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Influence of Protective Agents in the Toxicity of Cadmium to a Freshwater Fish (*Channa punctatus*)

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Toxicity of cadmium to fishes has stimulated considerable interest in recent years. Studies have shown that other metals, vitamins, chelating agents and protein diets which alter the physiological, biochemical and behavioral aspects in fish also influence cadmium toxicity (Carlson 1975; Mahajan and Agrawal 1978; Verma et al. 1981). Selenium, as a micronutrient, and zinc, as an essential metal, have been well known for many years for their role in animal physiology. Respiratory parameters of an animal are important for assessing the toxic stress, as they are valuable indicators of the functions of all vital life - sustaining processes. In the present study, the effects of cadmium on the rate of uptake of oxygen by the whole body and different tissues of the freshwater fish *Channa punctatus* were investigated. The influence of zinc, selenium and ascorbic acid on the toxicity of cadmium were also examined.

MATERIALS AND METHODS

Channa punctatus (15±2 cm in length and 60±5 g in weight) were obtained locally from a fish pond, brought to the laboratory, and held in tap water for 15 days. During this period, they were fed twice daily with commercial food. The test aquaria were renewed every 24 hr with water of following characteristics: temperature 20±3°C, pH 7.4, alkalinity 86 mg/L as $CaCO_3$, dissolved oxygen concentration 8.0±2 mg/L and hardness 165 mg/L as $CaCO_3$. Preliminary toxicity tests conducted in the laboratory showed that 11.2 mg/L of cadmium produced 50% mortality of the test fish in 96 hr. The test concentration of cadmium used here was 11.2 mg/L and the concentrations of ascorbic acid, zinc and selenium were varied until there was no mortality and the concentration was recorded as per the method of Schubert et al. (1978). The concentrations of ascorbic acid, zinc and selenium so obtained were 0.009 mg/g body weight, 0.004 mg/L and 0.011 mg/L, respectively. One-tenth of these concentrations was used for chronic exposure studies with the two metals.

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After washing with 0.1% $KMnO_4$ solution, three - hundred fish selected at random were divided into six groups. No food was provided during the acute exposure. The first group of fish was exposed to LC_{50} of cadmium for 96 hr and ascorbic acid was injected to each fish intramuscularly daily for four days. The second group of fish, also exposed to the LC_{50} of cadmium for 96 hr was injected with an equal volume of distilled water and served as the control for the first group. The third and fourth groups of fish were exposed to cadmium + zinc and cadmium + selenium combinations separately for 96 hr. The fifth group was exposed to the LC_{50} concentration of cadmium for 96 hr and served as the control for the third and fourth groups. The sixth group of fish was maintained in water without any metal solution.

For chronic exposure studies, four sets of experiments were conducted in which food was provided daily to the fishes at the rate of 3% of their body weight. In each set a group of 30 fish was exposed for 15, 30, 60 and 120 days. The first group of fish was exposed to a sublethal concentration of 1.12 mg/L cadmium. The second and third groups were exposed to cadmium + zinc (1.12+0.0004 mg/L) and cadmium + selenium (1.12+0.001 mg/L) combinations separately, while the fourth group was held as control.

At the end of each experiment, 15 fish were selected for oxygen consumption studies. Each fish was transferred to a respirometer. The dissolved oxygen content of the medium was measured immediately after transfer and after 1 hr with a dissolved oxygen meter and a modified Winkler's method (U.S. Environmental Protection Agency 1979). The difference in the oxygen content of medium was taken as the oxygen consumed/g body weight / hr. For tissue - respiration studies muscle and gill were finely teased while liver and kidney were homogenized. The rate of oxygen consumption was measured using Warburg's constant volume respirometer. Fish Ringer solution in phosphate buffer at pH 7.5 was used as the suspension medium for the tissues (Ekberg 1958) and the oxygen consumption by different tissues was measured at 23°C. The rate of oxygen consumption was calculated by the procedure given by Umbriet et al. (1959). Student's t-test given by Wardlaw (1985) was used to calculate the significance of the difference between the control and experimental means.

RESULTS AND DISCUSSION

Oxygen uptake by the whole body and different tissues of *C.punctatus* decreased following acute and chronic exposures to cadmium. The whole-body oxygen consumption decreased significantly by 12.0% after 96 hr, while in chronic exposures to a sublethal concentration of cadmium the rate of oxygen consumption decreased gradually from 15 days (61.1%) to 120 days (41.6%), possibly due to the onset of poisoning which primarily involves respiratory distress. Ascorbic acid failed to reduce the toxicity of cadmium after 96 hr, as there was only a 0.8% alteration in the decrease in oxygen uptake. Zinc was more effective in counteracting the decrease in oxygen uptake induced by cadmium in the two types of exposure, while selenium was effective only after 96 hr, 30 and 60 days.

