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Impact of heavy metal zinc on enzyme succinate dehydrogenase of zebrafish *(Danio rerio)*

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Abstract
Enzyme is an organic catalyst which induces biological reactions. The concept of biocatalyst is a major source of enzymes used common in animal cells. Succinate dehydrogenase is the oxidative enzyme it acts as a marker enzyme for detecting the presence of TCA cycle in tissues. It was aimed to assess the enzyme succinate dehydrogenase activities in gill, liver, kidney, brain and muscle of Zebrafish exposed to sublethal concentration of zinc. The 96 hour LC_{50} values for the period of 10, 20 and 30 days exposed to zinc showed a declined level of enzyme succinate dehydrogenase activities for 10, 20 and 30 days in the selected tissues. There is no information concerning the three different sublethal concentrations on the enzyme succinate dehydrogenase of Zebrafish. Hence, it was programmed to observe the effect of zinc on succinate dehydrogenase activities in gill, liver and kidney, brain and muscle of Zebrafish.

Keywords: effect of zinc on succinate dehydrogenase in zebrafish

Introduction
All biological organisms are exposed to variety of toxic substances in the environment. The occupational and accidental aspects of hazardous chemical exposure to human, contaminants influence water, soil and air (Moore and Ramamoorthy, 1984) [29]. Such exposures lead adverse health effects ranging from sublethal changes to death (Lu, 1985). Pollution due to toxic heavy metals in air, soil and water is a major global problem. Heavy metals are not easily degraded or destroyed; the reduction amount of heavy metals from effluents to a permissible limit before discharging them into the environment is very important for human health (Srividya and Mohanty, 2009) [50]. Water pollution is the major problem that needs keen attention and prevention method (Ali and Soltan, 1996; Handy, 1994; Osman, 2007) [4, 15 8]. The toxic condition was resulted from many sources, like spillage of chemical wastes, agricultural, domestic, and discharge of industrial effluents, (Handy, 1994; Ali and Soltan, 1996) [15, 4]. Water pollution is one of the major environmental and public health problems (Osman and Kloas, 2010) [4, 15]. The aquatic habitats are being contaminated by heavy metals due to anthropogenic activities and industrialization (Muthupriya and Altaff, 2010) [32].
All the Aquatic animals inhabiting polluted toxic environment tend to accumulate different types of many toxic substances in high concentrations. Even when the ambient contamination levels are low the hazardous situation for the entire ecosystem and food chain. There are 35 metals are associated with community and occupational exposure, 23 are described considers as heavy metals. The release of toxic substance is high with the growing technology and heavy metal application in industries (Sopha *et al.*, 2007). The contamination of freshwaters with a wide range of toxic substances has become a great concern throughout the world in the last few decades (Al-Weher, 2008) [5]. Heavy metals are the natural trace elements of the aquatic environment, but their concentrations have increased rapidly due to agricultural, mining, industrial and domestic activities (Leland *et al.*, 1978; Mance, 1987; Kalay and Canli, 2000) [23, 20]. Accumulation of heavy metals are more in fishes than the water and sediments (Olaifa *et al.*, 2004, Gumgum *et al.*, 1994) [37, 14]. The aquatic environments are changed rapidly due to the discharge of heavy metals into river or any aquatic environment (Heath, 1987) [17].
Among the heavy metals zinc is the fourth most widely used metal in the universe and also one of the major elements than the other. The concentration of zinc in the earth's crust was estimated as 72ppm (Abbasi, 1989) [1]. The concentration of zinc in various rocks and soils has been reported as 100 and 300 ppm (Adriano, 1980) [3]. The concentration of heavy metals zinc has rarely exceeded (0.5 ppm) in unpolluted springs, streams, river and lakes (Abbasi, 1989) [1]. In New York, Jamaica Bay sediments the zinc concentration have reported as 12.5 to 151 ppm (Abbasi *et al.*, 1998) [2]. Johansson, 1988 have reported that in Swedish forest lakes the zinc levels or ranging from 25 to 280 ppm. Input of zinc (annual in the environment due to erosion weathering and natural phenomenon is estimated at 8, 00,000 tonnes) (Nriagu, 1980) [36]. The annual deposition of zinc due to anthropogenic sources was estimated at 414,000 tonnes of zinc per annum. On the global basis, the primary zinc production was estimated at 99000 ton/year, waste incineration 37, 000 ton/year wood combustion 75,000 ton/year, iron and steel production 35,000 ton/year, other atmospheric emissions 68,000 ton/year and municipal waste water (1, 00,000 ton/year). Have observed an average the yearly concentration of zinc 19.80 ppb higher than that of cadmium, lead, copper, nickel and cobalt (Abbasi *et al.*, 1998) [2]. Zinc normally occurs in the environment in an oxidation state of Zn. The (Zn, Fe) S minerals sphalerite and wurtzit is by far the most predominant zinc are minerals (Kiekens, 1995) [21].
Zinc is an essential trace metal for terrestrial as well as aquatic organism. It is one of the less toxic heavy metals, but also one of the most widely occurring. However, at excessive uptakes, zinc can be toxic. Zinc is mobilized by acidification. Zinc may be considered, together with

