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INHIBITION OF HEART VENTRICULAR FUNCTION OF RAT BY BISPHENOL A THROUGH OXIDATIVE STRESS INDUCED INJURY OF VENTRICULAR TISSUE.

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ABSTRACT

The toxic effect of Bisphenol A (BPA) on heart ventricular function has not been reported till date. So the present study was undertaken to examine the effect of BPA on ventricular function in rat models. BPA (50mg/kgBW/day) was administered for 20 and 30 days by oral-gavage. The rats were sacrificed by cervical dislocation on 24th hour after last treatment. Paraffin embedded ventricular tissue sections were stained with hematoxylin-eosin. Significant degenerative changes were observed in hematoxylin-eosin stained ventricular tissue sections of test rats. The activities of antioxidant enzymes like- superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase were seen to be decreased significantly in both durations; while, the level of malondialdehyde, a biomarker of lipid peroxidation, was increased than dimethyl sulfoxide (DMSO, vehicle of BPA) treated rats. In conclusion, BPA inhibits ventricular function in rat presumably by producing oxidative stress induced injury of ventricular tissue.

KEY WORDS: **Bisphenol A, Ventricular myocytes, Antioxidant enzymes, Lipid peroxidation.**

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INTRODUCTION

Bisphenol A (BPA) is a man made compound which is considered as a potent environmental contaminant. It is a monomer of epoxy resins and used extensively to manufacture polycarbonate plastic products. It is hugely used to make a broad range of products including baby bottles, drinking glasses, food storage containers, microwave containers, water storage tanks and supply pipes, toys, sunglasses, lenses, the lining of food beverage containers, medical equipments, tubing, consumer electronics and dental sealants[1]. Widespread and continuous human exposure to BPA is believed to be mainly through dietary intake[2], with additional exposure through dental sealant, dermal exposure and inhalation of household dusts [3,4,5]. Polymers made from BPA can be hydrolyzed in high temperature, acidic and basic conditions, leading to leaching and migration of BPA into the internal food and beverage contents [6,7,8,9,10,11]. The widely used chemical has various effects on human physiological systems. It has been reported that BPA alters the function of coronary smooth muscle by activating Maxi-K (KCa.1.1) channels[12]. Recently a report has been published about the relationship between high urinary concentration of BPA and coronary artery disease [13,14,15]. The role of BPA as an oxidative stressor in liver, kidney, brain and other tissues has been reported in rat and mice models [16,17]. They suggested that BPA induces the formation of reactive oxygen species (ROS) in the cells[18,19]. Moreover, the study of Bindhumol et al.[18] revealed that low doses of BPA generate ROS by decreasing the activities of antioxidant enzymes and increasing lipid peroxidation thereby causing oxidative stress in liver of rats. An increase in oxidative stress is considered an important pathogenic mechanism in the development of various complications like cardio vascular, cerebro- vascular and peripheral vascular diseases [20, 21]. But there is no report regarding the effect of BPA on oxidative stress in cardiac tissues. So, in this study an attempt was taken to elucidate the possible effect of BPA on the ventricular function of heart of rat at the molecular physiological level.

MATERIALS AND METHODS

Animals

The study was conducted at the Environmental Physiology Laboratory in the Department of Physiology, following animal use protocols approved by the University of Kalyani Animal care committee in accordance with national guidelines. Studies were performed on 14-16 weeks old male white albino rats of Sprague-Dawley strain weighing about 150-200 gm. The animals were kept in a temperature, humidity controlled room with equal light-dark cycle (12 h light and 12 h dark) and fed standard laboratory chow and water *ad libitum.*

Reagents and Chemicals

All the reagents used were of analytical grade. Bisphenol A (BPA) was purchased from Sigma-Aldrich, USA and Dimethyl sulfoxide (DMSO), 5,5'-dithiobis-2-nitrobenzene (DTNB), oxidized and reduced glutathione, NADPH.Na_4 were procured from SRL, India. Eosin and Hematoxylin were procured from Merck, India.

Animal exposure and grouping

After one week of acclimatization to the environment the rats were randomly distributed to four groups of seven animals each. The rats of the first group were received 0.5 ml of 20% DMSO for 20 days and were designated as vehicle control group. The rats of the second group were received BPA at a dose of 50mg/kg body weight/day for 20 days and were marked as the treated groups. The rats of the third group were received same dose of DMSO for 30 days (i.e. vehicle control group) and the rats of fourth group were

received same dose of BPA for 30 days (i.e. treated group) by oral gavage.

