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Oxidative stress responses of a freshwater teleost, *Anabas testudineus*, to an endocrine disruptor, Bisphenol A

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Summary

Bisphenol-A (BPA), an industrial chemical used to manufacture polycarbonate plastic, is considered as a potent estrogenic endocrine disruptor. A majority of xenobiotics exert their toxic effect by causing generation of reactive oxygen species, leading to oxidative stress. Reports regarding oxidative stress responses to BPA in fish are scanty. From this viewpoint, in the present study, a freshwater fish, *Anabas testudineus*, was exposed to sub-lethal concentrations of BPA (2.5, 5.0, & 7.5 mg/l), for different time periods (7, 15 & 30 days). Four major enzymes of the fish's antioxidant defense system viz., catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (Se-GPx), and glutathione-S-transferase (GST), and the non-enzymatic antioxidant glutathione (GSH) were chosen as biomarkers to examine the effects of BPA. The activities of SOD, GPx and GSH were elevated to significant levels, while CAT and GST activities were decreased significantly suggesting oxidative stress in BPA-exposed fish. Reactive oxygen species are known to cause DNA fragmentation. Hence, DNA fragmentation assay was also done. The severity of DNA fragmentation in fish exposed to 7.5 mg/l BPA (30days) was remarkably increased ($p<0.05$ vs control) and this was revealed in gel electrophoresis analysis also. The results clearly show that BPA is a pollutant with oxidative potential, also with a potential for DNA fragmentation. The potential risks of this compound to nature and human populations are significant since the production of BPA related compounds is increasing dramatically each year.

Key words: Endocrine disruptor, Oxidative stress, DNA fragmentation, BPA, Antioxidants

Introduction

Bisphenol A (2, 2'-bis (4-hydroxyphenyl) propane (BPA) was developed in 1891 as a synthetic estrogen. It came into widespread use in the 1950s when scientists realized that it could be used to make polycarbonate plastic and some epoxy resins to line food and beverage cans. Moreover, large amounts of BPA have been used in the production of thermal receipt papers and these are produced in large quantities for use in cash register receipts, luggage tags and bus/train tickets (Mendum et al., 2010). Bisphenol A is the one of the highest volume chemicals produced worldwide (76.4 billion tonnes) with 6-8 % growth in demand expected per year (Burridge, 2003). Furthermore, the leaching potential of BPA in polycarbonate products and cans has been reported (Kang and Kondo, 2002; Howdeshell et al., 2003).

Bisphenol A is a renowned estrogen mimic (Letcher et al*.,* 2005; Oehlmann et al*.,* 2006), and the endocrine and reproductive disrupting activities of BPA have been ascertained in many organisms (Levy et al., 2004). Bisphenol A is known for causing oxidative stress in mammalian species, especially rat (Bindhumol et al., 2003; Rashid et al., 2009). But the oxidative potential of BPA has not yet been reported in sub-mammalian species, particularly in fish. In this aspect, it was thought worthwhile to investigate the oxidative potential of BPA, if any, in *Anabas testudineus*.

The solubility of BPA in water is 120-300mg/l (Lide, 2004). Rivers, lakes, and estuaries are major sinks for BPA. These surface waters accumulate BPA leached from plastic debris and landfill wastes along with BPA-containing sewage and effluent. The presence of BPA in aquatic habitat is well established (Suzuki et al*.,* 2004; Focazio et al*.,* 2008) and this has led to significant research efforts to investigate its biological effects in fish. Fish as a sentinel organism provide an early warning of

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effects that may later become apparent in other wild species and ultimately in humans (Cheek et al., 2001).

A large number of industrial compounds exert their toxic effect by generating reactive oxygen species (ROS), causing oxidative stress (Livingstone, 2001). Fish are endowed with defensive mechanisms to counteract the impact of ROS resulting from the metabolism of various chemicals. These systems include various antioxidant defense enzymes such as superoxide dismutases (SOD) which catalyze the dismutation of superoxide radical to hydrogen peroxide (H_2O_2), catalase (CAT) acting on hydrogen peroxide, glutathione S-transferase family (GST) possessing detoxifying activities towards lipid hydroperoxides generated by organic pollutants and glutathione peroxidase (GPx) which detoxifies H_2O_2 in the presence of glutathione (GSH) (Farombi et al., 2008; Di Giulio and Hinton, 2008).

