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EFFECT OF ACUTE DOSES OF BASIC VIOLET-1 (BV-1) ON ANTIOXIDANT/DETOXIFICATION ENZYMES OF *Labeo rohita*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Labeo rohita fingerlings (7.6 – 11.3 cm length and 16.1 – 26.7 g weight) were exposed for 96h to 0, 0.2 (LC_{10}), 0.4 (LC_{30}), 0.6 (LC_{55}), 0.8 and 1 mg/l of Basic violet-1 (BV-1, CI No. 42535), a widely used azo dye in dyeing and textile industries. Antioxidant/detoxification enzymes such as glutathione-s-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were estimated in liver, kidney, gill, muscle and brain of the fish as markers for the stress of BV-1. After 96h, the fish were kept for a recovery period of 30 days and activity of enzymes was determined at 15 day intervals. Significant dose dependent increase over control in the activity of GR was observed in all the tissues while a significant decrease over control was observed in SOD activity in all the tissues. The results indicate that the dye is very toxic to *L. rohita* as there was a marked change in the activity of selected enzymes in the exposed fish. Gill was maximally affected tissue whereas GR was maximally affected enzyme and the effect prolonged till the end of recovery period. So this enzyme in gill can be considered as best biomarker to determine toxicity of even very low doses of the azo dye BV-1 in fish.

Keywords: *L. rohita*; basic violet-1; azo dye; brain; antioxidant enzymes; detoxification.

1. INTRODUCTION

Azo dyes are the most important synthetic dyes which are extensively used in textile, pharmaceutical and printing industries. Discharge of these dyes from various industries especially textile industries has adversely affected water bodies and aquatic organisms. Azo dyes are aromatic hydrocarbon derivatives of benzene, toluene, naphthalene, phenol and aniline. They are important group of xenobiotic

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compounds as they are recalcitrant in biodegradation processes. They pose threat to aquatic organisms as well as terrestrial animals. The environmental and subsequent health effects of dyes released through textile industry waste water are increasingly becoming subject to scientific scrutiny as several dyes and their derivatives are potent carcinogens and mutagens. Approximately, 10,000 different dyes are used in industries, and over $7x10^5$ tons of synthetic dyes are produced annually worldwide. It is estimated that worldwide these industries discharge around 280,000 tons of dyes into the environment every year [1]. During dying process, a substantial amount of azo dye is lost in wastewater. At the same time even a very small amount of dye in water (10-50 mg/l) affects the transparency and gas solubility of water [2]. Zollinger [3] reported that about 10-15% of dyes were lost in effluent during dyeing process.

Aerobic organisms generate reactive oxygen species (ROS) such as superoxide anion radical ($O2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) as a result of oxidative metabolism. $^{\bullet}OH$ can initiate lipid peroxidation (LPO) in tissues [4]. To minimize the negative effects of ROS, fish, like other vertebrates, possesses an antioxidant defense (AD) and detoxification system, which utilizes enzymatic and non-enzymatic mechanisms. Some of the most important AD enzymes are glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD), while the nonenzymatic defenses include Vitamins E, C and A, glutathione, carotenes and ubiquinol10 [5]. When the activity of these antioxidant defense systems decreases or ROS production increases, oxidative stress may occur. A large number of pollutants generally disturb the equilibrium between ROS and the AD system therefore oxidative stress biomarkers are becoming increasingly important in the field of ecotoxicology.

Antioxidant/detoxification enzymes thus play a crucial role in maintaining cell homeostasis. Their induction reflects a specific response to pollutants [6] and they have been proposed as biomarkers of contaminant-mediated oxidative stress in a variety of marine organisms, including mussels [7]. Oxidant-mediated toxicities may result if these enzymes are inhibited [8] and DNA damage (e.g. DNA adduct formation), enzymatic inactivation and lipid peroxidation may occur if the system is impaired [4, 9].

In the present study acute toxic potential of basic violet-1 (BV-1) was evaluated in *Labeo rohita*, an Indian major carp. Fish are found abundantly in the rivers of India and are cultivated at a large scale in Punjab. *L. rohita* is considered as good experimental model because they are very sensitive to vast variety of pollutants as these may enter the fish body from water and affect biochemical and physiology of fish. It is a fish of great commercial value and forms an important component of aquatic ecosystem. The purpose of this study is to select the target tissue and biomarker enzyme for the toxicity of a highly toxic Basic violet-1 dye in *L. rohita.* Levels of antioxidant/detoxification enzyme, glutathione-s-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were estimated in liver, kidney, gill, muscle and brain after 96h of exposure as an indicator of the stress of the selected lethal doses of the dye. Little work has been done on the effect of the azo dyes on antioxidant/detoxification enzymes of fish. Therefore, in the present study, it is proposed to explore these biomarkers in fish as an indicator of toxicity of acute doses of BV-1.

2. MATERIALS AND METHODS

2.1 Chemicals

All AR grade chemicals used for the present study were purchased from SRL, Sigma-Aldrich and Himedia. Azo dye, BV-1 (CI: 42535) was purchased from the local market, Amritsar, Punjab, India.

2.2 Animal Care

Fingerlings of *L. rohita* (7.6 – 11.3 cm length and 16.1 – 26.7 g weight) were collected from the ponds of Government Fish Farm, Rajasansi, Amritsar. Fish was subjected to an acclimation period of three weeks in plastic pools of 200L capacity in the laboratory. Fish were fed on Toya floating pellets during acclimation as well as experimental period except for 24h preceding exposure and during the bioassay. Tap water was dechlorinated to remove chlorine from water so that chlorine will not interfere with the results obtained and also to make it safe for fish to consume. Tap water after dechlorination was used as diluent and control, test water was changed every day. Average of the physico-chemical parameters of the water was: pH- 7.3, temperature- 27^0C, electrical conductivity- 570 µmhos/cm, total dissolved solids (TDS)- 0.1 g/l, total solids (TS)- 0.6 g/l, total suspended solids (TSS)- 0.5 g/l, dissolved oxygen (DO)- 5.2 mg/l, free CO_2- 8.5 mg/l, total alkalinity- 332.6 mg/l.

