American Journal of Life Sciences
2014; 2(6): 389-394
Published online December 31, 2014 (http://www.sciencepublishinggroup.com/j/ajls)
doi: 10.11648/j.ajls.20140206.19
ISSN: 2328-5702 (Print); ISSN: 2328-5737 (Online)

Therapeutic efficiency of *Spirulina* against lead acetate toxicity on the fresh water fish *Labeo rohita*

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To cite this article:

Manjunatha Bangeppagari, Jaffer Mohiddin Gooty, Juan Ortiz Tirado, Selvanayagam Mariadoss, Selvaraj Thangaswamy, Naga Raju Maddela, Darwin Rueda Ortiz. Therapeutic Efficiency of *spirulina* against Lead Acetate Toxicity on the Fresh Water Fish *Labeo rohita*. *American Journal of Life Sciences.* Vol. 2, No. 6, 2014, pp. 389-394. doi: 10.11648/j.ajls.20140206.19

Abstract: The release of heavy metals in to the aquatic environment causes water pollution problems because of their toxicity, persistence and bioaccumulation. Lead has no known role to play in the human body that is physiologically relevant, and its harmful effects are myriad. Lead from the atmosphere and soil ends up in water bodies thus affecting the aquatic organisms. This situation has thus prompted numerous investigators to study on the effects of this heavy metal on the biological functions of aquatic organisms, particularly on the antioxidant enzyme activity in fish. In the present investigation the effect of lead acetate (heavy metal) on antioxidant enzyme activity was evaluated in the fresh water fish *Labeo rohita.* The experimental fish were treated with sub lethal concentration of lead acetate (0.015 mg/ L) for 120 hrs. *Spirulina* was used as supplementary feed during the experimental period. We observed various lead induced lipid peroxidation (LPO), antioxidant enzyme (SOD and CAT) changes and *Spirulina* supplementary feed therapeutic efficiency was observed in the gill and liver tissues of the fish. All the experimental data are statistically significant at $p<0.05\%$ level. The present study was under taken the toxic effect of lead acetate on *Labeo rohita* fish and chelating property of *Spirulina*.

Keywords: Antioxidant Enzymes, LPO, SOD, CAT, *Labeo rohita*, Lead Acetate, *Spirulina*

1. Introduction

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are indestructible and most of them have toxic effects on organisms [1]. Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be degraded or destroyed [2]. Among various toxic pollutants, heavy metals are particularly severe in their action due to the tendency of bio-magnification through food chain. The global heavy metal pollution of water is a major environmental problem. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated [3]. Industrial discharges containing toxic and hazardous substances, including heavy metals [4][5] contribute tremendously to the pollution of aquatic ecosystem. Dangerous pollutant that can be absorbed by fish when exposed to elevated levels in an aquatic environment is Lead (Pb). Absorption of lead occurs by different ways through gills and skin or by ingestion of contaminated water and food; and may lead to high mortality rate or cause many biochemical and histological alterations in survived fish [6].

Lead is one of the first metals used by humans. It is highly persistent, not involved in normal metabolism and very toxic [7][8]. Particularly in the 20th century, countless thousands of organic trace pollutants have been produced and in part released into the environment [9]. Lead has the potential to

adversely affect the human and animal health. It causes physiological, biochemical, and neurological dysfunctions in humans [10]. Mobarak [11] reported that low level of lead exposure during the early development produced long-lasting cognitive and neurobehavioral deficit, persistent immune changes, reduced fertility, a delay in sexual maturity, irregular estrus and reduced number of corpora lutea in human and experimental animals. Notable and recent reports have indicated that lead can cause neurological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical disorders in animals [12] [16].

