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ORIGINAL RESEARCH ARTICLE

Curative Efficacy of *Spirulina* against Lead Acetate Toxicity on the *Cyprinus carpio* (Linn.) Fresh Water Fish

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ABSTRACT

Fishes are the important source for bioindicators of various environmental pollutants. Among the pollutants, heavy metals are serious problem of aquatic life. In the present investigation, fresh water fish *Cyprinus carpio* was used as experimental animal, heavy metal like lead acetate (0.017 mg/lit) was used as sublethal concentration for 120 hrs experimental study. *Spirulina* was used as supplementary feed for the experimental period. In this study, we observed various lead induced lipid peroxidation, antioxidant enzyme (SOD and CAT) changes and biochemical (Protein, Glucose, Glycogen and Amino acid) alteration and *Spirulina* supplementary feed curative efficacy was observed in the gill and liver tissues of the fish. All the parameters are statistically significant at $p< 0.05\%$ level.

Key words: Lead acetate, *Spirulina*, *Cyprinus carpio*, Antioxtant and Biochemical parameters.

1. INTRODUCTION

Lead occurs naturally in the environment. However, most lead concentrations that are found in the environment are a result of human activities. Due to the application of lead in gasoline, an unnatural lead - cycle has consisted. In car engines lead is burned, so that lead salts (chlorines, bromines, and oxides) will originate. These lead salts enter the environment through the exhausts of cars. The larger particles will drop to the ground immediately and pollute soils or surface waters, the smaller particles will travel long distances through air and remain in the atmosphere. Part of this lead will fall back on earth when it is raining. This lead - cycle caused by human production is much more extended than the natural lead - cycle and has caused lead pollution to be a worldwide issue [1].

Lead intoxication is probably the most common form of heavy metal intoxication and is well documented as one of the most dangerous and insidious poisons to man. The absorbed lead is conjugated in the liver and is passed to the kidney, where a small quantity is excreted and the rest accumulates in the body [2]. Accumulated lead induced various hazards effects on living organisms. Fishes are the important environmental indicators for aquatic heavy metal pollution. Alterations of antioxtant and biochemical chances are important parameters for toxicity study [3]. Lead could also interact with biological membranes, inducing lipid peroxidation (LPO) [4].

Heavy metal could produce a decrease of free radical scavenging enzymes, such as catalase (CAT) and superoxide dismutase (SOD). Lead increased the level of lipid peroxidation [5]. Protein content was depleted in the liver and brain tissues of lead treated anabus [6]. Green supplementary feed studies are need for eliminating process of heavy metal as current trend. *Spirulina* is called micro vegetable. It is also widely used as an animal feed supplement [7]. *Spirulina* intake per day of 10 g per adult is widely recommended to maintain health. *Spirulina* is an Oscillatoriaceae family which grows naturally in countries which have a warm climate and has been considered as supplement in human and animal food [8]. They have been found to be a rich source of vitamins, minerals, essential fatty acids and antioxidant pigments such as carotenoids [9]. The protective effect of

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Spirulina against cadmium induced oxidative stress and also is attributed to its antioxidant and chelating effects [10]. In the present investigation, recovery ability of *Spirulina* against lead induced antioxidant and biochemical alteration in *Cyprinus carpio* (Linn) fresh water fish was studied.

2. MATERIALS AND METHODS

Collection of experimental fish

The fish *Cyprinus carpio* 75g±5 of weight 15±5 cm length were obtained from the Navarathna fish farm near by Pinnaloor; fishes were safely transferred to the laboratory. 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.

Lead acetate toxicity studies

Lead acetate was weighed accurately and dissolved in distilled water. The LC_{50} - 0.034 mg/L 120 hours, sublethal concentration 0.017 mg/liter was used for 24, 48, 72, 96 and 120 hours experimental study.

Supplementary feed preparation

The dried *Spirulina* was collected from aurospirul commercial form, Aurovill Village (away from 15 km) near to Pondicherry. The *Spirulina* was kept carefully. *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.

Experimental Design

After acclimation the *Cyprinus carpio* fresh water fish were divided into four groups. Each group consisted of 6 animals.

Group I : Control
Group II : Fish treated with lead acetate 0.017 mg/L sublethal concentration for 120 hours
Group III : Fish treated with lead acetate 0.017 mg/L sublethal concentration+ *Spirulina* 500 mg/fish for 120 hours.
Group IV : *Spirulina* 500 mg/fish for 120 hours.