2.3 Experimental Design

10 fishes were exposed in each concentration in duplicate to 0, 0.2 (LC_{10}), 0.4 (LC_{30}), 0.6 (LC_{55}), 0.8

and 1 mg/l of BV-1 for 96h and alive fish from each concentration were kept for 30 days in tap water for recovering from the stress of the dye. The level of the enzymes was estimated in the liver, kidney, gill, muscle and brain of the fish after exposure (96h) with Systronic dual beam spectrophotometer- Genesis 10UV. Tissues were dissected out and kept in respective buffers, dried, weighed and homogenized in cold buffer. Ice was kept around the tissues to avoid heating and denaturing of the enzymes. The homogenate was centrifuged at 10,000×g for 45 min at $+4^0$C and supernatant was collected for estimation of the levels of antioxidant/detoxification enzymes. The specific activity was reported as µM/min/mg protein for GST, GR and GPx and mM/min/mg protein for CAT and U/min/mg protein for SOD.

Glutathione- S-transferase (GST) activity, with the substrate 1-chloro-2,4-dinitrobenzene (CDNB), was measured by the method of Chien and Dauterman [10]. 10% homogenate was prepared in 0.1 M sodium phosphate buffer (pH 7.6) containing 1 mM phenylthiourea (PTU). Assay was performed in a reaction mixture containing 100 µl of 95% ethanolic CDNB solution, 100 µl 50 mM GSH solution and 25 µl of crude enzyme solution with 0.1 M sodium phosphate buffer (pH 7.6) containing 0.1 mM PTU in a total volume of 1 ml. Enzyme activity was determined by monitoring the increase in absorbance at 340 nm, at intervals of 1 min for a total time of 5 min.

Glutathione reductase (GR) activity was assayed according to Carlberg and Mannervik [11], with some modification, by measuring the oxidation of NADPH at 340 nm. 20% homogenate was prepared in 50 mM potassium phosphate buffer (pH 7.6). The reaction mixture consisted of 1.2 ml of 50 mM potassium phosphate buffer (pH 7.6), 0.2 ml of 3 mM EDTA, 0.2 ml of 0.1 mM NADPH and 0.2 ml of 1 mM GSSG.

Glutathione peroxidase (GPx) activity was determined according to Flohe and Gunzler [12] by employing H_2O_2 as substrate. 20% homogenate was prepared in 0.1 M sodium phosphate buffer (pH 7.0) containing 5.0 mM EDTA. The reaction mixture consisted of freshly prepared GR solution (2.5 U ml^{-1}) in 0.1 M sodium phosphate buffer (pH 7.0) containing 5 mM EDTA, 10 mM sodium azide, 1.6 mM NADPH and 4 mM H_2O_2, 10 mM GSH. The oxidation of NADPH was followed at 340 nm at intervals of 1 min for a total time of 5 min.

Catalase was measured according to Bergmeyer [13] by measuring decrease in absorbance at 240 nm at 25^0C. 5% homogenate was prepared in 0.05 M potassium phosphate buffer (pH 7.0). The reaction mixture consisted of 30% H_2O_2 in 0.05 M potassium phosphate buffer (pH 7).

Superoxide dismutase (SOD) was estimated by the method of Kono [14] by measuring an increase in absorbance at 540 nm. 25% homogenate was prepared in 50 mM sodium carbonate buffer (pH 10.0). The reaction mixture consisted of 50 mM sodium carbonate buffer (pH 10), 20 mM hydroxylamine hydrochloride, 96 µM NBT and 0.6% Triton X-100.

2.4 Protein Measurement

Concentration of protein in the extract was measured by the method of Lowry et al. [15] with Bovine serum albumin as standard.

2.5 Statistical Analysis

Data were subjected to ANOVA for finding out the differences in the activity of enzyme before and after exposure and within the groups. Tukey test was used for finding differences among the enzyme activity of the fish. The biochemical results are reported as Mean±S.E. The differences were regarded as statistically significant when $P<0.001$ to $P<0.05$.

3. RESULTS

On exposure to the dye the fish became restless, tried to jump out of the water and the response was dose-dependent, gradually they stopped swimming and remained static in a corner of the aquarium. Intermittently fish swim unsteadily with jerky movements gulping intensity increased and fish turned upside down before mortality. Mucus secretion increased in the exposed fish and dead fish had a thick coat of mucus on the body and gills. Color of the body, gills and viscera became bluish violet on exposure to higher doses of BV-1. Exposure to the dye also brought a decline in feeding intensity during recovery period.

Values for enzyme activity after 96h exposure and on the 15^{th} and 30^{th} day of recovery period are depicted in Fig. 1-5. Enough fish was not available, so the observations could not be recorded for 0.8 mg/l concentrations after 96h exposure and 0.6 mg/l concentration on the 15^{th} and 30^{th} day of recovery period.

Significant dose dependent increase ($p<0.05$) in GST activity was observed in kidney, muscle and brain over control and significant decrease ($p<0.05$) was observed in liver and gill. After 96h exposure, highest increase and decrease in GST activity were noticed in

kidney and liver, respectively on exposure to 0.6 mg/l dye (Fig. 1A). On the 15th and 30th day of recovery period, the trend of GST activity was same for all the tissues (Fig. 1B and 1C) with liver, kidney, gill and brain showing significant (P<0.01) change on the 30th day of recovery period. The highest increase was noticed in muscle and brain on the 15th and 30th day of recovery period, respectively but maximum decline (P<0.001) was observed in gill on both these durations.