Sample collection

After the completion of the treatment duration of 20 days and 30 days, the animals were sacrificed by cervical dislocation on the 24th hour after completion of the last dose. The heart was removed and a segment of the ventricle was washed in ice cold phosphate buffer solution, dried and stored in -20°C for further biochemical studies. The rest of the ventricle was washed in buffer and kept in Neutral buffer formalin for further histological study.

Homogenate preparation

Segments of heart from all the experimental groups were excised separately and minced in ice-cold saline. A known weight of tissue was homogenized in 10ml buffer (0.1M phosphate buffer, pH 8.0) with 2 mM EDTA and 0.5% Triton-X-100 by a tissue homogenizer on ice. The tissue homogenate was then centrifuged and the supernatant was collected and stored at -20°C for further study.

Biochemical assay

Superoxide dismutase (SOD) activity was measured as per the protocol of Marklund and Marklund, 1974[22]. 1 unit of SOD was defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50%. Catalase (CAT) activity was measured following the protocol of Sinha et al, 1972 [23] with slight modifications. Glutathione reductase (GR) activity was measured according to the method of Staal et al, 1969[24]. Glutathione peroxidase (GP_X) activity was estimated by the method of Rotruck et al, 1973[25]. The amount of Malondialdehyde (MDA) was estimated according to the protocol of Devasagayam and Tarachand, 1987[26]. Protein content of ventricular tissue of heart was measured by the method of Lowrey et al, 1951[27] with Bovine Serum Albumin as the standard.

Histological study

Histological technique for the morphological study of the ventricular tissue

NBF fixed and paraffin impregnated ventricular tissue sections were stained with normal hematoxylin-eosin stain according to the method of Bancroft et al, 2003[28] with slight modifications. Briefly, 5µm paraffin section was kept sequentially in xylene and graded ethanol and stained with hematoxylin for 2 minutes. After removing the excess colour the slide was counterstained with eosin and then the stained slides were dehydrated with graded ethanol, cleared with xylene and mounted with DPX and were observed under the microscope (400X magnification). Images were obtained by digital SLR Olympus Camera (E-620) fitted with Olympus light microscope (CH20i).

Statistical Analysis

All results were presented as mean ± SEM. Statistical comparisons between the values obtained in vehicle control and in treated rats were carried out by using a Student's t test for paired values. $P \leq 0.05$ was considered as significant (n=7).

RESULTS

Biochemical Study

The activities of antioxidant enzymes were seen to be decreased in a duration dependant manner in 20 days and 30 days exposure durations. The activity of SOD (Table 1, Figure 1), CAT (Table 1, Figure 2), GP_X (Table 1, Figure 3), GR (Table 1, Figure 4) were decreased significantly in both durations than the respective vehicle control group .The production of MDA, a biomarker of lipid peroxidation, was increased significantly in a duration dependant manner in 30 days exposure group of rats (Table 1, Figure 5).

Table 1

Showing activities of SOD, CAT, GPx, GR and amount of MDA of the microsomal fraction of the ventricular tissue of DMSO (vehicle) and BPA treated rats.

Biochemical variables	20days exposure		30 days exposure	
	Vehicle control	BPA treated	Vehicle control	BPA treated
SOD (U/mg protein)	7.08±0.354	6.10±0.225*	6.86±0.234	6.04±0.231*
CAT (U/mg protein)	27.06±0.512	25.18±0.453*	26.59±0.689	24.42±0.589*
GP_X (nmole GSH/mg protein)	4.78±0.355	3.61±0.385*	4.87±0.401	3.77±0.227*
GR (nmole/mg protein)	24.28±0.924	16.23±0.661***	23.94±0.827	11.48±0.56***
MDA (n mole MDA/mg protein)	14.96±0.577	16.66±0.548	13.98±0.703	19.74±0.204***

Values are represented as Mean±SEM (n=7), *p<0.05 vs. vehicle control, ***p<0.001 vs. vehicle control.