Antioxidant enzymes, such as catalase (CAT), glutathione peroxidase (GPX), glutathione S transferase (GST), glutathione reductase (GR) and superoxide dismutase (SOD) are of great importance in antioxidative stress to cope with free radicals leading several disturbances [17][18]. Fishes are the important environmental indicators for aquatic heavy metal pollution. Alterations of antioxidant and biochemical changes are important parameters for toxicity study [19]. Lead could also interact with biological membranes, inducing lipid peroxidation (LPO) [20]. Heavy metal could decrease free radical scavenging enzymes, such as catalase (CAT) and superoxide dismutase (SOD). Lead increased the level of lipid peroxidation [21]. Protein content was depleted in the liver and brain tissues of lead treated anabus [22]. *Spirulina* is called micro vegetable and it is also widely used as an animal feed supplement [23]. *Spirulina* intake per day 10g per adult is widely recommended to maintain health of the individual. *Spirulina* is an Oscillatoriaceae family member which grows naturally in countries which have a warm climate and has been considered as supplement in human and animal food [24]. They have been found to be a rich source of vitamins, minerals, essential fatty acids and antioxidant pigments such as carotenoids [25]. The protective effect of *Spirulina* against cadmium induced oxidative stress and it is also attributed to be antioxidant and chelating effects [26]. *Spirulina* is a cynobacterium classified as blue green algae. It has been used as a food [27] because of its quantity of proteins, vitamins, essentialamino acids, minerals and essential fatty acids [28]. It has been reported in some reviews that *Spirulina* have several pharmacological activities. Khan *et al.,* [29] reported that *Spirulina* have antioxidant properties especially some phycobiliproteins such as Cphycocyanin (CP) and allophycocyanin [30]. In view of the above, and considering the lack of sufficient knowledge about the toxic potential effect of lead acetate to freshwater fishes, the objective of this work was to evaluate its effect on enzymatic antioxidant profiles of *Labeo rohita.*

2. Materials and Methods

The fish *Labeo rohita* 75g±5 of weight 15±5 cm length were obtained from the Fisheries Department, Anantapur, Andhra Pradesh, India and were transferred to large cement tanks. They were kept in the cement tank filled with dechlorinated water and continuous aeration. Acclimatization to experimental condition for 15 days at room temperature, fishes were fed with artificial libitum during acclimatization and tank water was renewed every day after feeding, food was withheld from before 24 hours to the experiment.

2.1. Lead acetate Toxicity Studies

Lead acetate was weighed accurately and dissolved in distilled water. The LC_{50} - 0.034 mg/L 120 hours, sub lethal concentration 0.015 mg/liter was used for 24, 48, 72, 96 and 120 hours experimental study.

2.2. Supplementary Feed Preparation

The dried *Spirulina* was collected from Seesali Village, Kalla Mandal, West Godavari District, Andhra Pradesh, India. *Spirulina* and little quantity of distilled water used to make pellets. This pellet was dried at room temperature very hygienically. 500mg/fish, *Spirulina* pellets used as supplementary feed.

2.3. Experimental Design

After acclimation the *Labeo rohita* fresh water fish were divided into four groups. Each group consisted of 6 animals. Group I: Control Group II: Fish treated with lead acetate 0.015 mg/L sub lethal concentration for 120 hours Group III: Fish treated with lead acetate 0.015 mg/L sub lethal concentration+*Spirulina* 500 mg/fish for 120 hours. Group IV: *Spirulina* 500 mg/fish for 120 hours.

2.4. Antioxidant Enzymes Assay

The concentration of TBARS in the tissues (gill and liver) was estimated by the method of [31]. Superoxide dismutase (SOD) activity was determined following the procedure of [32]. The activity of catalase (CAT) was assayed by the method of [33].

2.5. Statistical Analyses

The data obtained from the quantitative study were expressed as the mean±SD. The mean values were calculated from 6 individual observations. $P<0.05$ values were calculated by the two tailed students 'T' test.

3. Results and Discussion

The antioxidant enzymes in fish could be used as biomarkers of exposure of aquatic pollution [34]. Many metals are known to be powerful oxidants. Redox active metals such as Cr, Cu and Fe, undergo redox cycling, though redox-inactive metals, such as Cd, Hg and Pb deplete major antioxidants in the cell, especially thiol containing antioxidants and enzymes [35]. One of the well-known and main mechanisms is a production of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion radical and hydroxyl radical induced by metals through various mechanisms such as Fenton- and Haber-Weiss type reactions. Metals can promote oxidative damage by directly increasing the cellular concentration of

ROS and by altering the cellular antioxidant capacity in fish [36] [37] [38]. Through ROS-mediated reactions, metals cause DNA damage, lipid peroxidation, and protein modification [39] [40]. These enzymatic responses are associated with increased ROS production leading to "oxidative stress" occurring when the ROS generation rate exceeds that of their removal [41] [35] [42].

