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A comparative study on the toxicity of Bisphenol A (BPA) and Bisphenol S (BPS) on heart ventricular muscle

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Abstract

Bisphenol S (BPS) is a synthetic chemical that is used as a substitute for toxic Bisphenol A (BPA) for the production of polycarbonate plastics, epoxy resins, and paper products. This study has been undertaken to perform a comparative analysis of the toxicity of BPS and BPA on heart ventricular musculature. Adult male albino rats were given BPS, BPA, and a combination of both BPS and BPA for 30 consecutive days via oral gavage. From the results, it can be observed that both BPS and BPA significantly decreased the activities of different antioxidant enzymes (SOD, CAT, GPx, GR, GST), whereas malondialdehyde level has been significantly increased compared to the control. The inhibition of the activities of antioxidant enzymes is more pronounced in BPA than BPS exposed groups. Moreover, BPA leads to a greater degree of degeneration of heart ventricular musculature than BPS. In conclusion, though BPS induces oxidative stress-mediated damages of heart ventricular musculature, the potency of BPS-induced toxicity is comparatively lower than BPA.

Keywords BPA, BPS, Heart, Oxidative stress.

Introduction

Plastic is one of the major inventions of modern science that has been used extensively to manufacture innumerable common consumer goods. Bisphenol A (BPA) is a synthetic industrial chemical that is extensively used in the industries for making polycarbonate plastics and epoxy resins.

Several toxicological studies have been performed over the last decade to examine the possible risk factors of using BPA. BPA is a potent endocrine disruptor and has adverse effects on different physiological systems. Due to estrogenic in nature, BPA disrupts the reproductive system functions to a great extent. It has been reported that BPA exposure interferes with ovarian follicular development and its relevant gene expression (Li et al., 2013). Neonatal exposure to BPA has been reported to be associated with the development of polycystic ovarian syndrome in a rat model (Fernández et al., 2010). It has also been observed that BPA decreases the semen quality and sperm count in rats (Jin et al., 2013).

BPA also exerts adverse effects on the normal functioning of the intestine. The inhibitory role of BPA on the duodenal movement has also been reported (Sarkar et al., 2015). Not only that, BPA has been reported to be an antagonist of T_3 , inhibiting the binding of T_3 to $TR\alpha$ and $TR\beta$, resulting in suppression of gene expression in *X. Laevis* tail tissue (Kashiwagi et al., 2009). Studies indicate that BPA is associated with the development of mammary glands, prostate, and neuroblastoma cancer (Acevedo et al., 2013; Ho et al., 2006; Zhu et al., 2009). Prenatal and early childhood exposure to BPA can lead to behavioral changes, neurodevelopmental disorders, immune disorders, and defects in secondary sexual development in children (Kumamoto & Oshio, 2013; Yoshino et al., 2004; Braun et al., 2011).

Considering the toxicity and adverse effects of BPA, several analogues chemicals have been synthesized as an alternative to BPA. One such chemical is 4, 4'- dihydroxyphenyl sulfone (BPS).

It has increased stability against high temperature and increased resistance to sunlight, hence less leachable compared to BPA. Plastic products labeled as “BPA free” are often made up of BPS (Grignard et al., 2012). BPS is commonly used in the making of polycarbonate plastics, polyethylene terephthalate plastics, epoxy resins, and thermal receipt papers. The utilization of BPS has been increased drastically in recent years.

Different monitoring studies have established that BPS shows the ubiquitous distribution in the environment and can be detected in river water, sediment, sewage, and even in indoor dust samples (Yang et al., 2014; Yamazaki et al., 2015; Ćesen et al., 2018; Liao et al., 2012). Moreover, BPS has also been detected in human blood and urine samples (Lehmle et al., 2018).

BPS shows estrogen-mimicking activity and acts as a thyroid hormone disruptor (Kang et al., 2014; Naderi et al., 2014). BPS exerts toxic effects on the nervous system, reproductive system, cardiovascular system, renal and hepatic system (Kinch et al., 2015; Nourian et al., 2017; Ullah et al., 2016; Gao et al., 2015; Zhang et al., 2016; Zhang et al., 2018). Besides, BPS can bind to cellular macromolecules like DNA, protein and alters their structure (Mokra et al., 2017; Schöpel et al., 2016).

Despite having several studies on BPS-induced toxicity, the comparative analysis of BPS and BPA-induced toxicity on heart ventricular musculature has not been conducted to date. Hence, this study has been designed to observe the comparative toxicological potency of BPS and BPA on the heart ventricle.

