

Responses of the antioxidant defences of *Labeo rohita* exposed to Basic violet-1 (BV-1)

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Abstract

Present study envisaged evaluating the effect of Basic violet-1 (BV-1, CI No. 42535), a widely used azo dye in dyeing and textile industries, on antioxidant enzymes of *Labeo rohita* fingerlings (7.6 – 11.3 cm length and 16.1 – 26.7 g weight). Antioxidant enzymes such as acetylcholinesterase (AChE), succinate dehydrogenase (SDH), aspartate transaminase (AST) and alanine transaminase (ALT) 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 AST (79.69%) and ALT (50.07%) was observed in all the tissues while a significant decrease over control was observed in AChE (717.43%) and SDH (173.07%) activity in all the tissues. Alterations in the activity of enzymes could probably be due to a defense against oxidative damage caused by the dye and prolonged effect till the end of the recovery period could be a metabolic adaptation of the fish to the stress of the present dye. 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 and the effect prolonged till the end of recovery period. So these enzymes in the selected tissues can be considered as best biomarker to determine toxicity of even very low doses of the azo dye BV-1 in fish.

Keywords: Antioxidant enzymes, Azo dye, Basic violet-1, Detoxification, Liver, *L. rohita*.

Article Info

DOI: [10.31018/jans.v10i4.1911](https://doi.org/10.31018/jans.v10i4.1911)

Received: October 2, 2018

Revised: November 8, 2018

Accepted: November 25, 2018

How to Cite

Kaur, S. *et al.* (2018).

Responses of the antioxidant defences of *Labeo rohita* exposed to Basic violet-1 (BV-1). *Journal of Applied and Natural Science*, 10(4): 1248-1253

INTRODUCTION

Anthropogenic activities are responsible for increasing levels of pollutants in the aquatic environment all over the world (Zaharia and Suteu, 2012). Discharge of wastewater loaded with azo dyes from textile and food industries has adversely affected water bodies. Azo dyes pose threat to aquatic organisms as well as terrestrial animals. Approximately, 10,000 different dyes are used in industries, and over 7×10^5 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 (Kumar *et al.*, 2009). During dyeing process, a substantial amount of azo dye (10-15%) is lost in wastewater (Hassaan and El Nembr, 2017). 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 (Banat *et al.*, 1996).

Molecular and cellular biomarkers measured in aquatic organisms respond rapidly to the stress caused by environmental contaminants, and can be used to assess the health status of organisms

and to obtain early-warning signals before irreversible damage occurs at a higher level of biological organization. Bioaccumulation of toxic substances triggers redox reactions generating free radicals, especially free oxygen radicals, but also other reactive oxygen species (ROS) are produced, that induce biochemical alterations in fish tissues (Woo *et al.*, 2006). To counteract the toxic effects of ROS, aerobic organisms use both enzymatic and non-enzymatic antioxidants to scavenge the free radicals. However, when ROS generation exceeds the capacity of the cellular antioxidants, it will cause oxidative stress and oxidative damage (Morena *et al.*, 2005). Therefore, oxidation-related biomarkers, including oxidative stress indices and antioxidant parameters, are used in environmental risk assessment (Li *et al.*, 2010). Changes in the levels of antioxidants have been proposed as biomarkers of contaminant-mediated prooxidant challenge in a variety of marine organisms, including fish (Regoli *et al.*, 2002). Defense systems that prevent the formation of ROS, include the antioxidant enzymes such as succinate dehydrogenase (SDH), aspartate transaminase

(AST) and alanine transaminase (ALT). Complex mixtures of pollutants cause deleterious effects on the quality of water as well as on fish and other aquatic organisms. Fish acts as an important indicator of water quality under such conditions as it remains in direct contact with water for food and oxygen and is highly sensitive to any change in its environment (Kaur *et al.*, 2013). Fish adjusts its metabolism to adapt to the altered environment, therefore biochemical markers such as enzymes, proteins and amino acids of fish have been widely used in the studies related to toxicology, ecotoxicology and pharmacology.

Fish, *Labeo rohita*, are found abundantly in the rivers of India and are cultivated at a large scale in Punjab. Levels of antioxidant enzymes, acetylcholinesterase (AChE), succinate dehydrogenase (SDH), aspartate transaminase (AST) and alanine transaminase (ALT) 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 enzymes of fish. In the present study, acute toxic potential of Basic violet-1 (BV-1) was evaluated in *L. rohita*. It will help to explore these biomarkers in the fish as an indicator of toxicity of acute doses of BV-1.

MATERIALS AND METHODS

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.