cadmium as very mobile a bio available metal. Mobility of zinc varies concentration of zinc and redox potential (indirectly). Zinc is due to mobilization by acidification transported downwards in the soil profile. Zinc adsorption is not anticipated below Ph 6.0. However, above Ph 7.0 metal oxides, cley and apatite are efficient adsorbents capable of binding 90-100% of the zinc (Kiekens 1995; WHO, 1996) [21, 51]. Zinc is used mainly for galvanization and Zinc oxide is used mainly for rubber processing units. Zinc chloride is used as wood preserver, in soldering fluxes, and as dry battery filler.

Normally high exposures of zinc may damage the lung tissues, damage to heart, muscle, liver and kidney (Al-Attar, 2007). Fish is the major supply of healthy protein to the world's population. Among the aquatic animals fishes are the bio indicator species which place and remarkable role in monitoring water pollution. The mortality of fish indicates heavy pollution that can be measured in terms of biochemical, physiological or histological responses of the fish organism (Mondon *et al.,* 2001 [27]. Succinate dehydrogenase is a key enzyme in the oxidation of sugars catabolism (Lehninger *et al.,* 1993). Hence, it was programmed to assess the impact of sub lethal concentration of zinc on succinate dehydrogenase in gill, brain liver, muscle, and kidney of Zebrafish.

Materials and Methods

The Zebrafish having mean length of 4 to 6 cm 20 to 50g weight were purchased from the Quality Aquarium, Kolathur, Chennai. They were fed with tubifix worms regularly. The unused foods were removed carefully. They were given 0.1% KMNO4 solution and then kept in plastic pools for acclimatization for seven days. The stock solutions were prepared by using zinc LC_{50} was calculated for 96 h (38.64 ppm) (Sprague, 1971) [49] and 1/5th (high), 1/10th (medium) and 1/15th (low) of the LC_{50} values were 6.528, 3.264 and 2.176 ppm respectively taken for this study. Forty fishes were selected then it was divided into 4 groups. The first group was maintained in free from treatment and served as the control. The other 3 groups were exposed to sub lethal concentration of zinc in 10 litter capacity glass tank. The 2nd, 3rd and 4th groups were exposed to zinc for 10, 20 and 30 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were dissected out for succinate dehydrogenase activity estimation. Fishes were exposed to three different concentrations separately in plastic troughs. The control fishes were also maintained separately. They were fed with tubifix worms as per the normal procedure. The medium was renewed daily with sublethal concentration of the zinc. The succinate dehydrogenase activities in all the tissues were estimated by the Nachales *et al.* method (1960) [33]. The data were analyzed by DMRT one way ANOVA and test the level of significance (Duncan, 1957) [9].

Results

Depletion of succinate dehydrogenase activities of the selected tissues of Zebrafish exposed to zinc for 10, 20 and 30 days in different concentrate of the LC_{50} values of sublethal concentrations were estimated. Among the values, the maximum depletion of succinate dehydrogenase was found in liver during 30 days. Depletion in succinate dehydrogenase activities is directly proportional to the exposure period of the toxicant. The observed values gill, liver, kidney, brain and muscle were subjected to statistical analysis (Table 1-5).