Several researchers have associated DNA damage to oxidative stress (Twigg et al., 1998; Donnelly et al., 1999). Peroxidation of DNA can lead to chromatin cross-linking, base changes, and DNA strand breaks (Twigg et al., 1998). In this context, DNA fragmentation, if any, was also investigated in the present study. Moreover, all these investigations were done in hepatic tissue as liver is the site of multiple oxidative reactions and maximal free radical generation (Gul et al., 2004; Avci et al., 2005). Hence, in the present study, an attempt was made to evaluate the impact of BPA on antioxidant system and DNA fragmentation in *A. testudineus.*

Materials and Methods

Experimantal design

Test chemical

In the previous studies conducted in our laboratory, the LC50 value of BPA was determined to be 10 mg/l. Hence, sub-lethal doses of BPA viz., 2.5, 5.0 and 7.5 mg were selected for exposure. 75 (2.5 mg/l), 150 (5.0 mg/l) and 225 (7.5 mg/l) mg of BPA were weighed in separate microfuge tubes and dissolved separately by adding about 1ml ethyl alcohol and diluted in 30 liters of dechlorinated tap water in separate aquarium tanks.

Fish and Aquaria

The fish, *A. testudineus* used in the experiment, were collected from local suppliers and brought to the laboratory. The fish were acclimatized for 30 days in large cement tanks filled with dechlorinated tap water under laboratory conditions such as natural photoperiod and temperature (26 ± 2°C). Fish were fed daily on feed prepared in the laboratory.

After acclimatization, fish weighing 45 ± 5g were divided into 10 groups of 10 each in separate glass tanks. The first group of fish served as control and were kept in normal dechlorinated tap water. Fish in groups II, III and IV were exposed to 2.5 mg BPA/l of water for the periods 7, 15 and 30 days, respectively. Similarly, fish in groups V, VI and VII were exposed to 5 mg BPA/l of water and fish in groups VIII, IX and X were exposed to 7.5 mg BPA/l of water for a period of 7, 15 and 30 days, respectively. Fresh water sample having the specified doses of BPA was replaced on alternate days.

Sampling

The fish were starved for twenty four hours before sacrifice for getting optimum experimental conditions. After stipulated periods of exposure, fish were sampled and killed by spinal concussion. A portion of fresh liver tissue from each fish was used for DNA extraction and the remaining liver tissue was frozen immediately at –80°C in an ultralow deep freezer [NBS, USA] for biochemical assays.

Sample preparation for enzyme assay and protein measurement

The liver was surface-dried with filter paper, thoroughly washed with 50 mM phosphate buffer pH 7.4 and homogenized with 1 ml Tris.HCl buffer (pH 7.2). Homogenization was carried out at 4° C and centrifuged at 10,000 rpm for 10 min at 4° C in

a refrigerated centrifuge (Eppendorf, Germany). The supernatant thus collected was used for assay of antioxidant enzymes.

Assay of antiodidant enzymes

A. Superoxide dismutase (EC.1.15.1.1)

Superoxide dismutase was estimated according to the protocol of Kakkar et al. (1984). The reaction mixture contained 1.2 ml sodium pyrophosphate buffer (0.052 M, pH 8.3), 100µl phenazine methosulphate (186 µM), 300 µl nitroblue tetrazolium (300 µM), 200 µl NADH (780 µM), appropriately diluted enzyme preparation and water in a volume of 3 ml. Reaction was started by the addition of NADH. After incubation at 30^0 C for 90 seconds, the reaction was stopped by the addition of 1 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml n-butanol. The mixture was allowed to stand for 10 min, centrifuged at 2500 rpm, for 10 min and the butanol layer was carefully separated out. Color intensity of the chromogen in butanol was measured at 560 nm against blank (butanol) using UV-Vis spectrophotometer (Perkin Elmer, USA). One unit of enzyme activity was defined as the enzyme concentration required to inhibit the optical density at 560 nm of chromogen production by 50% in one minute under the assay conditions. The specific activity was expressed as IU mg $protein^{-1}$.

B. Catalase (EC.1.11.1.6)

Catalase activity was measured according to Maehly and Chance (1954). The estimation was done spectrophotometricaly following the decrease in absorbance at 230 nm. The reaction mixture contained 3 ml phosphate buffer (0.01 M, pH 7), 20µl H_2O_2 (2 mM) and 1 ml of approximately diluted extract. A system devoid of the extract constituted blank. The specific activity was expressed in terms of *n* moles of H_2O_2 decomposed/minute/mg protein.

C. Glutathione peroxidase (EC.1.11.1.9)

Glutathione peroxidase was measured according to Lawrence and Burck, (1976) with slight modification (Agergaard and Jensen, 1982). The activity was measured in 2 ml phosphate buffer (0.01 M, pH 7.4) containing 300 µl EDTA, 300 µl sodium azide (1 mM), 100 µl reduced glutathione (1 mM), 100 µl NADPH (0.1 M) and 200 µl tissue extract. Blank contained all the reagents except tissue extract. Change in absorbance was measured at 340 nm at 15 seconds interval for 1 minute. Enzyme activity was expressed as IU /mg $protein^{-1}$.

