphenol A on spleen of mice. Thirty mice aged 5-6 weeks with a body weight of 28-30 grams used to this experiment. Mice were be divided into three groups 3 groups; one was control group and other two presented as experimental group. Control group was labeled as (C), other experimental group 1 marks as BPS 40/mg/kg body weight +diet and group 2 mark as BPA 40/mg/kg body weight +diet. Dose of Bisphenol S was prepared on 40mg/kg b.w. Bisphenol.S was dissolved in 9ml distilled water in such way that each 0.1 ml contained the desired dose of Bisphenol S (40mg/kg) concentration of required dose. Dose of Bisphenol A was prepared on 40mg/kg b.w. 1ml of Bisphenol A was dissolved in 9ml distilled water in such way that each 0.1 ml contained the desired dose of Bisphenol.A (40mg/kg) concentration of required dose.

Dose Organization

The animal kept under control condition then animal was marked as experimental and control group. The experimental group further divided into two group 1 and group 2. The control group received clean water and first treated group received Bisphenol S o.1ml of 40mg/kg b.w. the second treated group receive Bisphenol A 0.1ml of 40mg/kg b.w. Dose were given orally for the period of 21 days with the help insulin.

Tissue processing and sampling

To evaluate the damage caused by Bisphenol A and S the spleen of mice and sample of spleen taken. After taking of spleen sample, they were put into sterile petri dish and wash it with normal saline solution for few minutes. (Hussein, A. J., 2007) protocol was follow for histopathological study of sample of spleen taken.

Morphological investigation

Sample of spleen taken from treated mice ready for morphological examination. Vernier caliper was used to determine the length and width of spleen of treated group of mice and control group of mice. A computerized weight measuring machine was used to determine the weight difference between the spleen of the treated group and control group. The difference between spleen weights were noted and found statistically significant difference (Morphometric anomalies were seen among control and experimental groups).

Histopathological Examination

For histopathology of mice spleen sample fixed. The spleen flap removed and washed with normal saline solution 0.75% NaCl. Then the flap of spleen tissue sample fixed in 10% formalin solution for the period of 24 hours. Then samples of spleen tissue were dehydrated by passing through different concentrations of ethanol and then cleared with liquid xylene. After dehydration spleen tissue was impregnated with the liquid paraffin then allowed tissue to solidify. After paraffin impregnation the wax block containing spleen tissue was trimmed and the section of spleen was made with the help of microtome. Then the temperature of the water bath maintained to 55 to 56% for stretching of ribbons. These section ribbons were placed on sterile glass slide with using of albumin 2 to 3 drops on slide. The slides firstly air dried then placed in incubator for 15 minutes at 50-55 ^{O}C. After removal of paraffin wax, slides immersed with the help of xylene 1 and 2 for the period of 10 minutes. Slides that contain spleen tissue were transferred into descending alcohol having concentration 100%, 90%, 70%, 50% for the period of 10 minutes and then with 5 minutes respectively. Slides immersed in hematoxylin for approximately for the period of 5 minutes. Slides washed with running tap water. Slide dipped into alcoholic solution (5%) for the period of 30 seconds. Slides were stained with eosin for one minute. Slides again washed with running tap water. After staining slides was again dehydrated**.** These prepared slides were observed under light microscope with different magnification 10x,40x,100x.

3. RESULTS AND DISCUSSION

Morphological analysis

The control group of mice showed that there was a regular appearance of spleen. The spleen and other organs look smooth and shiny. The red pulp of spleen and the White pulp of spleen standard no kind irregularities found spleen of control group of mice average in size and shape. Several kinds of abnormalities or deformities were found. e.g the spleen looks dull in color and also its shine of treated with Bisphenol A (BPA 40mg/kg). The spleen of treated with Bisphenol A (BPA 40mg/kg) shown malformation like colors and wrinkles on its surface. The spleen also showed that much dark stain on its surface and also dark stain representing the degradation of marginal zones that present between the white pulp and red pulp of spleen of treated group of mice with Bisphenol A (BPA 40mg/kg). On the contrary the spleen of the Dose group III Bisphenol S (BPS 40mg/kg**)** shown that was comparatively less damage than the spleen of Dose group II Bisphenol A (BPA 40mg/kg) and more damages than the spleen of control group

