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Toxicological studies of Methanol on Glutathione PeroXidase (GPx) activity of freshwater fish *Cirrhinus mrigala*

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Abstract: **Toxicology is a discipline, overlapping with biology, chemistry, pharmacology, and medicine, that involves the study of the adverse effects of chemical substances on living organisms and the practice of diagnosing and treating exposures to toxins and toxicants. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. Factors that influence toxicity includes the dosage, route of exposure, species, age, sex, and environment. The goal of toxicity assessment is to identify adverse effects of a substance. Adverse effects depend on two main factors: i) routes of exposure (oral, inhalation, or dermal) and ii) dose (duration and concentration of exposure). To explore dose, substances are tested in both acute and chronic models. Methanol is an industrial chemical used after 1800s. It is a toxic. This chemical are used to produce biodiesel and also used in ethanol manufacturing industries as a denaturant additive. Glutathione peroxidase is associated with insurance against oxidative pressure, and accordingly utilizes glutathione as a substrate. Glutathione likewise goes about as a substrate in other detoxifying proteins against oxidative pressure, for example, glutathione transferees. The present study has been undertaken to understand effect of Methanol on Superoxide Dismutase (SOD) activity on freshwater fish *Cirrhinus mrigala*.**

Keywords: **Methanol, Superoxide Dismutase, *Cirrhinus mrigala*.**

1. INTRODUCTION

Toxicology is the part of science, which manages the investigation of unfriendly impacts of synthetics on living life forms. It likewise contemplates the unsafe impacts of organic, physical and substance specialists in natural frameworks. Substance poisonous quality is affected by numerous elements, for example, species, age, sex, day and age, dose and course of presentation. The point of danger assessment is to distinguish the antagonistic impacts of substance. Unfriendly impacts rely upon the courses (oral, inward breath, or dermal) and dosage (span and focus) of introduction.

Contamination with concoction contaminants is a standout amongst the most basic ecological issues of the century. Streams, lakes and other sea-going bodies are dirtied with mechanical waste water, squanders from family unit exercises and farming run offs. Unregulated development of urban regions, without infrastructural administrations for legitimate accumulation, transportation, treatment and transfer of household squanders prompts expanded sea-going contamination. Contaminations and their synthetic constancy from various mechanical territories in the earth and freshwater biological systems are looked with spatially or transiently alarmingly elevated amounts of xenobiotic synthetic compounds (Brack *et al*., 2002; Diez *et al*., 2002). A portion of these synthetics are biodegradable while others are non-biodegradable; they remain hazardously for quite a while interim. Methanol, the substance chose for present examination, is a dismal, fluid and combustible compound with the equation CH_3OH utilized as a mechanical concoction after 1800s. It is a lethal compound utilized as a dissolvable, radiator fluid and fuel. Methanol is incorporated from the damaging refining of wood and furthermore from carbon monoxide and hydrogen. Methanol is harmful, when 10 ml of the substance is devoured

incidentally by drinking, it cause visual deficiency and 100 ml results in the passing of a person. These synthetic concoctions are utilized to create biodiesel, and utilized in ethanol fabricating ventures as a denaturant added substance.

The U.S. Ecological Protection Agency (USEPA) has detailed that around 20% of the methanol is specifically released into the dirt, ground water and surface water. The different procedures in vegetation, microorganisms and other living being discharges moved methanol in the oceanic condition. Such discharged methanol adjusts the physicochemical properties of water (Balchandra and Lomte, 2001). Through the diverse exercises, huge measure of methanol goes into the regular waters which unwittingly influence harm to non-target living beings, for example, angles. Once discharged, the half existence of methanol relies upon the nature and amount of the discharge. Fishes are considered as a vital bio-marker in amphibian environments. Fishes are all of a sudden influenced by the smallest change in water contamination since it is in direct contact with the water. Fish liver and kidney are associated with detoxification, osmoregulation, biotransformation and discharge of xenobiotics (Vesey, 2010). Fishes are extremely delicate to defilement of water, in this manner; toxins may altogether adjust some biochemical and physiological systems when they go into the organs of fishes (Banaee *et al*., 2011, Shrivastava *et al*., 2004).