D. Glutathione-S-transferase (EC.2.5.1.18)

Glutathione-S-transferase was measured according to Habig et al. (1974). The cocktail contained 1 ml phosphate buffer (0.5 M, pH 6.5), 100 µl 1-chloro, 2, 4, dinitrobenzene (CDNB) (30 mM), 0.1 ml reduced glutathione (30 mM) and 100 µl tissue extract. The increase in optical density of the enzyme was measured against the blank at 340 nm. Enzyme activity was expressed as nmoles of CDNB-GSH conjugate formed/minute/mg protein for tissue samples.

E. Glutathione content

Glutathione content was measured according to Benke and Cheevar (1974). The reaction mixture contained 2 ml phosphate buffer (0.3 M, pH 7.4), 500 µl 5, 5'-Dithio-bis-2-nitrobenzoic acid (DTNB) (0.04 %) and 200 µl tissue extract. A system devoid of extract served as blank. Change in absorbance was measured at 412 nm within 10 minutes. The specific activity was expressed as nmol/ 100g tissue.

Estimation of protein

Protein content for all enzymes was estimated using the same tissue extract according to the protocol of Bradford (1976).

Determination of DNA fragmentation

DNA fragmentation was measured by Diphenylamine (DPA)-spectrophotometric method (Wolozin et al., 1996). Intact DNA was separated from fragmented DNA by centrifugal sedimentation followed by precipitation and quantification using DPA. To minimize formation of oxidative artifacts

during isolation, 2, 2, 6, 6-tetramethyl piperidinoxyl (20 mM) was added to all solutions and all procedures were performed on ice. Briefly, hepatocytes (1 x 10^6 in 1ml PBS) were put in a 1.5 ml centrifuge tube (tube B) and centrifuged (200 g, 4° C, 10 min) to obtain the cell pellet. The supernatants were transferred to fresh tubes (tube S). The obtained pellet (tube B) was suspended in 1 ml TTE buffer (Tris-Triton-EDTA: TE buffer with 0.2% Triton-X 100, pH 7.4) and centrifuged at 20,000 x g (4° C, 10 min). The supernatant was transferred to fresh tubes (tube T) and the resulting pellets were resuspended in TTE buffer. Trichloroacetic acid (TCA) was added to tubes T, B and S and vortexed vigorously. Tubes were kept overnight at 4°C followed by centrifugation at 20,000 x g (4° C, 10 min). The supernatant was discarded and the pellet was hydrolyzed by the addition of 160 µl of 5% TCA followed by heating at 90° C for 15 mins. This was followed by addition of 320 µl of freshly prepared DPA (150 mg DPA in 10 ml acetic acid containing 150 µl conc. H_2SO_4 and 50 µl acetaldehyde solution). The color was developed by incubation at 37° C for 4 hours. Optical density of the solution was determined at 600 nm using UV-Vis spectrophotometer. Percentage DNA fragmentation was calculated using the following formula: % fragmented DNA = [S + T/S+T+B] x 100.

Agarose gel electrophoretic detection of DNA damage

Isolation of DNA from liver tissue and electrophoresis was carried out according to the method of Iwasa et al. (1996). Briefly, the homogenized liver tissue was lysed with buffer containing 100 mM Tris-HCl (pH 7.2), 25 mM EDTA, 0.5% SDS and 1 mg/ml proteinase K and kept in a water bath for 12 hr at 37°C. DNA was extracted twice with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). To the aqueous phase, sodium acetate (3 M) was added and the DNA was precipitated with chilled isopropanol. Following a 70% ethanol wash, the precipitated DNA was resuspended in Tris-EDTA buffer. Five micrograms of DNA per sample were electrophoretically separated on 1% agarose gel containing 0.5 µg/ml ethidium bromide in submarine gel electrophoresis system. The stained gels were captured using Gel-doc (Gelstan, Medicare, Germany). The migration distance of the DNA molecules from the top of the gel was used as a measure of DNA damage. It is recognized that the highly fragmented, low molecular weight DNA strands will migrate farther than non-damaged high molecular weight DNA strands.

Chemicals

Agarose and Tris were purchased from USB Corporation (USA). Diphenylamine, isoamyl alcohol, isopropanol, chloroform and phenol were purchased from MERCK (Mumbai, India). EDTA and SDS were purchased from Amersham Biosciences (USA). All the other chemicals used were of analytical grade and purchased from SRL (Mumbai, India).

Statistics

Data analysis was done by ANOVA. The differences in means were tested by using Duncan (1955) analysis. Significant level used was 0.05. The association of variables was studied by Pearson correlation test. All the statistical analyses were performed using the software SPSS 10.0 for Windows.

