Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 605-609

Received: 01-03-2019
Accepted: 05-04-2019

Anand Kumar
Department of Zoology and Aquaculture, Chaudhary Charan Singh Haryana, Agricultural University, Hissar, Haryana, India

RK Gupta
Department of Zoology and Aquaculture, Chaudhary Charan Singh Haryana, Agricultural University, Hissar, Haryana, India

VP Sabhlok
Department of Zoology and Aquaculture, Chaudhary Charan Singh Haryana, Agricultural University, Hissar, Haryana, India

Correspondence
Anand Kumar
Department of Zoology and Aquaculture, Chaudhary Charan Singh Haryana, Agricultural University, Hissar, Haryana, India

Modulatory effects of Ascorbic acid in fresh water fish (*Cirrhinus mrigala*) following simultaneous exposure to heavy metals

Anand Kumar, RK Gupta and VP Sabhlok

Abstract
The present experiment was carried out in laboratory of department of zoology and aquaculture in CCS HAU, Hisar. The fresh water fish *Cirrhinus mrigala* were exposed to two sub lethal concentration of zinc and lead of three-dose levels i.e 0.01, 0.02, 0.04 ppm for 21 days with ascorbic acid feeding at the rate of 400 mg/kg of diet to find out the ameliorating effects of the ascorbic acid. The ascorbic acid content were analyzed and found decrease with an increase of the dose level of lead. It was observed 35.05% decreased at 0.01pm 43.38 % at 0.02ppm and 66.85 % at 0.04 ppm as compared to the control while in case of zinc treatment 66.07 % at 0.01ppm, 72.20 % at 0.02ppm and 84.04 % at 0.04 ppm as compared to the control and also showed 8% liver protein decreased at 0.04 ppm of lead, while serum protein decreased 11 % less as compared to the normal feeding experiment. In zinc treatment it was noted that 5 % liver protein decreased less but 10 % serum protein decreased less at 0.04 ppm dose level as compared to the normal feeding. The cholesterol level also increased less. In zinc treatment it was increased 17% less at 0.04 ppm but in lead treatment cholesterol level increased 22% less as compared to the normal feeding.

Keywords: Ascorbic acid, lead, zinc, *Cirrhinus mrigala*, tissue

Introduction

The effects of water pollution are not only devastating to people but also to the animals, fish and birds as it makes the water unsuitable for drinking, agriculture and the industry. The contaminated water destroys the aquatic life and reduces its reproductive ability. The heavy metals are kept today at top priority list among the water pollutants as they are water soluble, non-degradable, vigorously oxidizing agents and strongly bind to many biochemical units. They produce cumulative toxicity in small doses over long periods of time and acute toxicity in higher doses. The ingestion of contaminated food is an obvious means of exposure to metals (Hall, 1995) [5] with the exception of occupational exposure, fish are acknowledged to be the single largest source of mercury for man. In some of the instances, fish catches were banned for human consumption because their total mercury content exceeded the maximum limits recommended by the FAO & WHO (1972) [3]. The other way out is the involvement of antioxidants like ascorbic acid, better known as vitamin C. Antioxidants thus are known to prevent the physiological, hematological and the histological alterations in fish against the damaging effects of pesticides or metal pollutants in aquatic environment. The other such antioxidants are vitamins A and E, beta-carotene and selenium. It has been shown that the pollutants increase the rate at which the body excretes ascorbic acid in response to a reduced stress caused by physical and physiological changes in fish. In most cases, vitamin C (ascorbate) is rapidly depleted in fish exposed to the sub lethal concentration of several inorganic and organic substances (Hota *et al*., 1993) [7]. Palace *et al*., 1996 found a decreased level of ascorbic acid content in some fishes upon an exposure to the pollutants. Administering high ascorbic acid to environmental-pollutant-exposed fish considerably neutralizes the toxic effect of the chemicals many folds. Furthermore, a high ascorbic acid supplementation in diet is also known to reduce the accumulation of pollutants residue in fish tissue.

