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Heavy metal lead nitrate induced toxic effects on enzymatic and non-enzymatic antioxidant activities in selected tissues of the fish *Labeo rohita* (Hamilton, 1822) fingerlings

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Abstract
The objective of the present study is to know the effect of lead nitrate on enzymatic and non-enzymatic antioxidant activities in selected tissues of the fish *Labeo rohita* fingerlings (10 ± 2g). The fish were exposed to a sub-lethal concentration of 1.56mg L^{-1} ($1/25^{th}$ of 96 hrs Lc_{50} value of lead nitrate) for a period of 60 days. At end of experimental period, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in gill, liver, muscle and kidney tissues were assayed. The levels of oxidative stress biomarkers were found to be decreased significantly ($p<0.01$ and $p<0.05$) in gill, liver, muscle and kidney of the test fish. The changes in these parameters were intensified as period of exposure increases.

Keywords: lead nitrate toxicity, *labeo rohita* fingerlings, SOD, CAT, GSH

Introduction
In the most recent decades, most of the country is undergoing a rapid industrial development, urbanization, construction, mining activities and deforestation. These activities may contribute to the environmental problem such as land, air and water pollution. Water pollution is a major problem across the globe with the presence of harmful contaminants in the environment. Contaminants in aquatic systems, including heavy metals stimulate the production of reactive oxygen species (ROS) that can damage fishes and other aquatic organisms. These organisms have evolved a variety of defense mechanisms, including detoxification and antioxidant systems, which protect against environmental stress.

To counteract the toxic effects of ROS, aerobic organisms use both enzymatic and non-enzymatic antioxidants but when ROS generation exceeds the capacity of the cellular antioxidants, it causes oxidative stress and oxidative damage. Therefore, oxidation-related biomarkers, including oxidative stress indices and antioxidant parameters are used for environmental risk assessment (Li *et al.*, 2010) [25] and implementation of programs to control pollution.

The Antioxidant enzymes play a crucial role in maintaining cellular homeostasis. Antioxidant enzymes, such as SOD and CAT, the protein GSH act by detoxifying the ROS generated. Therefore, antioxidant enzymes are considered as sensitive biomarkers in environmental stress before hazardous effects occur in fish, and are important parameters for testing water for the presence of toxicants (Heath, 1987; Geoffroy *et al.*, 2004) [17, 14].

The heavy metal lead can deplete major antioxidants in the cell, especially thiol-containing antioxidants and enzymes, and can cause significant increases in an ROS production, followed by a situation known as ‘‘oxidative stress’’ leading to various dysfunctions in lipids, proteins and DNA (Ercal *et al.*, 2001) [12]. Antioxidants present in the cells, such as glutathione (reduced), give their reducing equivalents to ROS in order to make them stable and as such protect the cell from free radicals such as H_2O_2. However, under the influence of lead, the level of ROS increases while that of antioxidants decreases. As part of the antioxidant enzymes such as GPx, CAT, and SOD, which depend on trace elements and prosthetic groups to accomplish the enzymatic detoxification of ROS, they are also potent targets to Pb toxicity (Ahamed and Siddiqui, 2007) [1].

Fish are also known to accumulate any pollutants directly from the polluted water and indirectly from the food chain (Jabeen *et al.*, 2016; Chaudhry and Jabeen, 2011) [7, 19]. *Labeo rohita* is a widely consumed teleost fish that commonly inhabits native aquatic bodies. The higher consumer preference and market demand for *rohu* during recent years have led to an increased production by aquaculture in India. Hence, the aim of this present study is to assess the lead nitrate induced toxicity on enzymatic and non-enzymatic antioxidants activity in the fish *Labeo rohita* fingerlings.

Materials and Methods
Test chemical and stock solution
Stock solution was prepared using analytical grade Lead Nitrate [Pb $(NO_3)_2$]. Since nitrate also cause toxicity, Lead Nitrate as such was used as a test chemical. 10gm of Lead Nitrate was dissolved in1lit of distilled water and the required concentrations of the test media were prepared using chlorine free tap water as diluents. Stock solution was prepared every day.