Eaton (1973) investigated the chronic toxicity of copper, zinc and cadmium to the fathead minnow (*Pimephales promelas*) and observed that the toxic effects of the mixtures attributable to copper appeared to be increased (synergistic), but that attributable to cadmium were reduced (antagonistic). The present study revealed that the overall efficiency of metals and ascorbic acid to counteract the decrease in whole-body oxygen consumption in all the experimental groups was in the order zinc > selenium > ascorbic acid.

After 96 hr of cadmium exposure, the rate of oxygen uptake by various tissues of fish decreased significantly. Gills showed the maximum decrease in oxygen uptake (23.6%), which became insignificant in combination with zinc. Although selenium and ascorbic acid in combination with cadmium also produced a significant decrease in gill respiration, the percent decrease was lower in the two combinations as compared to that of cadmium alone. Zinc, ascorbic acid and selenium restored the rate of oxygen uptake almost to the control level in the case of muscle and liver. No significant effect was produced by cadmium on kidney-oxygen uptake and and in the case of the three combinations the trend was to restore the oxygen uptake to the control level. Among the three chemicals, zinc was the most effective in reducing the toxicity of cadmium and the protective effect in the tissues was in the order gill > liver > muscle > kidney.

Table 1. Oxygen consumption values (ml of O_2/g body weight / hr) in control *Channa punctatus* and those exposed to cadmium, cadmium + ascorbic acid, cadmium + zinc and cadmium + selenium. Values in parentheses indicate percent decrease from control.

Exposure period	Control	Cadmium	Cadmium + Ascorbic acid	Cadmium + Zinc	Cadmium + Selenium
96 hr	35.00±3.01	30.80±2.6* (12.0)	31.9±2.1* (11.2)	33.14±3.2 (5.3)	31.82±1.6 (9.1)
15 days	34.91±2.2	29.28±3.1* (16.1)	-	31.11±1.3 (10.9)	30.85±2.3* (11.6)
30 days	35.09±1.1	28.9±1.6* (17.6)	-	33.72±2.1 (3.9)	32.62±1.1 (7.0)
60 days	35.60±1.0	26.14±2.1* (26.6)	-	34.63±1.2 (2.7)	32.97±2.3 (7.4)
120 days	38.80±2.8	20.90±1.4** (41.6)	-	32.76±1.5 (8.5)	27.23±1.3* (23.9)

Values are mean ± SD for 12 observations
Values are significant at $^{*}P < 0.05$; $^{**} P < 0.01$

Oxygen uptake by the tissues of fish gradually decreased with an increase in the period of exposure to sublethal concentration of cadmium. The percent decrease was higher in gills in all the stages of chronic exposure as compared to other tissues, except at 15 and 60 days of exposure where liver showed the highest percent decrease (19.7% and 63.2%) respectively. Of the four tissues selected for study, the most affected tissue was gill followed by muscle, liver and kidney.

Table 2. Tissue respiration in control *Channa punctatus* and those exposed to cadmium, cadmium + ascorbic acid, cadmium + zinc and cadmium + selenium for 96 hr (µl of O_2 consumed /g/hr). Values in parentheses indicate percent decrease from control.

Tissue	Control	Cadmium	Cadmium + Ascorbic acid	Cadmium + Zinc	Cadmium + Selenium
Gill	1635±21.6	1248±16.5* (23.6)	1365±11.2* (16.5)	1517±14.3 (7.2)	1405±9.3* (14.1)
Muscle	286±8.4	268±5.4* (6.3)	270±2.3 (5.6)	280±4.1 (2.1)	276±3.2 (3.5)
Liver	786±16.3	692.±8.2* (12.0)	702±6.3 (10.7)	762±10.1 (3.1)	725±5.3 (7.8)
Kidney	1263±9.2	1122±11.6 (11.2)	1137±13.5 (10.1)	1217±11.2 (3.6)	1165±9.5 (7.8)

Values are mean ± SD for 12 observations
Values are significant at $^*P < 0.05$

Gill was the most affected tissue as it comes directly in contact with the ambient medium. Decrease in oxygen uptake adversely affects the aerobic metabolism of tissues and the fish is forced to favor anaerobic metabolism for the energy demands of the body. Decrease in oxygen uptake may possibly be due to the "coagulation film anoxia", in which mucus lost from gills formes a thin coating over the lamellae, thus adversely affecting the absorption of oxygen from the surrounding medium.

Table 3. Tissue respiration in control *Channa punctatus* and those exposed to cadmium, cadmium + zinc and cadmium + selenium for 15 days (µl of O_2 consumed /g/hr). Values in parentheses indicate percent decrease from control.