Materials and Methods

The experiment was carried out in laboratory of department of zoology and aquaculture in CCS HAU, Hisar. The materials for experiment, 6-8 inch long fingerlings of *Cirrhinus mrigala* were collected from the local fish farm and acclimated in large tanks in lab conditions for about two weeks. These were exposed to the sublethal concentrations (<0.1 ppm) of zinc and lead for 45 days. Following treatments were given in small plastic tanks of 40 liter capacity. A replicated control without metal treatment was given. Metal treatment at 3 dose levels (0.01, 0.02 and 0.04 ppm). Each tank contained 8-10 fishes per replicate. The fish in each treatment was fed with normal standard diet on alternate days at the rate of 2 per cent of total fish body weight per replicate. Treated fish were exposed to the heavy metals at the most effective dose level and was then fed with diet supplemented with ascorbic acid at the rate of 400 mg/kg of diet to find out the ameliorating effects of the ascorbic acid. The acclimation tanks and the treatment tanks were filled up with the chlorine free tap water and aeration was provided continuously throughout the day with aeration unit. Three fishes from each treatment replicate were taken at the end of 21 days of the treatment and were analyzed for the estimation of various biochemical parameters in their tissues. Liver tissue/serum was also analyzed for total proteins (Lowry's *et al*., 1951) [9], cholesterol (Zlatkis *et al*., 1953) [13]. The fishes were subjected to metal exposures, fed on food supplemented with ascorbic acid @ 400 mg/kg of diet and was analyzed after 21 days for assessing the ameliorating effects of ascorbic acid in metal contaminated water. Ascorbic acid contents were analyzed in liver/serum by the method of Palace *et al*., (1996).

Result and Discussion

Ascorbic acid (vitamin C) known as antitoxic substance decreases the effect of heavy metals and other pollutants. In the present study, the ascorbic acid was given to the fish as diet supplement at the rate of 400 mg/kg of diet to find out the ameliorating effects of ascorbic acid after 21 days treatment of lead (heavy metal). In case of lead treatment it was observed that the ascorbic acid contents decreased with an increased dose levels of lead as compared to the control (lead free water-treatment) and percentage of ascorbic acid contents decreased 35.05 % at 0.01ppm, 43.38 % at 0.02 ppm and 66.85 % at 0.04 ppm (Table 1 and fig. 1). In case of zinc treatment, ascorbic acid contents decreased with an increase in the dose level of zinc by 66.07 % at 0.01 ppm, 72.70 %, at 0.02 ppm and 84.04 % at 0.04 ppm as compared to control (zinc free water treatment) Table 2 (Fig. 2). All this data shows that the ascorbic acid content reduces with an increase of the dose level of heavy metals. Several authors studied the relationship between ascorbic acid content in different tissues and the environmental pollutants. In most cases vitamin C is rapidly depleted in the fish exposed to the sub lethal concentration of several inorganic and organic substances (Heath, 1995; Hota *et al.*, 1993) [6, 7]. Some workers however, found an increased level of ascorbic acid content in some fishes upon an exposure to some pollutants (Diwan *et al*., 1978; Heath, 1995) [2, 6]. There is a significant change in proteins level in liver tissues at different concentration of the heavy metal (lead) with normal feeding and with ascorbic acid feeding (Table 3 and fig. 3). So, estimation of vitamin C is an important index of the environmental stress. Fishes are unique among these animals in a way that they have a system to store a chemically stable form of vitamin C which appears to allow metabolism of this compound differently from other vitamin C requiring organisms (Tucker and Halver, 1984) [12]. With normal feeding, 21.29 % decline total protein at 0.01 ppm, 39.02 % decline at 0.02 ppm and 52.75 % decline at 0.04 ppm was noticed but with ascorbic acid feeding 24.14 % total protein decrease occurred at 0.01 ppm, 35.65 % at 0.02ppm and 47.64 % at 0.04 ppm. In this treatment it was observed that in presence of ascorbic acid total protein decrease less as compare to normal feeding diet. (Table 4 and fig. 4). The effect of lead on the total cholesterol after 21 days with normal and ascorbic acid feeding (at the rate of 400 mg/kg of diet) (Table 5 and fig. 3) was observed the cholesterol level increased because of lead and zinc treatments and decrease because of ascorbic acid feeding. Ascorbic acid thus plays a role in oxidant defense and therefore participates in protection against such contaminants. Contaminant induced abnormalities such as skeleton deformities, fin erosions and skin lesions in fish can further be related to dietary ascorbic acid deficiency and/or over utilization of the ascorbic acid stored in the defense mechanism against toxicants (Guha *et al*., 1993; Thomas and Wofford, 1993; Palace *et al*., 1996) [4, 11]. Table 6 (Fig.4) shows that the cholesterol level increased in liver tissue of the treated fish with normal feeding and decrease with ascorbic acid feeding after the zinc treatment. Similar trends also observed by other researcher like Oil and cadmium exposure may cause markedly decreased levels of ascorbic acid in fish (Thomas and Wofford, 1993) [11]. A high concentration of vitamin C in the cell membrane regenerates the reduced vitamin E during the oxidation and reduction processes. Vitamin C is highly interactive and may fortify antioxidant defense and enhance immune responses directly by maintaining optimal vitamin E level (Oritiz *et al*., 1999) [10].

