JEB
Journal of Environmental Biology

Journal Website : www.jeb.co.in
E-mail : editor@jeb.co.in
ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Ameliorative effects of *Rosmarinus officinalis leaf* extract and Vitamin C on cadmium-induced oxidative stress in Nile tilapia *Oreochromis niloticus*

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Publication Info

Paper received:
17 April 2014

Revised received:
09 December 2014

Accepted:
20 March 2015

Abstract

The present study was undertaken to assess the bioaccumulation potential of cadmium in liver, kidney, gills and muscles of freshwater fish, Nile tilapia *Oreochromis niloticus* and the changes in oxidative stress indices in liver and kidney with or without simultaneous treatment with waterborne vitamin C and rosemary leaf extract. Adult tilapia were divided into seven groups. Six groups were exposed to sublethal concentrations of Cd, three groups to 5ppm, while other three to 10ppm. Two groups from each of the Cd exposed groups were treated with Vitamin C (5ppm) and rosemary leaf extract(2.5ppm) for a period of 21 days. Cadmium concentration in liver, kidneys and gills was significantly higher in the cadmium exposed groups being invariably high in the groups exposed to 10ppm $CdCl_2.H_2O$.Treatment with Vitamin C and rosemary leaf extract significantly reduced cadmium concentration in comparison to non-treated Cd exposed groups. Treatment with Vitamin C and rosemary leaf extract significantly reduced oxidative stress in Cd exposed fish as evidenced from lower concentration of lipid peroxides and reduced activity of catalase and higher activity of superoxide dismutase in liver and kidney as compared to control fish. Reduction in Cd induced oxidative stress and bioaccumulation was comparable between the two antioxidant treatments, Vitamin C and rosemary leaf extract. The key findings suggest that both the antioxidants used showed ameliorative potential to reduce tissue accumulation of Cd and associated oxidative stress in fresh water fish, Nile tilapia.

Key words

Antioxidants, Nile tilapia, Oxidative stress, Rosemary leaf extract, Vitamin C

Introduction

Heavy metal contamination of aquatic ecosystem is one of the critical environmental issue which needs to be addressed. Heavy metal contamination causes detrimental effects on the ecological balance of the recipient environment and aquatic biodiversity (Vosyliene and Jankaite, 2006; Farombi *et al.,* 2007). Metals can accumulate in aquatic organisms, including fish and persist in water and sediments (Luoma and Rainbow, 2008). Fish are the top consumers in aquatic food chain and thus serve as ideal bioindicators of metal contamination. Since fish is an important component of human nutrition, a produce from contaminated sites presents a potential risk to human health. Cadmium is a non-essential toxic environmental pollutant. Over the years, anthropogenic activities have increased Cd levels in the environment. 10% of the total Cd in the environment is derived from natural sources which include volcanic activity as the major source, whereas remaining 90% comes from anthropogenic activity (Okada *et al.,* 1997). Cd, a cumulative pollutant found in aquatic ecosystem, makes fish particularly vulnerable to cadmium exposure (Bhakta and Munekage, 2008).

The major routes of Cd in aquatic environment are industrial, agriculture and communal wastewater containing alarmingly high level of heavy metals including Cd compounds (Kumar *et al.,* 2007). Freshwater ecosystems like lakes, rivers and reservoirs are more susceptible to Cd contamination. Studies on accumulation pattern of cadmium in tissues of different fish

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species *Cyprinus carpio* (Sumet and Blust, 2001) *Oreochromis niloticus* (Firat *et al.,* 2009) and *Paralichthy solivaceus* (Cao *et al.,* 2010)*Rhamdia quelen* (Pretto *et al.,* 2011) show that Cd accumulates mainly in metabolically active tissues, such as kidney, liver and gills and least in muscles and skin.