The Indian Major Carps are delicacy when contrasted with other freshwater angle species. *Cirrhinus mrigala*, the chose angle for present experimentation, is the benthopelagic, potamodromous, microscopic fish feeder and has a quick development rate. It flourishes in quick streaming streams and waterways. Bringing forth happens in the negligible regions of the water bodies with a profundity of 50 to100 centimeters over a sand or earth substrate amid rearing season i.e. storm season. The Cirrhinus mrigala neglects to breed normally in appropriated water bodies like lakes; henceforth they are subjected to instigate rearing (Rema Devi *et al*., 2011). *Cirrhinus mrigala* is a prominent nourishment angle. It is generally cultivated as a segment of a polyculture framework.

Glutathione peroxidase is associated with insurance against oxidative pressure, and accordingly utilizes glutathione as a substrate. Glutathione likewise goes about as a substrate in other detoxifying proteins against oxidative pressure, for example, glutathione transferees. It takes an interest in amino corrosive transport through the plasma film, rummages hydroxyl radical and singlet oxygen specifically, detoxifying hydrogen peroxide and lipid peroxides by the reactant activity of GPX. Glutathione can recover the most essential cancer prevention agents, vitamins C and E back to their dynamic structures.

Cysteine that includes a thiol gathering, glutathione is a vital intracellular non-enzymatic cancer prevention agent. Glutathione is exceptionally plentiful in the cytosol, cores and mitochondria and is a noteworthy dissolvable cancer prevention agent in cell compartments. The intracellular substance of glutathione relies upon natural factors and capacities as a harmony between its use and union. Introduction to ROS/RNS, or to mixes which can produce ROS, can build the substance of GSH by expanding the rate of GSH amalgamation. Essentially, GPX contends with catalase for H_2O_2 as a substrate. Glutathione redox cycle is a noteworthy wellspring of insurance against gentle oxidative pressure, though catalase turns out to be progressively imperative in assurance against extreme oxidative pressure.

2. MATERIALS AND METHODS

There are no major threats recognized for the species. There is no conservation efforts directed towards this species, nor is one required due to abundance of population. Hence for the present study freshwater fish *Cirrhinus mrigala* is selected. Analytical grade Methanol (CH_3OH, Ranbaxy, India; molecular weight 32.04; weight per milliliter at 20ºC is 0.790 - 0.793 g) was used as the test chemical. It is available in liquid form and was treated directly to the test medium. Required volume of the chemical was added to the test medium.

Estimation of Glutathione PeroXidase (GPx) by Beers and Sizer method (1952):

Estimation of Glutathione peroxidase was carried out with the help of method described by Beers and Sizer method (1952). Tissue was homogenized in phosphate buffer (0.067M, P^H 6.8) and centrifuged at 10,000rpm for 15 minutes. The supernatants were used as an enzyme source for determination of GPx activity. For the assay, 3 ml of 0.067M phosphate buffer (H_2O_2) were pipette out into the experimental cuvette, 0.05 ml of the sample and 0.01ml sodium azide (1mM) was added, mixed with a glass rod. The change in absorbance was measured after 30 seconds up to 3 minutes on UV-1800 spectrophotometer.

Statistical Analysis:

The values are expressed and were recorded and percent changes were calculated as compared with control. Results of the study were statistically analyzed and levels of significance were determined by using Student‘t’ test.

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3. RESULTS

Glutathione PeroXidase (GPx)

Acute exposure:

The observed mean and percent change values for Glutathione peroxidase in different tissues of fish *Cirrhinus mrigala* are given in Table and graphically represented in Figure.

Control group:

In control fish *Cirrhinus mrigala*,the Glutathione peroxidase was in the orderofLiver > Muscle > Brain > Gill.

Experimental group:

After exposure, there was significant incline in Glutathione peroxidase in the order of: Liver > Muscle > Brain> Gill in both LC_0 and LC_{50} group. During duration of exposure, Glutathione PeroXidase ranged within 5.17 <5.86 in Gill, 8.20<10.98 in Liver, 5.44<6.45 in Brain and 6.99<8.79 in Muscle. Due to LC_0 exposure, there was highly significant ($P<0.001$) activity observed in Liver (20.73) and Muscle (16.3) while significant activity was observed in Brain (11.39) and Gill (5.42). After exposure to LC_{50}, Glutathione PeroXidase significantly increased in Liver (33.9) followed by Muscle (25.75), Brain (18.56) and Gill (13.35).