Table 1: Ascorbic acid contents (µg/g) after 21 days treatment of lead in *C. mrigala* fed on ascorbic acid diet (400 µg/kg of diet)

Treatment dose (ppm)	Ascorbic acid contents (µg /g)
Control	21.30 ± 3.45
0.01	13.83 ± 1.30 (35)
0.02	12.06±1.09 (42)
0.04	7.06 ± 0.40 (66)
SE(m)	1.45
CD at 5%	6.30

Figures in parentheses are the percentage change over contr

Table 2: Ascorbic acid contents (µg/g) after 21 days treatment of zinc in *C. mrigala* fed on ascorbic acid diet (400 mg/kg of diet)

Treatment dose (ppm)	Ascorbic acid contents (µg /g)
Control	32.60±8.75
0.01	11.06±4.07 (66)
0.02	9.06±3.96 (72)
0.04	5.20±1.92 (84)
SE(m)	7.96
CD at 5%	N.S

Figures in parentheses are the percentage change over control

Table 3: Effects of lead on total tissue protein (µg/g) after 21 days feeding with ascorbic acid (400 mg/kg of diet)

Treatment dose (ppm)	Total protein (µg/g)			
	Total liver protein		Total serum protein	
	Normal feeding	Ascorbic acid feeding	Normal feeding	Ascorbic acid feeding
Control	67.22± 0.30	73.44 ± 0.20	10.80±0.25	5.09 ± 0.27
0.01	58.87 ±0.10 (12)	63.87 ± 0.32 (13)	8.74±0.13 (19)	4.98 ± 0.21 (2)
0.02	49.13 ±0.26 (26)	55.32±0.27 (24)	8.00 ±0.23 (25)	3.99 ± 0.35 (21)
0.04	42.00±0.24 (37)	51.90 ± 0.14 (29)	4.90 ±0.24 (54)	2.90 ± 0.18 (43)
SE(m)	0.26	0.27	0.29	0.27
CD at 5%	0.86	0.90	0.94	0.90

Figures in parentheses are the percentage change over control

Table 4: Effects of zinc on total tissue protein (µg/g) after 21 days feeding with ascorbic acid (400 mg/kg of diet)

Treatment Dose (ppm)	Total protein (µg /g)			
	Liver protein		Serum protein	
	Normal feeding	Ascorbic Acid feeding	Normal feeding	Ascorbic acid feeding
Control	71.90 ± 0.04	80.39 ± 0.30	11.37±0.14	6.90 ± 0. 04
0.01	56.59 ± 0.25 (21)	60.98 ±0.33 (24)	9.46±0.11 (16)	5.85 ± 0. 29 (15)
0.02	43.84 ± 0.32 (39)	51.73±0.15 (35)	7.65±0.36 (32)	4.80± 0. 05 (30)
0.04	33.97 ± 0.35 (52)	42.09±0.35 (47)	6.88±0.31 (39)	4.05 ± 0.12 (29)
SE(m)	0.27	0.44	0.25	0.16
CD at 5%	0.88	1.44	0.83	0.53

Figures in parentheses are the percentage change over control

Table 5: Effects of lead on total tissue cholesterol (µg/g) after 21 days feeding with ascorbic acid (400 mg/kg of diet)

Treatment dose (ppm)	Total tissue cholesterol (µg /g)	
	Normal feeding	Ascorbic acid feeding
Control	28.27 ± 0.09	28.35 ± 0.17
0.01	36.00 ± 0.08 (28)	27.10 ± 0.11(3)
0.02	47.00± 0.05 (67)	38.20 ± 0.17(35)
0.04	49.24 ± 0.08 (75)	43.10 ± 0.05(53)
SE(m)	0.08	0.14
CD at 5 %	0.27	0.46

Figures in parentheses are the percentage change over control

Table 6: Effects of zinc on total tissue cholesterol (µg / g) after 21 days feeding with ascorbic acid (400 mg/kg of diet).