Results

Activities of antioxidant enzymes

A. Superoxide Dismutase

There was a significant increase in SOD activity in liver tissue after 7, 15 and 30 days of exposure to 2.5 mg/l BPA (Fig. 1A). Exposure to 5.0 mg/l BPA significantly increased SOD activity after 7 and 15 days but the activity was unaffected after 30 days of exposure. There was no significant change in SOD activity after 7 days of exposure to 7.5 mg/l BPA but the activity increased significantly after 15 and 30 days of exposure.

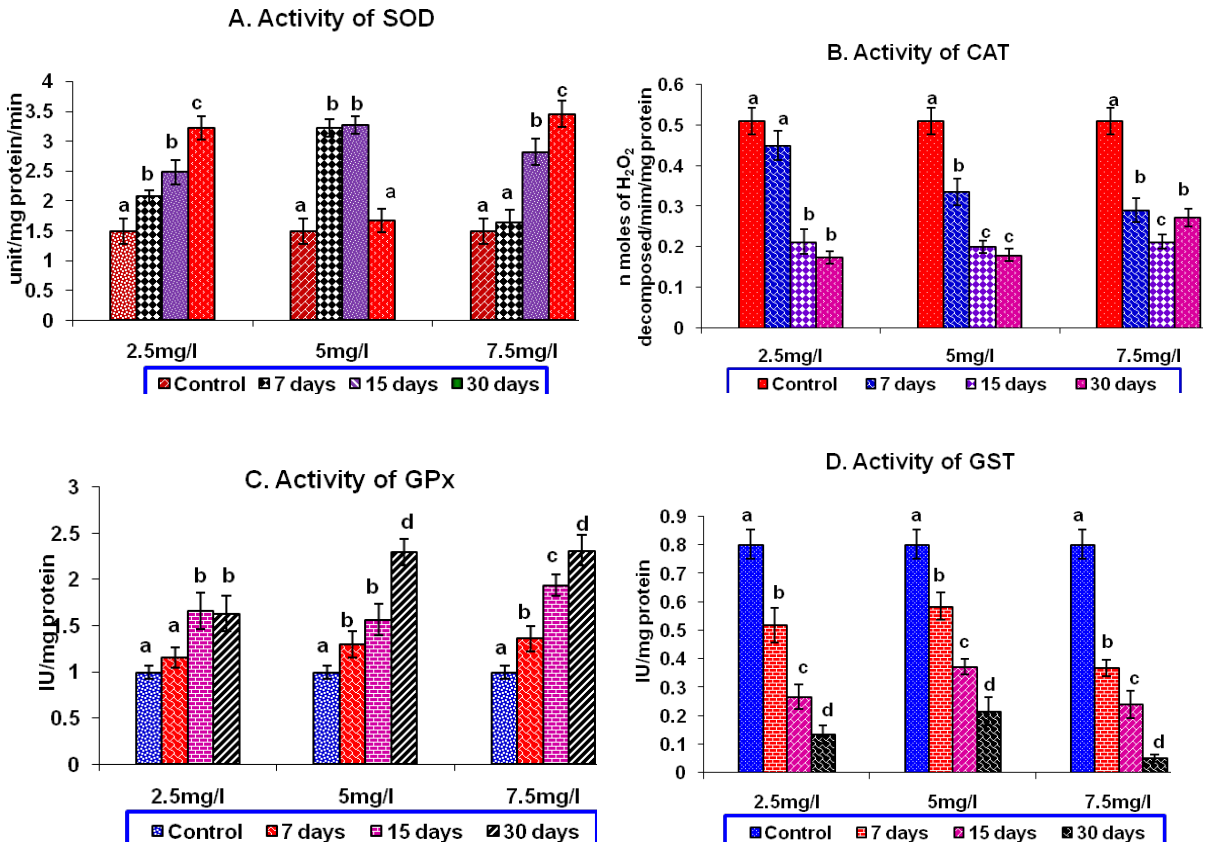

Fig. 1: The effect of sub-lethal doses of BPA (2.5, 5.0 and 7.5mg/l) on the activities of SOD (A), CAT (B), GPX (C) and GST (D) in the liver of ***A. testudineus*** in relation to control. Each column is mean ± SEM for six fish. Mean with different superscript letter for each parameter is significantly different at P<0.05.