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 and 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 preceeding exposure and during the bioassay. Tap water after dechlorination was used as diluent and control, test water was changed every day.

pH, Temperature and Electrical conductivity of water were recorded by using soil and water analysis kit (Decible-DB-1203) manufactured by Decible, India Ltd. TDS, TS, DO, free CO₂ and total alkalinity were determined according to APHA (1998). Average of the physico-chemical parameters of the water was: pH- 7.3, temperature- 27°C, 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 CO₂- 8.5 mg/l, total alkalinity- 332.6 mg/l.

Experiment design: 10 fishes were exposed in each concentration in duplicate to 0, 0.2 (LC₂₀), 0.4 (LC₄₀), 0.6 (LC₆₀), 0.8 (LC₈₀) and 1 (LC₁₀₀) 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.

Liver, kidney, gill, muscle and brain of the fish after exposure (96h) were dissected out, 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°C. Supernatant was collected and antioxidant enzymes were estimated with Systronic dual beam spectrophotometer-Genesis 10UV.

Acetylcholinesterase (AChE) was estimated according to the method given by Ellman *et al.* (1961) and absorbance was measured at 412 nm. The specific activity was reported as nM/min/mg protein.

Succinate dehydrogenase (SDH) was assayed by the method of King (1967). The decrease in absorbance at 420 nm was measured corresponding to the reduction of ferricyanide. The specific activity was calculated as mM/min/mg protein.

The method given by Wilkinson *et al.* (1972) was used for measuring the activity of Aspartate transaminase (AST) and Alanine transaminase (ALT). The decrease in absorbance was recorded at 340 nm. The specific activity was calculated as U/min/mg protein.

Protein measurement: Concentration of protein in the extract was measured by the method of Lowry *et al.* (1951) with Bovine serum albumin as standard.

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.

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 15th and 30th day of recovery period are depicted in Fig. 1-4. Enough fish was not available, so the observations could not be recorded for

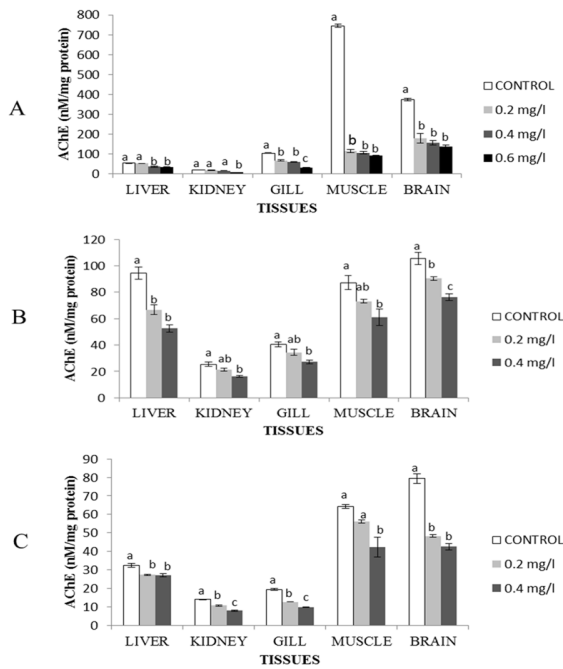


Fig. 1. Effect of BV-1 on the activity of AChE (nM/min/mg protein, Mean±S.E) after 96h exposure (A), on 15th day (B) and on 30th day of recovery period (C). Values with different superscript (a, b, c and d) are significantly different at P<0.05.

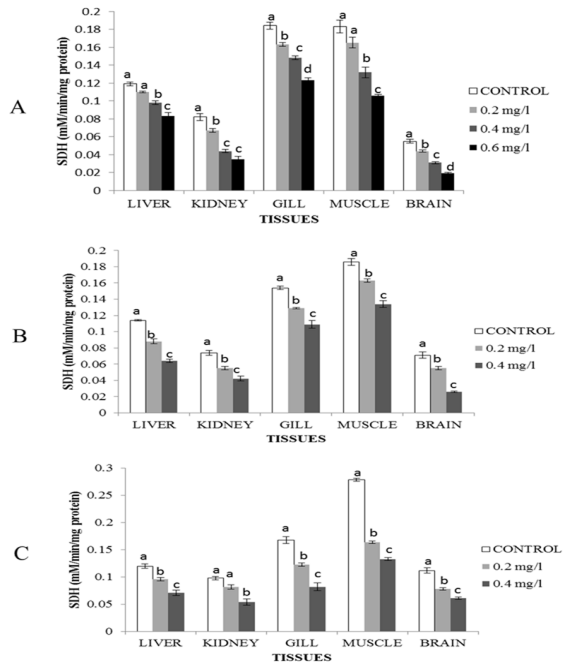


Fig. 2. Effect of BV-1 on the activity of SDH (mM/min/mg protein, Mean±S.E) after 96h exposure (A), on 15th day (B) and on 30th day of recovery period (C). Values with different superscript (a, b, c and d) are significantly different at P<0.05.

0.8 mg/l concentrations after 96h exposure and 0.6 mg/l concentration on the 15th and 30th day of recovery period.