Treatment dose (ppm)	Total tissue cholesterol (µg / g)	
	Normal feeding	Ascorbic acid feeding
Control	28.20 ± 0.04	21.16 ± 0.28
0.01	32.29 ± 0.31 (16)	22.10 ± 0.40 (4)
0.02	42.00 ± 0.11 (53)	35.01 ± 0.51 (42)
0.04	51.00 ± 0.17 (82)	41.19 ± 0.55 (65)
SE(m)	0.19	0.45
CD at 5%	0.63	1.47

Figures in parentheses are the percentage change over control

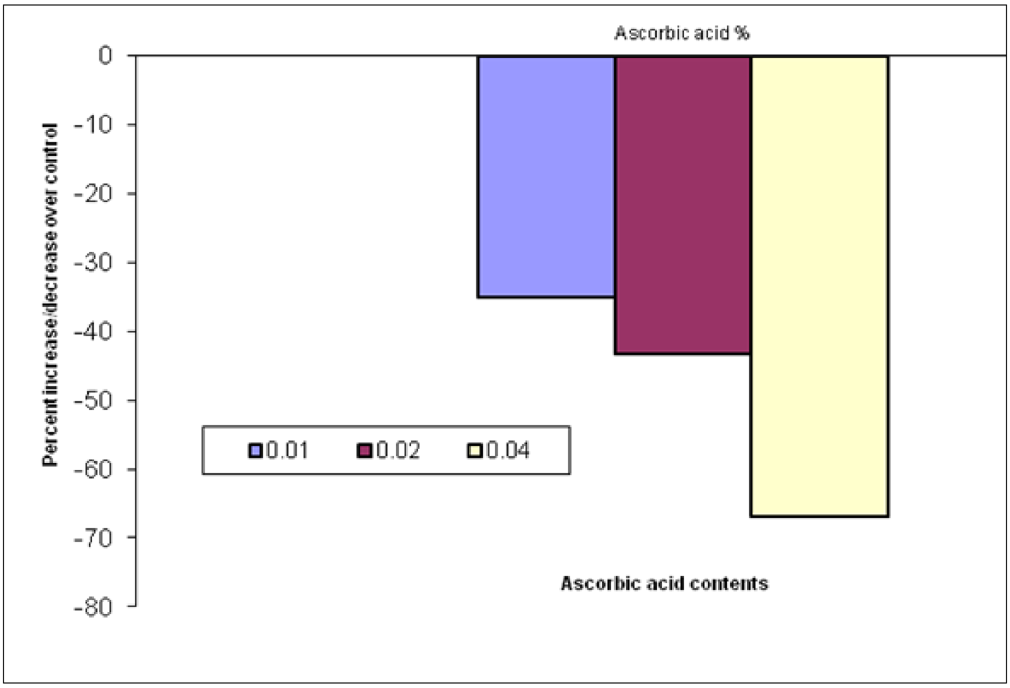

Fig 1: The ascorbic acid contents (SGLG) after 21 days treatment of lead in C. mrigala fed on Ascorbic acid (400mglkg of diet)

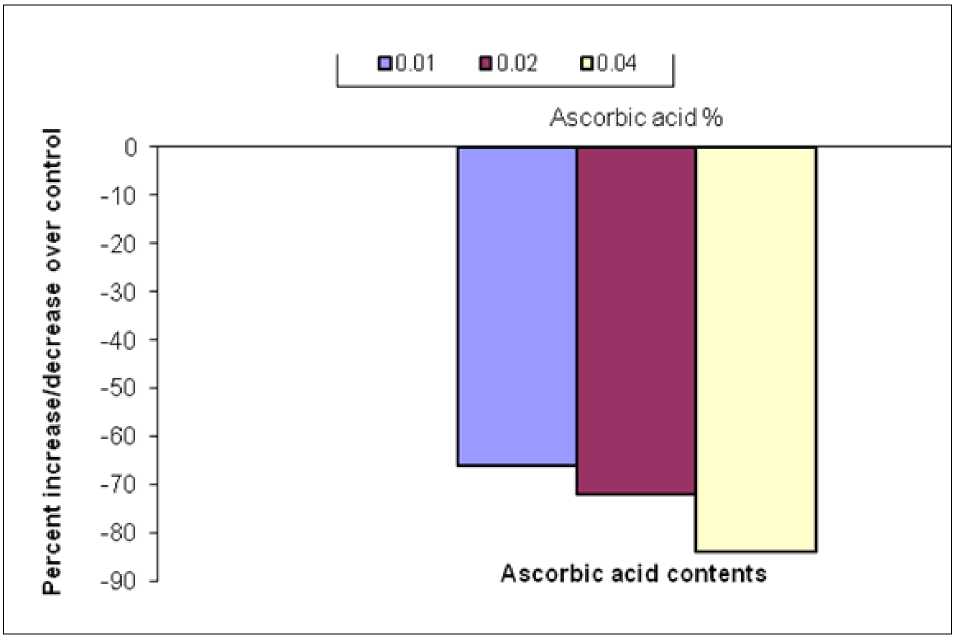

Fig 2: The ascorbic acid contents (Ng /g) after 21 days treatment of zinc in C. mrigala fed on Ascorbic acid (400mg/kg of diet)

