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Research Article

AMELIORATIVES ROLE OF VITAMIN C, RED CABBAGE EXTRACT (*BRASSICA OLERACEA*) AND TURMERIC (*CURCUMA LONGA*) RHIZOME EXTRACT ALLEVIATE CADMIUM-INDUCED OXIDATIVE STRESS IN FRESHWATER BLOCH (*HETEROPNEUSTES FOSSILIS*) IN LIVER, GILLS AND MUSCLE

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ABSTRACT

The aim of present study to investigate bioaccumulation potential of cadmium (Cd) and changes in oxidative stress indices in liver and muscle tissues from Cd-exposed Bloch (*Heteropneustes Fossilis*) with or without simultaneous treatment of water with vitamin C, red cabbage extract and turmeric. Bloch (*H. Fossilis*) with average length of 15 ± 5 cm and weight of 78 ± 6 g were used. Fishes were divided into nine groups (I to IX) each comprising 16 fishes. The fishes of groups II, III, IV and V were challenged with 3 ppm of cadmium chloride monohydrate ($CdCl_2.H_2O$), whereas groups VI, VII, VIII and IX were exposed to 6 ppm $CdCl_2.H_2O$ solution for a period of 45 days. Group I was kept as negative control and the fishes of this group were maintained in water containing no added Cadmium chloride. Group II and VI were maintained as Cd exposed non treated control to serve as positive controls. Fishes of III and VII, IV and VIII, V and IX received vitamin C (5 ppm), red cabbage extract (5 ppm) or turmeric extract (5 ppm), respectively during the entire experiment period. The concentrations of Cd in liver and gills increased significantly following exposure to Cd and the level continued to rise with the increase in exposure duration. The reduction in Cd induced oxidative stress was highest in vitamin C treated group followed by red cabbage extract and turmeric treatment. The results suggest that vitamin C, red cabbage extract and turmeric have potential to reduce tissue accumulation of Cd and associated oxidative stress in *Heteropneustes fossilis*.

Key Words: *Red Cabbage Extract, Turmeric Rhizome Extract, Bloch and Vitamin-C*

INTRODUCTION

Aquaculture is branch of science which provides the important source of protein rich food for humans and has become a popular component of human diet. Indian Bloch (*Heteropneustes fossilis*) is a hardy, bottom dweller and omnivorous fish. Natural water reservoirs are traditionally being used for aquaculture and they contribute significantly to total fish production across the world. Unfortunately, these natural resources are getting polluted with environmental pollutants and contaminants (Kumar *et al.*, 2007, 2008; Burger 2008). Industrial, agriculture and communal wastewater containing alarmingly high levels of heavy metals including cadmium (Cd) compounds enter into different water reservoirs without their prior treatment. This has resulted in higher concentrations of heavy metal residues in many fish culture ponds in different parts of India (Kumar *et al.*, 2007, Kumar *et al.*, 2008, Kumar and Singh 2011). Cd is a non-biodegradable heavy metal and is toxic to aquatic organisms at a low concentration in culture system (Burger 2008; Ng and Wood 2008). Major sources of this toxicant include Ni-Cd and Ag-Cd batteries manufacturing plants, sewage sludge and lead mining and processing units (Kumar *et al.*, 2007).

Phosphate fertilizers also contain high level of Cd, and therefore, run-off from agricultural land can also pollute the aquatic environment (Cherian and Goyer, 1989). Investigation of the chemical compounds within medicinal plants has become desirable. Therefore, it is essential to establish the scientific basis for their therapeutic actions as these may serve as the source for the development of effective drugs (Hossain *et al.,* 2012). It is bioaccumulation and biomagnification potential via food web pose serious public health risk to fish consumers (Viana *et al.,* 2005). Increased concentration of Cd has been found in liver, gills and muscle of freshwater Bloch *H. fossilis* procured from local markets of Western U.P., India (Kumar *et al.,* 2008; Kumar and Singh, 2011). Toxic effects of Cd are mostly linked to patho-physiological changes resulting mainly in muscle damage as expressed by depressed muscle function with rise in serum creatinine (Aoshima and Kasuya, 1987), disruption of normal calcium and iron metabolism, anaemia and inhibition of the activity of many important enzymes including alkaline phosphatase and Ca-ATPase (Thomas *et al.,* 1982). Besides, the increased utilization of vitamin C during Cd exposure causes reduction in tissue level of vitamins in rat (Chatterjee *et al.,* 1973) and juvenile mullet *Mugil cephalus* (Thomas *et al.,* 1982). The compare of dietary a-tocopherol with that of DL-tocopheryl acetate, both either alone or in combination with vitamin C (ascorbic acid), on the growth performance, survival, and stress resistance of angelfish was studied (Norouzitallab *et al.,* 2009). The nutritional effect of vitamin E in diets for Litopenaeus vannamei postlarve (PL19) was investigated (Ruff *et al.,* 2001). Free radicals and oxidative stress have been incriminated in the pathogenesis of several toxicities including Cd exposure in experimental rats (Oner *et al.,* 1995) and freshwater teleost, *Oreochromis mossambicus* (tilapia) (Basha and Rani, 2003) and goldfish (*Carassius auratus gibelio Bloch*.) (Zikic *et al.,* 2001) suggesting a beneficial role of antioxidants in the alleviation of Cd toxicity in fishes. The effects of aged garlic extract (AGE), establish the therapeutic window, and determine its protective mechanism in a cerebral ischemia model (Aguilera *et al.,* 2010). Therefore, this investigation was aimed to study the changes in oxidative stress indices in different tissues following Cd exposure to *H. fossilis* and ameliorative potential of vitamin C, red cabbage extract and turmeric in Cd-exposed fishes. Cured rhizomes provided high yield of volatile oil with appreciably high antioxidant potential (Gounder *et. al.,* 2012). The rate of accumulation which varied from tissue to tissue, may be attributed to the proximity of the tissues to the toxicant medium, their structural and functional organization and presence of various molecules having high affinity to cadmium. Five compounds from the red cabbage extract dimethyltrisulfide, acetic acid, dimethylsulfoxide, 4-(methylthio) butane nitrile and benzen epropanenitrile dhad the highest concentration when obtained by diethylether solvent extraction method (Ikeura *et al.,* 2012).