Experimental design
The fingerlings of the freshwater fish *Labeo rohita*, weighing 10 ± 2gms and length 7±1cm were procured from Venkatesh fish farm, at Pinnalur and transferred to the fibrous tank in the Department of Zoology, Annamalai University. The water in

the tank was aerated twice a day, the fish were fed daily with ground nut cake and rice bran. The physic-chemical properties of water used for experiments had pH 7.4± 0.2, dissolved oxygen 6-7 ml /l, hardness 160 ppm and temperature 28 ± 1ºC. Before experimentation has been executed, the fish were acclimated to the laboratory conditions for a period of 15 days. Later, a groups of 50 fish were exposed to the sub-lethal concentration of 1/25th of 96 h LC_{50} value of lead nitrate (i.e.; 1.56mg/lit) and a control group was also maintained for a period of 60 days. After the periods of 15, 30, 45 and 60 days of exposure, six individuals from control and treated groups were sacrificed and gill, liver, muscle and kidney tissues were removed for enzymatic analysis.

Assay of Superoxide dismutase (SOD) activity in tissue

SOD activity was assayed by the method of Kakkar *et al.* (1984) [23]. The assay of SOD was based on the inhibition of formation of NADH-phenazine-methosulphate-nitroblue tetrazolium formazone. The reaction was initiated by the addition of NADH. After incubation for 90 seconds, the reaction was stopped by the addition of glacial acetic acid. The color formed at the end of the reaction was extracted into butanol layer and measured.

Estimation of catalase (CAT) in tissue

The activity of catalase was determined by the method of Sinha (1972) [35]. Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the pressure of hydrogen peroxide. The chromic acetate formed was measured at 620 nm.

The catalase preparation was allowed to split hydrogen peroxide for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate/acetic acid mixture and the remaining hydrogen peroxide as chromic acetic acid is determined calorimetrically.

Estimation of reduced glutathione (GSH)

The level of reduced glutathione was determined by the method of Beutler and Kellay (1963) [6]. This method was based on the development of yellow color when dithio-dinitro bis benzoic acid (DTNB) was added to compounds containing sulfhydryl groups.

Statistical analysis of data

The data is expressed as mean ± Standard deviation (SD). Statistical comparisons were performed by one-way analysis of variance followed by Duncan's Multiple Range Test. The results were considered statistically significant if the P values were less than 0.01 and 0.05

Results

The results of this present investigation showed a decreasing trend in the level of SOD, CAT and GSH in the test fish after exposure to the sub-lethal concentration of lead nitrate after 60 days of exposure. A significant decrease of P<0.01 level was observed in all the studied antioxidant on 15 days of exposure period and a significant decrease of P<0.05 level was noticed during 30, 45 and 60 days of exposure periods (figs.1 to 3).

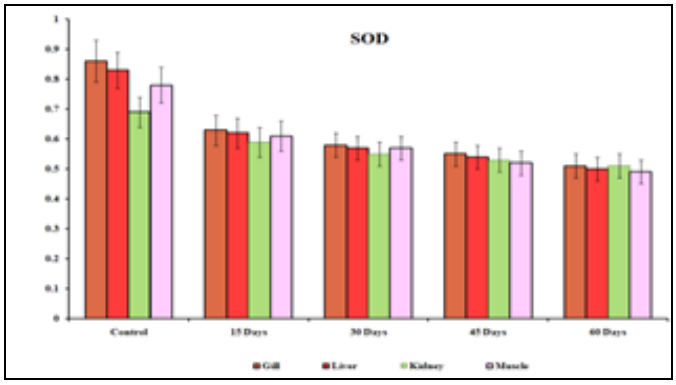

Fig 1: Effect of lead nitrate on Superoxide dismutase (SOD) activity in gill, liver, kidney and muscles of *Labeo rohita* (The values are mean ± S.D for six individuals).

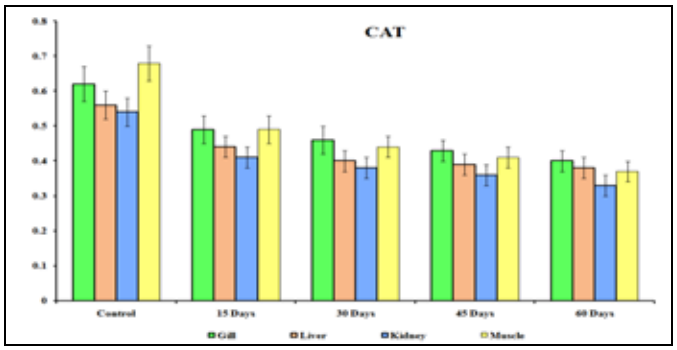

Fig 2: Effect of lead nitrate on catalase (CAT) activity in gill, liver, kidney and muscles of *Labeo rohita* (The values are mean ± S.D for six individuals).