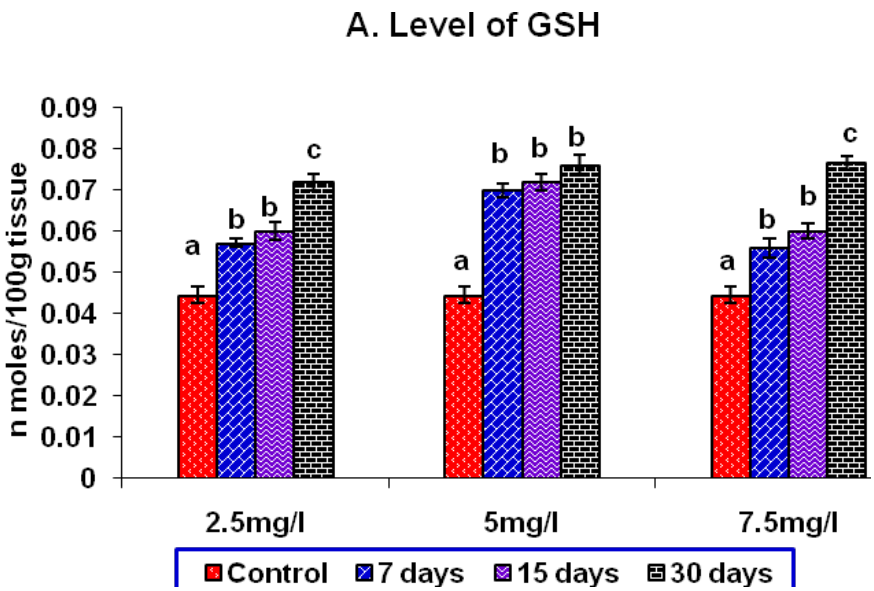

Fig. 2: The effect of sub-lethal doses of BPA (2.5, 5.0 and 7.5mg/l) on the level of GSH (A) in the liver of *A. testudineus* in relation to control. Each column is mean ± SEM for six fish. Mean with different superscript letter for each parameter is significantly different at P<0.05.