MATERIALS AND METHODS

Experimental Fishes

Bloch, *H. fossilis* with average length of 15 ± 5 cm and weight of 78 ± 6 g were procured from Nanak Matta Dam (Nanak Sager) Udham Singh Nagar, District Uttarakhand (U.K.) India. They were checked thoroughly for injury and disease conditions, and only healthy fishes were used for this study. After washing with 0.01% $KMnO_4$ solution for 15 min, they were placed in nine plastic pools (500 L) containing non-chlorinated water. Prior to the start of the experiment, the fishes were acclimatized to the food and laboratory conditions with 12 h dark and 12 height cycles , pH range of 6.95 to 7.60 and temperature ranging from 16 to 24°C for 15 days.

Experimental design

Fishes were divided into nine equal groups each comprising of 16 fishes (n=144). Each group was kept in separate plastic pools. Out of 9 groups, one group was kept as negative control; the fishes were maintained in water containing no added $CdCl_2.H_2O$ and without any treatment. The fishes of four groups were exposed to a sub-lethal concentration of 3 ppm solution of 98% pure $CdCl_2.H_2O$ (equivalent to 1.644 ppm Cd), where as remaining four groups were exposed to a sub lethal concentration of 6 ppm 98% pure $CdCl_2.H_2O$ (equivalent to 3.288 ppm Cd) added in the water for 45 days. Estimated concentrations of

Cd in plastic pool water were 1.643 and 3.285 ppm Cd for 3 and 6 ppm $CdCl_2.H_2O$ exposure, respectively. Among the Cd exposed groups, two groups, one from each of 3 and 6 ppm exposed groups were kept as non-treated control (positive control), whereas other exposed groups were treated with vitamin C (5 ppm), red cabbage extract (5 ppm) and turmeric extract (5 ppm), one from each of 3 and 6 ppm exposed groups during the entire experiment period. Chemicals used in the study $CdCl_2.H_2O$ (98% pure), vitamin C (99.7% pure) were of analytical grade and procured from Shanghai Renyoung Pharmaceutical Co. Ltd. The water, $CdCl_2$ and antioxidant treatments (vitamin C, red cabbage extract and turmeric) of each plastic pool were changed after 48 h. twelve fishes from each groups were scarified by decapitation on day 15, 30 and 45 of the experiment. Out of 12 fishes from each group, 6 fishes were used for estimation of antioxidant enzymes in muscle, liver, and gills and remaining 6 fishes were used for assessing Cd accumulation pattern in liver, gills and muscle.

Preparation of basal diet for fish

The basal diet for fishes was prepared as suggested by Datta and Kaviraj (2003) by mixing rice bran (25%), wheat flour (25%), mustard oil cake (22%), fish meal (26%) and mineral mixture (2%) containing copper (3.12%), cobalt (0.45%), magnesium (24.14%), iron (9.79%), iodine (1.56%), zinc (21.3%), calcium (30.0%), phosphorous (8.25%). Ingredients were mixed to form dough, passed through the sieve to prepare pellets and dried in hot air oven at 60-70 °C. The average concentration of Cd in feed was 0.009 ppm. A diet with an adequate vitamin C content to ensure normal growth and for batter growth use of mixture of mineral nutrient (Terova *et al.*, 1998)

Preparation of Red Cabbage Extract

First, prepare the red cabbage extract by either tearing or cutting three or four red cabbage leaves into small pieces and place into the blender. Add a small amount of hot distilled water and blend until the color is as dark as possible. This takes less than 2 minutes. Filter twice to allow collection of the red cabbage mush and the clean extract of anthocyanin. Discard the mush and set the extract aside and prepare the acid and base. The next step is to prepare the HCl and NaOH solutions. Muriatic acid tends to fume and is a strong acid. 0.1M solution sodium hydroxide. Dissolve 4.0 grams of NaOH (lye) in enough distilled water to make 1 liter. This has a pH of 13. 0.1M hydrochloric acid solution. Add 17 ml of muriatic acid (28% HCl) to make 1 liter of solution. This has a pH of 1.

Then we follow this serial dilution procedure:

1. Label 13 test tubes or other containers from 1 to 13.
2. Place 9.0 ml of distilled or deionized water in all test tubes except #1 and #13.
3. Prepare solutions in the acid range in the following manner:

a. Place 10.0 ml of 0.1M HCl in test tube #1. (pH = 1)
b. Transfer 1.0 ml of 0.1M acid from test tube #1 to test tube #2 and mix thoroughly. (pH = 2)
c. Transfer 1.0 ml of acid solution from test tube $2 to test tube #3 and mix thoroughly. (pH = 3)
d. Continue making the serial dilutions by transferring 1.0 ml of the most recently diluted acid solution to the next test tube until six acid solutions of pH 1 to 6 have been prepared. Be sure to mix each thoroughly before the transfer.

4. Add 10.0 ml distilled or deionized water to test tube #7. (pH=7)
5.Prepare solutions of base in the following way:

a. Place 10.0 ml of 0.1M NaOH in test tube #13.(pH=13)
b. Transfer 1.0 ml of 0.1M NaOH from test tube #13 to test tube #12 and mix thoroughly. (pH=12)
c. Continue making serial dilutions of the base going from pH 12 down to pH 8 by transferring 1.0 ml of the most recently diluted basic solution to the next test tube and mixing thoroughly each time. Then, add enough of the anthocyanin extracts to see the color change. To extract with acetone or ethyl alcohol, simply place a small amount of red cabbage leaves in a glass container and add the solvent to just cover the leaves. Allow 24 hours with occasional stirringto get the extraction. Filter and proceed with when required for experimentation (Figure A and Figure B).