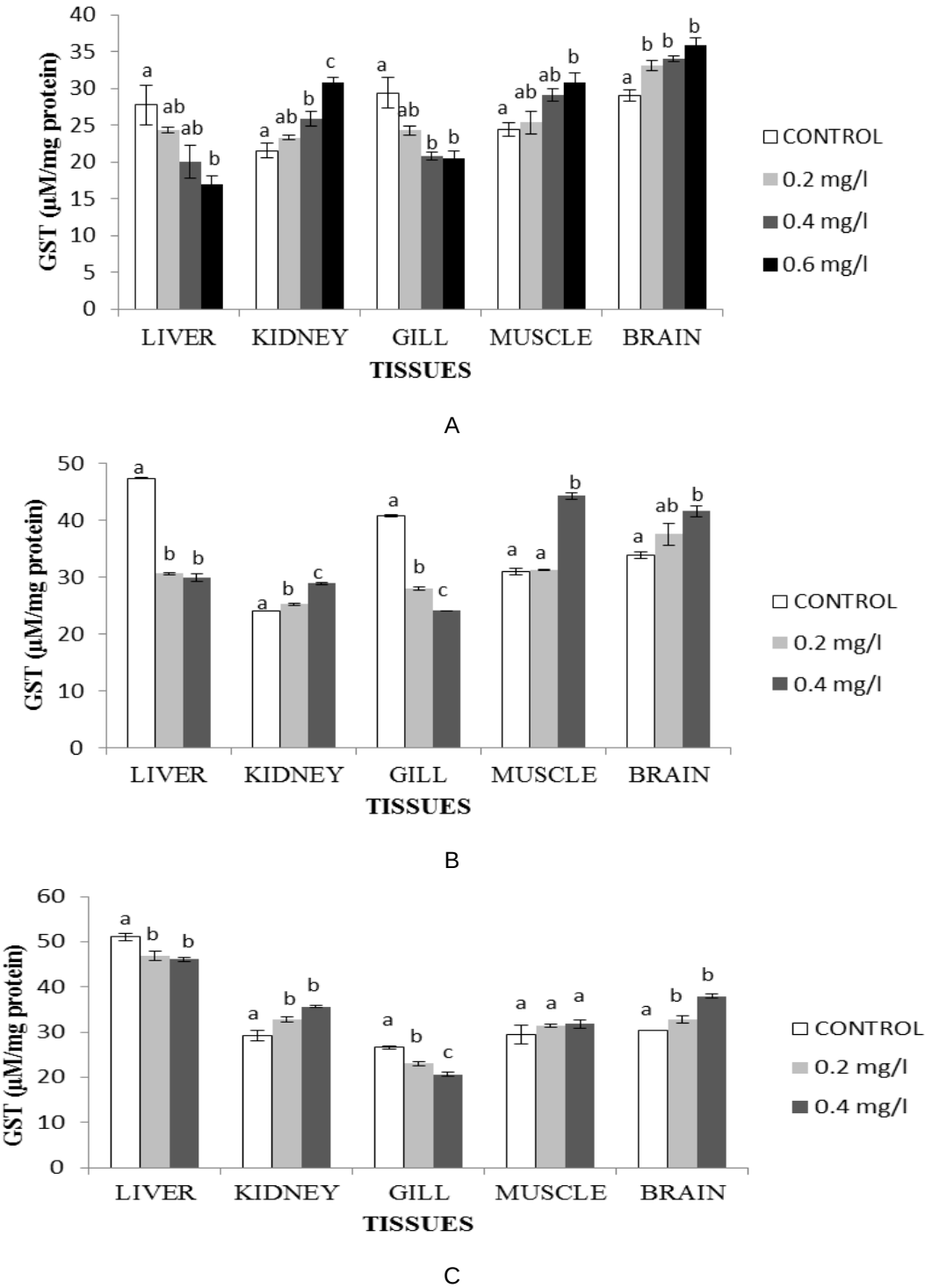

Fig. 1. Effect of BV-1 on the activity of GST (µM/min/mg protein, Mean±S.E) after 96 h exposure (A), on 15th day (B) and on 30th day of recovery period (C)

The activity of GR increased dose dependently ($p<0.001$) in all the tissues as compared to control after 96h (Fig. 2A), on the 15th ($P<0.01$) and 30th day ($P<0.05$) of recovery period (Fig. 2B and 2C). The highest increase in GR activity was noticed in gill, liver and kidney after 96h exposure, on the 15th and 30th day, respectively.

In liver, kidney and gill, the GPx activity decreases ($p<0.001$) as the concentration of the dye increases but in case of muscle and brain, the activity increased ($P<0.001$) dose dependently (Fig. 3A). The highest increase in GPx activity were noticed in muscle on exposure to 0.6 mg/l dye whereas highest decrease was noticed in liver after 96 h exposure, on the 15th and 30th day of recovery period. Same trend was observed on the 15th and 30th day of recovery period (Fig. 3B and 3C) with significant ($P<0.005$) change was observed in all the tissues except brain on the 30th day of recovery period. The highest increase was noticed in brain and muscle on the 15th and 30th day of recovery period, respectively.