In LC_0 group, percent change was maximum in Liver (20.73) and minimum in Gill (5.42). In LC_{50} group, maximum percent change was in Liver (33.9) and minimum in Brain (13.35) as compared to the control. In general, the Glutathione peroxidase was significant in Gill, Liver, Brain and Muscle tissues of *Cirrhinus mrigala* as compared with control after acute exposure to Methanol.

Table 1: Effect of Methanol on the total Glutathione PeroXidase (GPx) in various organs of the fish *Cirrhinus mrigala* after acute exposure

(µmol / mg protein/ min)

Sr. No.	Organs	Control	Exposure of Methanol	
			LC_0	LC_{50}
1.	Gill	5.17±0.074	5.45±0.20* (5.42)	5.86±0.07** (13.35)
2.	Liver	8.20±0.15	9.90±0.07*** (20.73)	10.98±0.12*** (33.9)
3.	Brain	5.44±0.61	6.06±0.48* (11.39)	6.45±0.55* (18.56)
4.	Muscle	6.99±0.42	8.13±0.25*** (16.3)	8.79±0.10*** (25.75)

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

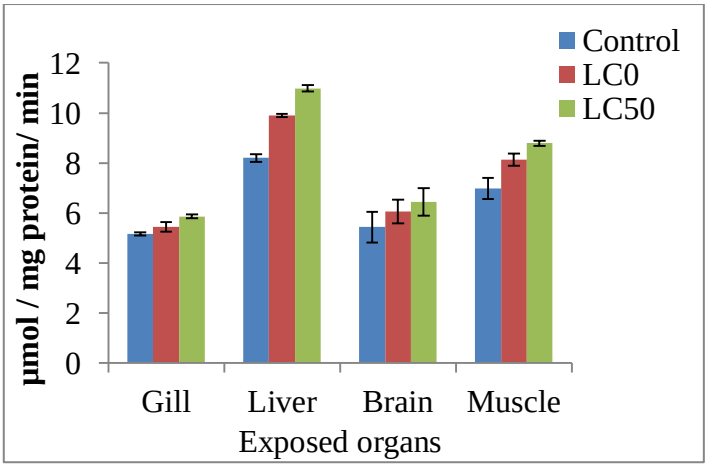

Figure 1: Effect on the Glutathione PeroXidase (GPx) of freshwater fish *Cirrhinus mrigala* after acute exposure to Methanol.

4. DISCUSSION

The level of toxicant in fish tissues is influenced by many factors such as biotic, abiotic and environmental which influence on fish growth and mortality rate. The fish species, age of fish, habitat, concentration of toxicant, exposure period, temperature, salinity of water, pH in water, dissolved oxygen in water and other physiological conditions modify toxicity to the fish.

Glutathione peroxidase

Glutathione peroxidase (GPx) (EC1.11.1.9) is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipidhydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Several isozymes are encoded by different genes, which vary in cellular location and substrate specificity. Glutathione peroxidase 1 (GPx1) is the most abundant version, found in the cytoplasm of nearly all mammalian tissues, whose preferred substrate is hydrogen peroxide. Glutathione peroxidase 4 (GPx4) has a high preference for lipid hydroperoxides; it is expressed in nearly every mammalian cell, though at much lower levels. Glutathione peroxidase 2 is an intestinal and extracellular enzyme, while glutathione peroxidase 3 is extracellular, especially abundant in plasma.