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Preparation of Turmeric (Curcuma Longa) Rhizome Extract

The collected Rhizomes of *Curcuma longa* were boiling for several hours and dry them in ovens, cut into small pieces. Extraction was done by using Soxhlet ethanol (hydro-alcoholic) as solvent. The extracts were concentrated under reduced pressure, dried and stored at 400C in air tight containers for further studies (99% pure). (Figure C and Figure D)

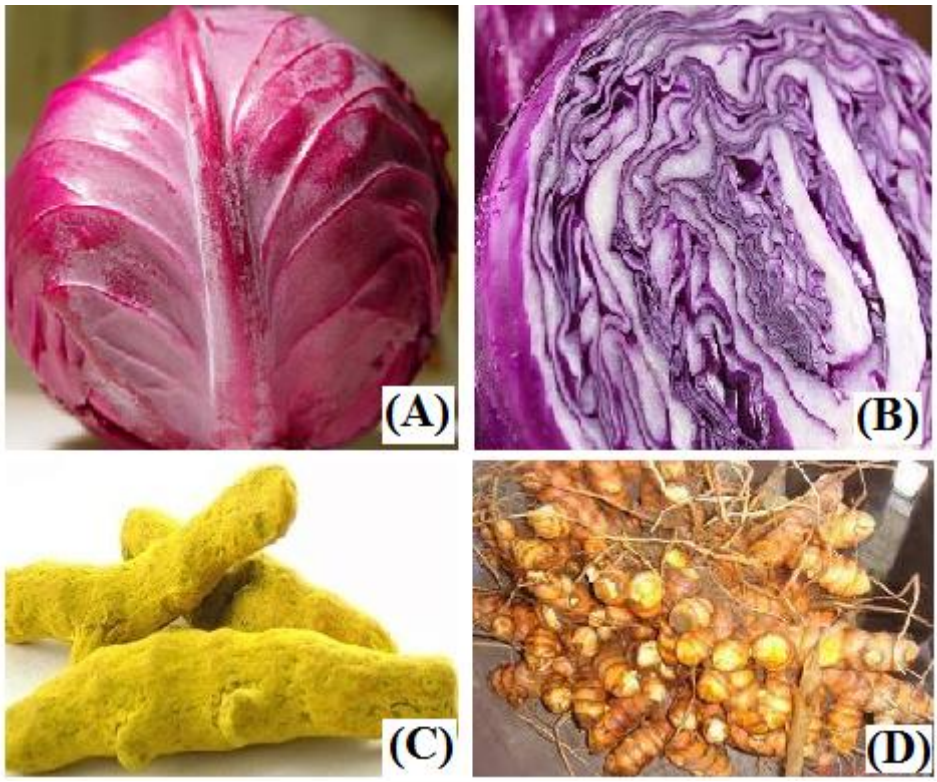

Figure 1: (A) Red Cabbage (B) Half part of Red cabbage (C) Dry Turmeric (Curcuma Longa) (D) Rhizone of turmeric (Curcuma Longa)

Biochemical analysis

Chemicals for analysis

Tris cacodylic acid, diethylenetriamine penta acetic acid (DTPA) (99% pure), nitro blue tetrazolium (98%), pyrogallol (N 98%), sodium dodecyl sulphate (N 99%), bovine serum albumin (97%), triton X-100 and thiobarbituric acid (99%) were purchased from Sigma Chemicals, USA. Nitric acid (69%), NaH_2PO_4 (98%), KH_2PO_4 (99.5%), perchloric acid (72%), sulphuric acid (98%), hydrochloric acid (36%) excelargrade and pyridine (99%), 1-butanol (0.99%) extra pure grade were purchased from sigma Aldrich.

Lipid peroxides

Endogenous lipid peroxide in 10% tissue homogenate of liver, gills and muscle was estimated following the method of Okhawa *et al.*, (1979). The homogenate was prepared in 1.15% Tris-KCl buffer and the protein content in the homogenate was measured by the method of Lowry et al., (1951). The level of endogenous lipid peroxide was expressed in nanomoles of malonyldialdehyde (MDA) per milligram of protein using 1.56×10^5 as the extinction coefficient (Utley *et al.*, 1967)

Superoxide dismutase and catalase

The activity of superoxide dismutase (SOD) was measured in the tissue supernatant following the method of Marklund and Marklund (1974) with certain modifications as suggested by Minami and Yoshikawa

(1979). Each unit of SOD activity is de fined as the quantity of the enzyme that inhibits auto-oxidation of pyrogallol by 50% in the assay mixture of 3 ml consisting of 50 mM of tris-cacodylic acid buffer (pH 8.2), enzyme preparation (sample) after suitable dilution of the sample and 0.2 mM of pyrogallol. The increase in absorbance due to auto-oxidation of pyrogallol was recorded at 420 nm wavelength in a spectrophotometer. The protein contenting the homogenate was estimated by the method of Lowry et al., (1951). Haemolysate was estimated spectrophotometrically at wavelength of 240 nm after appropriate dilution following the method of Cohen *et al.*, (1970). Briefly, the reaction was initiated by the addition of 50µl of diluted sample to 2.950 ml of phosphate buffer H_2O_2 solution. Initial absorbance was read against reference in which instead of H_2O_2, the same amount of phosphate buffer was added. Time required for the fall of absorbance by 0.50 of the initial absorbance was noted and catalase present in the assay mixture was expressed in units per milligram of protein or units per milligram of haemoglobulin for tissues and gills, respectively.