Accumulation of heavy metals including Cd in fish can stimulate the formation of reactive oxygen species (ROS) or other free radicals. Enhanced generation of ROS results in a condition known as "oxidative stress" which has been incriminated in pathogenesis of heavy metal toxicity (Basha and Rani, 2003). Metals like Cd are well-known inducers of oxidative stress and assessment of oxidative damage and antioxidant defense in fish can reflect metal contamination of the aquatic environment (Livingstone, 2003). Previous studies have reported that fish promptly responds to cadmium and other contaminants with modulation in detoxification enzymes (Kumar *et al.,* 2009).The activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT),glutathione (GSH) and glutathione S-transferase (GST) have been reported to alter in fish as a response to metal exposure to detoxify and clear ROS, and thus counteract the oxidative cellular damage caused (Ruas *et al.,* 2008).

Apart from antioxidant enzymes, effect of vitamins C, E and selenium as protective anti-oxidants Stohs *et al.,* 2001; Das and King, 2007) is well established. Currently, there has been an increased interest in identifying novel plant based antioxidants to combat oxidative stress. Since these phyto compounds are potent and have no or low side effects, they have garnered a position in food industry. The antioxidative efficacy of various plant-based compounds including flavonoids like quercitin, grape seed extract and leaf extract of holy basil, *Ocimum sanctum* have been reported in mammals, and that of taurine and garlic extract has also been reported Cd exposed in fish (Hwang and Wang ,2001;Banerjee *et al.,* 2003;Kumar *et al.,* 2009).

Extract of *Rosmarinus officinalis* (Rosemary) are natural antioxidants that are used in food, food supplements and cosmetic applications (Ibbara *et al.,* 2011).Rosemary leaf extract is one such plant source that has been proposed by the European Community Reference Laboratory (as a feed additive (Dossier no. FAD -*2004-0003*) in the class of antioxidants (ECR 2005). The antioxidative potential of rosemary is primarily due to phenolic diterpenes, carnosol and carnosic acid. The alcoholic leaf extract contains *10%* carnosic acid. Carnosic acid-rich rosemary extract has been reported to have antioxidant activity *in vitro* and *ex vivo*(Ibbara *et al.,* 2010). These antioxidant effects have been recapitulated *in vivo.* Consequently, carnosic acid-rich rosemary extract has been reported to reduce oxidative stress in aged rats(Posadas, 2009). A study by Ozugul *et al.* (2010) showed the potential of rosemary extract as a natural preservative agent in fish preservation owing to its antioxidant properties. Further, Ahmed and Abdella (2010) reported the efficacy of rosemary leaf extract as an antioxidant to combat doxorubicin-induced oxidative stress in rat models. Carnosic acid rich leaf extract of rosemary has been reported to exhibit marked anti-inflammatory and anti-hyperglycaemic effects in mice thus proving to be a potential preventive treatment against oxidative damage attributed to the pathogenesis of metabolic disorders (Ibbara *et al.,* 2011).

With this premise and aiming at a better understanding of the ameliorative effect of exogenous antioxidants such as, vitamin C and rosemary leaf extract on cadmium-induced oxidative stress in fish, the present study was designed to investigate the changes in oxidative stress indices and tissue accumulation in liver kidney, gills and muscle following Cd exposure to a freshwater teleost, Nile tilapia *Oreochromis niloticus*.

Materials and Methods

Experimental design : Mature Nile tilapia *Oreochromis niloticus*, with average length of 20 cm and weight of 70-100 g were procured from a fish farm near Riyadh city in March 2012.They were checked thoroughly for injury and disease conditions, and only healthy fish were used for the study. After washing with 0.01% $KMnO_4$ solution for 15 min, they were placed in seven glass aquaria (100l) containing non-chlorinated water. Prior to the start of the experiment, the fish were acclimatized to food and laboratory conditions with 12 hr dark and 12 hr light cycles, pH range of 6.95 to 7.60 and temperature ranging from 16 to 24°C for 15 days. Experimental fish were fed once daily and the residuals were removed after 48 hr by siphoning. Each group comprising of 15 fishes, was kept in separate glass tanks for a period of 21 days.

