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Research Article
Effect of Bisphenol-A on Antioxidant Enzymes and Lipid Peroxidation in Liver of Chick Embryos

[1]J. Sravani, [1]K. Padmaja, [1]P. Eswara Prasad and [2]B. Punya Kumari

[1]Department of Veterinary Biochemistry, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, India
[2]Department of Animal Genetics and Breeding, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, India

Abstract

Objective: The aim of the study was to estimate the intensity of oxidative damage and alterations in antioxidant enzyme activities in liver of bisphenol-A (BPA) treated chick embryos. **Methodology:** An experiment was conducted to study the effect of BPA on thiobarbituric acid reacting substances (TBARS), glutathione (GSH) and antioxidant enzymes during embryonic development in chicks. Group I served as control, groups II-IV were injected with BPA dissolved in distilled water in three concentrations as 100, 250 and 500 μM, respectively, separately to 11th and 14th day old chick embryos. The developed embryos were sacrificed after 24 and 48 h of BPA exposure to collect liver tissues for estimation of TBARS, GSH and antioxidant enzyme activities (superoxide dismutase, catalase, glutathione peroxidise and glutathione-S-transferase). **Results:** The results showed a significant dose dependent increase in TBARS, GSH, GP_X, GST and SOD after 24 and 48 h in both 11th and 14th day treated embryos. Whereas, CAT activity was not altered much after 24 h, but a significant increase was noticed after 48 h in liver of 11th and 14th day BPA treated chick embryos. The results revealed that increased levels of TBARS after 24 h of BPA treatment and were decreased after 48 h due to increased levels of antioxidant enzyme activities. **Conclusion:** Finally, it was concluded that BPA has induced oxidative stress and it was reduced by antioxidant enzyme activities, which indicates protective mechanism against the induced oxidative stress. The BPA is more toxic at early stages of embryonic development.

Key words: BPA treatment, oxidative stress, TBARS, GH, GPx, SOD, GST

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Corresponding Author: J. Sravani, Department of Veterinary Biochemistry, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, India Tel: 8143243805

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Environmentally released manmade chemicals like polychlorinated biphenyls and bisphenol-A has serious effects on endocrine and reproductive systems in domestic animals. It is one of the most common chemicals exposed in everyday life. It is used in a wide variety of consumer products like food storage containers, hard plastic water bottles, medical equipment, canned foods, PVC pipes, dental sealants, nail polish, electronic compact discs and digital versatile discs etc.[1]. It comes into contact with, heating plastic bottles, presence of acidic or basic foods and beverages in plastic and repeated washing increases the rate of BPA leaching from bottles[2].

Bisphenol-A causes a wide range of adverse effects at low doses in both terrestrial and aquatic animals[3] and is associated with endocrine, immune and metabolic effects[4]. Highest concentrations of BPA were found in adipose tissue and liver[5]. It is a potential risk to early stages of mammalian life[6]. The aim of the study was to estimate the intensity of oxidative damage and alterations in antioxidant enzyme activities in liver of bisphenol-A (BPA) treated chick embryos.

MATERIALS AND METHODS

Source of fertilized eggs and incubation conditions: The present study was conducted at the Department of Veterinary Biochemistry, College of Veterinary Science, Tirupati. Freshly laid wild Bobcock strain zero day old fertilized eggs were procured from Department of Poultry Science, College of Veterinary Science, Tirupati. They were incubated at 37.5±0.5°C with a relative humidity of 65% in an egg incubator.

Experimental groups:

Group I : Control group (distilled water)
Group II : Bisphenol-A (100 µM)
Group III : Bisphenol-A (250 µM)
Group IV : Bisphenol-A (500 µM)

The embryos were sacrificed after 24 and 48 h for the collection of liver tissue samples.

Biochemical analysis: Thiobarbituric acid reacting substances in tissues were estimated by the method of Ohkawa *et al.*[7].

Assay of antioxidant enzymes

Preparation of enzyme extract: Liver tissues were blotted dry, thawed and homogenized at 4°C in 3 volumes of 0.25 M sucrose containing 0.07 M phosphate buffer (pH 7.2), 10 mM EDTA and 0.1% triton X-100. Post mitochondrial supernatant was prepared by centrifuging at 12,000 g for 15 min at 4°C using REMI refrigerated centrifuge.