Cadmium concentration

Tissue samples of liver and muscle (0.5 g each) were wet digested with nitric and perchloric acids (3:1) mixture (Kolmer *et al.*, 1951). Two analytical blanks were run simultaneously with each batch of digestion with deionized triple distilled water as biosample. Equal amount of acid mixture was added in duplicate test samples, blanks and standard reference materials during digestion. The concentration of Cd in digested tissue samples was analyzed in an atomic absorption spectrophotometer (AAS 4141, ECIL, Hyderabad, India) at wavelength of 229.5 nm (detection limit 0.0 05 µg/ml) with 6 mA current. A recovery experiment was carried out by adding measured volumes of standard solution (Merck Pvt. Ltd., Mumbai, India) of Cd to measured volumes of a solution containing the samples dissolved in the acid mixtures. The spiked concentration was 1 ppm, while the average recovery of added Cd was 91 ± 0.35%. The presented data has not been corrected for this recovery. The average reading of blanks was subtracted from standard and test sample and then final concentration (µg/g) was calculated.

Statistical analysis

One way of analysis of variance (ANOVA) of parametric test followed by Tukey HSD test (SPSS, 2001) was performed to find out the significant differences among treatment groups within each period of Cd exposure.

RESULTS AND DISCUSSION

Concentration of Cd in Liver, Gills and Muscle

Tables 1 and 2 show the concentrations of Cd in the muscle and liver from the fishes exposed to Cd with or without treatment of water with vitamin C, red cabbage extract and turmeric. The concentration of muscle and liver was significantly ($P<0.05$) higher in Cd exposed groups than unexposed group, and the concentration continued to increase with the increase in duration of exposure. Fishes exposed to 3 ppm $CdCl_2.H_2O$ for 15 days had significantly ($P<0.05$) higher concentrations of Cd in liver and gills than those treated with vitamin C, red cabbage extract and turmeric. However, after 15 days of exposure, treatment with turmeric did not reduce Cd concentration significantly ($P<0.05$) in liver and muscle, although treatment with vitamin C and red cabbage extract reduced Cd concentration in the tissues compared to Cd exposed non-treated group. The concentration of Cd in liver and gills from fishes exposed to higher level of Cd together with vitamin C and red cabbage extract treatment was significantly lower on 30th and 45th day than those exposed to Cd alone. In general, turmeric treatment did not reduce Cd accumulation significantly in the tissues. The cadmium concentrations in muscles were not detectable range.

Lipid peroxides (LPO)

Table 3 shows the tissue LPO levels in liver, gills and muscles at different observation periods. The level of LPO in muscle and liver was significantly ($P<0.05$) greater in the group exposed to 3ppm level of $CdCl_2.H_2O$ and given no treatment than non-exposed group on day 45. However, 6 ppm exposure of

$CdCl_2.H_2O$ significantly (P<0.05) increased the LPO levels on day 15 onwards in liver and day 30 in gill. The LPO level increased with increasing duration of exposure in non-treated groups. The LPO level in Liver reduced significantly (P<0.05) in vitamin C and red cabbage extract groups as compared to non-treated group at both 3 and 6 ppm exposure levels on day 45. However, LPO level in fishes exposed to 6 ppm and treated with vitamin C reduced by day 30. LPO levels in gills of treated fishes exposed to 3 ppm $CdCl_2.H_2O$ were similar to that in negative control, but were greater in treated fishes exposed to 6 ppm $CdCl_2.H_2O$ than negative control on day 15 and 30.

Superoxide dismutase (SOD)

The SOD activity was significantly higher in liver and gills in 3 ppm $CdCl_2.H_2O$ exposed groups till 15th and 45th day respectively, as compared to respective control levels (Table 4). The subsequent observations in 3 and 6 ppm exposure groups revealed a comparable (P<0.05) SOD activity in liver between positive and negative controls. In general, treatment groups had higher SOD activity in tissues compared to group without Cd exposure. The SOD activity in non-treated Cd exposed group reduced gradually in both liver and gills, which was more evidenced in higher level of exposure, although reduction was not significant. However, red cabbage extract and turmeric treatment maintained the SOD activity in tissues. Overall, the activity of SOD in liver improved due to treatment with antioxidants

Catalase

Table 5 shows the catalase activity at different observation periods in different groups. The catalase activity reduced significantly in liver and gills with increasing duration of Cd exposure in fishes, which did not receive any treatment. However, vitamin C treatment with both level of exposures increased catalase activities in both the tissues. Red cabbage extract and turmeric treatment also improved catalase activity compared to non-treated Cd exposed group, especially by day 45. The catalase activity in muscles decreased gradually following Cd exposure both the levels. On day 15, the catalase activity in liver were greater in 3 ppm $CdCl_2.H_2O$ exposed groups compared to negative control, but the levels were similar among 3 ppm $CdCl_2.H_2O$ exposed groups. On day 30 and 45 for lower level of Cd exposure and from day 15 onward for higher level of Cd exposure, the activity of catalase in muscles increased due to treatment with antioxidants compared to non-treated groups, which was either higher or similar to negative control.