The seven experimental groups included of negative control; containing dechlorinated tap water with no added $CdCl_2.H_2O$ and without any antioxidant treatment. Three groups were exposed to sub-lethal concentration of 5 ppm $CdCl_2$ solution. The remaining three groups were exposed to sub-lethal concentration of 10 ppm of $CdCl_2$ soution. Among the Cd exposed groups, two groups one from each of 5 and 10 ppm exposed groups were kept as non-treated control (positive control).Two exposed groups were treated with ascorbic acid (5 ppm) and other two groups with rosemary leaf extract of (2.5 ppm), from each of 5 and 10 ppm exposed groups during the entire experimental period. Antioxidants were added in tank water.

After exposure period, fish were sacrificed from each group. Out of 15 fish from each group, 8 fish were used for estimation of antioxidant enzymes in liver and kidney and remaining 7 fish were used for assessing Cd accumulation pattern in kidney, liver, gills and muscle. All the tissues were preserved at -80°C till further analysis.

Analysis of physico-chemical parameters : Water samples from each tank were collected at weekly interval to analyse the

basic physico-chemical parameters of water. The pH of water samples was recorded using a digital pH meter (PB-*11* Sartorius).Dissolved Oxygen of water samples was recorded using a DO meter with Galvanic Probe (Adwa AD610).

Analysis of cadmium :

Water : Once a week during 21 days of experimental period, water samples from each experimental tank was analyzed, to check equivalence between nominal and actual Cd concentrations by atomic absorption spectrophotometer.

Tissue : Samples of kidney, liver, gills and muscle (0.5 g each) were wet digested with 3.5ml of HNO_3 *65*% and 0.5 ml of H_2O_2 30% in a microwave digestor (Milestone, Italy). Cd concentration in digested tissue samples and tank water was analyzed by atomic absorption spectrophotometer (220 FS Varian, Australia) at 228.8 nm wavelength (detection limit 0.002 µg ml^{-1}) with 4.0 mA current. A calibration curve with standard solutions was plotted. Average reading of blank was subtracted from standard and test sample and then final concentration (µg g^{-1}) was calculated.

Biochemical analysis : Tissue homogenate of liver and kidney from each experimental group was prepared in accordance with the protocol provided with the enzyme assay kits (Cayman Chemicals, USA).Cayman's TBARS Assay Kit was used to measure lipid peroxidation in terms of MDA (malondialdehyde) following a modified method. For this assay, 25 mg of tissue was weighed and 250 µl RIPA buffer was added with 50 µl EDTA. The tissue was then sonicated over ice. After this the contents were centrifuged at 1,600 x g for 10 mins at 4 ºC.The supernatant was stored in ice and used for assay. Cayman's Superoxide Dismutase Assay kit was used to measure SOD activity. For this 0.5 g of tissue was homogenized in 3-6 ml of cold 20 mM HEPES buffer, pH *7.2* containing 1mM EDTA, 210mM mannitol and 70 mM sucrose.

The contents were then centrifuged at 1500 x g for *5* min at 4 ºC. The supernatant was stored on ice and used for assay. Cayman's Catalase Assay kit was used to measure CAT activity. For this 0.5 g was homogenized on ice in 2-5 ml of cold PBS(phosphate buffered saline). Thereafter, the contents were centrifuged at 10,000 x g for 15 mins at 4 ºC.The supernatant was stored in ice and used for assay. The assays were performed in accordance with the protocol provided with the kit. TBARS values in terms of MDA equivalents were expressed in µ mg^{-1} wet wt. SOD and CAT activities were expressed as units per gram of wet tissue (Crapo *et al.,*1978;Okhawa *et al.,* 1979; Cohen *et al.,* 1970).

Statistical analysis : All the presented data are expressed as mean values ±SE. One-way analysis of variance (ANOVA)was performed followed by an unpaired Student's t-test to analyze group differences.Numerical data was correlated with SPSS 16.0 statistical software (Chicago, IL, USA), and significance level was set to $p \leq 0.05, p \leq 0.01, p \leq 0.001$, respectively.

