SUMMARY

In the present study *L. rohita*, an Indian major carp, and an important food fish, was given acute (96h) and subchronic (150 days) exposures to an azo dye, Acid black-1 (AB-1, CI: 20470) and then kept for a recovery period of 90 days for observing the time for prolongation of the stress post exposure. The concentrations of AB-1 were 0, 4, 6, 8 and 10 mg/l dye for acute exposure and 0, 0.625, 1.25 and 2.5 mg/l dye for the subchronic exposure. During the recovery period, fish were kept in dechlorinated tap water without dye. Detoxification enzymes and enzymatic/non-enzymatic antioxidants were estimated in liver, kidney, gill, muscle and brain while proteins, free amino acids and secondary structure of protein were estimated in liver, blood and muscle at the end of 96h exposure, at 50 days intervals during subchronic exposure, and on 45th and 90th day of the recovery period. The present dye was observed to have a strong affinity for the tissues of test fish as there was a dose and duration dependent increase in the intensity of black color on the body, gills and viscera of the exposed fish. Color intensity of the tissues remained almost same till the end of the recovery periods post exposure.

The enzymatic defense system of the test fish was strongly affected by AB-1 because it caused significant changes in the activities of antioxidant enzymes as well as glutathione-mediated detoxification enzyme in all the tissues of *L. rohita*. The data clearly show that GR, GPx, CAT, SOD, AST, ALT, GST, AcP, AKP and AChE were affected more (higher change over control in all the tissues during the exposures as well as the recovery periods) by the present dye in comparison to SDH, LDH, GSH, GSSG and MDA. GR, GPx, AcP, GSSG and MDA increased while SOD, SDH, LDH, ALT and AChE decreased in all the tissues throughout the study. GST, CAT, AST, AKP and GSH did not show a constant trend on exposure to this dye. Maximum effect of the dye was observed on SOD, liver was observed to be the most sensitive tissue to the stress of the doses selected for acute exposure (4-10 mg/l dye) while brain and muscle were affected more by the doses (0.625-2.5 mg/l dye) selected for the subchronic exposure. A dose dependent decrease was observed in water soluble antioxidants of blood and muscle after both the exposures as well as till the end of the recovery periods. On the other hand, lipid soluble antioxidants increased over control in all the tissues after 96h exposure and the
recovery period following it but showed a dose dependent increase in liver and blood during the subchronic exposure and the following recovery period. AB-1 affected antioxidants of blood more than that of liver and muscle.

The protein content decreased and free amino acids increased dose and duration dependently in all the tissues after both the exposures (96h and 150 days) as well as till the end of the recovery periods. Maximum effect of AB-1 was observed on the protein content of muscle throughout the study. Concentrations of various amino acids were affected differently by the doses of the dye as tryptophan increased over control after 96h exposure while isoleucine increased throughout the recovery period following it. However, during the subchronic exposure and the recovery period following it, valine was maximum in all the tissues of the dye exposed fish.

FTIR spectra showed a shift in the peaks along with a decline in the area in the tissues of the AB-1 exposed fish. Maximum shift in the bands at ~1654 cm\(^{-1}\) and ~1450 cm\(^{-1}\) was observed for blood (1649-1660 cm\(^{-1}\) and 1448-1458 cm\(^{-1}\)) after 96h exposure to dye. However, for ~1543 cm\(^{-1}\) band it was maximum for liver (1531-1550 cm\(^{-1}\)) during the recovery period after 96h exposure. Shift in the band at ~1385 cm\(^{-1}\) was maximum in muscle after 96h exposure (1384-1412 cm\(^{-1}\)). Comparable shift in all these bands was observed after subchronic exposure to even very low doses of AB-1 but it showed slight improvement during the recovery period post subchronic exposure. Area of the bands for amide I, II and III, except for an increase in muscle (~1450 cm\(^{-1}\)) and liver (~1385 cm\(^{-1}\)), declined over control on exposure to dye and prolonged till the end of the recovery period. The trend of decline over control was ~1654 cm\(^{-1}\) (2830.07%) > ~1543 cm\(^{-1}\) (1392.86%) > ~1450 cm\(^{-1}\) (1044.87%) > ~1385 cm\(^{-1}\) (102.72%) in the dye exposed fish during the recovery period.

Data obtained in the present study showed that the activities of all the selected detoxification enzymes, enzymatic/non-enzymatic antioxidants, contents of protein and free amino acids and secondary structure of proteins were affected by AB-1. Present dye had a long lasting effect as the alterations prolonged till the end of the recovery period of 90 days. It can be concluded that SOD, valine and amide I region (~1654 cm\(^{-1}\)) in the liver and muscle can act as biomarkers to the stress of acute and subchronic exposure to
AB-1, respectively. This study will help in determining the consequences of direct as well as indirect exposure of man to AB-1 as the fish respond to toxins in a manner similar to higher vertebrates and form an important link in the food chain of man.