Assay method

The concentration of TBARS in the tissues was estimated by the method of [11]. Superoxide dismutase activity was determined following the procedure of [12]. The activity of catalase (CAT) was assayed by the method of [13]. Protein content in the tissues was determined after trichloro acetic acid precipitation by the method of [14]. Total free amino acids and content of the tissue were estimated by the method of [15]. Quantitative estimation of tissue glucose and glycogen were done following method of Kemp and Kit [16].

Statistical calculation

The data obtained from the quantitative study were expressed as the mean ± S.E. 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

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. 120 hours treated group TBARS level increased in gill (53%) and Liver (33.34%). Increasing of TBARS elevating the ROS level in the tissues leads to cellular damage, [17] 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 [18] and [19], 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 decrease at 120 hrs gill (29.99%) and liver (37%). 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·-, H_2O_2 or the products of its decomposition [20]. SOD plays an important role in protecting tissues against oxygen free radicals. Catalase level in treated fish decreased gradually during exposure period. CAT decreased in the 120 hrs gill (19%), liver (32%). 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. Inhibition of haem synthesis by lead is well reported and since CAT is a heme-containing enzyme, its activity decreases [21]. Total protein

content in treated fish gradually decreased during exposure period when compared to control group protein content decrease at 120 hours gill (29.99%), liver (37%). Lead induces stress caused protein depletion. Similarly the maximum amount of depletion was recorded in the liver and the lowest amount depleted in the brain was observed [6].

The level of free amino acid were increased in the treated group when compared to control, group III free amino acid level gradually changed in 24 hrs to 120 hrs statically significant at $p \leq 0.05$ level. 120 hours treated group free amino acid level increased in gill (53%) and liver (33.34%). The amino acids are the building blocks of protein. Amino acids which cannot be synthesized in the body must be supplemented through diet. Since, the food value of fish is directly depending on their protein content, the contamination by the toxic substance will reduce their nutritive value [22]. An increase in amino acid content in liver tissue might be due to enhanced proteolysis and decreased utilizations of amino acid for protein synthesis. Increased free amino acids levels have been reported in liver, muscle and brain tissue of *Cyprinus carpio* exposed to sublethal concentration of mercury [23]. The level of glucose were increased in the treated group when compared to control, group III glucose level gradually changed in 24 hours to 120 hrs statically significant at $p \leq 0.05$ level. 120 hours treated group glucose level increased in gill (53%) and liver (33.34%). Glycogen content in treated fish decreased gradually during exposure period. Glycogen content was decreased in the 120 hours gill (19%), liver (32%). The increased in the glucose level of the tissue while decrement in tissue glycogen in exposed fish makes it clear that the glycogen reserves are being used to meet the stress caused. Increased in serum glucose level in fish under stress was reported by [24, 25]. In the case of group III parameters like TBARS, CAT, SOD, total protein, glucose, amino acid and glycogen 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 thereby 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 [26]. Vitamin C and vitamin E reduced lead induced oxidative stress [27, 28]. The antioxidant mechanism of β-carotene has been suggested to be single oxygen qenching, free radical scavenging and chain breaking during lipid peroxidation [29]. The metalloprotective role of *Spirulina* may be attributed due to the presence of β-carotene [30]. *Spirulina* have rich content of vitamin C, E and β-carotene 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

In the present observation, clearly indicated that lead induced oxidative stress and inhibiting the protein metabolism. 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.

Table 1: Changes in the level of lipid peroxidation (n mol/mg of protein) content in the freshwater fish *Cyprinus carpio* on the effect of lead acetate and *Spirulina* exposed to 120 hours

Organs	Groups	Exposure hours				
		24	48	72	96	120
Gill	Group I	10.35± 0.01	10.38 ± 0.02	10.49 ± 0.05	10. 78 ± 0.02	10.83 ± 0.04
	Group II	10.96 ± 0.10	11.36 ± 0.048*	12.69 ± 0.1*	13.45 ± 0.23*	14.56 ± 0.32*
	Group III	10.55 ± 0.02	10.65 ± 0.03*	10.78 ± 0.05*	10.86 ± 0.10*	10.56 ± 0.02*
	Group IV	10.41 ± 0.03	10.45 ± 0.03	10.43 ± 0.02	10.39 ± 0.02	10.36 ± 0.05
Liver	Group I	12.78 ± 0.03	12.83 ± 0.02	12.85 ± 0.01	12.93 ± 0.04	12.97 ± 0.04
	Group II	13.89 ± 0.02	14.78 ± 0.04*	15.37 ± 0.04*	16.65 ± 0.20*	17.43 ± 0.01*
	Group III	13.33 ± 0.03	13.65 ± 0.02*	13.74 ± 0.03*	13.82 ± 0.12*	13.93 ± 0.04*
	Group IV	12.81 ± 0.01	13.10 ± 0.02	12.75 ± 0.01	12.33 ± 0.03	12.45 ± 0.02