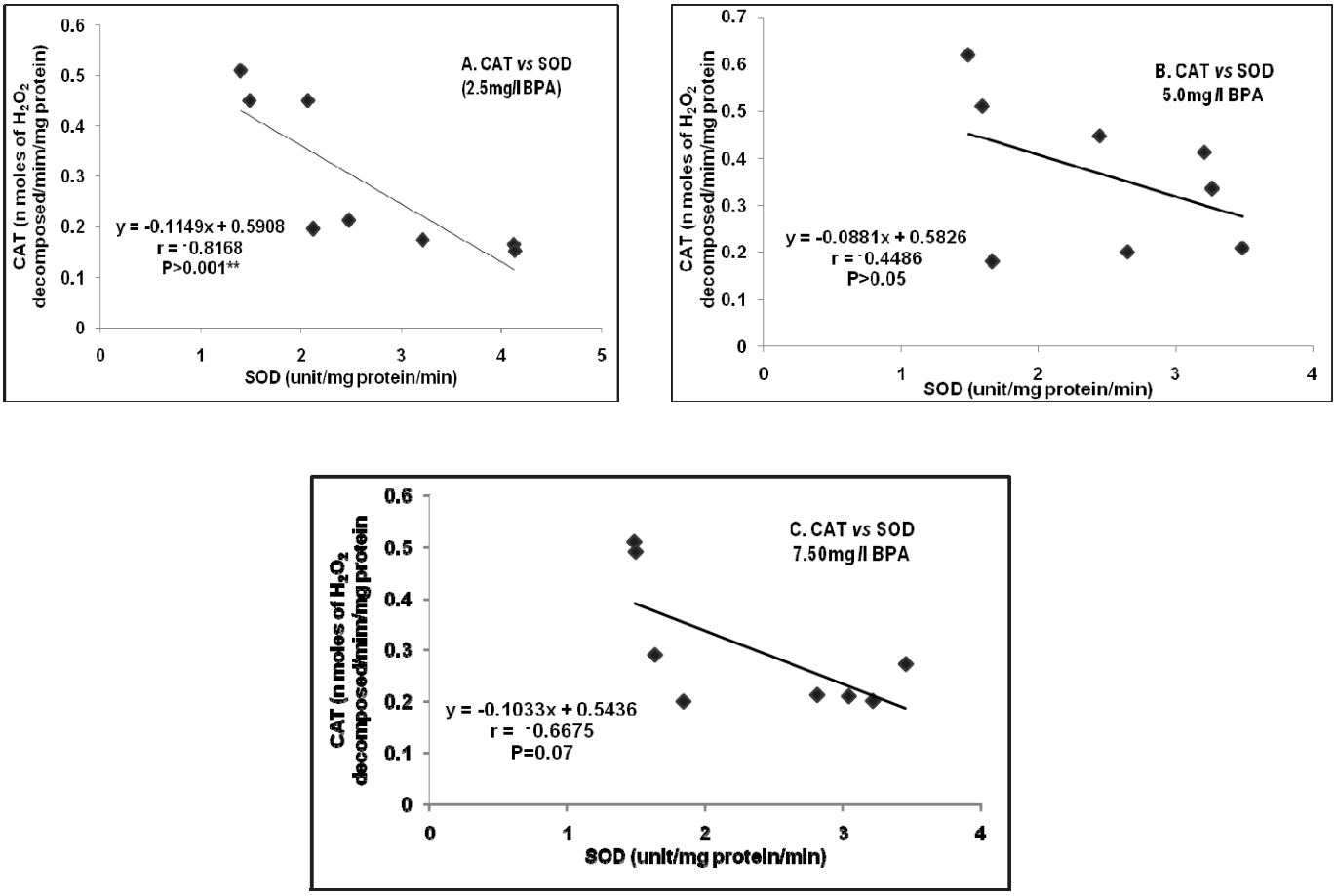

Fig. 3: Correlation between CAT activity and SOD activity in the liver of *A. testudineus* exposed to 2.5mg/l BPA (A) ((P>0.001**), 5.0mg/l BPA (B) (P>0.05) and (C) 7.5 mg/l BPA (P>0.05).

B. Catalase

Catalase activity did not change significantly after 7 days of exposure to 2.5 mg/l BPA (Fig. 1B). However, significant decrease in CAT activity occurred after 15 and 30 days of exposure to 2.5 mg/l BPA. A significant decrease in CAT activity was observed after exposure to 5.0 mg/l BPA in a time-dependent manner. Likewise, exposure to 7.5 mg/l BPA significantly decreased CAT activity in the fish after 7, 15 and 30 days. A significant negative correlation was found between SOD and CAT activities after exposure to 2.5 mg BPA/l ($r = -0.8168$; $P>0.001$**) (Fig. 3A). A negative correlation was observed after 5.0 mg BPA/l ($r = -0.4486$; $P>0.05$) (Fig. 3B) and 7.5 mg BPA/l ($r = -0.6675$; $P>0.05$) (Fig. 3C) exposure.

C. Glutathione peroxidase

One way ANOVA showed that there was significant increase in GPx activity after 15 and 30 days of exposure to 2.5 mg/l BPA while there was no significant change in GPx activity after 7 days of exposure (Fig. 1C). A significant increase in GPx activity was observed in fish exposed to 5.0 mg/l and 7.5 mg/l BPA after 7, 15 and 30 days.

Glutatione-S-transferase

Glutatione-S-transferase activity decreased significantly in hepatic tissue of fish exposed to 2.5, 5.0 and 7.5 mg/l BPA (7, 15 & 30 days) in a dose- and time-dependant manner at all exposures (Fig. 1D).

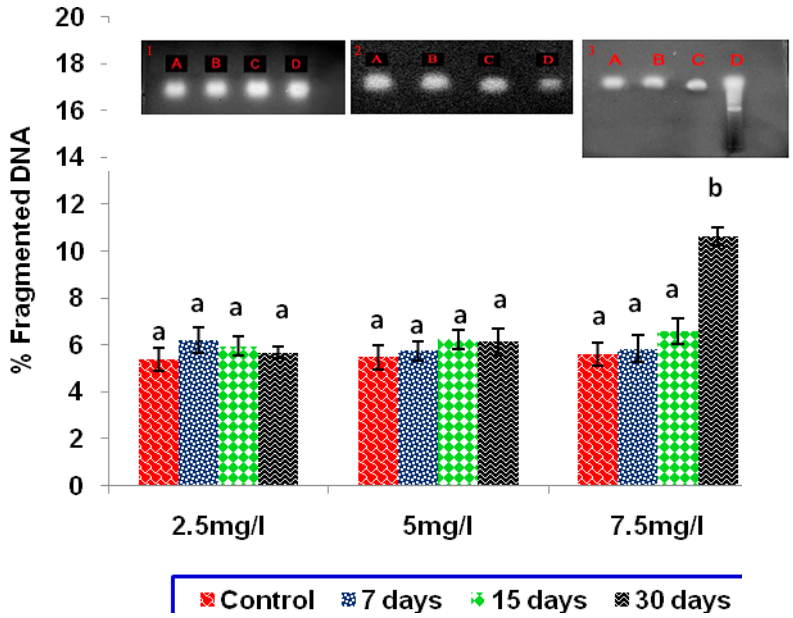

Fig. 4: Influence of BPA on DNA damage in liver of *A. testudineus* by agarose gel electrophoresis and DPA method. In electropherograms 1 (2.5mg/l BPA), 2 (5.0mg/l BPA) and 3 (7.5mg/l BPA), Lane A represents control, Lane B – 7 days, Lane C – 15 days and Lane D – 30.

Glutathione content

Fish exposed to 2.5, 5.0 and 7.5 mg/l BPA had significantly elevated hepatic concentration of GSH after 7, 15 and 30 days as compared to the control group (Fig. 2).