Glutathione peroxidase activity was assayed by the method of Rotruck *et al.*[8]. Glutathione-S-transferase activity was determined by the method of Habig *et al.*[9]. Superoxide dismutase activity was measured according to Misra and Fridovich[10]. Catalase activity was measured by the method of Beers and Sizer[11]. Glutathione content was determined according to the method of Ellman[12].

Statistical analysis: Statistical significance between the groups was analysed by one way ANOVA followed by Tukey's *post hoc* test using statistical package for social sciences (SPSS 15.0 version).

RESULTS

The results showed a significant dose dependent increase in TBARS in both 11th and 14th day BPA treated chick embryos compared to control. Maximum induction was observed after 24 h, which was found to be nearly 2 and 2.5 fold in group III and IV, respectively in 11th day treated embryos. In 14th day BPA treated embryos, it was found to be 8, 25 and 62% increase after 24 h in group II, III and IV, respectively. The percentage of increase was decreased after 48 h of treatment in both 11th and 14th day treated embryos (Fig. 1 and 2).

The levels of nonenzymatic antioxidants GSH and enzymatic antioxidants SOD, CAT, GST and GP_X activities were measured to evaluate the stability of ROS production in liver.

The GP_X activity was found to be decreased with increase in development of the embryo (Fig. 3 and 4). Significant elevation was observed after 48 h compared to 24 h in both 11th and 14th day treated embryos. It was found

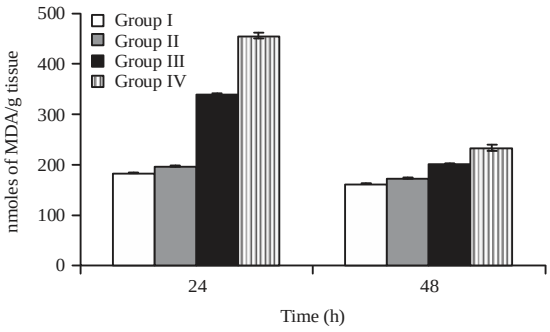

Fig. 1: TBARS in liver of 11th day treated chick embryos

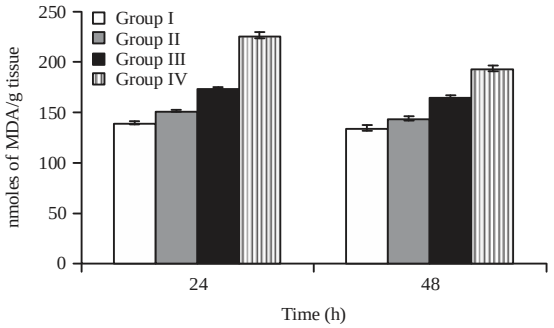

Fig. 2: TBARS in liver of 14th day treated chick embryos

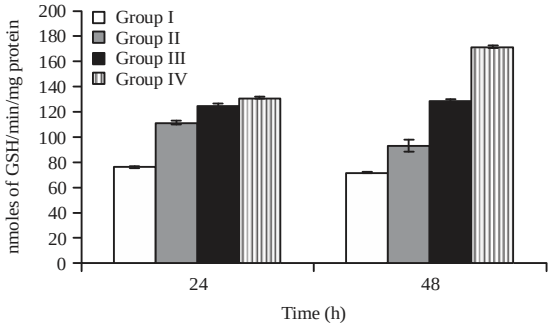

Fig. 3: Glutathione peroxidase activity in liver of 11th day treated chick embryos

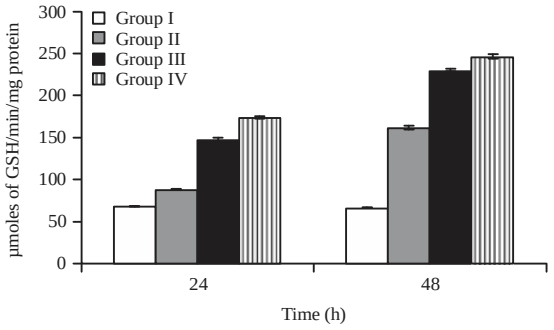

Fig. 4: Glutathione peroxidase activity in liver of 14th day treated chick embryos

to be 1.75 and 2.5 fold induction after 48 h in 11th day in group III and IV, respectively compared to group I. Whereas, it was 2.5 and 3.5 fold induction after 48 h in 14th day in group II and III, respectively compared to group I. The results showed significant increase in GST activity after 48 h compared to 24 h in both 11th and 14th day BPA treated chick embryos. It was observed nearly 3 and 4 fold induction in 11th day and 2 and 3.5 fold induction in group III and IV, respectively compared to group I after 48 h in 14th day treated embryos (Table 1).