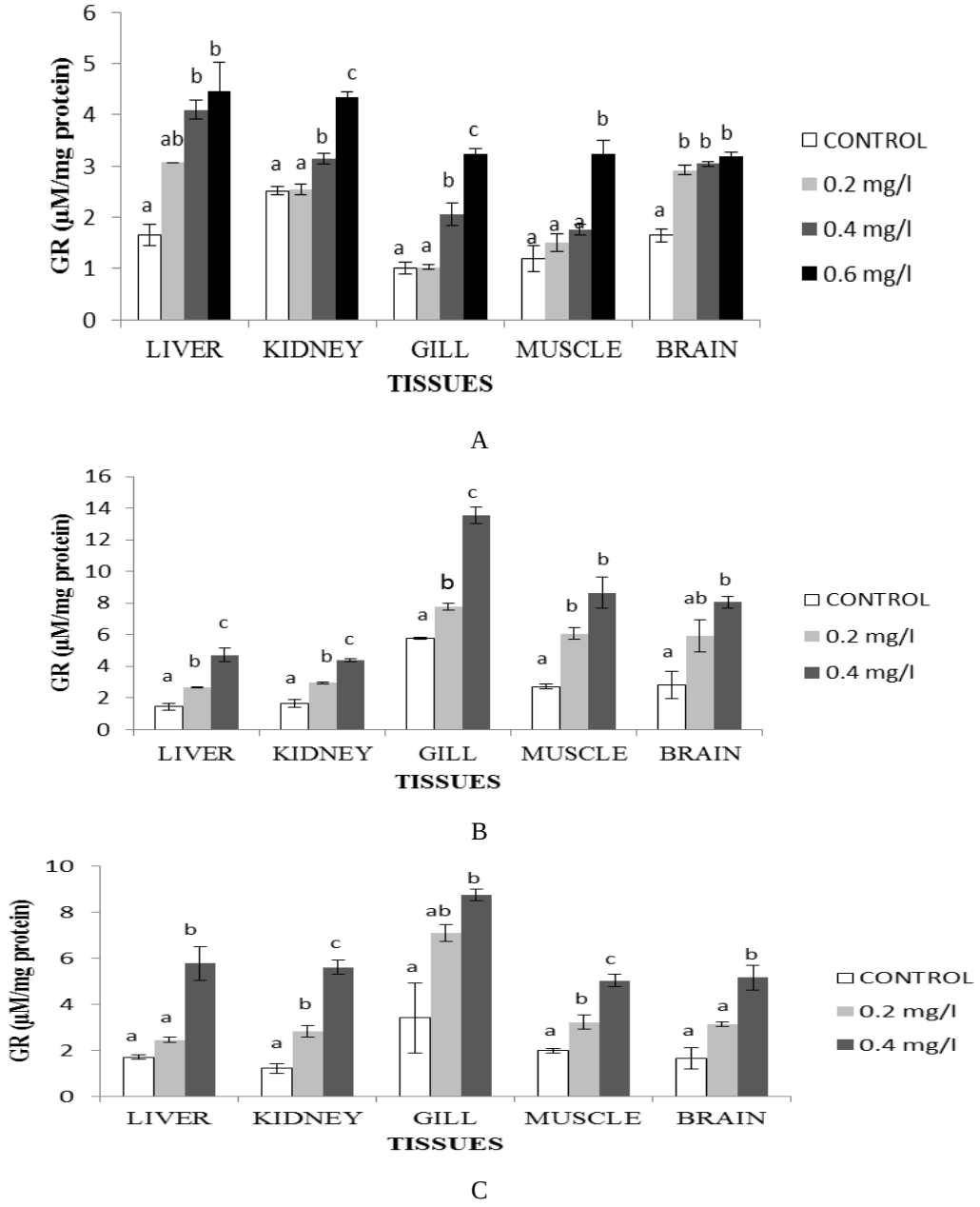

Fig. 2. Effect of BV-1 on the activity of GR (µM/min/mg protein, Mean±S.E) after 96 h exposure (A), on 15th day (B) and on 30th day of recovery period (C)

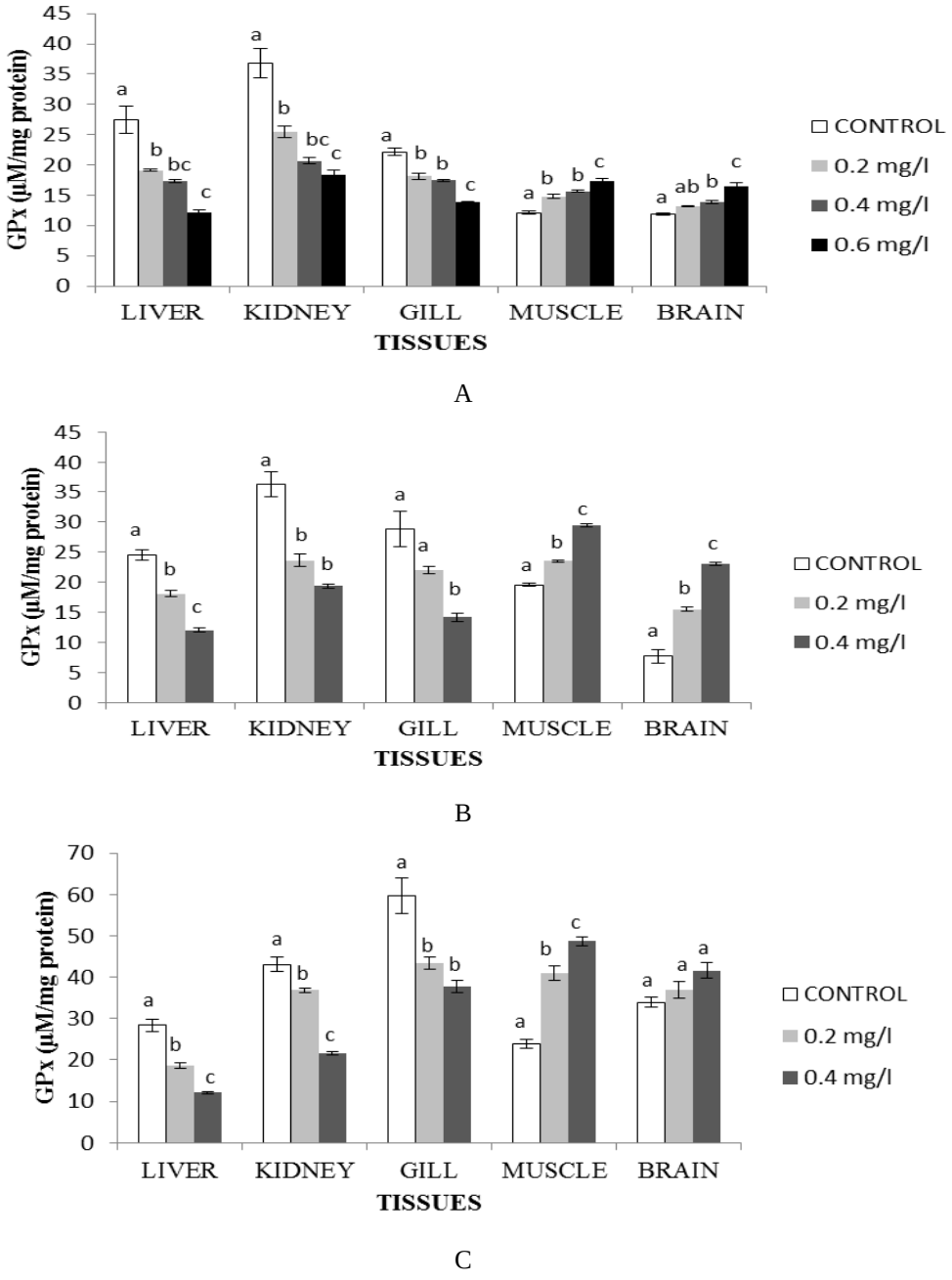

Fig. 3. Effect of BV-1 on the activity of GPx (µM/min/mg protein, Mean±S.E) after 96 h exposure (A), on 15th day (B) and on 30th day of recovery period (C)