Evaluation of DNA fragmentation

There was no significant change in % fragmented DNA after 7, 15 and 30 days of exposure to 2.5 and 5.0 mg/l BPA as compared to control (Fig. 4). A significant increase in % fragmented DNA was observed only at higher concentration of 7.5 mg/l BPA, after prolonged period of exposure (30 days) and this appeared also on gel electropherogram. In the agarose gel, fragmented DNA (low molecular weight with greater migration) was detected only in fish exposed to 7.5 mg/l BPA for 30 days whereas no significant change was detected at any other concentrations, in which all the bands appeared as single high molecular weight intact DNA.

Discussion

The present study revealed that BPA induced oxidative stress in the liver of *A. testudineus*. It was obvious that SOD and GPx activities increased significantly while the activities of CAT, GST and GSH decreased significantly at all exposures to BPA. But DNA fragmentation was observed only on exposure to higher concentration of BPA.

Superoxide dismutase catalytically scavenges superoxide radical, which appears to be an important agent of toxicity of oxygen and this provides a defense against oxygen toxicity (Kadar et al., 2005). In our study, the hepatic SOD activity was increased significantly on exposure to sub-lethal doses of BPA as compared to that of control. Comparable increased activity of SOD was observed in some fish exposed to various types of endocrine disruptors such as butachlor in *Clarias gariepinus* (Farombi et al., 2008) and paclobutrazol in Zebra fish, *Danio rerio* (Ding et al., 2009). Exposure to heavy metals resulted in increased hepatic SOD activity in catfish, *Chrysichthys nigrodigitatus* and tilapia, *Oreochromis niloticus* (Doherty et al., 2010), in brown trout, *Salmo trutta* (Hansen et al., 2006) and in *Pagellus acarne*, *Sarpa salpa* and *Liza saliens* (Metwally and Fouad, 2008).

Catalase activity is considered as a sensitive biomarker of oxidative stress in fish (Gul et al*.,* 2004; Sanchez et al*.,* 2005). Catalase activity was found to be significantly decreased in the liver of freshwater fish, *Channa punctatus* exposed to endosulfan (Pandey et al*.,* 2001) and *O. niloticus* exposed to Ag^{+} and Cd^{2+} (Atli et al*.,* 2006). In the present study also, the activity of CAT decreased significantly at all exposures to sub-lethal doses of BPA.

The CAT-SOD system is considered the first line of defense against oxyradical formation (Pandey et al., 2001). Our results are similar to those of Yilmaz et al. (2006), by. who found significantly higher SOD activity and significantly low CAT activity in the liver of carp, *C. carpio* from the contaminated waters in the Karakaya Dam Lake, Turkey. Exposure to heavy metals produced increased SOD activity and decreased CAT activity in African cat fish, *C. garienpinus* (Farombi et al*.,* 2007). Moreover, significant negative correlation was found between the SOD and CAT activities after 2.5 mg BPA/l ($r = -0.8168$), 5.0 mg BPA/l ($r = -0.4486$) and 7.5 mg BPA/l ($r = -0.6675$) exposure in our study.

The apparent increase in SOD activity in the liver of the fish may be due to the production of superoxide anions which might have led to the induction of SOD to convert the superoxide radical to H_2O_2. The increase in SOD activity indicates that more protein is required to protect against superoxide radicals, whereas a decrease in CAT activity indicates reduced ability to protect cells against H_2O_2 (Papagiannis et al*.,* 2004). The lowest activity of CAT was explained by the increased generation of H_2O_2 by SOD (Ahmad et al., 2005) or the flux of superoxide radicals which have been shown to inhibit CAT activity (Kamunde et al*.,* 2002**;** Stanic et al*.,* 2006). Low CAT activity might also be due to increased GPx activity, both of which use H_2O_2 as substrate (Yilmaz et al*.,* 2006). The responses found on SOD and CAT strongly indicated that oxidative stress might be a potential hazard for *A. testudineus* exposed to sub-lethal doses of BPA.

Glutathione peroxidase, a selenium-dependent enzyme (Orbea et al., 2000), inactivates organic ROS and H_2O_2 with reduced GSH (Ansaldo et al*.,* 2000). A significant increase of GPx activities was observed in *C. punctatus* (Pandey et al., 2001) and *O. mykiss* exposed to Pb^{2+} and Cu^{2+} (Ates et al*.,* 2008), *Anguilla anguilla* exposed to β-naphthoflavone (Ahmad et al., 2005) and in *O. mykiss* exposed to sodium selenite (Orun et al., 2005). The increased GPx activity in the present study may possibly due to the increased production and enzyme inducing effect of H_2O_2 derived from $O_2^{\cdot-}$ as previously reported by Pandey et al. (2001) in fish exposed to endosulfan. Moreover, environmental pollutants increase GPx activity progressively in response to increased H_2O_2 production (Ahmad et al*.,* 2005).

