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Protective responses of antioxidant enzymes against bisphenol-A induced oxidative stress in Asian Seabass *Lates calcarifer*

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Bisphenol A (BPA) is an endocrine disruptor used as plastic monomer and plasticizer widely present in the aquatic environment. In the present study, the effect of BPA on the antioxidant system in *Lates calcarifer* was documented. Three different concentrations such as 1, 10, 100 µg/l were selected and exposed to fishes for 30 days. After BPA exposure, antioxidant parameters were estimated against control. The levels of marker enzymes were assessed in the gill, muscle, liver and brain tissues. The results showed that the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxidation (LPx) were increased in treated groups than control. On the other hand, reduced glutathione (GSH) level was decreased in BPA exposed groups compared to control. Based on the results, imbalance in the antioxidant defence system clearly indicated that the BPA toxicity could lead to susceptible oxidative stress in various tissues of the fish *L. calcarifer*.

[**Keywords**: Antioxidant system, Bisphenol A, *Lates calcarifer*, oxidative stress]

Introduction

Endocrine disruptors (EDs) are the exogenous substance that interferes with the endocrine system causing harmful effect in living organisms[1]. The developing endocrine disruptors are Bisphenol A (BPA), Nonylphenol (NP) and Octylphenol (OP)[2]. BPA is used as an intermediate in the production of plastics and resins[3]. NP is used in the manufacturing of detergents, paints, pesticides and personal care products[2]. OP is involving in rubber processing to make tyres and inks[4]. Many researches evidenced the presence of BPA in aquatic environments[5].

Estuaries are the most vulnerable zone that accumulates the agricultural, industrial and urban pollution[6]. Now-a-days, industries produced large quantities of effluents[7]. Fishes are the sentinel species that could be used as a biomonitor of land-based pollution because they may accumulate hydrophobic organic compounds from water and sediments through their diets.

In animals, series of enzyme catalysed biotransformation reactions were carried out to detoxify the xenobiotics which entered to body. Hence, activation of these enzymes may provide more substantiation for pollution exposure[8]. Fishes are the major portion of fisheries; are widely used for *in vitro* toxicity assessment because of its extensive wild distribution, sensitivity to toxicants and fairly easy to perform *ex situ* experiments. In recent years, fishes acted as a potential bioindicators for environmental pollution[9]. Fishes are threatened due to aquatic pollution with long-term exposure in natural habitat develops apt biomarker for environmental contamination[10]. Alkylphenols generates 'reactive oxygen species' (ROS) in animals exposed to xenobiotics.

As first level of cellular responses, ROS homeostasis has been altered against the accumulated free radicals and the antioxidant defense cum repair systems minimize the level of damage[11]. Oxidative deterioration of polyunsaturated fatty acids was the result of lipid peroxidative damage to gill membrane and led to impaired solute and osmoregulatory function in gills[12].

The common potential antioxidants enzymes are superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase and these enzymes could be effectively used as biomarkers for contaminants.

Many researches on oxidative stress in fish focus on toxicological aspects, such as the effects of different xenobiotics on antioxidant-enzymes[13] and are crucial in the effort to counteract the oxidative stress caused by toxicants[14].

Increased oxidative stress attributes to neuro degeneration[15]. Enhanced production of ROS and reactive nitrogen species (RNS) mediated by pollutants results in oxidative and nitrative stress[16]. Seabass, *Lates calcarifer* is a commercially imperative fish species which is commonly present in seawater and also harbors estuaries and exposed to river pollution. *L. calcarifer* is able to accumulate the toxicants and so highly suitable for biomarker studies[17]. The sublethal effects of the pollutants could be monitored in aquatic organisms through the considerable alterations in biomarkers of fish captured from stressed environments[18]. In the above context, this study aims to determine the effects of BPA on oxidative stress mediated biomarker responses in brain, liver, muscle and gills of *Lates calcarifer*.

Material and methods

Seabass *Lates calcarifer*, weaned to artificial feed with the average body weight of 30 ± 1.0 g were collected from the S.K. Aquafarm, Tiruvarur, Tamil Nadu, India. Then the fishes were transported to the laboratory in aerated plastic buckets containing habitat water. Afterwards, the fishes were acclimatized for two weeks in laboratory using plastic tanks with ample aeration.