Significant ($p<0.005$) dose dependent decrease in CAT activity was observed in gill, muscle and brain as compared to control whereas the activity of CAT in liver and kidney increased ($P<0.001$) dose dependently (Fig. 4A). The highest increase and decrease in CAT activity were noticed in liver and brain, respectively on exposure to 0.6 mg/l dye. Same trend was observed on the 15th (Fig. 4B) and 30th (Fig. 4C) day of recovery period. The highest increase ($P<0.01$) was noticed in kidney on both the recovery period durations whereas maximum decline was observed in gill and brain ($P<0.005$) on the 15th and 30th day of recovery period, respectively.

The activity of SOD decreased significantly ($p<0.05$) in all the tissues (except brain) at dose dependent manner after 96h exposure (Fig. 5A) as well as on the 15th and 30th day of recovery period (Fig. 5B and 5C, respectively) with significant ($P<0.05$) change in liver, kidney and gill only on both these durations.

The highest decrease in SOD activity were noticed in gill after 96h exposure and on the 15th day of recovery period whereas maximum decline was observed in kidney on the 30th day of recovery period.

4. DISCUSSION

Biochemical responses observed in liver, kidney, gill, muscle and brain of *L. rohita* against Basic-violet-1 are regarded as early warning indices of pollution in the environment. The toxicity of the dye prolonged for a long time as the fish was not able to recover from the stress even 30 days after the exposure. Antioxidant enzymes play important roles in adaptation to these environmental stress conditions [16]. GST is a multicomponent enzyme involved in the detoxification of many xenobiotics by conjugating with glutathione, which plays an important role in protecting tissues from oxidative stress [17]. Fish cells usually try to remove pollutants by means of GSTs which could be the reason for the increased activity of the enzymes in the present fish. An increase in the activity of GST has been observed in rat and fish exposed to azo dye [18, 19, 20]. Raised GST activity could probably be due to defensive adaptation of organisms to the presence of a variety of organic compounds in the environment [21]. GST induction has also been reported in fishes exposed to organic contaminant or pesticides [22, 23, 24]. In the present study, GST activity decreased in liver and gill of *L. rohita* on exposure to BV-1 and this may be due to the stress caused by the dye. Lower GST activity has been observed in fish exposed to DCA and textile effluents [25, 26]. GST inhibition has also been noticed in fishes exposed to pesticide [27, 28].

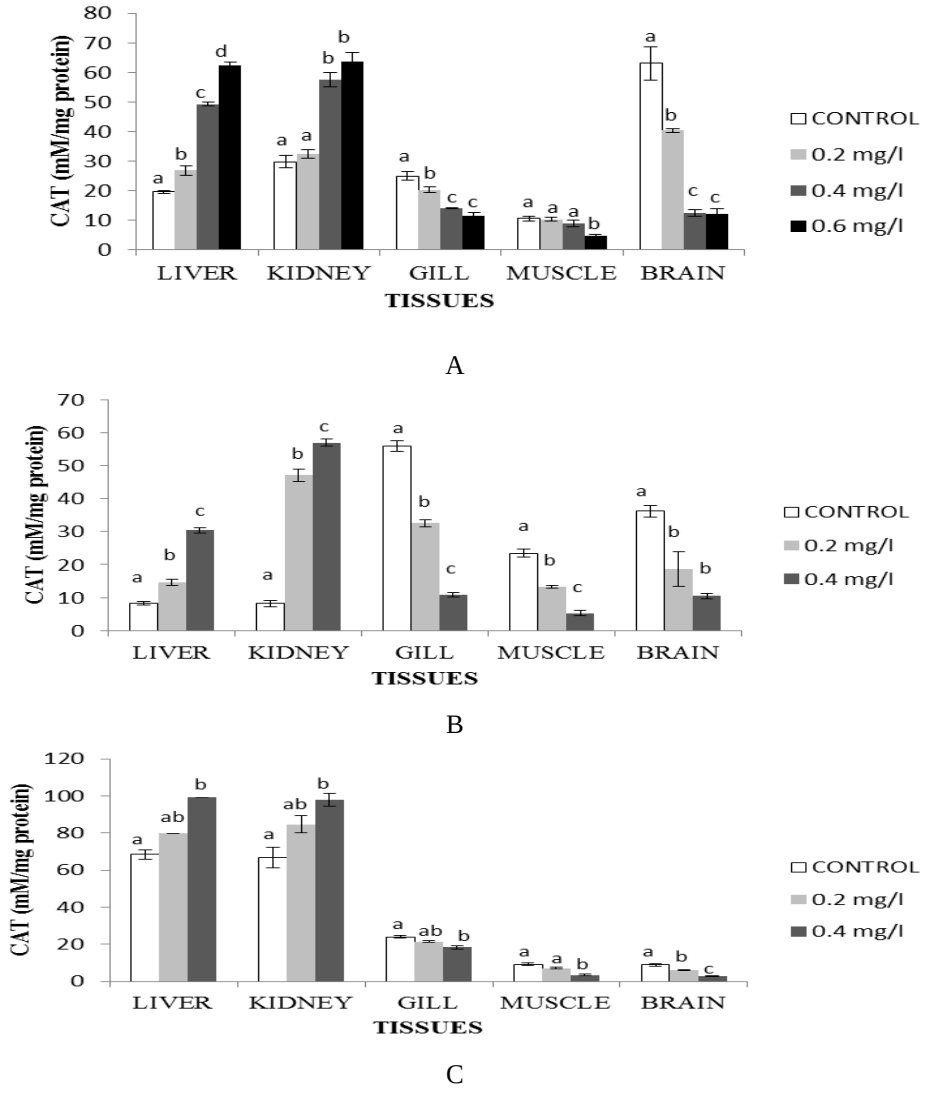

Fig. 4. Effect of BV-1 on the activity of CAT (mM/min/mg protein, Mean±S.E) after 96 h exposure (A), on 15th day (B) and on 30th day of recovery period (C)