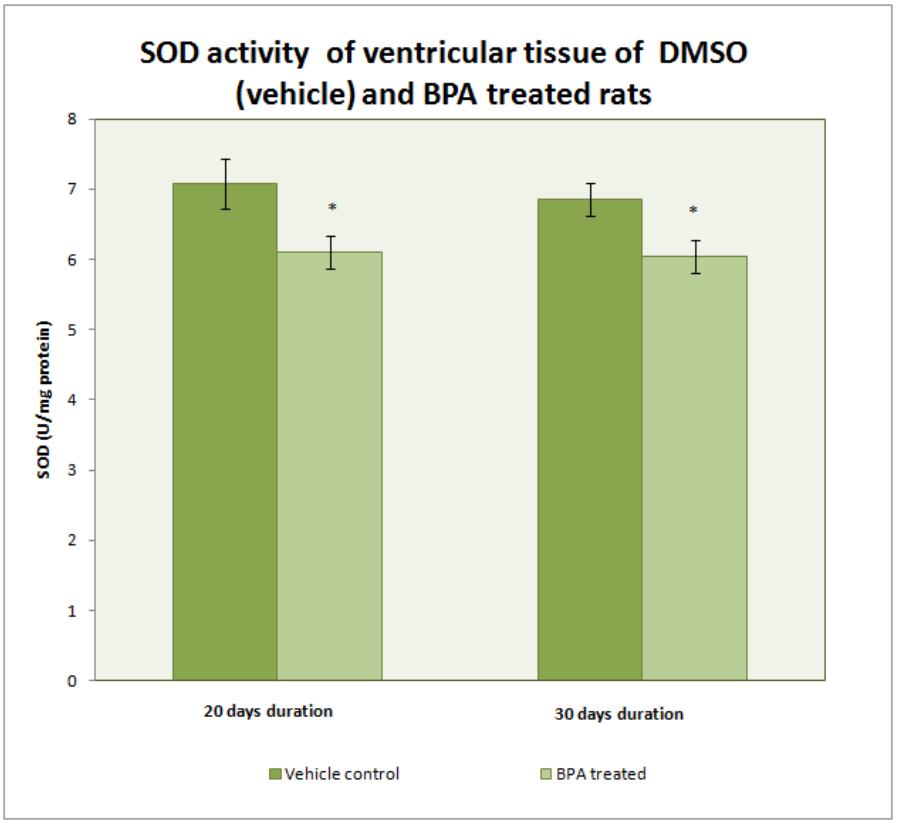

Figure 1

Showing SOD activity of ventricular homogenate of DMSO (vehicle) and BPA treated groups of rats of 20 and 30 days durations. Values are represented as Mean±SEM (n=7), *p<0.05 vs. vehicle control.

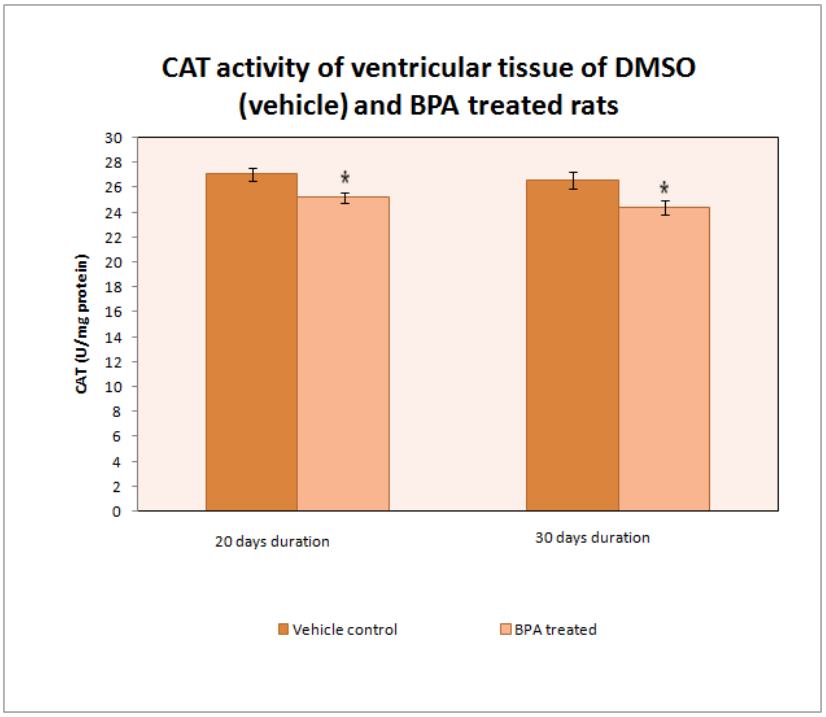

Figure 2

Showing CAT activity of ventricular homogenate of DMSO (Vehicle) and BPA treated groups of rats of 20 and 30 days durations. Values are represented as Mean±SEM (n=7), *p<0.05 vs. vehicle control.