Table 2: Changes in the level of superoxide dismutase (U/min/mg of protein) activity in the freshwater fish *Cyprinus carpio* on the effect of lead acetate and *Spirulina* exposed to 120 hours

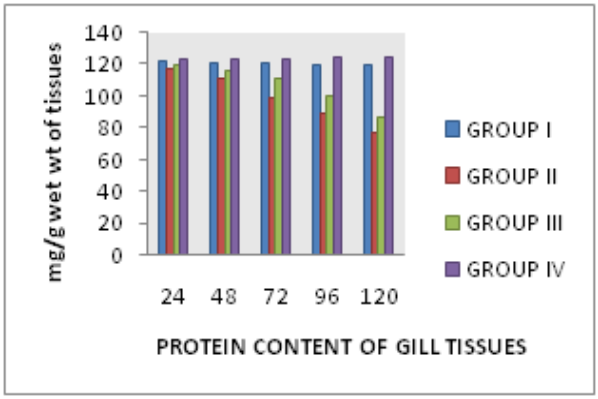
Organs	Groups	Exposure hours				
		24	48	72	96	120
Gill	Group I	39.15 ± 0.24	39.35 ± 0.21	39.45 ± 0.03	39.63 ± 0.27	39.75 ± 0.35
	Group II	34.24 ± 0.1	31.34± 0.35*	28.75 ± 0.19*	26.34 ± 0.27*	23.45 ± 0.24*
	Group III	38.45 ± 0.35	37.78 ± 0.23*	36.89 ± 0.34*	37.34 ± 0.34*	38.56 ± 0.12*
	Group IV	39.23 ± 0.23	39.35 ± 0.15	39.55 ± 0.32	39.71 ± 0.16	39.82 ± 0.24
	Group I	43.45 ± 0.04	43.56 ± 0.25	43.61 ± 0.23	43.72 ± 0.32	43.82 ± 0.16
	Group II	41.43 ± 0.12*	38.19 ± 0.26*	36.76 ± 0.14*	37.20 ± 0.17*	34.40 ± 0.25*

Liver	Group III	42.37 ± 0.32*	41.56 ± 0.34*	41.18 ± 0.23*	41.76 ± 0.26*	41.98 ± 0.32*
	Group IV	43.56 ±0.12	43.72 ± 0.22	43.81 ± 0.15	43.95 ± 0.13	44.24 ± 0.26

Table 3: Changes in the level of catalase (µ mol of H_2O_2 consumed/ min/mg of protein) activity in the freshwater fish *Cyprinus carpio* on the effect of lead acetate and *Spirulina* exposed to 120 hours

Organs	Groups	Exposure hours				
		24	48	72	96	120
Gill	Group I	9.10 ± 0.16	9.35 ± 0.02	9.41 ± 0.01	9.44 ± 0.02	9.46 ± 0.03
	Group II	8.75 ± 0.16	8.35 ± 0.34*	7.36 ± 0.25*	7.12 ± 0.14*	6.47 ± 0.04*
	Group III	8.93 ± 0.16	8.76 ± 0.23*	8.35 ± 0.15*	8.05 ± 0.04*	8.27 ± 0.23*
	Group IV	9.15 ± 0.33	9.45 ± 0.24	9.55 ± 0.15	9.63 ± 0.32	9.67 ± 0.02
Liver	Group I	12.65 ± 0.21	12.68 ± 0.14	12.72 ± 0.23	12.75 ± 0.25	12.81 ± 0.18
	Group II	11.89 ± 0.15*	11.23 ± 0.15*	10.13 ± 0.34*	9.56 ± 0.32*	9.12 ± 0.23*
	Group III	12.34± 0.34	11.86 ± 0.24*	11.45 ± 0.25*	11.64 ± 0.12*	11.74 ± 0.15*
	Group IV	12.73 ± 0.16	12.83 ± 0.06	12.91 ± 0.14	13.10 ± 0.13	13.23 ± 0.15*

Figure 1: Changes in the level of protein (mg/g wet wt. of tissue) content in the freshwater fish *Cyprinus carpio* on the effect of lead acetate and *Spirulina* exposed to 120 hours