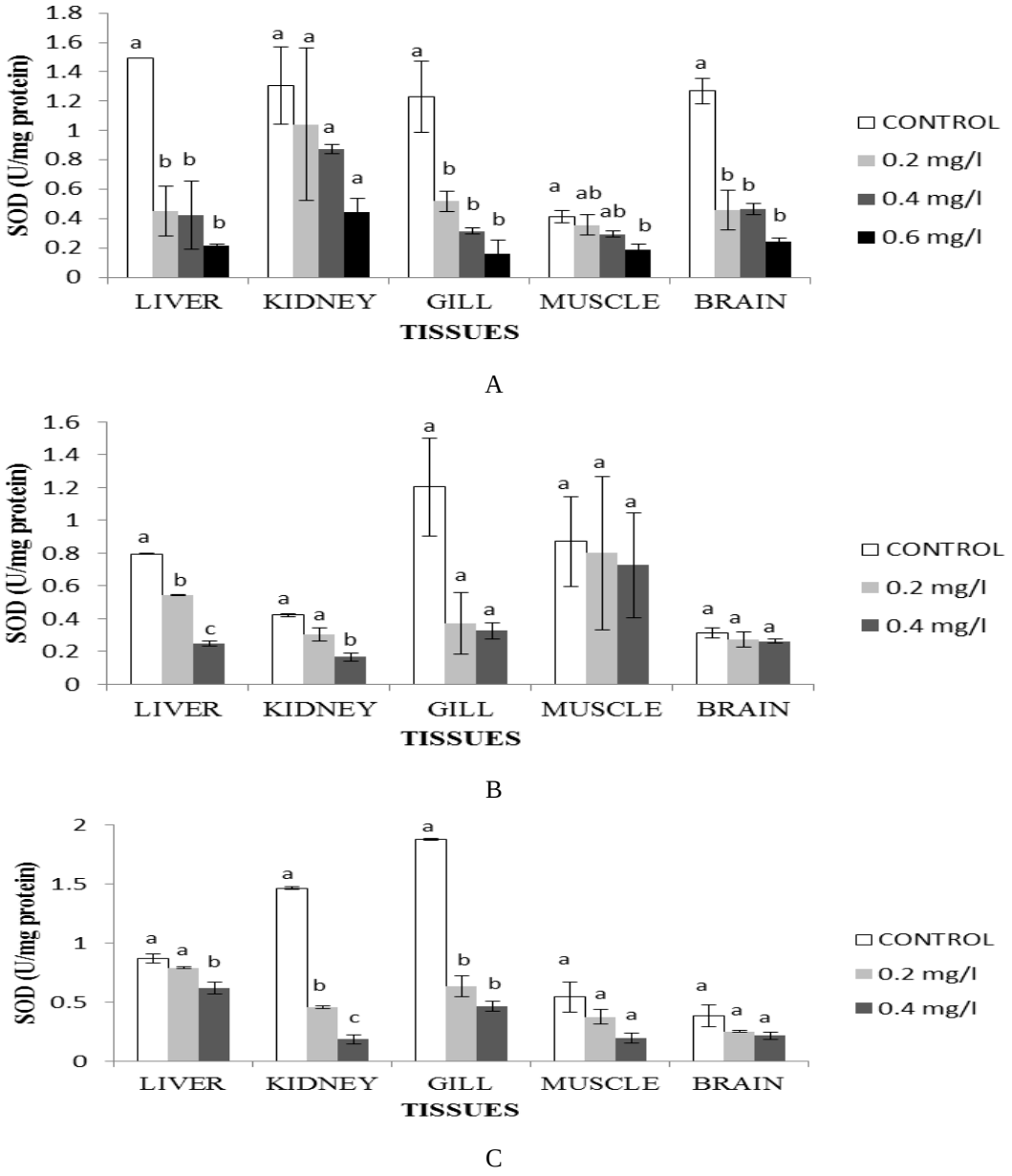

Fig. 5. Effect of BV-1 on the activity of SOD (U/min/mg protein, Mean±S.E) after 96 h exposure (A), on 15th day (B) and on 30th day of recovery period (C)

GR plays an important role in cellular antioxidant protection and adjustment processes of metabolic pathways under stress [29]. An increase in the activity of GR in the present study may be due to the stress of the dye as the increase was directly proportional to the increase in the dose of the dye. The induction of GR activity is considered a potential biomarker of oxidative stress in living organisms [8]. Oh and Lee [18] observed enhanced GR activity in liver of DAB and BHA treated rats. Fluctuations in GR activity has been reported in dye exposed fish [30]. Petrivalsky et al. [31] reported a significant increase in hepatic GR activity in rainbow trout exposed to Phenobarbital.

GPx catalyzes the reduction of hydrogen peroxide derived from oxidative metabolism as well as peroxides from oxidation of lipids and is considered the most effective enzyme against lipid peroxidation [32]. Induction of GPx was observed in muscle and brain of the fish, this indicates that the antioxidant pathway was stimulated, probably due to the increased production of peroxides [28]. The increase in GPx activity probably reflects an adaptation to the oxidative conditions to which the fish had been exposed [33]. Decreased GPx activity was observed in liver, kidney and gill of the present fish which hints towards involvement of the tissue in neutralizing the

impact of peroxides formed under the stress of the dye. A reduced GPx activity in a given tissue could also indicate a failure of the antioxidant system. Visweswaran and Krishnamoorthy [30] observed fluctuations in the GPx activity in testis of Tartrazine treated rats. Decline in GPx activity in the present fish can be correlated to reduced enzyme synthesis or more $O_2^{\cdot}$ production under the stress of the dye as suggested by Vijayavel et al. [34] in naphthalene exposed *Scylla serrate*. Santos et al. [35] discussed that the reduction of GPx activity in kidney and liver may be attributed to the longer influence of various organic and inorganic redox active contaminants.