Bisphenol A (BPA) (purity) was purchased from Himedia (Himedia, India). BPA dissolved in 0.001 % dimethyl sulfoxide (DMSO) as the working solutions *viz*. 1, 10, 100 µg/l and stored at 4 °C. Fishes were segregated into 4 groups each group contains 10 fishes. Various concentrations of BPA (1, 10, 100 µg/l) were selected and exposed for 30 days. The exposure concentrations were fixed based on the sub lethal concentration of BPA in fishes like medaka[19], fathead minnow[20] and *C. riparius*[21]. The fishes reared without BPA treatment served as control group. At the end of experiment, the gill, muscle, liver and brain tissues were carefully dissected out from all treated and control groups. All the tissue samples rinsed with cold phosphate buffer saline (PBS, pH 7.0) and weighed. Then samples stored in liquid nitrogen for enzyme activity detection.

Tissue samples were dissected and homogenized using 100 mM sodium phosphate buffer (pH 7). The homogenates were centrifuged at 750 × g for 15 min and the resultant supernatant was centrifuged at 10,000 × g for 90 min. Afterwards, the supernatant was collected and utilized as source of enzyme. The whole protocol was done below 4 °C.

Superoxide dismutase was assayed following the protocol of Misra & Fridovich[22]. The activity of catalase was assayed by the method of Takahara *et al.*[23]. The level of reduced glutathione was determined in line with Moron *et al.*[24]. The activity of glutathione peroxidase was assayed by the method of Rotruck *et al.*[25]. The lipid peroxides content was measured by the procedure of Ohkawa[26].

Data obtained in the antioxidants analyses of both control and treated fishes were subjected to statistical analyses with one way analysis of variance (ANOVA) using SPSS 16.0 to determine the statistical significance between the groups.

Results

In this investigation, the antioxidant system of seabass *L. calcarifer* was assessed based on the marker enzymes such as SOD, CAT, GSH, GPx and LPx in both the control and BPA treated fishes.

In BPA treated fish *L. calcarifer*, SOD level was elevated in gill, muscle, liver and brain tissues.

Significant increase in the level of SOD at 10 µg/l BPA treated group was noted when compared with other treated groups. The maximum level of SOD 80.25 ± 3.63 unit/mg protein was recorded in liver tissue of 100 µg/l and the minimum value of SOD 3.17 ± 0.2 unit/mg protein was noticed in brain tissue of BPA treated *L. calcarifer* (Fig. 1).

Catalase level was augmented in the tested tissues of *L. calcarifer* treated with BPA. A substantial increase in the level of CAT (89.55 ± 4 unit/mg protein) was observed in liver than other tissues. Increased in catalase level based on the dose-dependent manner was inferred when the *L. calcarifer* exposed to BPA (Fig. 2).

In contrast, GSH level was gradually decreased from 5.73 ± 0.37 nmol/min/mg protein to 3.58 ± 0.33 nmol/min/mg protein in gills. 5.27 ± 0.31 nmol/min/mg protein to 3.49 ± 0.16 nmol/min/mg protein in muscle, 6.89 ± 0.53 nmol/min/mg protein to 4.07 ± 0.27 nmol/min/mg protein in liver and 9.08 ± 0.61 nmol/min/mg protein to 7.63 ± 0.44 nmol/min/mg protein in brain tissues of fishes exposed to 1 to 100 µg/l of BPA respectively (Fig. 3).