Morphometric analysis

The initial body weight of control group was noted that was 16.1 ± 4.6g, the final weight of the control group was noted after the administration of oral dose. It's found that the average body weight of mice found that 17.4 ± 4.8g. The calculated increase in the percentage of body weight was 51.5. The initial body weight of mice is noted to determine the percentage body weight gain mice after treatment of Bisphenol A (BPA 40mg/kg). The initial body weight of Bisphenol A (BPA 40mg/kg) treated group of mice was 16.1 ± 4.6g, the average final weight of mice noted that was 17.4 ± 4.8g. After calculating the percentage of body weight increased was 28.73g. The percentage of values shown that a significant loss in the percentage of weight increase as compared to control group. The initial body weight of Bisphenol S (BPS 40mg/kg) dose III treated group of mice was 18.1 ± 5.3g, the average final weight of treated mice group was 26.4 ± 4.2g, its shown that after calculation the percentage of body weight the gain in the body weight was 45.8 its determine that a significant higher than the percentage body weight of mice of Bisphenol A (BPA 40mg/kg) 28.73g.

Histological Analysis of spleen

Histological investigation shows that there was clear alteration of splenic tissue of treated groups of mice as compared to control group. splenic tissue of BPA and BPS treated group of mice showed that highly activated germinal centers of spleen white pulp with minimal apoptotic feature and prominent megakaryocytosis and lymphohistiocytic infiltrate of the red pulp, and comparatively increased the count of eosinophils and mature lymphocytes were noted detected. On other hand there was abnormalities also occurred in spleen due to occurrence and accumulation of iron in the spleen that increase in red pulp and microphage count increase in white pulp. There were apoptotic changes that occurred in the germinal center of white pulp of the spleen both BPA and BPS treated group of mice. Histological study also shown that the wall of central arterioles was ruptured and shown that increasingly narrow lumens.

4. CONCLUSION

Bisphenol A and S is used in many edibl plastic products that used daily in life like baby bottles, baby feeders, plastic bottles, hard and soft plastic, water bottles, storage containers, food containers and paper industry, protective coating for pipeline. Overuse of these products such as bisphenol A and S has many healthcare issues to humans as well as affecting children's health. In the present study we try to find toxicological evaluation the harmful effects of bisphenol A and S spleen of the mice.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. A Comparison of average weight of mice of dose II groups Bisphenol A (BPA 40mg/kg) and control group of mice

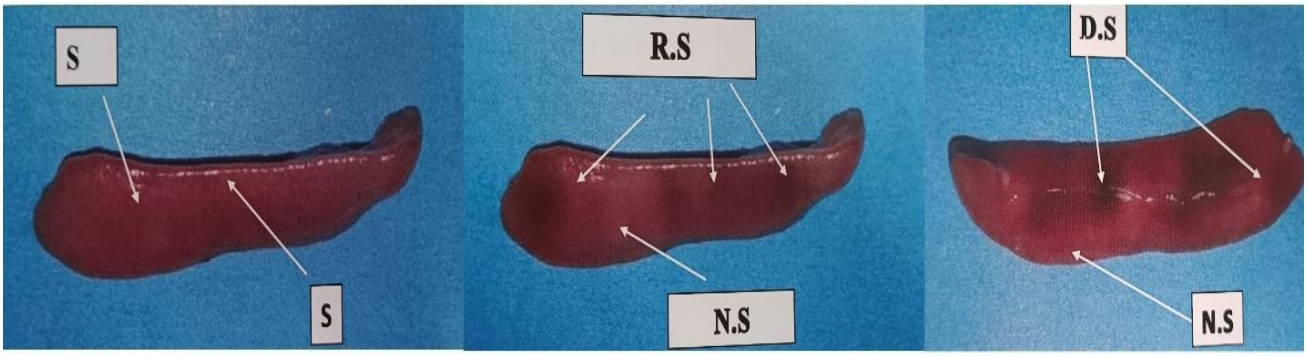
Parameter	Control group (mean ± SEM)	BPA II (BPA 40mg/kg) (mean ± SEM)
Initial Body weight (g)	16.1 ± 4.6	17.4 ± 4.8
Final body weight (g)	24.4 ± 4.8	22.4 ± 6.2***
Spleen weight	0.37 ± 0.43	0.13 ± 0.43***

Note: The results of table shown as ***= p <0.001

Table 2. A Comparison of average weight of mice of dose groups Bisphenol A (BPA 40mg/kg) and Bisphenol S (BPS 40mg/kg).