The mean cadmium concentration in the liver of different treatment groups at a given observation day was the least in fishes treated with vitamin C. For example, on day 45, Cd concentration in liver of vitamin C treated fishes were 6.69 (for 3 ppm $CdCl_2$ exposure) and 6.32 (for 6 ppm $CdCl_2$ exposure) percent lower than corresponding positive control levels. A careful observation of the data revealed that vitamin C had maximum mitigating ability, followed by red cabbage extract and turmeric. Ameliorative effect of vitamin C in Cd toxicity had also been reported in rats (Flora *et al.,* 1986; Kannan and Flora, 2004). The reduction in Cd accumulation by vitamin C might be due to the presence of C=O and -OH groups as side chain of sulfur. It reduces intestinal uptake of Cd in mammals (Sahagian *et al.,* 1967) an also accumulation (Fox, 1975), possibly by competing for the sulphydryl binding sites on metallothioneins (Evans *et al.,* 1970; Thomas *et al.,* 1982; Erdogan *et al.,* 2005). During chronic exposure, much of the metal is excreted in the bile, bound to glutathione (Cherian and Vostal, 1977; Arizono *et al.,* 1995) that may require vitamin C for its synthesis (Deana *et al.,* 1975). The vitamin C restores iron, zinc and calcium levels in Cd intoxicated quail (Fox 1975) and has been shown to be important for calcium uptake in trout (Mahajan and Agrawal, 1980). It has been suggested that partial occupation of sulphydryl binding sites by the less toxic metal than the highly toxic metal may block the action and deposition of the more toxic metals (Sastry and Shukla, 1994; Santos *et al.,* 2005). Significantly (P<0.05) higher levels of LPO in liver and gills of Cd exposed fishes indicated the occurrence of oxidative damage in the lipid membranes. It might be due to the toxic insult of excess Cd that was accumulated in different parenchymatous organs. Manca *et al.,* (1991) and Koizumi and Li (1992) also reported elevated LPO levels in various tissues of rats exposed to acute as well as chronic Cd toxicity. Gagne *et al.,* (2008) also reported increased LPO

Table1: Cadmium concentrations (ppm) in liver and gills of fishes exposed to 5 ppm cadmium chloride ($CdCl_2$) and treated with vitamin C, red cabbage extract and turmeric rhizome extract

Tissue	Periods (D)	Group					P Value
		None	$CdCl_2$	$CdCl_2$+ Vit-C	$CdCl_2$ + red cabbage extract	$CdCl_2$ + turmeric rhizome extract	
Liver	15	0.08 ± 0.04^{a}	37.30 ± 0.50^{dx}	26.60 ± 0.44^{bx}	28.26 ± 0.71^{bx}	31.16 ± 1.52^{cx}	<0.001
	30	0.08 ± 0.04^{a}	75.24 ± 0.51^{cy}	60.51 ± 0.69^{by}	60.71 ± 0.65^{by}	73.73 ± 4.07^{cy}	<0.001
	45	0.09 ± 0.00^{a}	119.22 ± 1.45^{cz}	101.39 ± 1.99^{bz}	103.51 ± 3.63^{bz}	112.47 ± 6.07^{bcz}	<0.001
	P Value	0.89	<0.001	<0.001	<0.001	<0.001	
Gills	15	0.05 ± 0.01^{a}	23.93 ± 0.51^{dx}	15.61 ± 1.51^{bx}	15.32 ± 1.17^{bx}	20.15 ± 1.89^{cx}	<0.001
	30	0.08 ± 0.03^{a}	46.91 ± 0.93^{dy}	28.17 ± 0.91^{by}	32.83 ± 0.61^{cy}	43.86 ± 3.28^{dy}	<0.001
	45	0.08 ± 0.01^{a}	77.15 ± 0.87^{dz}	40.93 ± 0.85^{bz}	54.32 ± 0.76^{cz}	71.19 ± 4.71^{dz}	<0.001
	P Value	0.44	< 0.001	<0.001	<0.001	<0.001	

a, b, c Values bearing different superscripts in a row differ significantly (Pb 0.05).
x, y, z Values bearing different superscripts in a column within tissues differ significantly (Pb 0.05)

Table:-2 Cadmium concentrations (ppm) in in liver and gills of fishes exposed to 10 ppm cadmium chloride ($CdCl_2$) and treated with vitamin C, red cabbage extract and turmeric rhizome extract

Issue	Periods (d)	Group					P value
		None	$CdCl_2$	$CdCl_2$+ Vit-C	$CdCl_2$ + red cabbage extract	$CdCl_2$ + turmeric rhizome extract	
Liver	15	0.07 ± 0.03^{a}	44.92 ± 0.57^{dx}	35.52 ± 0.59^{bx}	40.49 ± 0.59^{cx}	41.59 ± 3.716^{cx}	<0.001
	30	0.07 ± 0.03^{a}	83.45 ± 0.98^{dy}	75.57 ± 1.07^{by}	64.54 ± 1.16^{cy}	77.64 ± 4.936^{dy}	<0.001
	45	0.08 ± 0.00^{a}	151.73 ± 1.79^{dz}	124.85 ± 1.14^{bz}	142.03 ± 3.23^{cz}	140.95 ± 3.865^{cz}	<0.001
	P Value	0.88	<0.001	<0.001	<0.001	<0.001	
Gills	15	0.05 ± 0.01^{a}	36.93 ± 2.02^{cx}	33.95 ± 0.78^{bcx}	30.56 ± 1.38^{bx}	32.95 ± 2.27^{bcx}	<0.001
	30	0.07 ± 0.03^{a}	70.78 ± 0.78^{cy}	75.27 ± 0.77^{by}	56.28 ± 0.99^{by}	85.87 ± 1.69^{cy}	<0.001
	45	0.08 ± 0.01^{a}	94.87 ± 1.06^{dz}	79.87 ± 1.19^{bz}	85.03 ± 0.96^{cz}	86.27 ± 3.07^{dz}	<0.001
	P Value	0.43	< 0.001	<0.001	<0.001	<0.001	

Table 3: Lipid peroxides levels (nmol MDA/milligrams of protein or nmol MDA/milligrams of Hb) in liver, gills and muscle of cadmium chloride ($CdCl_2$) exposed fishes treated with vitamin C, red cabbage extract and turmeric rhizome extract.