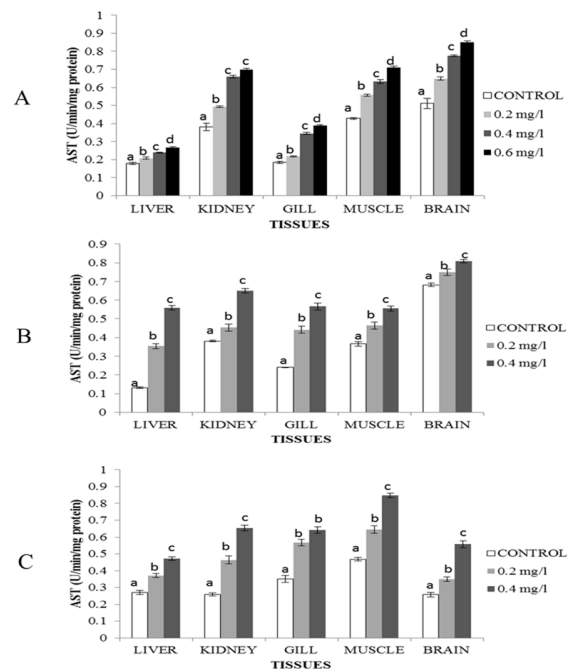


Fig. 3. Effect of BV-1 on the activity of AST (U/min/mg protein, Mean±S.E) after 96h exposure (A), on 15th day (B) and on 30th day of recovery period (C). Values with different superscript (a, b, c and d) are significantly different at P<0.05.

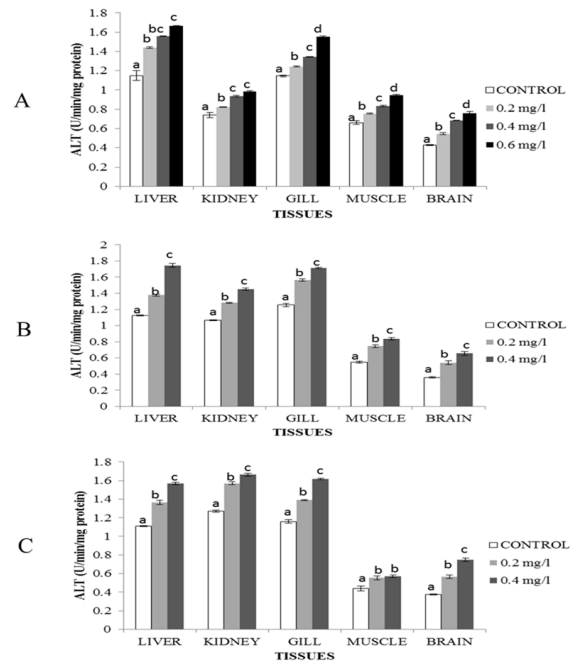


Fig. 4. Effect of BV-1 on the activity of ALT (U/min/mg protein, Mean±S.E) after 96h exposure (A), on 15th day (B) and on 30th day of recovery period (C). Values with different superscript (a, b, c and d) are significantly different at P<0.05.

The activity of AChE decreased significantly over control ($p < 0.05$) in all the tissues in a dose dependent manner after 96h exposure (Fig. 1A) as

well as on the 15th and 30th day of recovery period (Fig. 1B and 1C, respectively). The highest decrease in AChE activity was noticed in muscle (717.43%), liver (79.47%) and gill (97.96%) after 96h exposure, on the 15th and 30th day of recovery period, respectively.

The activity of SDH decreased dose dependently over control ($P < 0.001$) in all the tissues after 96h exposure to the dye and on 15th and 30th day of recovery period (Fig. 2A, 2B, 2C). The maximum decrease in SDH activity over control was observed in muscle (109.77%, 72.64%) and brain (173.07%) at 0.6 mg/l on the 30th day of recovery period and after 96h, on the 15th day of recovery period, respectively.

The activity of AST increased over control ($P < 0.001$) in all the tissues after 96h as well as on the 15th and 30th day of recovery period. Maximum increase was observed in liver (79.69%), kidney (60.40%) and gill (52.58%) on 15th day, 30th day and after 96h exposure, respectively (Fig. 3A, 3B, 3C).

A dose dependent increase over control ($P < 0.01$) in ALT activity was observed in all the tissues after 96h exposure as well as on the 15th and 30th day of recovery period. Maximum increase over control was noticed in brain (43.89%, 45.19% and 50.07%) on these durations (Fig. 4A, 4B, 4C).