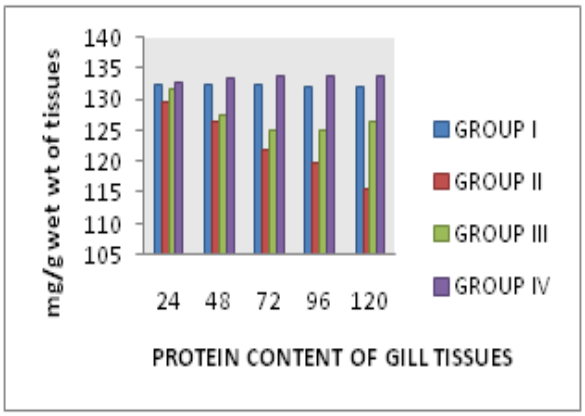
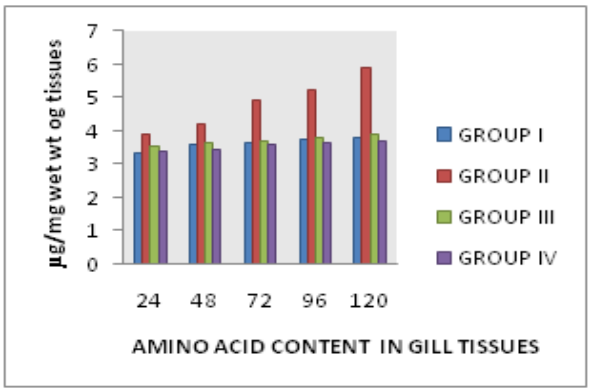


Figure 2: Changes in the level of amino acid (µg/mg wet wt. of tissue) content in the freshwater fish *Cyprinus carpio* on the effect of lead acetate and *Spirulina* exposed to 120 hours



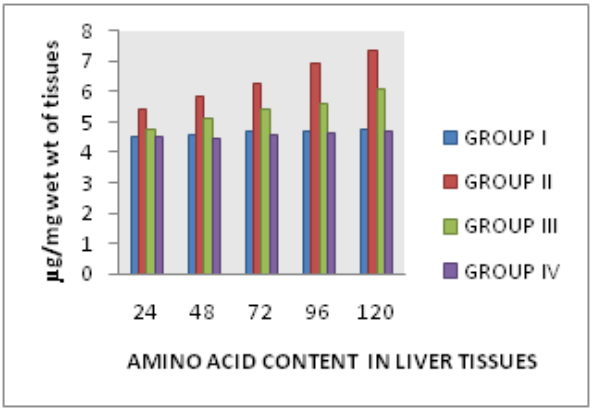
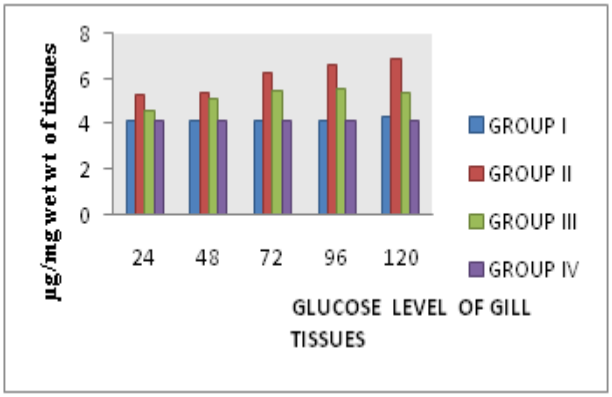
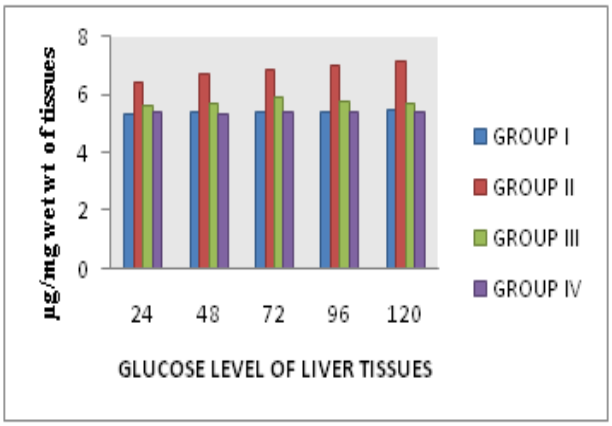


Figure 3: Changes in the level of glucose (µg/mg wet wt. of tissue) content in the freshwater fish *Cyprinus carpio* on the effect of lead acetate and *Spirulina* exposed to 120 hours





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