Materials and Methods

Chemicals and reagents

Analytical grades of chemicals have been considered to be used for this study. Bisphenol S (BPS) has been purchased from Sigma Aldrich, USA (CAS No. 80-09-1, purity 98%). Dimethyl sulfoxide (DMSO) and Bisphenol A (CAS No. 80-05-7) were purchased from Merck and Sigma Aldrich, USA respectively.

Animal model for the study

Adult male albino rats of the Sprague-Dawley strain were chosen for this study. Healthy male rats weighing about 110-140 gms were purchased from an authorized breeder and kept in the animal house of Molecular Neurotoxicology Laboratory, Department of Physiology, University of Kalyani. After the end of the acclimatization period of one week in the new environment, the rats were divided into 4 groups containing 8 rats each. The experimental animals were maintained at a standard ambient temperature of 21-25°C and 12h light-dark cycle. The animals were provided with an abundant supply of standard rodent food and water. Animal maintenance and study were conducted very carefully as per the guidelines of the Kalyani University Animal Ethics Committee.

Animal grouping and dose selection

The rats were given test elements by the way of oral gavage for 30 consecutive days. The first group received 0.5 ml of 20% DMSO and subsequently designated as a vehicle control group. The remaining groups received BPS, BPA, and a combination of BPS and BPA respectively.

	Animal grouping
Control	Received 20% DMSO (Vehicle for BPS and BPA, 0.5 ml) for 30 consecutive days
BPS	Received BPS (120 mg/kg body weight/day, 0.5 ml) for 30 consecutive days
BPA	Received BPA (120 mg/kg body weight/day, 0.5 ml) for 30 consecutive days
BPS+BPA	Received BPS (120 mg/kg body weight/day, 0.5 ml) and BPA (120 mg/kg body weight/day, 0.5 ml) for 30 consecutive days. BPS was given for 15 days and BPA was given for other 15 days. Both the drugs were administered on alternative days

Sample collection and processing

After the end of the test duration, the control and exposed rats were sacrificed by cervical dislocation preferably 24 hours after the last applied dose. A part of the heart ventricular tissue was washed in ice-cold phosphate-buffered saline and kept at -20°C for further biochemical assay. Another part of the heart ventricular tissue was fixed in neutral buffered formalin (NBF) and paraffin-embedded for histological observation.

Tissue homogenization

Heart ventricular sections of all the groups of rats were minced in ice-cold phosphate buffer. A known weight of tissue was homogenized in phosphate buffer (0.1 M, pH 8.0) with 2mM EDTA and 0.5% Triton-X-100 with the help of a tissue homogenizer (Tissue Homogenizer, RQ-127A, REMI, India) on ice. After that, the homogenate was centrifuged and the supernatant was collected. Then it was preserved at -20°C for further biochemical analysis.

Assay for oxidative stress-related variables

The activities of the antioxidant enzymes were examined following standard protocols using a spectrophotometer. Superoxide dismutase (SOD) activity was assayed following the method Marklund & Marklund, 1974. Catalase (CAT) activity was examined as per the method of Sinha, 1972, whereas glutathione peroxidase (GP_X) activity was assayed following the method of Rotruck et al., 1973 with a little

modification. The activities of Glutathione reductase (GR) and glutathione-s-transferase (GST) were estimated as per the protocol of Staal et al., 1969 and Habig et al., 1974 respectively. The level of malondialdehyde (MDA) was measured following the method of Devasagayam & Tarachand, 1987.

Hematoxylin and Eosin staining

Paraffin-embedded heart ventricular tissue sections were stained with hematoxylin-eosin stain following the procedure of Bancroft & Gamble, 2008 with a little modification. The sections were observed under a light microscope (400 X magnification). Images were captured by a digital SLR Olympus camera (E-620) coupled with Olympus light microscope (CH20i).

Statistical analysis

Data obtained from control, BPS, BPA, and a combination of BPS and BPA exposed groups of rats were presented as Mean±SEM. Statistical analysis of all the data was carried out by Student's t-test followed by one-way analysis of variance (ANOVA). Data were analyzed by GraphPad Prism software version 5.03 for Windows (GraphPad Software Inc.). p<0.05 was considered as the level of significance.