Activity level of CAT increased significantly in liver and kidney of the present fish but decreased in gill, muscle and brain. Oh and Lee [18] observed increased CAT activity in the liver of DAB and BHA treated rats. The increased CAT activity has also been observed in *Carassius auratus* exposed to cadmium and naphthalene, phenanthrene, 3,3'-dimethylbenzidine, nitrobenzene and textile mill effluents [36, 37, 38, 20, 39]. Superoxide anion dismutation to the CAT substrate H_2O_2 [32, 40] may account for increase in CAT activity in tissues of the present fish. Decline in CAT activity was observed in *C. auratus* exposed to HC Orange No. 1 [19], *Oncorhynchus mykiss* exposed to Malachite green [41], *L. rohita* exposed to anthracene [42] and Tartrazine treated rats [30]. CAT reduction was probably due to damage to the CAT active site [43, 44].

On exposure to BV-1, the activity of SOD decreased in all the tissues of the present fish. Reduced SOD activity was observed in Tartrazine exposed Wistar rats [30]. Decline SOD activity has also been reported in *C. auratus* exposed to DMBz and nitrobenzene [38, 20], *Clarias gariepinus* exposed to textile effluents [45] and *L. rohita* exposed to anthracene [42]. SOD is inhibited by its own catalytic activity since the H_2O_2 produced by the enzyme reduces Cu^{2+} to Cu^{+} in the active site, thereby inducing a series of Fenton reactions. The end products of this cascade (Cu^{2+} $^{\cdot}OH$ and its ionized forms) attack the histidine adjacent to the active site through oxidation and inhibit the enzyme [46].

5. CONCLUSION

The present findings strongly suggest that BV-1 exposure caused a generalized oxidative stress in *L. rohita*. A short term exposure to BV-1 provoked peroxidative damage in all the tissues and gill in particular and revealed an organ specific antioxidant response involving a differential modulation of the enzyme activities. GR, CAT and SOD were affected more (change over control in all the tissues) but GR was maximally affected by the dye. Liver, kidney and gill were affected more but gill was observed to be the most sensitive tissue to the stress of the present dye. The information presented in this study will be helpful in fully understanding the mechanism of BV-1 toxicity in fish.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1 Puvaneswari N, Muthukrishnan J, Gunasekaran P. Toxicity assessment microbial degradation of azo dyes. Indian J. Exp. Biol., 2006;44(8):618-626.

2 Banat IM, Nigam P, Singh D, Marchant R. Microbial decolorization of textile-dye containing effluents – a review. Bioresour. Technol. 1996;58:217–227.

3 Zollinger H. Colour chemistry- synthesis, properties and applications of organic dyes and pigments. VCH, New York. 1987;92.

4 Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Clarendon Press, Oxford, 1989;543.

5 Wilhelm Filho D. Fish antioxidant defenses: A comparative approach. Braz. J. Med. Biol. Res., 1996;29:1735-1742.

6 Doyotte A, Cossu C, Jacquin M, Babut M, Vasseur P. Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater

bivalve Unio tumidus. Aquat. Toxicol. 1997;39:93–110.

7 Gamble SC, Goldfarb PS, Porte C, Livingstone DR. Glutathione Peroxidase and other antioxidant enzyme function in marine invertebrates (*Mytilus edulis, Pecten maximus, Carcinus maenas and Asterias rubens*). Mar. Environ. Res. 1995;39:191–195.

8 Stegeman JJ, Brouwer M, Di Giulio RT, Fo¨ rlin L, Fowler BA, Sanders BM, Van Veld PA. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. In: Huggett RJ, Kimerle R, Mehrle PM, Bergman H.L. (Eds.), Biomarkers, Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. Lewis, USA. 1992;235–335.

9 Xu L, Zheng GJ, Lam PKS, Richardson BJ. Relationship between tissue concentrations of polycyclic aromatic hydrocarbons and DNA adducts in green-lipped mussels (*Perna viridis*). Ecotoxicology. 1999;8:73–82.

10 Chien C, Dauterman WC. Studies on glutathione-S-transferase in *Helicoverpa* (Heliothis) zea. Insect Biochem. 1991;21(8):857-864.

11 Carlberg I, Mannervik B. Purification and characterization of flavoenzyme glutathione reductase from rat liver. J. Biol. Chem. 1975;250:5475-5480.

12 Flohe L, Gunzler WA. Glutathione Peroxidase. In: Packer, L. (Ed.), Methods in Enzymology. Academic press, New York. 1984; 105:115-121.

13 Bergmeyer HU. Methods of enzyme analysis. Vol I. Academic press. Inc. New York. 1974;1:438.

14 Kono Y. Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys. 1978;186:189-195.

15 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J. Biochem. 1951;193:265–275.

16 Zhu LS, Shao B, Song Y, Xie H, Wang J, Wang JH, Liu W, Hou XX. DNA damage and effects on antioxidative enzymes in zebra fish (Danio rerio) induced by atrazine. Toxicol Mech. Methods. 2011;21(1):31–36.

17 Fournier D, Bride JM, Poirie M, Berge JB, Plapp FW. Insect glutathione-s-transferases: biochemical characteristics of the major forms of houseflies susceptible and resistant to insecticides. J. Biol. Chem. 1992;267:1840-1845.

18 Oh SH, Lee MH. Effect of p-Dimethylaminoazobenzene and 2(3)-tert-Butyl-4-hydroxyanisole on lipid peroxidation, Glutathione-S-transferase, peroxidase and reductase in rat liver. Yonsei Med. J. 1981; 22(2):95-100.

19 Sun Y, Yu H, Zhang J, Yin Y, Shen H, Liu H, Wang X. Bioaccumulation and antioxidant responses in goldfish Carassius auratus under HC Orange No. 1 exposure. Ecotoxicol. Environ. Saf. 2006;63:430-437.

20 Peng S, Xing Y, Rui L, Jian-ping C. Effects of Nitrobenzene on Liver Antioxidant Defense System of Carassius auratus. Chem. Res. Chin. Uni. 2010;26(2):204—209.

21 Vander oost R, Beyer J, Vermeulen PE. Fish bioaccumulation and biomarkers in enviormental risk assessment: a review. Environ. Toxicol. Pharmacol. 2003;13:57-149.