Lipid peroxidation is the initial step of cellular membrane damage caused by pesticides, metals and other xenobiotics [43]. Lipid peroxidation is considered to be a valuable indicator of oxidative damage of cellular components. Most components of cellular structure and function are likely to be potential targets of oxidative damage, and the most susceptible substrates for auto oxidation, polyunsaturated fatty acids of the cell membrane, which undergo lipid peroxidation. In the present investigation, the level of TBARS were increased in the treated group when compared to control, group III TBARS level gradually changed in 24 hrs to 120 hrs statically significant at $p \leq 0.05$ level. Increasing of TBARS elevating the ROS level in the tissues leads to cellular damage, [44] who found significant increase in the lipid peroxidation and decrease in the level of endogenous antioxidants in the liver of lead exposed animals. In another study of [45][46], Lipid peroxidation is a biochemical marker for the free radical mediated injury. The results show an increase in the level of lipid peroxides due to lead intoxication.

SOD activity in treated fish decreased gradually during exposure period when compared to control group. SOD enzyme converts the superoxide radicals into $H_2O_2$the reduced activity of SOD in presence of lead acetate may cause accumulation of $O_2 \cdot -$, H_2O_2 or the products of its decomposition [47]. SOD plays an important role in protecting tissues against oxygen free radicals. SOD is a group of metalloenzymes that plays a crucial antioxidant role and constitutes the primary defense against the toxic effects of superoxide radical in aerobic organism [48]. Depletion of antioxidant enzyme activity could be caused by a down-regulation of transcription and translation process.

Catalase level in treated fish decreased gradually during exposure period. Antioxidant enzyme CAT removed the SOD generating H_2O_2 by converting H_2O_2 into O_2 and water molecule. The inhibition of CAT activity may be due to enhanced production of O_2 - and peroxyl radicals during the chronic administration of lead. CAT is an enzyme located in peroxisomes and facilitates the removal of H_2O_2 [59][50]. Inhibition of heme synthesis by lead is well reported and since CAT is a heme-containing enzyme, its activity decreases [51].

Table 1. *Changes in the level of lipid peroxidation (n mol/mg of protein) content in the fresh water fish Labeo rohita on the effect of lead acetate and Spirulina exposed to 120 hours*

Organs	Groups	Exposure period				
		24h	48h	72h	96h	120h
Gill	Group I	10.31 ± 0.01	10.34 ± 0.01	10.45 ± 0.03	10.74 ± 0.01	10.79 ± 0.02
	Group II	10.92 ± 0.08	11.32± 0.043*	12.65 ± 0.1*	13.41 ± 0.21*	14.53 ± 0.31*
	Group III	10.51 ± 0.01	10.61 ± 0.02*	10.73 ± 0.04*	10.82 ± 0.09*	10.52 ± 0.02*
	Group IV	10.37 ± 0.02	10.42 ± 0.02	10.39 ± 0.01	10.35 ± 0.01	10.31 ± 0.04
Liver	Group I	12.74 ± 0.02	12.79 ± 0.01	12.82 ± 0.01	12.89 ± 0.03	12.93 ± 0.03
	Group II	13.85 ± 0.01	14.74 ± 0.03*	15.33 ± 0.03*	16.61 ± 0.18*	17.39 ± 0.01*
	Group III	13.29 ± 0.02	13.61 ± 0.01*	13.69 ± 0.02*	13.78 ± 0.11*	13.88 ± 0.03*
	Group IV	12.77 ± 0.01	13.08 ± 0.01	12.71 ± 0.01	12.29 ± 0.02	12.41 ± 0.01

Table 2. *Changes in the level of superoxide dismutase (U/min/mg of protein) activity in the fresh water fish Labeo rohita on the effect of lead acetate and Spirulina exposed to 120 hours*