DISCUSSION

Cellular oxidative stress is established when the pro-oxidant forces overwhelm the antioxidant defences. These antioxidant defences comprise enzymatic and non-enzymatic mechanisms. These systems can prevent the formation of oxyradicals or intercept oxidative propagation reactions promoted by the oxyradicals once formed (Bainy *et al.*, 1996). Since many environmental contaminants exert toxic effects related to oxidative stress, antioxidant enzymes activities are being studied as potential biomarkers in environmental risk assessment programs (Vander Oost *et al.*, 2003). In the present study, on exposure to Basic violet-1 (BV-1), biochemical responses observed in liver, kidney, gill, muscle and brain of *L. rohita* exposed for 96h indicated that 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.

A dose and duration dependent increase in bluish violet colour on the body, gills and viscera of the exposed fish indicates absorption/adsorption of present dye by the test fish. Only a slight change in the intensity of bluish violet color from the tissues till the end of the recovery period hints towards strong affinity of the dye for the tissues of the fish. Several dyes have been reported to have high affinity for the tissues of fish (Tonogai *et al.*, 1979, 1980).

AChE is a serine hydrolase whose primary role is

to hydrolyze and modulates the amount of neurotransmitter acetylcholine in cholinergic synapses (O' Brien 1967) and it is also concerned with the ionic content (Vander Kloot, 1956). Many workers have reported that dyes and their derivatives are complex inhibitors of AChE (Mansour *et al.*, 2010). The inhibition of AChE and elevation of Ach content may be due to the decreased ionic composition in the tissues of *L. rohita* (David *et al.*, 2009). Malla Reddy *et al.* (1992) suggested that the inhibition of AChE activity with a concomitant increase in acetylcholine (Ach) content in the tissues is an implication of greater disruption to the integratory activity of the central nervous system. Damage to the central nervous system might have caused uncontrolled hormonal release and the toll of an animal may be possible by the degeneration of many biochemical and physiological functions (Corbett, 1974). Inhibition of AChE in fish was accompanied by an increase in acetylcholine levels that can be dangerous since it will impact feeding capability, swimming activity, identification, avoidance of predators and spatial orientation of the species (Uner *et al.*, 2006).

SDH is an exclusively mitochondrial marker enzyme located in the mitochondrial inner membrane, is part of both the TCA cycle and respiratory electron transfer chain (Rutter *et al.*, 2010). SDH activity decreased in *Spiralothelphusa hydrodroma* exposed to textile dye industry effluent (Sekar *et al.*, 2008) and in *Labeo rohita* exposed to AB-1 dye (Kaur and Kaur, 2015). Decrease in SDH activity in the tissues of fish has also been reported on exposure to other pollutants like quinolphos and zinc (David *et al.*, 2010; Zheng *et al.*, 2011). Decline in the SDH activity in the current study may be due to an impairment of aerobic respiration. It is pointed out by Tripathi and Shasmal (2011) that utilization of lactate aerobically by the tissues was impaired on exposure of the fish to quinolphos.

Transaminases are mitochondrial and cytosolic enzymes, involved in the catabolism of amino acids and an increase in transaminase activity could either be due to their possible leakage from the cytosol across damaged plasma membrane into the general blood circulation or increase in their synthesis as a result of the organ dysfunction (Vasanth *et al.*, 2012). Amin *et al.* (2010) and Himri *et al.* (2011) observed a significant increase in AST and ALT activities in Tartrazine treated rats. A significant increase in AST and ALT activities has also been observed in *Clarias lazera* exposed to dyestuff and chemical wastewater and *L. rohita* exposed to anthracene by Abdel-Moneim *et al.* (2008) and Vasanth *et al.* (2012), respectively. Increase in the activity of transaminases may be due to either increased operation of transamination or increased synthesis of amino acids from one of sources like glucose during exposure (Tilak

et al., 2005). In present study significant dose dependent decrease over control in AChE and SDH activity while a significant dose dependent increase over control in AST and ALT activity of all the tissues of the dye exposed fish till the end of the recovery period clearly indicates that such fish will be at a greater risk if there is a slight change in their environment in future.

Conclusion

Fish is a rich source of protein but contaminated fish is a direct threat for man. At levels safe for survival pollutants cause changes in the proteins and DNA of the organisms. The changes in the antioxidant enzymes can be used as biomarkers of toxicity in the absence of mortality. The present study strongly suggests that exposure of BV-1 caused a generalized oxidative stress and damage in all the tissues of *L. rohita* and revealed an organ specific antioxidant response involving a differential modulation of the enzyme activities. AChE was affected more (change over control in all the tissues) and maximum effect was observed in liver. The information presented in this study will be helpful in fully understanding the mechanism of BV-1 toxicity in fish. This study will help in determining the consequences of direct as well as indirect exposure of man to azo dyes as the fish respond to toxins in a manner similar to higher vertebrates and form an important link in the food chain of man.

ACKNOWLEDGEMENTS

Financial support from University with potential for Excellence (UPE), University Grants Commission (UGC), New Delhi, India is greatly acknowledged.

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