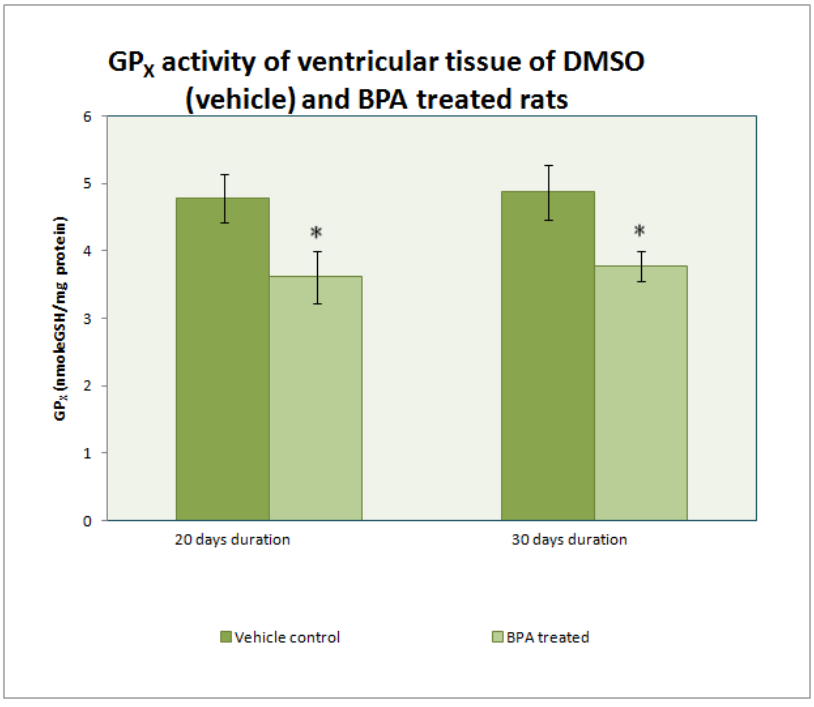

Figure 3

Showing GP_X activity of ventricular homogenate of DMSO (Vehicle) and BPA treated groups of rats of 20 and 30 days durations. Values are represented as Mean±SEM (n=7), *p<0.05 vs. vehicle control.

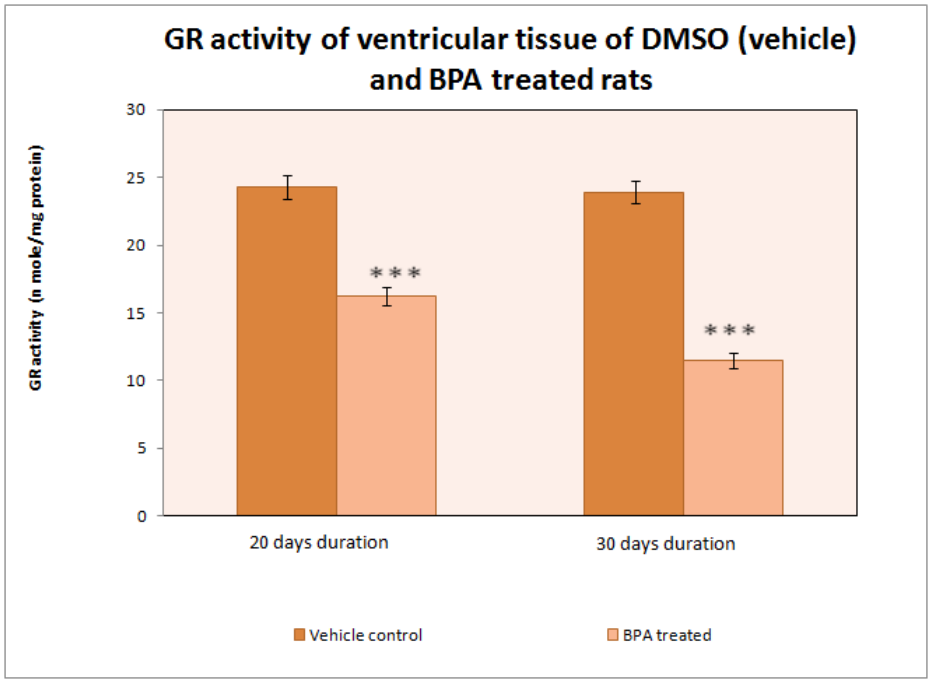

Figure 4

Showing GR activity of ventricular homogenate of DMSO (Vehicle) and BPA treated groups of rats of 20 and 30 days durations. Values are represented as Mean±SEM (n=7), ***p<0.001 vs. vehicle control.