22 Beyer J, Sandvik M, Hylland K, Fjeld E, Egaas E, Aas E, Skare JU, Goksoyr A. Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus* L.) and Atlantic cod (*Gadus morhua* L.) exposed by caging to polluted sediments in Sorfjorden, Norway. Aquat. Toxicol. 1996;36:75-98.

23 Grinwis GCM, Besselink HT, Van der Brandof EJ, Bulder AS, Engelsma MY, Kuiper RV, Wester PW, Vaal MA, Vethaal AD, Vos JC. Toxicity of TCDD in European flounder (Platichthys flesus) with emphasis on histopathology and cytochrome P450 1A induction in several organ systems. Aquat. Toxicol. 2000;50:387-401.

24 Stephensen E, Svavarsson J, Sturve J, Ericson G, Adolfsson-Erici M, Forlin L. Biochemical indicators of pollution exposure in Shorthorn sculpin (*Myoxocephalus scorpius*) caught in four harbours on the Southwest coast of Iceland. Aquat. Toxicol. 2000;48:431-442.

25 Monteiro M, Quintaneiro C, Pastorinho M, Pereira ML, Morgado F, Guilhermino L, Soares AMVM. Acute effects of 3,4-dichloroaniline on biomarkers and spleen histology of the common goby Pomatoschistus microps. Chemosphere. 2006;62:1333–1339.

26 Wenjuan, Z., Wei, L., Jing, Z., Huimin, Z., Yaobin, Z., Xie, Q. and Yihe, J. (2012), Characterisation of acute toxicity, genotoxicity and oxidative stress posed by textile effluent on zebrafish. J. Environ. Sci., 11, 1963-1971.

27 Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere. 2009;76:932–937.

28 Modesto KA, Martinez BBR. Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and Acetylcholinesterase activity. Chemosphere. 2010;81:781-787.

29 Li ZH, Zlabek V, Velisek J, Grabic R, Machova J, Randak T. Modulation of antioxidant defense system in brain of rainbow trout (Oncorhynchus mykiss) after chronic carbamazepine treatment. Comp. Biochem. Physiol. C. 2010;151:137–141.

30 Visweswaran B, Krishnamoorthy G. Oxidative Stress by Tartrazine in the Testis of Wistar Rats. J. Pharm. Biol. Sci., 2012;2(3):44-49.

31 Petrivalsky M, Machala M, Nezveda K, Piacka V, Svobodova Z, Drabek P. Glutathione-dependent detoxifying enzymes in rainbow trout liver: search for specific biochemical markers of chemical stress. Environ. Toxicol. Chem.1997;16(7):1417-1421.

32 Winston GW, Di Giulio RT. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 1991;19:137-161.

33 Lenartova V, Holovska K, Pedrajas JR, Martinez-Lara E, Peinado J, Lopez-Barea J, Rosival I, Kosuth P. Antioxidant and detoxifying fish enzymes as biomarkers of river pollution. Biomarkers, 1997;2:247-252.

34 Vijayavel K, Gomathi RD, Durgabhavani K, Balasubramanian MP. Sublethal effect of naphthalene on lipid peroxidation and antioxidant status in the edible marine crab Scylla serrate. Mar. Pollut. Bull. 2004; 48:429–433.

35 Santos MA, Pacheco M, Ahmad I. Anguilla Anguilla L. antioxidants responses to in situ bleached kraft pulp mill effluent outlet exposure. Environ. Int. 2004;30:301.

36 Shi H, Sui Y, Wang X, Luo Y, Ji L. Hydroxyl radical production and oxidative damage induced by cadmium and naphthalene in liver of Carassius auratus. Comp. Biochem. Physiol. C. 2005;140:115–121.

37 Yin Y, Jia H, Sun Y, Yu H, Wang X, Wu J, Xue Y. Bioaccumulation and ROS generation in liver of Carassius auratus, exposed to phenanthrene. Comp. Biochem. Physiol. C. 2007;145:288–293.

38 Liu H, Yu H, Giesy JP, Xu T, Zhou Y. Response of antioxidant parameters to 3,3'-dimethyl-benzidinein goldfish (carassius auratus) liver. Fresen. Environ. Bull. 2009; 18:737-743.

39 Zagal A, Mazmanci B. Oxidative stress response in Nile tilapia (Oreochromis niloticus) exposed to textile mill effluent. Toxicol. Ind. Health. 2011;27(1):81-85.

40 Vega-Lopez A, Jimenez-Orozco FA, Jimenez-Zamudio LA, Garcia-Latorre EA, Dominguez-Lopez ML. Prooxidant and antioxidant sex-linked response and its relationship to mixed oxidase function enzymes in liver of Ameca splendens, an endangered goodied fish exposed to PCBs. Toxicol. Environ. Chem., 2009;91(2):315-330.

41 Yonar ME, Yonar SM. Changes in selected immunological parameters and antioxidant status of rainbow trout exposed to malachite green (Oncorhynchus mykiss, Walbaum, 1792). Pestic. Biochem. Physiol. 2010;97:19-23.

42 Vasanth S, Ganesh A, Vijayakumar TS, Karthikeyeni S, Manimegalai M, Subramanian P. Assessment of anthracene on hepatic and antioxidant enzyme activities in Labeo rohita (Hamilton, 1822). Int. J. Pharm. Life Sci. 2012;3(5):1696-1704.

43 Kono Y, Fridovich I. Superoxide radical inhibits Catalase. J. Biol. Chem. 1982;257 :5751-5754.

44 Bainy ACD, Saito E, Carvalho PSM, Junqueira VBC. Oxidative stress in gill, erythrocytes, liver and kidney of Nile tilapia *(Oreochromis niloticus*) from a polluted site. Aquat. Toxicol. 1996;34:151-162.

45 Ayoola SO, Bassey BO, Alimba CG, Ajani EK. Textile effluent induced genotoxic effects and oxidative stress in Clarias gariepinus. Pak. J. Biol. Sci. 2012;15(17):804-812.

46 Hodgson EK, Fridovich I. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: Inactivation of the enzyme. Biochemistry. 1975;14:5294-5299.