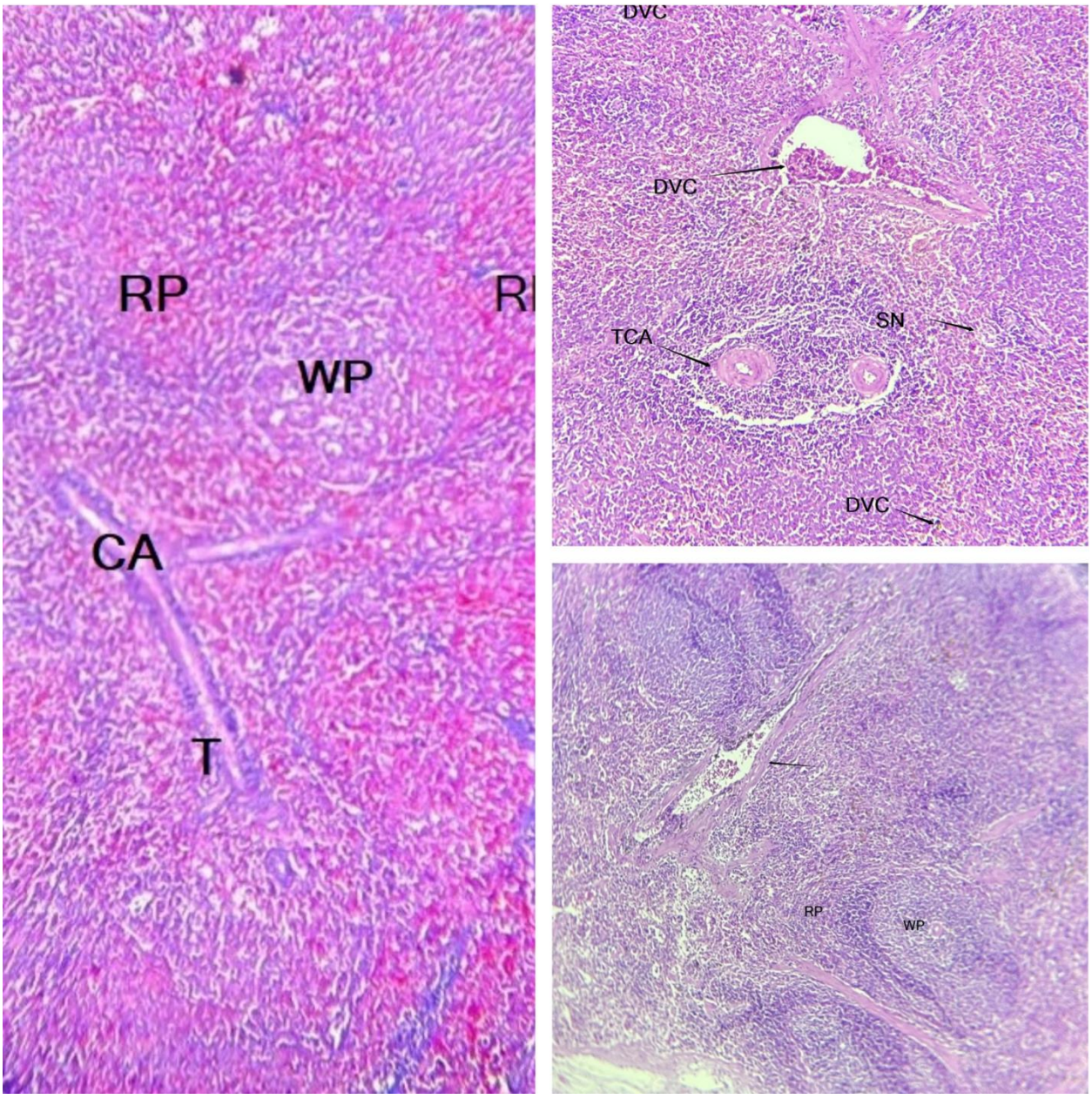
Parameter	BPA II (BPA 40mg/kg) (mean ± SEM)	BPA III (BPA 40mg/kg) (mean ± SEM)
Initial Body weight (g)	17.4 ± 4.8	18.1± 5.3
Final body weight (g)	22.4 ± 6.2***	26.4 ± 4.2***
Spleen weight (g)	0.13 ± 0.43	0.31 ± 0.43***

Note: Results were represented as ***p<0.001.



(A) (B) (C)

Figure 1. Spleen of control group shown (A) Bisphenol A treated group mice spleen (C) Bisphenol S treated group mice spleen



(A) (B & C)

Figure 2. H & E-Stained spleen section of control, Bisphenol A and Bisphenol S treated group for period of 21 days. Photomicrographs were taken at 10x,40x 100x using *optika- B-350 microscope.*