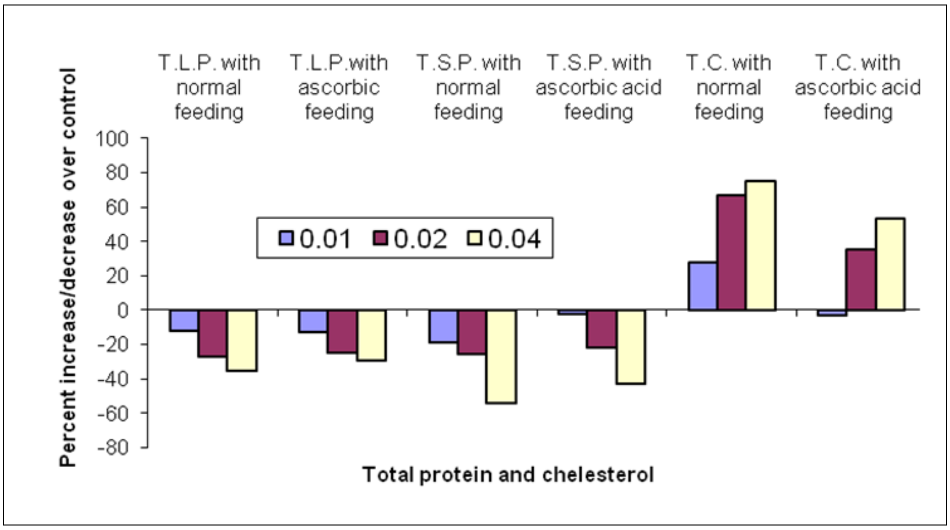

Fig 3: The effect of lead on total protein (pg g) and total chelesterol (p.g1g) in C. mrigala fed on Ascorbic acid (400 mg ikg of diet) 21 days treatment.

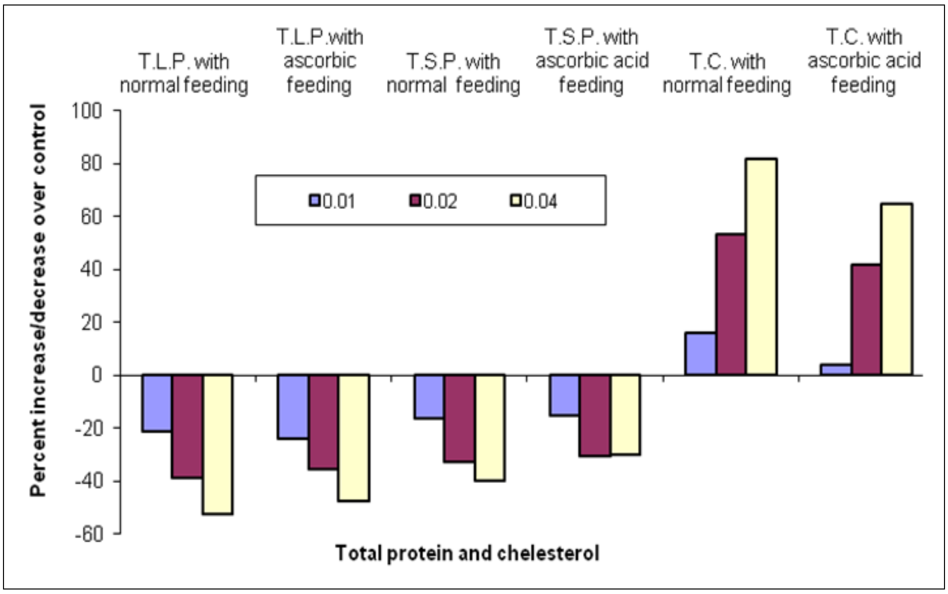

Fig 4: The effect of zinc on total protein (pg /g) and total chelesterol (p.glg) in C. mrigala fed on Ascorbic acid (400 mg /kg of diet) 21 days treatment

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