Organs	Groups	Exposure period				
		24h	48h	72h	96h	120h
Gill	Group I	9.8 ± 0.14	9.33 ± 0.02	9.40 ± 0.01	9.42 ± 0.02	9.43 ± 0.03
	Group II	8.72 ± 0.14	8.33 ± 0.32*	7.34 ± 0.23*	7.10 ± 0.12*	6.45 ± 0.04*
	Group III	8.89 ± 0.14	8.72 ± 0.21*	8.32 ± 0.13*	8.02 ± 0.02*	8.25 ± 0.21*
	Group IV	9.11 ± 0.29	9.41 ± 0.21	9.49 ± 0.12	9.57 ± 0.28	9.63 ± 0.01
Liver	Group I	12.61 ± 0.19	12.65 ± 0.12	12.69 ± 0.21	12.72 ± 0.23	12.79 ± 0.17
	Group II	11.85 ± 0.13*	11.21 ± 0.13*	10.11 ± 0.32*	9.53 ± 0.29*	9.8 ± 0.21*
	Group III	12.30 ± 0.29	11.81 ± 0.21*	11.38 ± 0.22*	11.58 ± 0.9*	11.69 ± 0.12*
	Group IV	12.68 ± 0.14	12.79 ± 0.04	12.87 ± 0.12	12.98 ± 0.11	13.17 ± 0.13*

Table 3. *Changes in the level of catalase (μ mol of H_2O_2 consumed/ min/mg of protein) activity in the fresh water fish Labeo rohita on the effect of lead acetate and Spirulina exposed to 120 hours*

Organs	Groups	Exposure period				
		24h	48h	72h	96h	120h
Gill	Group I	39.11 ± 0.21	39.31 ± 0.19	39.40 ± 0.02	39.58 ± 0.25	39.70 ± 0.32
	Group II	34.20 ± 0.1	31.29 ± 0.31*	28.70 ± 0.1*	26.32 ± 0.25*	23.42 ± 0.21*
	Group III	38.41 ± 0.32	37.75 ± 0.20*	36.85 ± 0.31*	37.31 ± 0.31*	38.54 ± 0.10*
	Group IV	39.19 ± 0.20	39.31 ± 0.12	39.52 ± 0.29	39.67 ± 0.13	39.78 ± 0.21
Liver	Group I	43.41 ± 0.02	43.52 ± 0.22	43.58 ± 0.20	43.69 ± 0.28	43.78 ± 0.13
	Group II	41.39 ± 0.09*	38.15 ± 0.21*	36.71 ± 0.12*	37.18 ± 0.15*	34.38 ± 0.21*
	Group III	42.35 ± 0.29*	41.52 ± 0.31*	41.15 ± 0.21*	41.72 ± 0.24*	41.95 ± 0.29*
	Group IV	43.51 ± 0.09	43.68 ± 0.20	43.78 ± 0.12	43.91 ± 0.10	44.21 ± 0.24

In the case of group III parameters like TBARS, CAT and SOD level recovery result was observed. This recovery may due to *Spirulina* supplementary feed. It was observed that gill and liver increase SOD, CAT activity as antioxidant potential and there by declines the level of lipid peroxidation. *Spirulina* is considered a valuable additional food source of some macro and micronutrient including high quality protein iron, gamma linolenic fatty acids, carotenoids and vitamins [52]. Vitamin C and vitamin E reduced lead induced oxidative stress [53][54]. The antioxidant mechanism of β-carotene has been suggested to be single oxygen quenching, free radical scavenging and chain breaking during lipid peroxidation [55]. The metalloprotective role of *Spirulina* may be attributed due to the presence of β-carotene [56]. *Spirulina* have rich content of vitamin C, E and β-carotene and this phytochemical constituent may reduce the lead toxicity and enhance the radical scavenging property. *Spirulina* alone treated group near to normal better anti oxidant defense were observed.

4. Conclusion

The antioxidant enzymes are of great importance against the effects of metals in fish. We conclude that antioxidant enzyme activity in the gill and liver clearly indicated that lead induced oxidative stress. Whereas *Spirulina* enhance antioxidant enzymes and reducing the lead toxicity in the recovery group fish. In this study suggested that *Spirulina* supplementary feed enhance health of the fish.

Conflict of Interest

The authors declare that we have no conflict of interest.

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