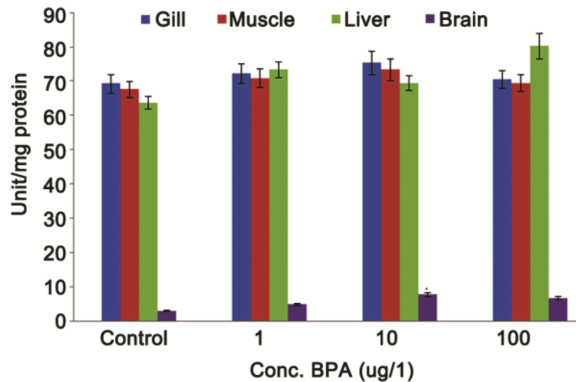

Fig. 1 — Impact of BPA on the SOD level in different tissues of *L. calcarifer*

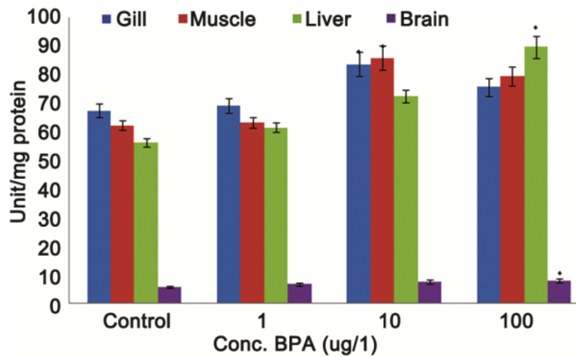

Fig. 2 — Impact of BPA on the CAT level in different tissues of *L. calcarifer*

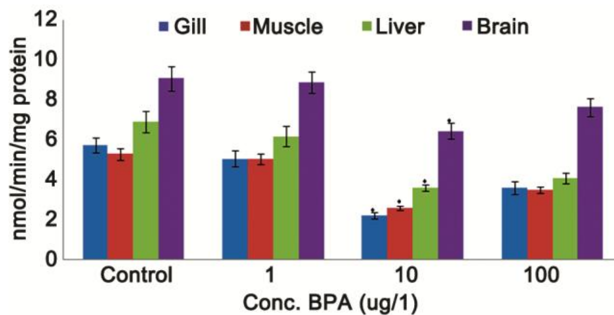

Fig. 3 — Impact of BPA on the GSH level in different tissues of *L. calcarifer*

Results clearly indicated that, the BPA exposure in seabass resulted in dose dependent decrease in GSH level. The GPx level was elevated in all the experimental groups from 1 µg/l to 100 µg/l of BPA. Moreover, the highest value of GPx (18.57 ± 1.8 µmol/min/mg protein) was registered in the liver of 100 µg/l of BPA and the least value of GPx (0.32 ± 0.03 µmol/min/mg protein) in the brain of control *L. calcarifer*. In general, the level of GPx was remarkably increased in brain tissue rather than the other tested tissues (Fig. 4).

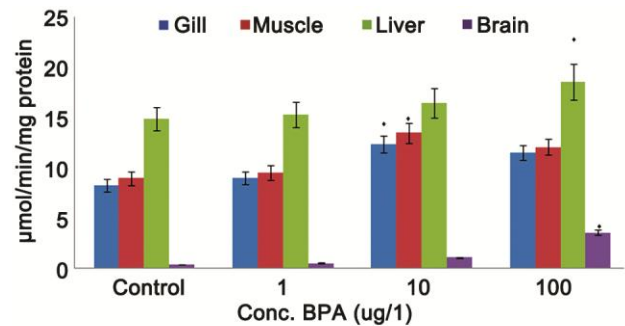

Fig. 4 — Effect of BPA on the GPx level in different tissues of *L. calcarifer*

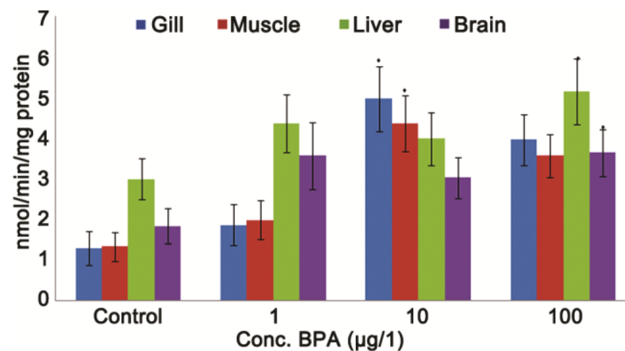

Fig. 5 — Impact of BPA on the LPx level in different tissues of *L. calcarifer*

In the BPA exposed groups, lipid peroxidase level was increased in all the test tissues. The considerable elevation in LPx level of 5.13 ± 0.81 nmol/min/mg protein was noticed in liver during 100 µg/l of BPA exposure and the lowest LPx level of 1.27 ± 0.42 nmol/min/mg protein was observed in gill of control fish. The increment in the LPx content of *L. calcarifer* was tissue and BPA dose dependent from 1 µg/l to 100 µg/l respectively (Fig. 5). Statistical analyses indicate that changes in the SOD, CAT, GSH, GPx and LPx levels in BPA treated groups differed significantly from the control group ($P < 0.05$).

Discussion

Fishes inhabiting in aquatic system are exposed to a different mixtures of contaminants, pollutants and pesticides may either overcome the threat of environmental stressors or may develop pathological conditions which alter the routine operations of the body.

The situation when the steady-state ROS concentration is acutely or chronically increased leading to oxidative modification of cellular constituents and resulting in disturbance of cellular metabolism and regulatory pathways[27]. The

mechanisms of antioxidant defences include enzyme systems that act to remove ROS, low-molecular-weight compounds that directly scavenge ROS (in animals, some produced endogenously and others obtained from the diet), and proteins that act to sequester pro-oxidants, particularly iron and copper[28].

