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Toxicological studies of methanol on superoxide dismutase (SOD) activity of freshwater fish *Cirrhinus mrigala*

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Abstract
Superoxide dismutase is an enzyme that alternately catalyzes the dismutation of the superoxide (O_2^-) radical into two less damaging species: either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. The present study was designed to evaluate the effect of Methanol on the Superoxide dismutase (SOD) activity on freshwater fish, *Cirrhinus mrigala*. Alteration in fish anti-oxidant enzyme i.e., SOD concentration was measured in tissues exposed to lethal and sublethal concentration of Methanol for 4(acute) and 30(chronic) days. LC_{50} was measured and was observed to be 12.50 ml/l. Superoxide dismutase (SOD) activity tend to increase in different tissues of exposed fish in order of Liver > Muscle > Gill > Brain at both the lethal and sublethal concentrations of Methanol. The increased SOD activity in *Cirrhinus mrigala* reflects a toxicant induced damages in fishes.

Keywords: Methanol, *Cirrhinus mrigala*, superoxide dismutase (SOD) activity

1. Introduction
Aquatic organisms inhabiting polluted water ways tend to accumulate toxic chemicals on high concentrations even when the ambient environmental concentrations are low. Environmental pollutants are becoming toxicants due to their adverse effects on living organisms. Pollutants can induce oxidative stress by generating free radicals. Fish tissues have antioxidant defense systems to protect them from oxidative stress caused by pollutants [1, 2]. Superoxide dismutase is an enzyme that alternately catalyzes the dismutation of the superoxide (O_2^-) radical into two less damaging species: either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes numerous types of cell damage. SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. Therefore, the present study was undertaken to assess the impact of Methanol on the cell reinforcement status in the different tissues of freshwater fish *Cirrhinus mrigala*.

2. Materials and Methods
Methanol CH_3OH, molecular weight 32.04 was used as the test chemical. It is available in liquid form. The required volume of the chemical was added to the test medium. The experiment was initiated by collecting a healthy set of *Cirrhinus mrigala* weighing an average of 8-10g and 10-12cm in lengths, which were procured from Government fish seed production center, Dhom, Tal -Wai, Dist- Satara. The fishes were acclimatized and exposed to lethal (LC_0-11ml/l, LC_{50}-13.05ml/l) for 96 hrs and sub lethal concentrations ($1/20^{th}$- 0.65ml/l, $1/10^{th}$-1.30ml/l) for 30 days of Methanol.
At the end of exposure period, the live fishes were sacrificed. The tissues such as gill, liver, brain, and muscle were collected, washed with distilled water, blotted and weighed before homogenization. The homogenate (10mg/ml) was prepared in phosphate buffer (P^H 7.8) and centrifuged at 10,000 rpm for 25 minutes. After centrifugation, the supernatant was taken as the source of the enzyme for estimation of SOD activity. Super Oxide Dismutase (SOD) activity was determined by method of Beauchamp and Fridovich [3]. The optical density was measured at 560nm on a UV-1800 spectrophotometer and the activity of SOD was expressed as the amount of enzyme per mg of protein. The protein content was determined by Lowry *et al.*, [4] using Bovine serum Albumen as a standard.

2.1 Statistical analyses
In the present study, obtained data was expressed as a means ± SE. The values were statically analyzed by the student t test and results were considered significant at (P<0.05), (P<0.01) and (P<0.001) level.

3. Results
Changes in the Superoxide dismutase (SOD) activity and percent change of different tissues in freshwater fish *Cirrhinus mrigala* exposed to acute exposure of Methanol are shown in Table 1 and graphically represented in figure 1.

Table 1: Effect of Methanol on the Superoxide dismutase (SOD) in various organs of the fish *Cirrhinus mrigala* after acute exposure (% inhibition)

Tissue	Control	LC_0	% change over control	LC_{50}	% change over control
Gill	20.6±1.96	23.21±2.53*	12.67	24.19±2.19*	17.42
Liver	28.23±2.56	35.02±2.70*	24.1	37.63±2.33***	33.35
Brain	14.28±1.23	15.97±1.13*	11.83	16.76±1.11*	17.37
Muscle	24.76±1.65	28.30±2.29*	14.3	32.25±1.21**	30.25

Values are the mean of (n=5) ± SD, *= P<0.05; ** = P<0.01; ***= P<0.001

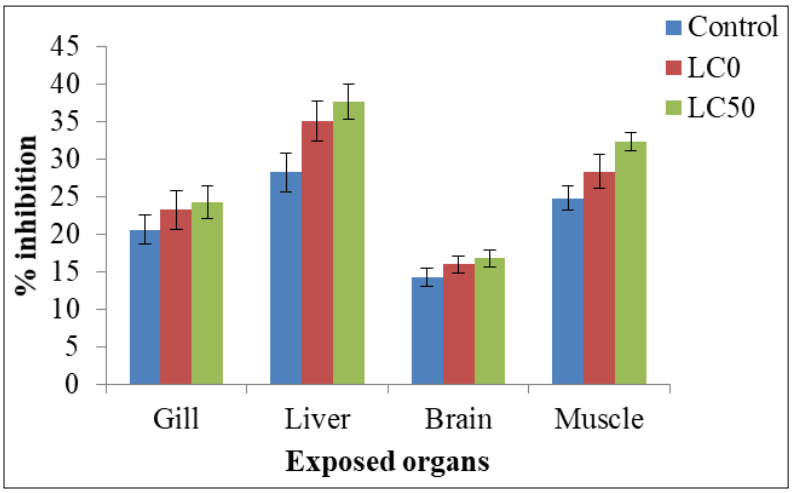

Fig 1: Effect on the Superoxide dismutase of freshwater fish *Cirrhinus mrigala* after acute exposure to Methanol.