Results and Discussion

Exposure to sublethal concentrations of cadmium chloride (5ppm and 10ppm) did not cause any mortality during the exposure period of 21 days. The mean values of basic physico-chemical parameters like DO (5.0 mg l^{-1}), Temperature (29-30°C) and pH (7.1) of water in the experimental tanks was within the conducive range during the experimental period. The actual concentration of Cd in water in the experimental tanks was recorded weekly for 21 days. The estimated Cd concentration in water in positive control group was 0.00 ppm, while for the groups exposed to Cd at 5ppm and10 ppm was 4.98 and 7.94 ppm. The estimated Cd concentration in water in Cd exposed groups (5ppm and 10 ppm) treated with ascorbic acid (5 ppm) was 3.22 and 6.89 ppm. While Cd exposed groups (5ppm and 10ppm) treated with rosemary leaf extract(2.5ppm) recorded Cd concentration of 3.22 and 6.87 ppm respectively.

Cadmium level in liver, kidney and gills was significantly ($p \geq 0.001$) higher in cadmium exposed groups in comparison to control group. Cadmium concentration in liver, kidney and gills was invariably and significantly higher($p \geq 0.001$) in the groups exposed to higher dose of cadmium (10ppm) than lower dose (5ppm). Overall, the accumulation pattern of Cd in the target tissues did exhibit a dose dependent effect (Fig.1 a,b,c). It has been reported earlier (Kumar *et al.,* 2009; Firat *et al.,* 2009; Cao *at al.,* 2010) that Cd accumulation increased with increasing Cd concentration and our findings are in consistent to these reports. Kumar *et al.* (2005,2009) also reported a similar Cd accumulation pattern in *C. batrachus* with significant increase in Cd concentration in liver and kidney after 15 days of exposure, which was further enhanced with time. The results of the present study showed that there was no detectable increase in Cd level in the muscle of fish from Cd exposed groups as compared to control (Fig.1d). In line with this, Sumet and Blust (2001) reported that Cd accumulated in the tissues of *C. carpio* in the following order: kidney > liver>gills>muscles. Firat *et al.* (2009) also reported that Cd exposure to *O. niloticus*for 28 days showed a significant increase in Cd concentration in liver and gills, highest in liver. Further more, subchronic cadmium poisoning in seabass *Dicentrarchus labrax* showed that the primarily affected organs were kidneys and liver whereas gills were affected to a lesser extent (Thophon *et al.,* 2003).

Overall, in the present study the effect of rosemary leaf extract on bioaccumulation was more pronounced in groups exposed to higher level of Cd (10 ppm). After 21 days of exposure, treatment with Rosemary leaf extract (2.5 ppm) did not significantly reduce Cd concentration in liver, however treatment with Vitamin C significantly ($p \geq 0.001$) reduced Cd concentration in Cd exposed group (5ppm) in comparison to non-treated group.