Glutathione-S-transferase activity decreased significantly in BPA exposed fish in a time and dose-dependent manner. In fish, GST is used primarily as a biomarker indicating aquatic environment pollution with wastewater of municipal, industrial, agricultural or mining origin (Tjalkens et al., 1998). Total GST activity decreased in the liver tissue in the guppy, *Poecilia reticulate* following exposure to dimethoate (Frasco et al*.,* 2002) and in Atlantic cod exposed to an estrogenic mimetic, nonylphenol (Hasselberg et al*.,* 2004a, b). This decrease in GST activity can be explained by an accumulation of metabolites of the first or the second detoxification stage that may cause the inhibition of the total GST activity (Krea et al., 2007).

The change in GPx activity observed above is generally accompanied by changes in the level of GSH, which is the co-substrate for H_2O_2 decomposition by GPx (Sies, 1999). Accordingly, GSH concentration increased in the fish exposed to BPA as compared to that of controls at all exposures. As an antioxidant, GSH plays a major role in cellular metabolism and free radical scavenging (Barata et al*.,* 2005; Athansios et al*.,* 2006).

An estrogenic mimetic, nonylphenol increased GSH in Atlantic cod (Hasselberg et al.,

2004a,b), in largemouth bass, *Micropterus salmoides* (Hughes and Gallagher, 2004) and in rainbow trout, *O. mykiss* (Uguz et al., 2003). Endosulfan exposure resulted in a significant increase of GSH levels in all the organs of *C. punctatus* (Pandey et al*.,* 2001). Exposure to heavy metals resulted in increased activity of hepatic GSH content in catfish, *C. gariepinus*, and *C. nigrodigitatus* and tilapia, *O. niloticus*, and *T. guinensis* (Doherty et al., 2010). Increase in GSH has been described as one of the protective mechanisms that fish adopt in the initial phases of exposure to aquatic pollutants (Sagara et al*.,* 1998). As intracellular non-enzymatic antioxidant, GSH status is critical to resistance of oxidative stress in cells and organisms (Biswas et al*.,* 2006; Lushchak and Bagnyukova, 2006); the apparent increase in GSH levels in the liver of *A. testudineus* suggested an adaptive and protective role of this biomolecule against oxidative stress induced by the BPA.

The previous studies conducted in our laboratory in *A. testudineus* after exposure to sewage containing estrogenic chemicals (Soorya et al*.,* 2012) and Arochlor-1245 (Amrutha et al*.,* 2014) also are comparable to the results of the current study.

In the present study, when % fragmented DNA was quantified by DPA method, significant increase in % fragmented DNA was observed only in liver of *A. testudineus* exposed to 7.5 mg/l BPA after 30 days of exposure. Accordingly, this appeared on agarose gel electropherogram as low molecular weight band with greater migration whereas no significant change was detected at any other concentrations, in which all the bands appeared as single high molecular weight intact DNA. DNA fragmentation represents an integrative response to the impact of multiple toxic and environmental factors. However, this response is dependent on both the toxicant concentration and exposure duration (Chaudhry and Jabeen, 2010).

It has been demonstrated that pollutant exposure does lead to corresponding increases in DNA damage (Steinert, 1999). Any changes to DNA may have long-lasting effects but the self-repairing capability of DNA may affect the precise interpretation of the relevant bioassays (Connell et al*.,* 1999). According to Black *et al.* (1996), the presence of high molecular weight DNA may not necessarily be a result of the exposure to low impact areas but rather, due to the induction of DNA repair mechanisms after the exposure to a highly impacted area. In fact, many authors have demonstrated that toxicant burdens or exposure duration must reach a threshold level before such repair systems can be initiated (Pavlica et al*.,* 2001; Hoff et al., 2003). The increase in DNA damage can be explained by an increase in oxidative stress (Almeida et al*.,* 2005) and lipid peroxidation (Twigg et al*.,* 1998; Potts et al*.,* 2000). Thus the imbalance in antioxidant enzymes system as observed in our study might have led to DNA fragmentation at higher concentration of BPA (7.5 mg/l, 30 d). It is assumed that at lower concentration of BPA, the induction of DNA repair mechanisms might have resulted in high molecular weight intact DNA, hence no change was observed.

In the present study, the results indicated that BPA induced oxidative stress in the liver of *A. testudineus* by decreasing the antioxidant enzymes. Hence, it is evident that the delicate balance among the antioxidant enzymes is disturbed which may lead to perturbations of redox status. The results clearly showed that BPA is a pollutant with oxidative potential. Moreover, at higher concentration of BPA exposure (7.5 mg/l 30 d), DNA fragmentation was also observed.

This study shows that fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water-borne pollutants. It is also evident that antioxidant enzyme activity levels can be utilized as molecular biomarkers of oxidative stress to investigate the mechanisms of environmental toxicity in aquatic organisms. Monitoring of the biomarkers in living organisms including fish is a validated approach and serves as early warning of adverse changes and damage resulting from chemical response.

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