Tissue	Control	Cadmium	Cadmium + Zinc	Cadmium + Selenium
Gill	1638±21.2	1401±20.9* (14.5)	1592±16.2 (2.8)	1446±11.3 (11.7)
Muscle	283±7.0	257±5.1* (9.2)	2.72±4.3 (3.9)	263±3.9 (7.1)
Liver	785±11.7	630±7.4* (19.7)	713±9.7 (9.2)	682±8.6 (13.1)
Kidney	1261±22.1	1195±20.7 (5.2)	1226±11.5 (2.8)	1207±13.2 (4.3)

Values are mean ± SD for 12 observations
Values are significant at $^*P < 0.05$

Table 4. Tissue respiration in control *Channa punctatus* and those exposed to cadmium, cadmium + zinc and cadmium + selenium for 30 days (µl of O_2 consumed /g/hr). Values in parentheses indicate percent decrease from control.

Tissue	Control	Cadmium	Cadmium + Zinc	Cadmium + Selenium
Gill	1631±14.6	1170±20.2* (28.3)	1584±12.1 (2.9)	1298±16.2* (20.4)
Muscle	282±8.2	210±5.6* (25.5)	269±7.1 (4.6)	229±3.4* (18.8)
Liver	787±11.4	603±7.6* (23.4)	748±5.3 (5.0)	648±3.2* (17.7)
Kidney	1264±24.5	1090±17.9* (13.8)	1229±3.1 (2.8)	1139±5.1 (9.9)

Values are mean ± SD for 12 observations
Values are significant at $^{*}P < 0.05$

Table 5. Tissue respiration in control *Channa punctatus* and those exposed to cadmium, cadmium + zinc and cadmium + selenium for 60 days (µl of O_2 consumed /g/hr). Values in parentheses indicate percent decrease from control.

Tissue	Control	Cadmium	Cadmium + Zinc	Cadmium + Selenium
Gill	1631±26.4	743±18.6* (54.4)	1578±20.3 (3.2)	869±11.2** (46.7)
Muscle	284±6.2	146±7.1** (48.6)	273±3.2 (3.9)	198±5.4* (30.3)
Liver	782±10.2	494±6.8** (63.2)	716±5.4 (8.4)	576±3.2* (26.3)
Kidney	1262±21.6	936±18.6** (25.8)	1185±11.7 (6.1)	995±2.1* (21.2)

Values are mean ± SD for 12 observations
Values are significant at $^{*}P < 0.05$; $^{**}P < 0.01$

Inclusion of zinc in the ambient medium along with cadmium had a protective effect on the toxicity of the latter as no significant difference was observed in the rate of oxygen consumption by all the tissues in all the chronic stages of exposure. Selenium exerted its protective effect only after 15 days.

Table 6. Tissue respiration in control *Channa punctatus* and those exposed to cadmium, cadmium + zinc and cadmium + selenium for 120 days (µl of O_2 consumed /g/hr). Values in parentheses indicate percent decrease from control.

Tissue	Control	Cadmium	Cadmium + Zinc	Cadmium + Selenium
Gill	1633±24.1	486±20.6** (70.2)	1502±11.3 (8.0)	681±13.8** (58.3)
Muscle	281±7.2	89.6±4.2** (68.1)	207±3.2* (26.3)	182±3.4* (35.2)
Liver	780±11.2	328±7.6** (58.1)	665±3.1 (14.7)	476±3.9* (39.1)
Kidney	1261±19.8	681±11.6** (46.1)	1129±11.3 (10.5)	891±10.5* (29.3)

Values are mean ± SD for 12 observations
Values are significant at $^{*}P < 0.05$; $^{**} P < 0.01$

In chronic exposure, zinc showed maximum protection against cadmium toxicity in all the tissues and it was more marked after 120 days. Restoration in tissue respiration was highest in case of gill. The protective action of zinc on cadmium - induced decrease in tissue respiration was in the order gill > liver > muscle > kidney, whereas in case of selenium the protective action was in the order liver > muscle > gill > kidney. Among the two metals, zinc was more effective than selenium in counteracting the toxicity of cadmium.

A combination of non - ionic detergent and mercury or copper produced a less than additive effect to rainbow trout (Calamari and Marchetti 1973). Regarding the mechanism of protective action of zinc and selenium on cadmium toxicity, all the metals occupy the same or neighboring receptor sites in the plasma membrane. According to Friberg et al. (1974) and Verma et al. (1982) the receptor sites are most likely the -SH groups. Partial occupation of these sites by the less toxic metal earlier than the highly toxic metal blocks the action and deposition of the more toxic metal. This results in an antagonistic effect of a combination. The existence of a protective mechanism against the toxic effects of heavy metals was suggested by the finding of simultaneous uptake, sometimes in equimolar amounts, of selenium and heavy metals in the fishes (Ganther et al. 1982; Froslie et al. 1985). The exact mechanism of counteracting the toxic effect of cadmium on fish after application of these protective agents is not clear and merits further study.

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