Antioxidant enzymes are the efficient biomarker to determine the oxidative stress owing to xenobiotic toxicity. The present results demonstrate chronic revelation to sub-lethal concentration of BPA induced antioxidant enzymatic alterations in Asian Seabass *L. calcarifer*. Superoxide dismutase together with catalase and peroxidases converts ROS molecules in less reactive species restricts the formation of radical species and stands as primary layer of antioxidant enzymes.

In this study, SOD of *L. calcarifer* was hiked in the vital organs like gills, muscle and brain during 0.1 µg/l BPA exposures and the SOD was found to be augmented in liver of *L. calcarifer* during 10 µg/l BPA exposure. However, subsequent acceleration in the concentration of BPA recorded reduction in SOD content in the tested organs because of exceeding the threshold limit of BPA.

Superoxide anions are responsible for increased SOD activity[29]. Correspondingly, *in vitro* treatment of BPA resulted in increased in SOD activity in sterlet (*Acipenser ruthenus*) spermatozoa[30]. In this study, the catalase activity was registered the increasing trend on dose dependent manner based on the threshold limit of vital organs of *L. calcarifer*.

Catalase is a first active enzyme that shows changes owing to oxidative stress[31]. Various studies indicated that the catalase activity is decreased in *Channa punctatus* exposed to deltamethrin[32], in bullhead *Ameiurus nebulosus* by the heavy metal contamination[32]. Besides, reduced catalase activity in freshwater fish *Sarotherodon mossambicus* was observed due to the exposures of endosulfan and Chlorpyriphos[33]. Similarly, CAT activity was induced at 1, 10, and 100 µg/l of BPA in adult Medaka *Oryzias latipes* by Wu *et al.*[34]. Whereas, the present increment in catalase activity was supported by Rudneva-Titova and Zherko[35] who reported increase in hepatic CAT activity in some experiments with fish exposed to PCBs-containing sediments.

A significant decline in GSH level in experimental tissues under the present study may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated as a result of BPA toxicity. Accordingly, Rao[36] reported the decrease in GSH level along with the consequent increase in the concentration of organophosphate (OP) insecticide treated to *Oreochromis mossambicus*. Similarly, decline in the level of GSH was noticed in different tissues of *Ctenopharyngodon idellus* when exposed to Chlorpyrifos[37].

Accordingly the findings of Korkmaz *et al.*[38], BPA treatment resulted in decreased level of GSH in liver of male rats. Glutathione peroxidase enzymes (GPx) are widely disseminated in animal tissues neutralize the hydrogen peroxide by pairing its reduction to H_2O_2 through oxidation of reduced glutathione. GPx can also act on peroxides other than hydrogen peroxide[39]. In our results, the GPx reported to increase in dose and organ dependant manner based on its threshold limit. Similarly, the increased quantities of hydrogen peroxide and lipid peroxides were resulted in increased GPx activity[40]. The lessening of GPx activity is caused owing to the inhibition enzyme by BPA.

In this study, a remarkable increase in lipid peroxidation (LPx) in *L. calcarifer* during BPA exposure was noticed in organ wise dose dependant manner. Increased level of lipid peroxidation was recorded in common carp *Cyprinius carpio* exposed to BPA[41]. Suneetha[42] found a significant induction of LPx in the gill, kidney and liver of *Labeo rohita* treated with endosulfan and fenvalerate which may be due to reactive oxygen species (ROS). Increased LPx in *Channa punctatus* exposed to butachlor is the result of oxidative stress due to accumulation of free radicals that resulted in cellular damage[43]. Amin and Hashem[44] reported the increase in lipid peroxidation in the liver of *Clarius gariepinus* treated with deltamethrin. Elevation in the level of lipid peroxidation was observed in Cyhalothrin treated *Oreochromis mossambicus*[45].

Conclusion

Antioxidant responses in the fish *L. calcarifer* due to exposure of BPA in different organs such as gill, muscle, liver and brain were illustrated in this study. The concentration-dependent increase of superoxide dismutase, catalase, glutathione peroxidase and lipid peroxidase in all tissues was recorded. Whereas, reduced glutathione level decreased in treated groups compared to control. The oxidative stress and the defence responses were varied among the tissues owing to BPA toxicity in the fish *L. calcarifer*. Further studies are being directed in our laboratory in order to find out the molecular pathways behind the

changes in antioxidant defenses in Asian seabass, *L. calcarifer* due to BPA toxicity.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All the authors contributed equally for the experimental design, observation, analysis, manuscript preparation and correction. Also, all the authors read and approved the final manuscript.

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