The results indicated significant dose dependent increase in SOD activity after 24 h compared to 48 h both in 11th and 14th day BPA treated chick embryos. There was nearly 3 and 5 fold induction in11th day and 2.5 and 4 fold induction in 14th day in group III and IV, respectively compared to group I (Fig. 5 and 6). There is no significant change in CAT

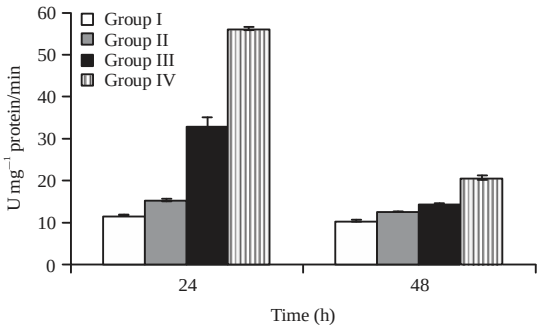

Fig. 5: SOD activity in liver of 11th day treated chick embryos

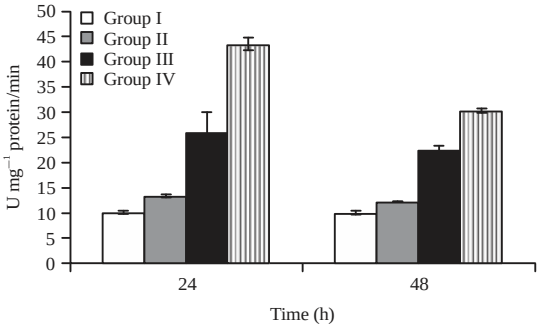

Fig. 6: SOD activity in liver of 14th day treated chick embryos

Table 1: Mean values of GST activity (U mg^{-1} protein) in liver tissue

	11th day		14th day	
Treatment	24 h	48 h	24 h	48 h
Group I	8.75±0.22^{a}	11.77±0.16^{a}	12.69±0.04^{a}	12.12±0.23^{a}
Group II	10.38±0.21^{b}	14.32±0.22^{b}	12.48±0.09^{a}	21.51±0.26^{b}
Group III	12.51±0.27^{c}	36.03±0.57^{c}	13.71±0.14^{b}	26.49±0.88^{c}
Group IV	13.88±0.12^{d}	47.57±0.36^{d}	15.00±0.16^{c}	42.15±0.77^{d}
df	(3, 20)	(3, 20)	(3, 20)	(3, 20)
F	113.81	2228.48	95.45	422.60

Values are Mean±SE (n = 6), Means with different superscripts differ significantly with in the column ($p<0.05$)

Table 2: Mean values of CAT activity (U mg^{-1} protein) in liver tissue

	11th day		14th day	
Treatment	24 h	48 h	24 h	48 h
Group I	11.10±0.46[a]	12.03±0.28[a]	9.96±0.13[b]	8.80±0.27[a]
Group II	13.35±0.34[b]	16.86±0.18[b]	10.16±0.31[b]	15.01±0.28[b]
Group III	12.50±0.35[b]	21.88±0.42[c]	9.75±0.20[b]	22.73±0.19[c]
Group IV	13.79±0.33[c]	27.90±0.64[d]	8.97±0.22[a]	26.24±0.53[d]
df	(3, 20)	(3, 20)	(3, 20)	(3, 20)
F	10.14	265.64	5.28	517.18

Values are Mean±SE (n = 6), Means with different superscripts differ significantly with in the column (p<0.05)

