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Influence of Protective Agents on Metal Induced Respiratory Distress in *Labeo rohita* (Ham)

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Aquatic pollution has become a global problem and is posing a serious threat to the survival of aquatic organisms. Persistent presence of pollutants like heave metals in aquatic ecosystems has reportedly caused metabolic stress in organisms even to the extent of mortality in some cases. Investigations till date regarding the effect of such pollutants and the means to counteract this effect are insufficient (Bhattacharya 1995; Carlson 1975; Domingo, 1995; Ekberg 1958; Froslie et al.1974; Kukreja and Khan 1997; Liu et al.1995; Mahajan and Agarwal (1978; Pizzi et al. 1995). The present investigation thus attempts to take a step forward in this direction so that aquatic ecosystems can be protected and their economic value will be enhanced.

The aquatic organism used for the purpose of this investigation is Labeo rohita (Ham) which is a nutritionally and economically important culture fish. Respiratory parameters are key indicators to assess the toxic influence on the energy metabolism and physiological status of an organism, so the oxygen uptake of Labeo rohita has been considered as a measure of its metabolic rate. Thus the investigation involves the study of the toxic impact of cadmium on the whole body oxygen uptake and the oxygen uptake of different visceral organs (gills, muscle, liver and kidney) in L. rohita (Ham) under acute (96 hrs) and chronic exposure. The investigation also attempts to find out the influence of presence of protective agents against cadmium toxicity. The protective agents used for the study are selenium and zinc. Selenium is known to prevent cadmium caused necrosis, blood pressure, injury to pancreatic beta cells and induction of hepatoglucogenic enzymes. Zinc is known to neutralize the toxic effect of cadmium, prevent tissue necrosis and influence the susceptibility to cadmium because zinc appears to be competitive to cadmium.

MATERIALS AND METHODS

Healthy, disease free and alive Labeo rohita (18-20 cm in length, 80-90 gms in weight) were procured locally from specific water bodies and kept in aquaria containing laboratory tap water for 10 days. They were fed *ad libitum* (thrice a day), a commercial food, during this period. The water in aquaria was changed daily. The physico-chemical parameters of the aquaria water was also recorded (temperature 22 °C ± 1.5 °C, pH 7.4, alkalinity 88 mg/l as $CaCo_3$, D.O. 8.4 ± 2 mg/l and hardness 172 mg/l as $CaCo_3$). After random sampling and mild $KMno_4$ wash (to remove surface infectants), around 400 Labeo rohita were selected and divided into eight groups each of 50; four groups for acute exposure and four groups for chronic exposure.

Exposure of Labeo rohita to 11.2 mg/l of cadmium for 96 hours resulted in 50% mortality. Thus 11.2 mg/l was determined to be the sub-lethal concentration of Cd by probit analysis method (Finney 1971; Shaffi et al .1999). 0.003 mg/l of zinc and 0.014 mg/l of selenium were used as the test concentrations (Schubert et al. 1978).

Acute exposure was carried out for 96 hrs. The first group of L. rohita was subjected to

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acute exposure (96 hrs) of sub-lethal concentration of Cd (11.2 mg/l) alone. The second group of L. rohita was subjected to acute exposure of the test concentration of Zn 0.003mg/l) along with the sub-lethal concentration of Cd. The third group of Labeo rohita was subjected to acute exposure of the test concentration of Se (0.014 mg/l) along with the sub-lethal concentration of Cd. The fourth group of Labeo rohita was kept in water without any metal concentration and treated as control under acute exposure. During acute studies Labeo rohita was not provided any kind of food (Shaffi and Dubey 1989).

Chronic exposure was carried on for terms of 15, 30, 60, 120, and 150 days. The first group of Labeo rohita was subjected to chronic exposure (15, 30, 60, 120 and 150 days) of sub-lethal concentration of Cd (11.2 mg/l) alone. The second group of Labeo rohita was subjected to chronic exposure of the test concentration of Zn (0.003 mg/l) along with the sub-lethal concentration of Cd. The third group of Labeo rohita was subjected to chronic exposure of the test concentration of Zn (0.014 mg/l) along with the sub-lethal concentration of Cd. The fourth group of Labeo rohita was kept in water without any metal concentration and treated as control under chronic exposure. During chronic studies food was provided at the rate of 4% of their body weight.

Fifteen Labeo rohita from each of the four groups of acute exposure and the four groups of chronic exposure were selected for oxygen consumption studies and transferred to a respirometer. The dissolved oxygen (D.O) of the medium was recorded immediately after transfer and also after one hour of transfer with a dissolved oxygen meter using the modified Winkler`s method. The difference between the two readings was taken to be the oxygen uptake/g body weight/hr. This quantity is a measure of the whole body oxygen uptake and indicates the impact of the presence of metals in medium.

To study the oxygen uptake of different visceral organs, tissues from gills and muscles were finely teased and the tissues from liver and kidney were homogenised. Oxygen consumption was measured by using Warburg constant volume respirometer. Fish ringer solution in phosphate buffer at pH 7.5 was used as the suspension medium for the organs (Ekberg 1958). The temperature during measurement was kept at 27 °C (Umbriet et al. 1959). The experiment was repeated 10 times with different Labeo rohita each time.