Table 1-5: Succinate dehydrogenase activity in gill, liver, kidney, brain and muscle of Zebrafish exposed to sublethal concentration of zinc.

Gill				
Treatments	**Gill control**	**Low concentration**	**Medium concentration**	**High Concentration**
10 days	0.058 ± 0.004^{b}	0.055 ± 0.004^{b}	0.053 ± 0.004^{ab}	0.047 ± 0.003^{a}
20 days	0.061 ± 0.004^{c}	0.052 ± 0.003^{b}	0.047 ± 0.003^{a}	0.040 ± 0.003^{a}
30 days	0.059 ± 0.004^{d}	0.048 ± 0.003^{c}	0.041 ± 0.003^{b}	0.031 ± 0.002^{a}
Liver				
Treatments	Liver control	Low concentration	Medium concentration	High Concentration
10 days	0.049 ± 0.003^{c}	0.046 ± 0.003^{c}	0.041 ± 0.003^{b}	0.035 ± 0.002^{a}
20 days	0.050 ± 0.003^{d}	0.041 ± 0.003^{c}	0.033 ± 0.002^{b}	0.026 ± 0.001^{a}
30 days	0.049 ± 0.003^{d}	0.034 ± 0.002^{c}	0.028 ± 0.001^{b}	0.020 ± 0.001^{a}
Kidney				
Treatments	Kidney control	Low concentration	Medium concentration	High Concentration
10 days	0.041 ± 0.003^{c}	0.039 ± 0.002^{bc}	0.038 ± 0.002^{b}	0.033 ± 0.002^{a}
20 days	0.040 ± 0.003^{d}	0.037 ± 0.002^{c}	0.031 ± 0.002^{b}	0.025 ± 0.001^{a}
30 days	0.039 ± 0.002^{d}	0.033 ± 0.002^{c}	0.029 ± 0.002^{b}	0.018 ± 0.008^{a}
Brain				
Treatments	Brain control	Low concentration	Medium concentration	High Concentration
10 days	0.053 ± 0.004^{c}	0.049 ± 0.003^{c}	0.047 ± 0.003^{b}	0.041 ± 0.003a
20 days	0.053 ± 0.004^{d}	0.044 ± 0.003^{c}	0.041 ± 0.003^{b}	0.033 ± 0.002a
30 days	0.052 ± 0.003^{d}	0.039 ± 0.002^{c}	0.033 ± 0.002^{b}	0.021 ± 0.001a
Muscle				
Treatments	Muscle control	Low concentration	Medium concentration	High Concentration
10 days	0.060 ± 0.004^{c}	0.059 ± 0.004^{c}	0.054 ± 0.004^{b}	0.049 ± 0.003^{a}
20 days	0.059 ± 0.004^{c}	0.052 ± 0.003^{b}	0.048 ± 0.003^{b}	0.039 ± 0.002^{a}
30 days	0.060 ± 0.004^{d}	0.045 ± 0.003^{c}	0.039 ± 0.002^{b}	0.025 ± 0.001^{a}

All the values are mean± SD of six observations; Values which are not sharing common superscript differ significantly at 5% level ($p < 0.05$); Duncan's multiple range test (DMRT)

Discussion

In general the constant usage of heavy metals alters the physiological and biochemical changes, organs it may result with adaptive significance to the life of an animal. Enzyme

is an organic catalyst which accelerates all the biological reactions. The concept of organic catalyst is a very major source of enzyme used common in animal cell. Pollution by heavy metals is an important global problem is aquatic animals and environment. The aquatic ecosystem is the ultimate recipient for the pollutants which was carried by anthropogenic and natural agents. The accumulation, of heavy metals in the aquatic ecosystem constitutes a formidable threat to biological life (George, 1989; Gagne *et al*., 1996; Fleeger *et al.,* 2003; Aramphongphan *et al*., 2009) [13, 12, 11, 6]. Heavy metals are one of the most-active polluting agents which cause serious to, metabolic and physiological problem (Shugart *et al.,* 1992) [46]. The heavy metals are considered as a most common group of pollutants, which cause different problems in freshwater animals, and therefore they have to be treated separately (Lloyd, 1992) [24]. The occurrence of heavy metals in all the environmental compartments including food chain of aquatic animal, despite their declining level as the point of source increased and remained within the permissible limit, was responsible for the toxicity that may affected the succinate dehydrogenase enzyme activity of fish (Mukherjee and Jana, 2007). In toxicological studies of acutet his exposure of heavy metal influences the cell damage in specific organs (Casillas *et al.,* 1983) [8]. The succinate dehydrogenase is an important enzyme of kreb's cycle whose qualitatine changes are significant during certain pathological conditions (Harper *et al.,* 1978) [16]. Sucinate dehydrogenase is the oxidative enzyme which was drastically affected by the action of heavy metals. Succinic acid dehydrogenase is selected as a key representative of metabolic enzyme. It is a marker enzyme for detecting the presence of TCA cycle in tissues (Natarajan, 1979) [34].