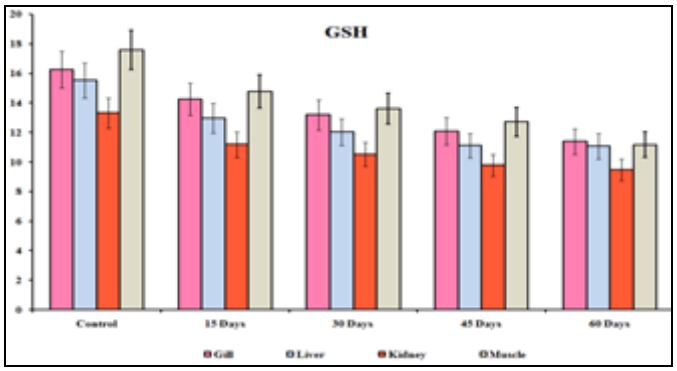

Fig 3: Effect of lead nitrate on Reduced glutathione (GSH) activity in gill, liver, kidney and muscles of *Labeo rohita* (The values are mean ± S.D for six individuals).

Discussion

Both laboratory and field experiments have revealed various enzymatic responses in animals including freshwater fish exposed to various pollutants and, in particular, the heavy metals depending on the dose, species and means of exposure (Wong and Wong, 2000; Nemcsok *et al.*, 1981; Jiraungkoorskul *et al.*, 2003; De Smet and Blust, 2001; Begum and Vijayaraghavan, 1995) [40, 29, 21, 10, 5]. The response to pollution is reflected as changes in some enzyme activities, especially key enzymes of biotransformation systems of

organisms, which can be used as biomarkers that are sensitive to pollution. These biomarkers, therefore, provide a tool for specific early warning sign for aquatic pollution also in fish species (Strmac and Braunbeck, 2000) [36].

In the present study, a significant decrease was noticed in the activity of antioxidants in gill, liver, muscle and kidney of *Labeo rohita* exposed to sub-lethal concentration of lead nitrate for a period of 60 days (figs, 1 to 3).

Superoxide dismutase, the first enzyme in the line of antioxidant defense, is responsible for catalyzing the conversion of the superoxide ions into water and molecular oxygen via catalase (Maran *et al.*, 2009) [26]. The accumulation of heavy metals might have led to the production of superoxide anions which led to the induction of SOD to convert the superoxide radical to H_2O_2. SOD catalytically scavenges superoxide radical which appears to be an important agent of toxicity of oxygen and this provides a defense against this aspect of oxygen toxicity (Kadar *et al.*, 2005) [22].

The enzyme SOD is known to provide cytoprotection against free radical induced damage by converting superoxide radicals (O_2^-) generated in peroxisomes and mitochondria to hydrogen peroxides. In the present study, a significant decrease ($P<0.05$) was noticed in the activities of SOD in all the studied tissues. This results was indicates the failure of defense mechanism to protect the test fish. Decreased SOD activity in the present study might be an indicator of damage in the antioxidant mechanisms caused by lead nitrate exposure. Similar to our findings Garcia Sampaio *et al.* (2008) [18] have showed a significant decrease in SOD level in the fish *Piaractus mesopotamicus* after exposure to copper.

Jianga *et al.*, (2011) [20] have reported that, the SOD, CAT and GSH levels were reduced in copper induced oxidative stress in *Cyprinus carpio* than control fish. The decreases in the level of SOD and CAT were observed in liver, brain and kidney of *Channa punctatus* during sub-lethal concentration of triazophos for 24, 48, 72 and 96 hrs. of exposure periods (Naveed *et al.,* 2010). A significant decrease in the level of SOD in this present study is consistent with previous research on brown trout (*Salmo trutta*) exposed to different heavy metal levels in rivers (Tamizhazhagan *et al.*, 2017: Hansen *et al.,* 2006) [38, 16], Zebrafish (*Danio rerio*) exposed to copper (Craig *et al.,* 2007) and *Oreochromis mossambicus* exposed to copper and cdmium (Dilek Saglam *et al.,* 2014) [11].