Tissue	$CdCl_2$	Periods (D)	Group					P Value
			None	$CdCl_2$	$CdCl_2$+ Vit-C	$CdCl_2$ + red cabbage extract	$CdCl_2$ + turmeric rhizome extract	
Liver	5 ppm							
		15	5.17 ± 0.08	5.77 ± 0.35^{x}	5.88 ± 0.23	6.15 ± 0.29	6.30 ± 1.01	0.56
		30	5.36 ± 0.11	6.19 ± 0.42^{xy}	5.59 ± 0.28	6.27 ± 0.32	5.47 ± 0.99	0.60
		45	5.05 ± 0.12^{a}	6.85 ± 0.27^{by}	5.25 ± 0.26^{a}	5.81 ± 0.32^{a}	6. 07 ± 1.17^{a}	0.05
		P Value	0.11	0.05	0.25	0.53	0.84	
	10 ppm	15	5.19 ± 0.08^{a}	7.17 ± 0.22^{b}	6.78 ± 0.17^{by}	7.06 ± 0.28^{by}	7.14 ± 1.07^{b}	0.04
		30	5.36 ± 0.11^{a}	7.47 ± 0.29^{c}	6.08 ± 0.16^{abx}	6.67 ± 0.32^{bcxy}	7.05 ± 0.63^{bc}	0.002
		45	5.08 ± 0.13^{a}	7.88 ± 0.31^{c}	5.78 ± 0.14^{ax}	6.05 ± 0.11^{abx}	6.89 ± 0.73^{b}	<0.001
		P Value	0.11	0.21	< 0.0 01	0.03	0.97	
Gills	5 ppm	15	4.44 ± 0.07	4.89 ± 0.33^{x}	4.79 ± 0.29	4.70 ± 0.30	4.79 ± 0.60	0.92
		30	4.59 ± 0.10	5.07 ± 0.29^{xy}	4.75 ± 0.27	4.44 ± 0.37	4.88 ± 0.57	0.61
		45	4.6 4 ± 0.09^{ab}	5.75 ± 0.23^{b}	4.6 6 ± 0.32^{ab}	4.07 ± 0.31^{a}	4.38 ± 0.86^{ab}	0.05
		P Value	0.21	0.05	0.96	0.31	0.86	
	10 ppm	15	4.44 ± 0.06	6.55 ± 0.29^{x}	5.37 ± 0.07^{z}	5.34 ± 0.17	5.50 ± 0.75	0.18
		30	4.57 ± 0.11^{a}	6.24 ± 0.30^{cxy}	5.07 ± 0.13a^{by}	5.75 ± 0.29^{bc}	6.04 ± 0.61^{bc}	0.009
		45	4.65 ± 0.11^{a}	6.87 ± 0.28^{bz}	4.70 ± 0.09^{ax}	5.24 ± 0.22^{a}	5.59 ± 0.67^{a}	0.001
		P Value	0.21	0.02	0.0 01	0.27	0.82	
Muscles	5 ppm	15	2.44 ± 0.12	3.26 ± 0.27	2.65 ± 0.28	3.26 ± 0.29	3.24 ± 0.48	0.18
		30	2.45 ± 0.09^{a}	3.60 ± 0.32^{b}	3.33 ± 0.08^{ab}	2.66 ± 0.30^{ab}	3.13 ± 0.35^{ab}	0.03
		45	2.79 ± 0.10^{a}	4.03 ± 0.33^{b}	2.83 ± 0.28^{a}	2.85 ± 0.26^{a}	3.18 ± 0.37^{ab}	0.03
		P Value	0.07	0.21	0.13	0.31	0.98	
	10 ppm	15	2.44 ± 0.12^{a}	4.25 ± 0.30^{b}	3.87 ± 0.33^{by}	4.29 ± 0.21^{by}	4.12 ± 0.82^{b}	0.04
		30	2.46± 0.09^{a}	4.58 ± 0.26^{c}	3.54 ± 0.13^{by}	4.24 ± 0.21^{bcy}	3.97 ± 0.62^{bc}	0.002
		45	2.79 ± 0.10^{a}	5.15 ± 0.28^{b}	2.78 ± 0.13^{ax}	3.37 ± 0.36^{ax}	3.78 ± 0.97^{ab}	0.02
		P Value	0.07	0.10	0.007	0.04	0.96	

a, b, c Values bearing different superscripts in a row differ significantly (Pb 0.05).
x, y, z Values bearing different superscripts in a column within each level of $CdCl_2$ of tissues differ significantly (Pb 0.05)

Table 4: Superoxides dismutase activities (unit/milligram of protein or unit/milligram of Hb) in liver, gills and muscle of cadmium chloride ($CdCl_2$) exposed fishes treated with vitamin C, red cabbage extract and turmeric rhizome extract