Table 3: Mean values of glutathione (mg g^{-1} tissue) in liver tissue

	11th day		14th day	
Treatment	24 h	48 h	24 h	48 h
Group I	0.32±0.006[a]	0.33±0.02[a]	0.43±0.01[a]	0.45±0.02[a]
Group II	0.43±0.008[a]	0.65±0.01[b]	0.79±0.01[b]	0.65±0.01[b]
Group III	0.62±0.02[b]	0.74±0.01[c]	1.18±0.01[c]	0.83±0.01[c]
Group IV	1.41±0.11[c]	0.89±0.02[d]	1.41±0.05[d]	0.90±0.02[d]
df	(3, 20)	(3, 20)	(3, 20)	(3, 20)
F	76.76	252.47	236.54	136.69

Values are Mean±SE (n = 6), Means with different superscripts differ significantly with in the column (p<0.05)

activity after 24 h but a significant increase was noticed after 48 h in both 11th and 14th day BPA treated chick embryos. The activity increased upto 40% in 11th day and 70% in 14th day after 48 h in group II compared to group I (Table 2). A dose dependent increase in glutathione levels was observed with BPA treatment. Significant increase was observed after 24 h compared to 48 h in both 11th and 14th day BPA treated embryos. The levels increased to 34% in 11th day and 84% in 14th day in group II compared to group I (Table 3).

DISCUSSION

Significant increase in TBARS was observed after 24 h in liver with BPA injections on 11th and 14th day of embryonic development. Present study results are in accordance with Chen and Schopfer[13] who reported that BPA shows its toxicity by increasing H_2O_2. The increased levels of TBARS after 24 h decreased after 48 h in both 11th and 14th day BPA treated embryos, which may be due to high capacity of chick embryo protective pathways against induced oxidative stress. Korkmaz *et al.*[14] also reported increased TBAR levels in liver of rats exposed to BPA.

In response to elevated TBARS, significant increase in GP_X activity was observed in 11th day compared to 14th day BPA injected embryos. Activity increased significantly after 24 h compared to 48 h of BPA exposure. The increased GP_X activity after 24 h of BPA treatment may be responsible for reduction in TBARS (due to removal of H_2O_2 by GP_X) after 48 h in liver. Similar results were observed in selenium treated chick embryos in response to increased MDA formation[15]. Immediate response in GP_X activity after BPA exposure shows major role of this enzyme in converting H_2O_2 radicals compared to catalase.

Activity of GST increased significantly after 48 h compared to 24 h of BPA treatment. It was found to be high on 11th day compared to 14th day BPA treated chick embryos. These results are in agreement with Padmaja and Ramamurthi[16] where zinc treatment increased GST activity in response to induced oxidative stress. Earlier it was reported that GST as a detoxifying enzyme plays an important protective role in embryonic tissue and liver is found to be one of the main target organ for environmental pollutants[17]. It protects cells or tissues against oxidative stress and damage by detoxifying various toxic substrates derived from cellular oxidative processes[18].

Present results showed increased activity of SOD after 24 h of BPA treatment indicating excess production of H_2O_2 radicals which only be effectively detoxified by increased GP_X activity after 48 h in BPA exposed chick embryos. The results in the present study are in accordance with Kabuto *et al.*[19], who observed that BPA injection induces over production of H_2O_2 in mouse organs and H_2O_2 is readily converted to hydroxyl radicals. There is no significant change in catalase activity after 24 h, because major role was played by GP_X in detoxifying H_2O_2 radicals. In the present study the CAT activity was increased after 48 h of BPA exposure showing its role in converting H_2O_2 radicals produced after 24 h due to SOD activity both in 11th and 14th day BPA treated embryos. The results showed elevated levels of GSH after BPA

treatment, which is important in the regulation of redox state and prevention of the cell damage induced by oxidative stress[20]. Similar results are noticed by Gualtieri *et al.*[21] where high doses of BPA increase cell content of GSH owing to increased GSH synthesis.

CONCLUSION

The results revealed that increased levels of TBARS after 24 h of BPA treatment were decreased after 48 h in both 11th and 14th day old chick embryos which may be due to increased levels of antioxidant enzyme activities after 48 h of BPA treatment. Finally, it was concluded that BPA has induced oxidative stress at early stages of chick embryonic development and it was reduced by antioxidant enzyme activities which indicates protective mechanism against the induced oxidative stress. The BPA is more toxic at early stages of embryonic development.

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