Catalase is a major antioxidant enzyme found in virtually all aerobic organisms. The activity of the enzyme varies in different tissues, being higher in organs with high oxidative potential (Halliwell and Gutteridge. 1984) [15]. CAT was also found to be the most sensitive antioxidant enzyme when compared to others. A significant increase in the activity of CAT was observed in the test fish during the study periods. The increase in CAT activity may be related to cope with the increased oxidative stress caused by metal exposures, while the decrease may be related to possible direct binding of metal ions to -SH groups on the enzyme molecule. Higher CAT activity was also recorded in different fish species after Cu and Cd exposures (Basha and Rani, 2003; Dautremepuits *et al.*, 2004; Sanchez *et al.*, 2005) [4, 9, 33].

According to Atli *et al.* (2006) [3], in Nile tilapia (*Oreochromis niloticus*) exposure to heavy metals and the highest decreases in CAT level was noticed in liver when compared to other tissues. In this present investigation also, the similar trend in decreases in the level of CAT was observed in liver than other studied tissues. This decreasing trend in the level of CAT in all the studied tissues can be collaborated with the findings of previous study of *Oreochromis niloticus* exposed to heavy metals, where an early increase in activity of catalase was observed, which scavenges the hydrogen peroxide in the case of heavy metals exposure, thus making the hydrogen peroxide unavailable to induce tissue damage. Sayeed *et al.* (2003) [34] have also reported a decrease in CAT activity in the test fish exposed to deltamethrin. A decrease in the activity of CAT has been previously reported in *Cyprinidae* fish living in Seyhan dam Lake of Turkey (Kono and Fridovich, 1982) [24]. Thirumavalavan (2014) [39] also found variations in SOD and CAT activity in *Catla* after mercuric chloride exposures depending upon metal concentrations. They concluded that toxicants may induce different antioxidant/pro oxidant responses depending on their ability to produce ROS. The response of the antioxidant system could differ when organisms exposed to metals and some other factors.

Glutathione plays an important role in the detoxification and excretion of xenobiotics (Reed and Beatty, 1980) [30]. Protective and adaptive roles of GSH against oxidative stress-induced toxicity are well established in aquatic animals (Regoli and Principato, 1995; Ahmad *et al.*, 2000) [31, 2]. In the present study, a significant decrease ($P<0.01$ and $P<0.05$) in the level of GSH in all the studied tissue were observed during the periods of exposure. Similar to our present study, Tanu Allen *et al.*, (2004) [37] have reported the high rate of GSH reduction in the gill, liver, kidney and brain of freshwater fish, *Channa punctatus* exposed to arsenic. Mather-Mihaich and DiGiulio (1986) [27] have shown a decrease in GSH level in channel catfish exposed to bleached kraft mill effluent. However, its level recovered after long-term exposure to the effluent. Furthermore, the apparent decrease in glutathione detoxification system in the gill, the first point of contact with environmental xenobiotics indicates that this system is a sensitive biochemical indicator of environmental pollution (Kono and Fridovich, 1982) [24]. Several studies have demonstrated that glutathione levels in various tissues or cells, which have low GSH concentrations are highly vulnerable to heavy metal toxicity, because, GSH plays a major role in antagonizing the oxidative action of heavy metal (Hasspieler *et al.*, 1994; Roy, 2004 and Thirumavalavan, 2014) [32, 39]. A reduction in the enzymatic and non enzymatic activities in the present investigation in *Labeo rohita* suggests that the fish is not in a healthy condition.

Conclusion

Pollution impact on ecosystem and human health is an urgent and worldwide issue since there are an ever increasing number of examples of environmental disturbances, likely to affect the biota and humans by both natural and anthropogenic stress. The assessment of environmental status has become an important issue in the striving for a sustainable society and use of natural resources. Hence, the findings of the present study unequivocally establish the detrimental effects of the lead nitrate in fingerlings of the freshwater fish. The alterations in the level of enzyme and non-enzymatic antioxidants in the

present investigation reflects the differential effects of pollution stress, which can be considered as biomarkers of exposure and subsequently as tools for biomonitoring in the assessment of environmental pollution.

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