Table 2: Changes in the activity levels of Superoxide dismutase (SOD) in different tissues of Methanol treated fish *Cirrhinus mrigala* after chronic exposure (% inhibition)

Tissue	Control	LC_0	% change over control	LC_{50}	% change over control
Gill	22.56±2.53	25.92±1.33*	14.89	28.45±2.19**	26.1
Liver	24.57±2.20	29.27±1.17**	19.12	33.37±3.47***	35.81
Brain	16.50±1.23	18.65±0.69*	13.03	20.17±3.09*	22.24
Muscle	21.23±1.14	24.68±1.11**	16.3	26.66±3.26***	25.58

Values are the mean of (n=5) ± SD, *= P<0.05; ** = P<0.01; ***= P<0.001

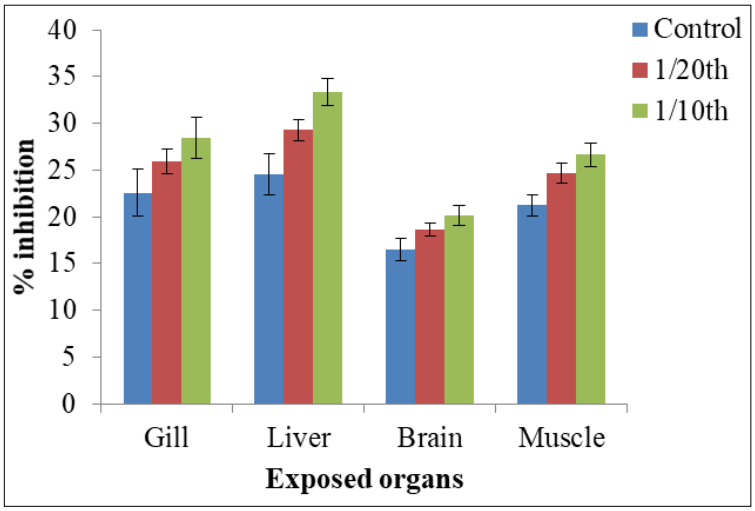

Fig 2: Effect on the Superoxide dismutase of freshwater fish *Cirrhinus mrigala* after chronic exposure to Methanol.

In acute exposure, the Superoxide dismutase activity of control fish was in the sequence of Liver > Muscle > Gill > Brain. In LC_0 group, percent change was maximum in Liver (24.1) and minimum in Brain (11.83). In LC_{50} group, maximum percent change was in Liver (33.35) and minimum in Brain (17.37) as compared to the control.

In chronic exposure, SOD activity of control was in the order Liver > Muscle > Gill > Brain. During exposure duration, maximum percent change was observed in Liver (19.12) at $1/20^{th}$ group and (35.81) at $1/10^{th}$ group. The minimum percentage was observed in Brain (13.03) and (22.24) in Gill at $1/20^{th}$ and $1/10^{th}$ group respectively as compared to the control.

4. Discussions

Superoxide dismutase (SOD, EC 1.15.1.1) is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging and is degraded by other enzymes such as catalase. Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. SOD enzymes deal with the superoxide radical by either adding or removing an electron from the superoxide molecules it encounters, thus changing the O_2^- into one of two less damaging species: either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). SOD out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin-forbidden. In biological systems, this means that its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal. The superoxide anion radical (O_2^-) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly (~10^5 $M^{-1}s^{-1}$ at pH 7). SOD is necessary because superoxide reacts with sensitive and critical cellular targets. For example, it reacts with the NO radical, and makes toxic peroxynitrite.

In the present study an increase in superoxide dismutase activity was observed when *Cirrhinus mrigala* was subjected to acute and chronic exposure to methanol for 4 and 30 days respectively. Similar result observed by Dimitrova *et al*., (1996) [5] he reported increase level of superoxide dismutase activity in *Cyprinus carpio* after exposure to zinc and lead. Studies conducted by Farombi and Adelowo (2008) [6] on *Clarias gariepinus* reported SOD activities increases in the liver and kidney treated with butachlor. Alteration in the SOD was supported by Stara *et al.* (2012) [7] who noticed similar changes in the muscle of common carp *Cyprinus carpio* treated with pesticide simazine. Increase or decrease in activity of antioxidant enzymes reflects a toxicant induced damages in fish. The increased SOD activity in *Cirrhinus mrigala* indicates an elevated antioxidant status attempting to neutralize the impact of the ROS. These findings are also supported by Andy (2013) [8] who observed a time dependent elevation in superoxide dismutase activity in *Channa striatus* exposed to 2, 4-D pesticide. Hemalatha *et al.* (2015) [9] reported sublethal effect of quinalphos on SOD activity of freshwater fish *Cyprinus carpio*.

Present study reveals the increased SOD level in gill, liver, brain and muscle tissue of Methanol treated fish indicates a detoxifying mechanism against the toxicity. Yonar Enis *et al*., (2015) [10] supports the present data an increase in SOD activity in *Cyprinus carpio* subjected to propolis. Abhijit and Poopal (2016) [11] reported elevation in activity of SOD in the Gill, Liver and Plasma of *Catla Catla* exposed to Methyl Parathion. Naz (2017) [12] observed an increase level of SOD activity in three indian major carps *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* after exposure to pesticide mixture.

5. Conclusions

From the observations of present study it can be concluded that the response of antioxidant enzyme (SOD) confirms that the fishes are under severe oxidative stress. The enzymatic and the non-enzymatic antioxidant machinery are interacting in a concentrated manner to eliminate ROS and prevent damage to cellular components. This suggests that Methanol at lethal and sublethal levels is capable of causing oxidative damage in *Cirrhinus mrigala.*

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