The impact of pollutants on aquatic ecosystem can be assessed by the measurement of different biochemical parameters in fish (Petrivalsky *et al*., 1997) [41]. Gills are the major vital organs in fish which IS having direct contact with the medium through which toxic substances enter into the body (Edwards, 1973) [10]. Succinate dehydrogenase enzyme is concentrated in chloride cells of fish gills, which was used as an indicator of osmoregulatory activity (Langdon and Thorpe, 1984) [22]. Liver is one of the most multi-faceted organs in higher animals. It is almost important target organ which acts as a chief metabolic and detoxification center (Bhattacharya and Mukherjee, 1976) [7]. Kidney is almost important organ for excretion and osmoregulation which is indirectly affected by toxic substance through blood circulation (Newman and MacLean, 1974) [35]. Fish muscles are edible and economically important.

In the present investigation the activity of SDH shows declined nature in gill, liver, kidney, brain and muscle of Zebrafish exposed to zinc. This suggests that an inhibited mitochondrial oxidation, which may lead to declined level in energy production. This indicates the impairment of oxidative metabolic cycle and the anaerobic glycolysis may be increased to meet out the energy demand during the stress period. There are evidences that the succinate dehydrogenase enzyme activities in the liver and muscle tissues of tilapia decreased when they were exposed to pesticide thiodon (Rajeswari *et al.,* 1989) [44]. It was reported that the decrease in the succinateactivity and lactate dehydrogenase in the different tissues *Oreochromis mossambicus* when exposed to pesticide methyl parathion. Similarly, Sastry and Subhadra, (1982) [45] have reported that declined level in the succinate dehydrogenase activity in liver tissue of *Channa punctatus* exposed to treated with cadmium and copper. Mary chandravathy and Reddy, (1994) [26] have reported that a decreased in succinate dehydrogenase activity in gill and liver tissues of Anabas scandens treated with lead nitrate. James *et al.* (1992) [18] have observed that the level of succinate dehydrogenase activity decreased in liver exposed to heavymetal. Mercury they reported that the metabolic shift from aerobiosis to an anerobiosis due to metal actions. More *et al.* (2005) [30] have observed that the level of succinate dehydrogenase activity decreased in Lamellidens marginalis exposed to heavy metal. The anaerobic activity is due to toxic stress which has reversed physiological and biochemical adaptation. Rajamanickam, (1992) [43] has observed a reduction level in SDH activity in the liver tissue of *Mystus vittatus* treated with copper. Radhakrishnaiah *et al.* (1992) [42] have reported that the suppression in succinate dehydrogenase activities in liver tissues of Zebrafish exposed to copper. The present study shows that the significant alterations in succinate dehydrogenase enzyme due to intoxication of zinc stress in Zebrafish. The decrease in succinate dehydrogenase enzyme activity may reflect decreased dependence on anaerobic carbohydrate metabolism in the selected tissues of Zebrafish when exposed to zinc.

Conclusion

The SDH activity showed the declined level in the selected tissue which indicates the alterations in enzyme synthesis due to heavy metal stress. The present study suggests that the decreased activity of SDH, the oxygen loading capacity may be responsible for the depletion level in respiration. Succinate dehydrogenase belongs to complex-II in the respiratory chain which is present in inner mitochondrial membrane. All the above findings suggest that he heavy metal affects the TCA cycle.

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