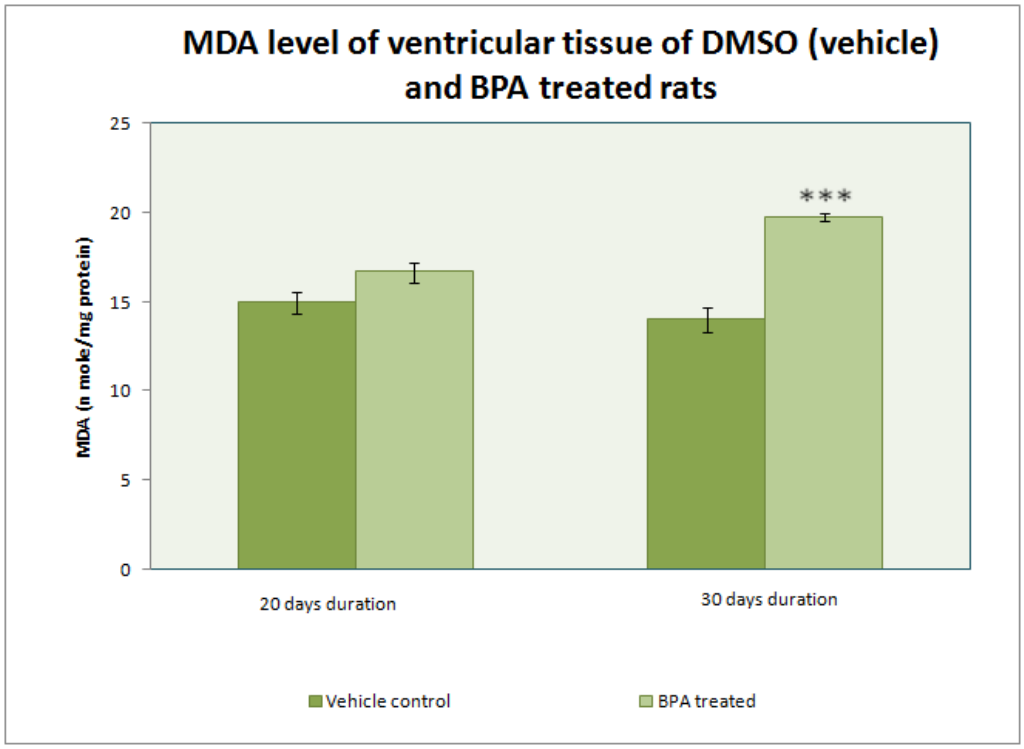

Figure 5

Showing MDA level of ventricular homogenate of DMSO (vehicle) and BPA treated groups of rats of 20 and 30 days durations. Values are represented as Mean±SEM (n=7), ***p<0.001 vs. vehicle control.

Histological study

Morphological study of ventricular tissue of DMSO and BPA treated rats

We found distinguishable degenerative changes in ventricular tissues of the hematoxylin-eosin stained ventricular tissue sections of treated rats (Figure: 6A-6D). Numerous lacunae were seen in the matrix of stained ventricular tissue section. Moreover, the size of the nucleus of the myocytes was enlarged and the shape became rounded in BPA treated group than the vehicle control group.