During present study, glutathione peroxidase activity was found to be increased significantly ($p<0.05$) in the gill, liver, brain and muscle tissue of fish *Cirrhinus mrigala*. Ahmad et al., (2000) reported a time dependent increase of GPx activities in freshwater catfish *Channa punctatus* in response to paper mill effluents. Significant induction in the level of glutathione peroxidase was observed in the liver of *Cyprinus carpio* after exposure to heavy metals reported by Vinodhini and Narayanan (2009). An increase in glutathione peroxidase activity was observed in different fishes such as *Clarias gariepinus*, *Cyprinus carpio*, *Cyprinus carpio* by (Farombi *et al*. 2007; Vinodhini and Narayanan 2009; Jastrzebska 2010). Monteiro *et al*., (2010) observed increase activity of glutathione peroxidase in gill, liver, muscle and heart of mercury chloride stressed fish *Brycon Amazonicus*. Present results are also parallel with the findings of Jastrzebska (2010) who reported increase in peroxidase activity in lead stressed fish *Cyprinus carpio* than the unstressed fish. The present results are in-conformity with the findings of Baysoy *et al*. (2012) who observed that lead stressed *Oreochromis niloticus* fish exhibited significantly increase glutathione peroxidase activity than the control fish. Peroxidase can act as a scavenger to reduce the harmful effects of ROS and converts the H_2O_2 into water and oxygen stated by Aruljothi and Samipillai (2014). Rahimikia (2017) also observed increased activity of glutathione peroxidase in the tissues of nickel exposed goldfish.

Li *et al*., (2003) observed increase level of superoxide dismutase, catalase and glutathione peroxidase activities in *Cyprinus carpio* exposed to microcystin. Sanchez *et al*., (2005) observed increase level of enzyme activities of the SOD, CAT and GPx in liver of *Gasterosteus aculeatus* exposed to copper. Present results are also parallel with the findings of Farombi *et al*., (2007), who reported that lead stressed fish *Clarias gariepinus* exhibited significantly higher glutathione peroxidase activity than the control fish. Ruas *et al*., (2008) showed increase in GPx and SOD activities in *Oriochromus niloticus* exposed to polluted river.

Monteiro *et al*., (2010) recorded increase in SOD, CAT and GPx activity in liver, gill and muscle of *Brycon amazonicus* exposed to mercury chloride. Jastrzebska (2010) found that lead exposed *Cyprinus carpio* exhibited significantly higher peroxidase activity than that of control fish.The present result are parallel with Li *et al*., (2011) who reported that fungicide propiconazole stressed fish *Oncorhynchus mykiss* exhibited significantly higher SOD, CAT and GPx activity than that of the control fish. Baysoy *et al*., (2012) observed a significant increase in the glutathione peroxidase activity in the Liver of *Oreochromis niloticus* exposed to Lead. Abedi *et al*., (2013) who observed that significantly higher peroxidase activity in the lead exposed fish *Cyprinus carpio* than that of unstressed fish. Increased activity of peroxidase in the liver of Methanol stressed *Cirrhinus mrigala* may be explained as a defensive mechanism against oxidative stress, which was similar to the findings of Vinay and Yadav (2014) who reported that the liver is a major organ for the production of antioxidant enzymes and therefore protects organisms from oxidative stress. The present results are inconformity with the earlier studies on the fish *Cirrhinus mrigala* exposed to lead chloride Raza *et al*., (2016) who reported a significant increase in glutathione activity under chronic exposure.

5. SUMMARY AND CONCLUSION

Toxicology is the branch of biology, which deals with the study of adverse effects of chemicals on living organisms. It also studies the harmful effects of biological, physical and chemical agents in biological systems. Chemical toxicity is influenced by many factors such as species, age, sex, time period, dosage and route of exposure. In acute toxicity experiments the freshwater fish *Cirrhinus mrigala* were exposed to organic solvent Methanol which showed that experimental fishwas sensitive to Methanol. In the present study, the 96 hr LC_{50} value of Methanol to was observed to be 12,250 mg/L with 95% confidence limit ranging between 14,230 to 11,270 mg/L.

Effect of Methanol on the antioxidant enzyme activity of fishes is one of the least studied aspects. In the present study GPx activities of fishes were increased on the exposure of acute concentrations of Methanol. This increase was more significant in liver as compared to muscle, gill and brain at both lethal and sublethal concentrations. Increased Glutathione peroxidase activity in the liver, muscle and intestine of Methanol stressed *Cirrhinus mrigala* may be because of a defensive mechanism against oxidative stress.

Alterations' in GPX activity, confirms the sensitivity and severity of damage caused to various tissues of experimental fish *Cirrhinus mrigala* to toxicant Methanol.

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