Tissue	$CdCl_2$	Periods (D)	Group					P Value
			None	$CdCl_2$	$CdCl_2$+ Vit-C	$CdCl_2$ + red cabbage extract	$CdCl_2$ + turmeric rhizome extract	
Liver	5 ppm							
		15	6.13 ± 0.18^{a}	7.48 ± 0.22^{b}	7.60 ± 0.14^{bx}	7.60 ± 0.44^{b}	7.67 ± 0.79^{b}	0.06
		30	6.84 ± 0.44	7.16 ± 0.47	8.43 ± 0.26^{y}	7.82 ± 0.70	7.32 ± 0.98	0.41
		45	6.45 ± 0.34^{a}	6.86 ± 0.45^{a}	8.66 ± 0.37^{by}	8.75 ± 0.34^{b}	7.15 ± 0.52^{a}	0.002
		P Value	0.33	0.53	0.03	0.26	0.89	
	10 ppm	15	6.13 ± 0.17	6.85 ± 0.57	7.14 ± 0.27x	7.38 ± 0.69	7.21 ± 0.64	0.48
		30	6.84 ± 0.44	6.30 ± 0.46	7.27 ± 0.26xy	6.90 ± 0.73	7.77 ± 0.91	0.85
		45	6.45 ± 0.34^{ab}	5.50 ± 0.74^{a}	8.04 ± 0.29^{cy}	7.47 ± 0.69^{b}	5.90 ± 1.19^{ab}	0.06
		P Value	0.33	0.29	0.05	0.82	0.60	
Gills	5 ppm	15	11.48 ± 0.29^{a}	13.62 ± 0.47^{b}	13.31 ± 0.21^{bx}	15.63 ± 0.37^{c}	15.79 ± 0.81^{c}	<0.001
		30	10.87 ± 0.44^{a}	14.33 ± 0.31^{b}	14.45 ± 0.29^{cy}	15.88 ± 0.53^{ab}	15.27 ± 1.06^{ab}	<0.001
		45	11.13 ± 0.35^{a}	14.63 ± 0.64^{b}	16.73 ± 0.18^{by}	15.64 ± 0.68^{b}	15.73 ± 1.24^{b}	<0.001
		P Value	0.95	0.21	< 0.0 01	0.14	0.82	
	10 ppm	15	11.48 ± 0.29^{a}	13.64 ± 0.98^{ab}	13.49 ± 0.53^{abx}	13.88 ± 1.12^{ab}	14.39 ± 1.23^{b}	0.06
		30	10.87 ± 0.44^{a}	12.89 ± 1.38^{ab}	15.06 ± 0.28^{bxy}	14.58 ± 0.67^{b}	14.90 ± 1.08^{b}	0.01
		45	11.13 ± 0.35^{a}	11.90 ± 0.75^{a}	16.30 ± 0.79^{by}	15.50 ± 1.38^{b}	14.10 ± 0.96^{b}	0.001
		P Value	0.95	0.87	0.05	0.46	0.7	
Muscles	5 ppm	15	3.40 ± 0.16	4.48 ± 0.76	4.91 ± 0.10^{x}	4.89 ± 0.11^{x}	4.60 ± 0.77	0.21
		30	3.52 ± 0.26^{a}	4.23 ± 0.70^{ab}	5.67 ± 0.26^{by}	4.92 ± 0.25^{ab}	5.14 ± 0.69^{b}	0.04
		45	3.82 ± 0.35^{a}	3.88 ± 0.29^{a}	5.87 ± 0.31^{by}	5.73 ± 0.22^{by}	5.31 ± 0.82^{b}	0.004
		P Value	0.51	0.79	0.03	0.01	0.78	
	10 ppm	15	3.40 ± 0.16^{a}	4.15 ± 0.40^{ab}	4.86 ± 0.09^{bcx}	4.92 ± 0.14^{bc}	5.42 ± 0.77^{c}	0.01
		30	3.52 ± 0.25	3.93 ± 0.59	5.05 ± 0.23^{x}	4.78 ± 0.71	5.02 ± 0.74	0.21
		45	3.82 ± 0.35^{a}	3.25 ± 0.39^{a}	4.74 ± 0.16^{by}	4.83 ± 0.52^{ab}	4.27 ± 0.94^{ab}	0.03
		P Value	0.51	0.38	0.0 04	0.98	0.61	

a, b, c Values bearing different superscripts in a row differ significantly (Pb 0.05).
x, y, z Values bearing different superscripts in a column within each level of $CdCl_2$ of tissues differ significantly (Pb 0.05).

Table 5: Catalase activities (unit/milligram of protein or unit/milligram of Hb) in liver, gills and muscle of cadmium chloride ($CdCl_2$) exposed fishes treated with vitamin C, red cabbage extract and turmeric rhizome extract

Tissue	$CdCl_2$	Periods (D)	Group					P Value
			None	$CdCl_2$	$CdCl_2$+ Vit-C	$CdCl_2$ + red cabbage extract	$CdCl_2$ + turmeric rhizome extract	
Liver	5 ppm							
		15	151.7 ± 1.11^{a}	154.1 ± 2.64^{aby}	174.8 ± 1.52^{cx}	161.5 ± 2.32^{bx}	155.9 ± 5.57^{ab}	<0.001
		30	149.1 ± 0.88^{ab}	146.5 ± 1.14^{axy}	181.2 ± 1.88^{dy}	163.7 ± 2.42^{cx}	158.2 ± 6.52^{bc}	<0.001
		45	150.6 ± 0.77^{b}	140.2 ± 4.52^{ax}	184.7 ± 0.82^{dy}	172.3 ± 3.35^{cy}	167.4 ± 3.34^{c}	<0.001
		P Value	0.44	0.01	0.001	0.03	0.31	
	10 ppm	15	151.6 ± 1.11	148.7 ± 1.94^{z}	160.9 ± 2.37^{x}	154.10 ± 2.14^{x}	151.2 ± 7.64	0.24
		30	149.9 ± 0.88^{ab}	138.5 ± 5.14^{ay}	172.3 ± 3.00^{cy}	158.5 ± 3.81^{b}	148.8 ± 7.31^{ab}	<0.001
		45	150.5 ± 0.77^{c}	122.9 ± 0.72^{ax}	183.6 ± 1.09^{ez}	172.9 ± 1.83^{dy}	136.9 ± 5.67^{b}	<0.001
		P Value	0.44	<0.001	<0.001	0.001	0.29	<0.001
Gills	5 ppm	15	246.4 ± 1.17^{a}	284.5 ± 3.53^{bz}	287.6 ± 3.47^{bx}	283.6 ± 1.77^{b}	286.7 ± 4.27^{by}	<0.001
		30	248.4 ± 0.91^{a}	273.5 ± 1.19^{by}	294.1 ± 0.83^{dy}	284.8 ± 4.08^{c}	273.7 ± 1.88^{bx}	<0.001
		45	245.6 ± 0.88^{a}	262.2 ± 0.94^{bx}	308.1 ± 0.51^{dz}	290.3 ± 1.07^{c}	288.7 ± 4.02^{cy}	
		P Value	0.14	0.001	<0.001	0.13	0.04	
	10 ppm	15	246.4 ± 1.17^{ab}	251.5 ± 3.47^{ay}	274.4 ± 2.95^{cx}	254.5 ± 2.29^{bx}	244.7 ± 6.23^{ab}	<0.001
		30	248.4 ± 0.91^{b}	230.9 ± 3.18^{ax}	270.6 ± 0.90^{cx}	267.5 ± 1.58^{cy}	236.4 ± 6.63^{a}	<0.001
		45	243.6 ± 0.88^{b}	222.7 ± 1.06^{ax}	290.6 ± 1.86^{dy}	266.5 ± 1.69^{cy}	243.2 ± 6.76^{b}	<0.001
		P Value	0.14	0.001	<0.001	<0.001	0.64	
Muscles	5 ppm	15	166.6 ± 0.71^{a}	181.2 ± 1.89^{bz}	182.2 ± 0.84^{by}	182.7 ± 1.28^{by}	184.8 ± 1.08^{by}	<0.001
		30	168.4 ± 0.69^{c}	152.5 ± 0.34^{ay}	167.8 ± 1.24^{cx}	161.0 ± 0.85^{bx}	174.8 ± 4.03^{dx}	<0.001
		45	167.5 ± 0.36^{b}	120.6 ± 1.32^{ax}	154.7 ± 0.36^{dy}	182.6 ± 1.66^{dy}	172.4 ± 3.05^{cx}	<0.001
		P Value	0.12	<0.001	<0.001	<0.001	0.02	
	10 ppm	15	166.6 ± 0.71^{e}	132.1 ± 0.87^{az}	154.6 ± 0.39^{cx}	164.6 ± 1.18^{dy}	144.4 ± 2.29^{bx}	<0.001
		30	168.4 ± 0.69^{c}	121.9 ± 0.80^{ay}	187.5 ± 0.63^{dy}	151.9 ± 2.27^{bx}	148.6 ± 3.80^{bx}	<0.001
		45	167.5 ± 0.36^{b}	96.5 ± 0.85^{ax}	183.9 ± 2.98^{cy}	182.6 ± 0.93^{cz}	180.8 ± 3.64^{by}	<0.001
		P Value	0.12	0.001	<0.001	<0.001	<0.001	