The significance of the difference between the oxygen uptake of control and treated Labeo rohita was calculated using students "t" test.

RESULTS AND DISCUSSION

Both acute and chronic exposure to Cd markedly reduced the total body oxygen uptake in Labeo rohita. But the decrease in the body oxygen uptake rate was less in case of Se treated Labeo rohita of the third group (8.55% to 35.38%) as compared to the Zn treated fish of the second group (11.16% to 35.38%). This indicates that Se was more effective in counteracting the toxic effect of Cd (Tables 1-7).

Organ oxygen uptake in Labeo rohita registered maximum decrease in gills (26.63%) followed by the liver (22.07%), muscles (13.18%) and kidneys (11.33%). This pattern of results continued even when Zn and Se were used as the protective agents. Highest decrease in organ respiration was noticed in gills at a 15 day exposure (Tables (2-7).

The exposure to Cd decreased the whole body oxygen uptake in Labeo rohita due to poisoning and subsequent hypoxial condition followed by respiratory distress (Shaffi and Dubey 1989; Shaffi 1993; Shaffi 1995; Shaffi et al. 1999; Shaffi 1999a; Shaffi 1999b). The maximum decrease in organ oxygen uptake was registered in gills as compared to liver, muscle and kidney in Labeo rohita as the gills are in direct contact with the ambient medium.

The presence of Cd also induces the formation of a mucous envelope on the entire body surface and particularly in gills and that affects the absorption of oxygen from water (Bhattacharya 1995; Carlson 1975; Domingo, 1995; Ekberg 1958; Froslie et al.1974; Kukreja and Khan 1997; Shaffi 1993 ; Shaffi 1995 ; Shaffi et al. 1999; Shaffi 1999;

Table 1. Whole body oxygen uptake values (ml of oxygen/g. Body weight/hr) in control Labeo rohita and those exposed to cadmium, cadmium + zinc and cadmium + selenium.

Exposure Period	Control	Cadmium Treated	Cadmium + Selenium	Cadmium + Zinc
96 hr	42.10 ± 5.04	37.40* (11.16) ± 3.96	39.60 (5.93) ± 4.44	38.50 (8.55) ± 3.39
15 days	41.60 ± 4.64	35.20* (15.38) ± 5.26	37.40 (10.09) ± 6.24	36.30 (12.74) ± 4.96
30 days	42.80 ± 5.48	34.40* (19.62) ± 3.96	36.70 (14.25) ± 2.98	35.20* (17.75) ± 2.68
60 days	40.98 ± 3.88	31.30 (23.62) ± 2.84	34.10* (16.78) ± 3.12	32.70* (20.20) ± 4.10
120 days	41.79 ± 5.16	27.10* (35.15) ± 2.56	31.40* (24.86) ± 1.98	28.40** (32.04) ± 2.42
150 days	41.94 ± 4.24	25.60** (38.96) ± 1.75	30.90* (26.32) ± 2.16	27.10** (35.38) ± 1.38

Values are mean ± SDM for 10 observations and significant at * $p<0.05$ p**<0.01

Table 2. Organ oxygen uptake in control Labeo rohita and those exposed to cadmium, cadmium + zinc and cadmium + selenium for 96 hrs (ml of oxygen consumed/g/hr) values in parenthesis indicate percentage of fall from control (acute exposure)

Organs exposed	Control	Cadmium Treated	Cadmium + Selenium	Cadmium + Zinc
Gill	1840 ± 10.21	1350* (26.63 ± 96.12	1470* (20.10) ± 124.48	1390* (24.45) ± 148.26
Muscle	364 ± 49.36	316* (13.18) ± 32.48	342 (6.04) ± 42.56	324 (10.98) ± 38.16
Liver	924 ± 58.24	720* (22.07) ± 69.10	768 (16.88) ± 74.16	744 (19.48) ± 42.18
Kidney	1375 ± 76.18	1215 (11.63) ± 82.12	1295 (5.81) ± 98.24	1274 (7.20) ± 89.24

Values are mean ± SDM for 10 observations and significant at * $p<0.05$ p**<0.0

Table 3. Organ oxygen uptake in control Labeo rohita and those exposed to cadmium, cadmium + zinc, cadmium + selenium for 15 days (ml of oxygen consumed /g/hr) values in parenthesis indicate percentage of fall from control (chronic exposure)

Organs exposed	Control	Cadmium Treated	Cadmium + Selenium	Cadmium + Zinc
Gill	1815 ± 150.26	1365* (24.79) ± 110.16	1615 (11.01) ± 138.24	1470* (19.00) ± 152.18
Muscle	356 ± 54.48	294 (17.41) ± 38.28	336 (5.61) ± 52.39	334 (6.17) ± 44.26
Liver	936 ± 69.62	760* (18.80) ± 75.64	866 (7.46) ± 84.41	846 (9.61) ± 56.14
Kidney	1356 ± 98.24	1294 (4.49) ± 116.76	1310 (3.39) ± 105.28	1301 (4.05) ± 96.38