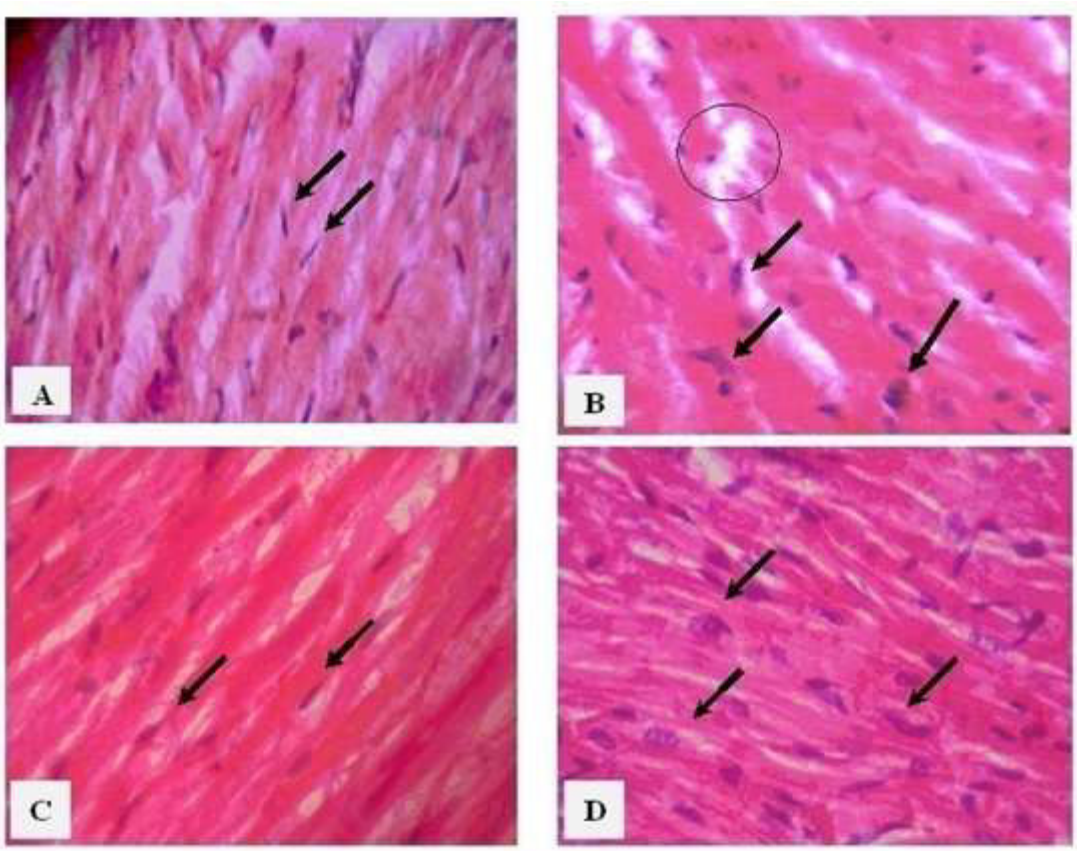

Figure 6

Hematoxylin and Eosin stained ventricular section of DMSO (Vehicle) and BPA treated rats (400X magnification). A) Histology of the ventricular tissue of the vehicle control group of rats of 20 days duration, B) Histology of the ventricular tissue of the BPA treated rats of 20 days duration, C) Histology of the ventricular tissue of the vehicle control group of rats of 30 days duration D) Histology of the ventricular tissue of the BPA treated rats of 30 days duration. Arrows in both sections of tissues indicate the changes in morphology of nucleus of ventricular myocytes. Circle indicates the appearance of lacunae in the matrix of ventricular tissue. Images were obtained by digital SLR Olympus Camera (E-620) fitted with Olympus light microscope (CH20i).

DISCUSSION

Some free radicals are generated as byproduct of metabolism and are extremely reactive. However, biological systems have evolved endogenous defense mechanisms against these free radical induced cell damage. SOD, CAT and GP_X are the primary antioxidant enzymes which directly eliminate reactive oxygen species viz. hydroxyl radical, superoxide radical, hydrogen peroxide etc. The stress conditions depend on the degree of production or inactivation of these antioxidant enzymes[29,30]. Our aim was to study the effects of BPA on antioxidant defense mechanisms in ventricular myocytes. In the present study the activities of antioxidant enzymes like SOD, CAT, GPx, GR were decreased and the amount of malondialdehyde, a biomarker of lipid peroxidation, was increased in BPA treated rats

compared to the respective vehicle control group of rats. From our study it is suggested that BPA might induce the oxidative stress in ventricular myocytes by inhibiting the activities of antioxidant enzymes as a result of the accumulation of reactive oxygen species (ROS) in ventricular myocytes. The accumulated ROS in turn induces lipid peroxidation in the biological membranes [31,32,33,34] as revealed by the increase in MDA level in our study. In order to study the oxidative stress induced ventricular tissue damage we have studied the degenerative changes in the ventricular tissue in histological sections of ventricle of heart in BPA and DMSO treated animals. The sizes of the nuclei were enlarged and the shapes were rounded in BPA treated rats. Numerous lacunae were also seen in the matrix of the ventricle. This result suggests that BPA inhibits the ventricular function of heart presumably by inducing the ventricular tissue damage through elevation of ROS levels in myocytes as a result of inhibition of antioxidant enzymes.

CONCLUSION

From this study it may be concluded that BPA inhibits the ventricular functions of heart presumably by inducing degeneration of ventricular tissue through oxidative stress of ventricular myocytes.

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