a, b, c, d, e Values bearing different superscripts in a row differ significantly (Pb 0.05).
x, y, z Values bearing different superscripts in a column within each level of $CdCl_2$ of tissues differ significantly (Pb 0.05).

concentrations and DNA strand breaks in gills and digestive glands of freshwater mussels, Elliption complanata exposed to Cd salts. There are several pathways by which Cd is thought to induce oxidative stress. It inhibits the mitochondrial electron-transfer chain reaction, leading to accumulation of semi ubiquitous, which enables it to transfer one electron (e−) to molecular oxygen to form superoxide radicals (Wang *et. al.,* 2004). Further, it may also interference with cellular antioxidant defense system via alteration in activities of antioxidant enzymes viz. SOD and catalase and status of glutathione (Sandrini *et. al.,* 2006). LPO level in the liver and gills of fishes treated with vitamin C, red cabbage extract and turmeric were comparable to control, suggesting that the Cd acts as a catalyst in the oxidative deterioration of biological macromolecules and this effect could be minimized by treatment with antioxidants. Vitamin C was more effective followed by red cabbage extract and turmeric in reducing LPO activity in tissues, especially at high level of Cd exposure. The higher activities of SOD and catalase in liver than gills and muscles of different treatment groups suggested that liver is an active site for synthesis of these antioxidant enzymes. SOD and catalase activities were greater in Cd exposed non-treated fishes than in fishes without Cd exposure, and the activity tended to decrease from day 15 onward in Cd exposed fishes. In experimental Cd toxicity in Nile tilapia (*Oreochromis niloticus*), Almeida *et al.,* (2002) also observed increased activities of SOD and catalase in liver and muscles. Basha and Rani (2003) also noted significant elevations of SOD and catalase activities in liver and muscle from day 7 onward, and these activities were maintained until day 15 and then decreased slightly on day 30 of exposure. Increased activities of SOD and catalase in tissues might be due to the detoxification mechanisms under long term exposure of Cd to protect animals from free radicals. Basha and Rani (2003) suggested that up regulation of enzyme production might be a defense mechanism, providing first line of defence against Cd toxicity before the induction of metallothionein synthesis. The profiles of LPO and activities of SOD and catalase suggested that vitamin C had maximum efficacy followed by red cabbage extract and turmeric in mitigating Cd induced oxidative stress. Vitamin C scavenges aqueous reactive oxygen species by rapid electron transfer and there by acts as an important antioxidant defence against oxidative damage (Halliwell *et al.,* 1987). The present findings also revealed higher SOD activities in Cd exposed and red cabbage extract treated fishes as compared to control. These constituents might have involved in scavenging free radicals and alleviation of Cd induced oxidative stress. Both Cd bioaccumulation and oxidative stress data suggested that turmeric is the least effective among all the ameliorating agents tried in the present study. Its efficacy in cat fish seems to be less pronounced as compared to that of vitamin C, red cabbage extract.

CONCLUSION

Bioaccumulation of Cd in liver and gills of freshwater Bloch *H. fossilis* their associated oxidative stresses increase in a dose dependent manner. Treatment with vitamin C, red cabbage extract and turmeric potentially reduces the Cd accumulation in tissues and ameliorate oxidative stress as evidenced from lower concentrations of lipid peroxides and higher activities of superoxide dismutase and catalase in liver, gills and muscles. The efficacy to ameliorate Cd-induced oxidative stress was maximum with vitamin C treatment followed by red cabbage extract and turmeric.

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Competing Interests

The authors declare that there are no conflicts of interest

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