Results and Discussion

Effects of BPS, BPA separately and BPS and BPA in combination on oxidative stress-related variables of heart ventricular muscle

The data obtained from the study on oxidative stress variables in control, BPS, BPA, a combination of BPS and BPA exposed groups are presented in Fig.1

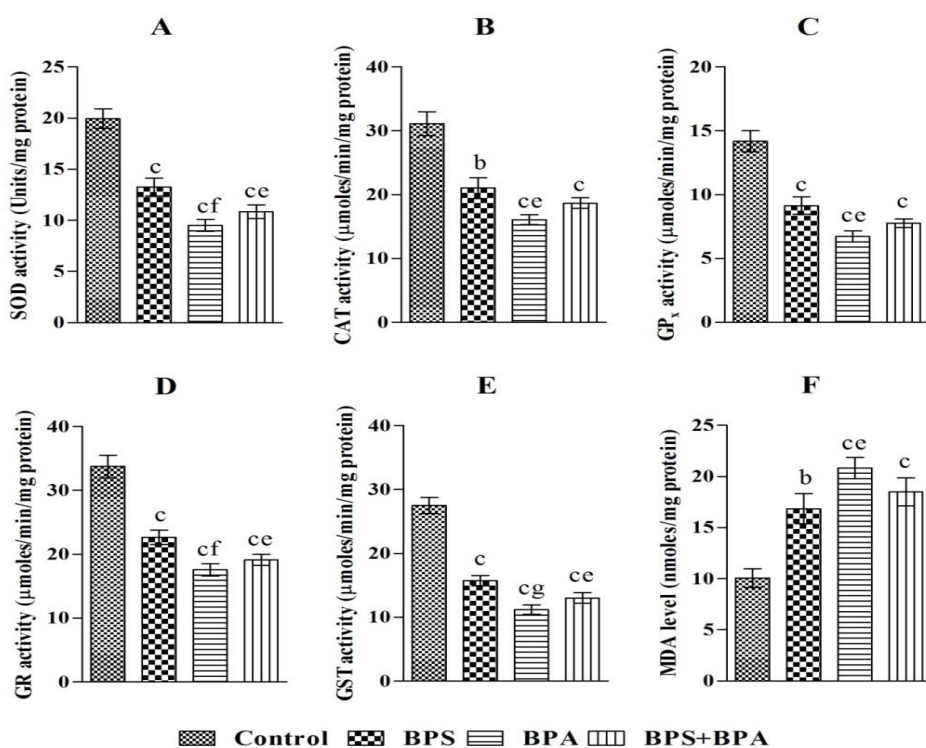


Fig. 1 Graphical representation showing the activities of (A) superoxide dismutase (SOD), (B) catalase (CAT), (C) glutathione peroxidase (GP_x), (D) glutathione reductase (GR), (E) glutathione-S-transferase (GST), and the level of (F) malondialdehyde (MDA) in control, BPS, BPA and combination of BPS and BPA exposed groups of rats after 30-day exposure duration. Values are presented as Mean±SEM (n=8). ^{b,c}p<0.01, 0.001 vs. Control, ^{e,f,g}p<0.05, 0.01, 0.001 vs. BPS

Both BPS and BPA significantly decrease the activity of superoxide dismutase (SOD) (p<0.001) in comparison to control (Fig.1A). Though SOD activity in BPA exposed group is lower than BPS exposed group (p<0.01). Catalase (CAT) activity showed a marked reduction in BPS and BPA group (BPS: p<0.01; BPA: p<0.001) than the Control group (Fig. 1B). However, CAT activity is greater in the BPS group than

in the rats exposed only to BPA (p<0.05). As demonstrated in Fig.1 glutathione peroxidase (GP_x) activity is markedly reduced in BPS and BPA exposed groups (p<0.001) with BPA showing more depression in GP_x activity than BPS (p<0.05) (Fig.1C). Similarly, BPS and BPA depress the activity of glutathione reductase (GR) (p<0.001) (Fig.1D). Again BPA revealed higher potency than BPS (p<0.01). Glutathione-s-

transferase (GST) activity was also significantly reduced after BPS and BPA administration compared to the control group ($p < 0.001$). But GST activity was greater in BPS exposed group than the group exposed only to BPA ($p < 0.001$). Combined exposure of BPS and BPA produced a significant decrease in the enzymatic activities and the enzymatic activities were lower than the enzymatic activities of BPS exposed rats but greater than enzymatic activities of the rats exposed only to BPA. On the other hand, the level of malondialdehyde (MDA), a lipid peroxidation marker, is significantly increased in ventricular muscle tissue homogenate in both BPS and BPA exposed group of rat in comparison to control (BPS: $p < 0.01$; BPA: $p < 0.001$).