Values are mean ± SDM for 10 observations and significant at * $p<0.05$ p**<0.01

Table 4. Organ oxygen uptake in control Labeo rohita and those exposed to cadmium, cadmium + zinc, cadmium + selenium for 30 days (ml of oxygen consumed/g/hr) values in parenthesis indicate percentage of fall from control (chronic exposure)

Organs exposed	Control	Cadmium Treated	Cadmium + Selenium	Cadmium + Zinc
Gill	1820 ± 165.12	1290* (29.12) ± 235.48	1440 (20.87) ± 170.14	1370* (24.72) ± 188.36
Muscle	340 ± 40.46	276* (18.82) ± 46.52	300 (11.76) ± 38.24	286 (15.88) ± 40.46
Liver	924 ± 72.28	720* (21.21) ± 88.96	780 (15.58) ± 96.12	740 (19.91) ± 68.24
Kidney	1340 ± 110.32	1135* (15.29) ± 120.48	1226 (8.50) ± 80.50	1180 (11.94) ± 105.10

Values are mean ± SDM for 10 observations and significant at * $p<0.05$ p**<0.01

Table 5. Organ oxygen uptake in control Labeo rohita and those exposed to cadmium, cadmium + zinc, cadmium + selenium for 60 days (ml of oxygen consumed/g/hr) values in parenthesis indicate percentage of fall from control (chronic exposure)

Organs exposed	Control	Cadmium Treated	Cadmium + Selenium	Cadmium + Zinc
Gill	1380 ± 145.10	980** (46.44) ± 120.42	1390* (24.40) ± 138.42	1190* (34.07) ± 162.10
Muscle	357 ± 35.28	290 (18.76) ± 30.18	317 (11.20) ± 38.24	302 (15.40) ± 30.48
Liver	920 ± 72.28	680 (26.08) ± 89.29	760 (15.58) ± 20.12	730 (20.65) ± 75.75
Kidney	1360 ± 105.49	1130* (16.91) ± 101.38	1260 (7.35) ± 70.10	1190 (12.50) ± 95.125

Values are mean ± SDM for 10 observations and significant at * $p<0.05$ p**<0.01

Table 6. Oxygen oxygen uptake in control Labeo rohita and those exposed to cadmium, cadmium + zinc, cadmium + selenium for 120 days (ml of oxygen consumed/g/hr) values in parenthesis indicate percentage of fall from control (chronic exposure)

Exposure Period	Control	Cadmium Treated	Cadmium + Selenium	Cadmium + Zinc
Gill	1830 ± 170.64	586** (67.97) ± 80.56	1280* (30.05) ± 65.10	940* (48.63) ± 70.10
Muscle	360 ± 72.16	240* (33.33) ± 32.42	290 (19.44) ± 40.24	270* (25.00) ± 64.24
Liver	940 ± 78.28	460* (51.06) ± 28.12	700 (22.53) ± 39.64	540* (42.55) ± 28.28
Kidney	1360 ± 110.42	980* (27.94) ± 42.42	1160 (14.70) ± 68.12	1120 (17.64) ± 84.16

Values are mean ± SDM for 10 observations and significant at * $p<0.05$ p**<0.01

Table 7. Organ oxygen uptake in control Labeo rohita and those exposed to cadmium, cadmium + zinc, cadmium + selenium for 150 days (ml of oxygen consumed/g/hr) values in parenthesis indicate percentage of fall from control.

Organs exposed	Control	Cadmium Treated	Cadmium + Selenium	Cadmium + Zinc
Gill	1850 ± 195.86	460** (75.13) ± 65.24	960* (48.10) ± 75.45	720* (61.08) ± 82.42
Muscle	350 ± 70.70	285 (32.85) ± 32.16	270 (22.85) ± 40.42	255 (27.50) ± 47.20
Liver	940 ± 85.12	410* (57.29) ± 54.26	608 (36.66) ± 39.32	480** (50.00) ± 52.10
Kidney	1340 ± 124.11	1090 (18.65) ± 102.32	1160* (13.43) ± 104.10	1140* (14.92) ± 78.10

Values are mean ± SDM for 10 observations and significant at * $p<0.05$ $p^{**}<0.01$

Shaffi 1999; Schubert et al. 1978). Separate addition of Se and Zn to the ambient medium lowers the Cd toxicity because these being less toxic metals occupy the SH groups which act as the receptor sites on the plasma membrane. The partial occupation of these receptor sites block them and prevents their occupation by more toxic metals like Cd and thus promoting the oxygen uptake even in the toxicated conditions. Influence of non-metals in rainbow trout, was also reported and can be taken as a corollary to present investigation (Schubert et al. 1978; Umbriet et al. 1959; USEPA 1979; Verma et al. 1981). Moreover, the accumulation of CO_2 and its occupation of hemoglobin surface area due to the presence of Se or Zn can also be considered responsible for counteracting the Cd poisoning.

Acute and chronic exposure to sub-lethal Cd induces respiratory distress in Labeo rohita and to a maximum in gills as compared to other organs. Se and Zn help in counteracting this effect of Cd. Among the two Se is found to be more effective.

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