A decrease in antioxidant enzyme activity or increase in the production of reactive oxygen species (ROS) leads to oxidative stress. Oxidative stress is one of the major reasons for the degeneration of cellular components disrupting both their structure and normal functioning. To examine the comparative toxicity between BPS and BPA, the effects of BPS and BPA on antioxidant enzyme activities have been evaluated. Results indicate that both BPS and BPA decrease the antioxidant enzyme activities, however, the degree of inhibition of these enzymatic activities is greater in BPA exposed group of rats comparing to the group of rats exposed only to BPS. Besides, the group of rats exposed to both BPA and BPS shows a decrease in the activities of antioxidant enzymes, and the enzymatic activities are lower than the enzymatic activities of BPS exposed rats but greater than enzymatic activities of the rats exposed only to BPA (Fig.1) The differences in the effects of BPA and BPS might be due to their structural differences and differences in the mode of actions in inducing oxidative stress. Wang et al., 2021 reported that both BPS and BPA down-regulate the activity of SOD in human neuroblastoma cell lines, IMR-32 and SK-N-SH and BPA exerted more suppression in SOD activity. Our study outcome is also supported by Huang et al., 2020. They ascertained that SOD activity was markedly decreased by BPS and BPA exposure with a concomitant rise in MDA content in human granulosa KGN cells. BPA revealed more toxic potency than BPS.

Effects of BPS, BPA separately and BPS and BPA in combination on the histo-anatomical structure of heart ventricular tissue

Histologically fixed and stained tissue sections were viewed under a microscope and captured images are summarized in Fig.2. The Control group shows the normal structure of heart musculature with even distribution of muscle fiber (Fig.2A). Both BPS and BPA produced significant damage in the histological structure of heart ventricular muscle (Fig.2 B, C). The necrosis in the ventricular muscle tissue was evident in the respective images. The size of the nucleus in the muscle fiber was enlarged in BPS and BPA exposed heart ventricle compared to the control. The inter-fibrillar spaces were also enhanced in BPS and BPA exposed groups of rats. Although the damage caused by BPA was more severe than BPS exposure. In the group exposed to both BPS and BPA, the degree of degeneration of ventricular musculature was higher

than BPS exposed rats but lower than BPA exposed rats (Fig.2D).

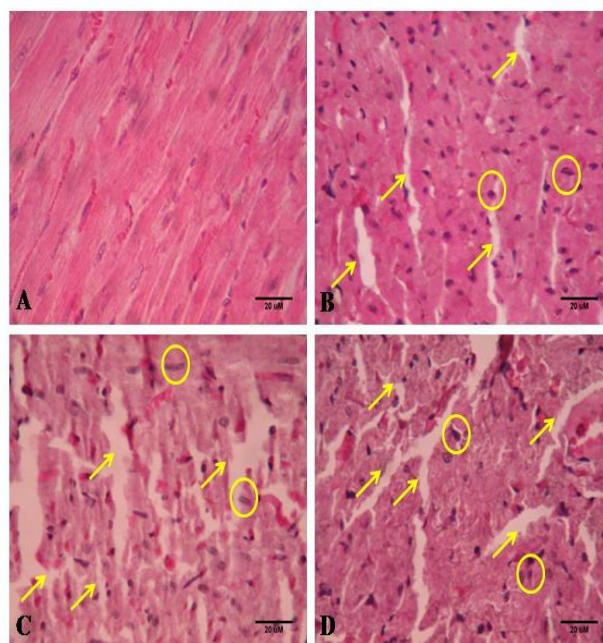


Fig. 2 Photomicrographs of hematoxylin and eosin-stained longitudinal sections of heart ventricular muscle of control, BPS, BPA, and combination of BPS and BPA exposed groups of rats after 30-day exposure duration (400X magnification). A. Heart ventricular wall section of control rats, B. Heart ventricular wall section of BPS exposed rats, C. Heart ventricular wall section of BPA exposed rats, D. Heart ventricular wall section of the combination of BPS and BPA exposed rats. Arrow heads indicate the occurrence of lesions in the heart ventricular musculature. Circles indicate the enlarged nuclei. Images were captured by a digital SLR Olympus camera (E-620) coupled with Olympus light microscope (CH20i).

Oxidative stress can damage cellular macromolecular resulting in deformed morphological and structural patterns in biological organs. The suppressing action of BPS and BPA on antioxidant enzymes can be corroborated with histological findings in this section.

Conclusion

After considering all the results, it can be concluded that BPS and BPA are both toxic to the structure of the heart ventricular muscle. They produce oxidative stress-mediated structural damages to the heart ventricular muscle, but the degree of toxicity is less pronounced in BPS than BPA.

Conflict of Interest

We declare no conflict of interest.

Consent for Publication

All the authors have given their consent for publication.

Ethical Considerations

The study was approved by the ethical committee.

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