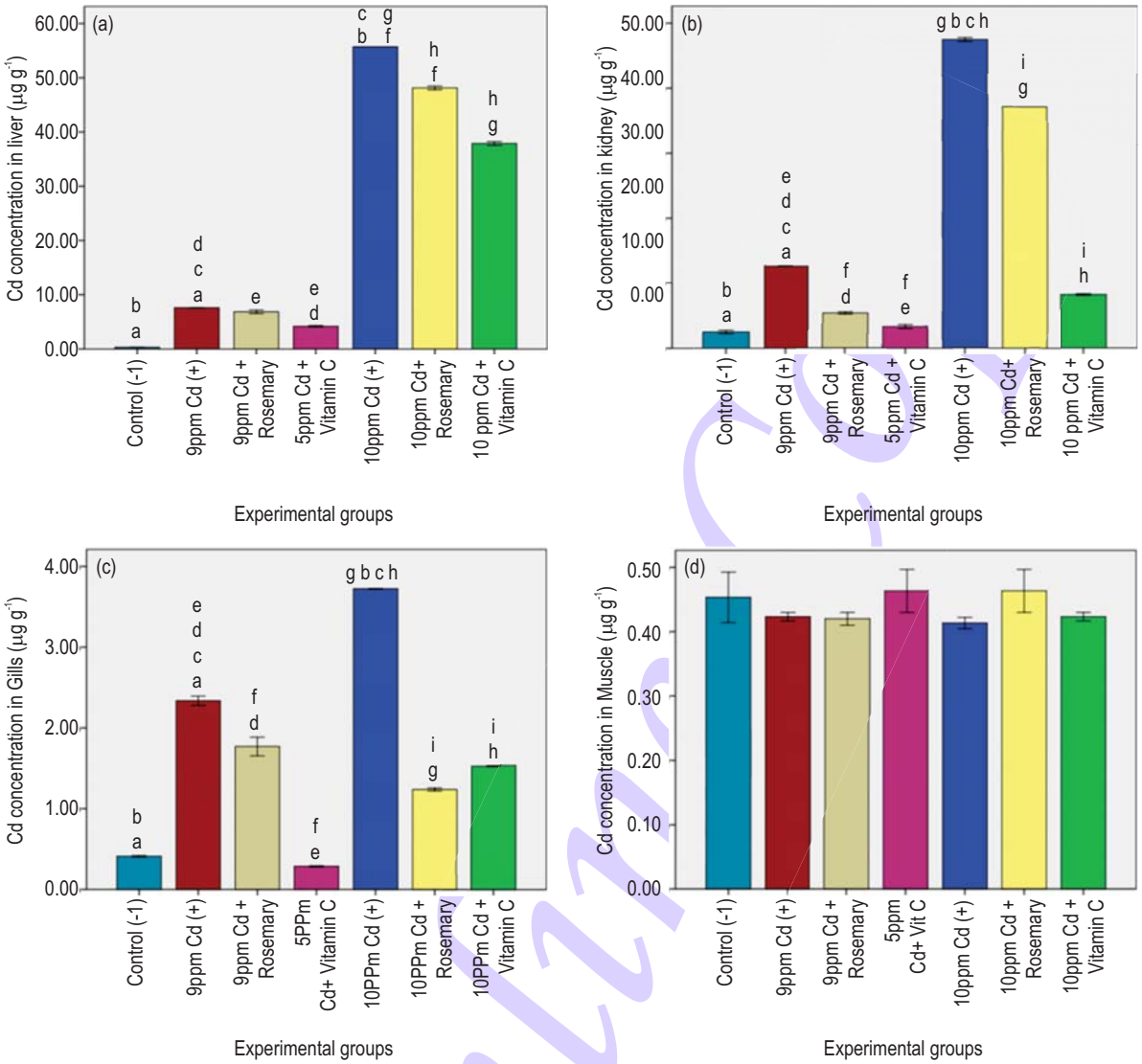

Fig. 1 : Cadmium concentrations (ppm) in tissue of fish exposed to 5ppm and 10 ppm cadmium chloride and treated with Vitamin C and Rosemary leaf extract. (a) liver; (b) kidneys; (c) gills (d) muscles. Values are expressed as mean of four replicates ± SE. Same letters indicated significant differences between the groups ($p \geq 0.001$)

Groups exposed to higher dose of Cd (10ppm) on treatment with Rosemary leaf extract and Vitamin C showed significant($p \geq 0.001$) reduction in Cd concentration in liver after the exposure period (Fig.1a). While post exposure period of 21 days ,treatment with Rosemary leaf extract (2.5ppm) and Vitamin C showed a significant ($p \geq 0.001$) reduction in Cd concentration in kidney in comparison to non–treated groups (Fig.1b). While in gills, 21 days of Cd exposure to 5ppm Cd showed a significant ($p \leq 0.01$) reduction in Cd concentration in gills on treatment with Rosemary leaf extract, while treatment with Vitamin C showed a highly significant ($p \leq 0.001$) decrease in comparison to non-treated group. Groups exposed to higher dose of Cd (10 ppm) showed a similar trend with highly significant ($p \leq 0.001$) decrease in Cd concentration on treatment with both Rosemary leaf extract and Vitamin C (Fig.1c). In consensus with the results of the present study, an experimental study on the assessment of ameliorative ability of Vitamin C on cadmium exposed freshwater catfish *C. batrachus* showed a detectable decrease in Cd concentration in liver and kidneys (Kumar *et al.,* 2009). Vitamin C is a well known antioxidant and its role in alleviating cadmium toxicity has been previously reported in rats (Kannan and Flora, 2004). Reduction in Cd accumulation by Vitamin C may be attributed to the

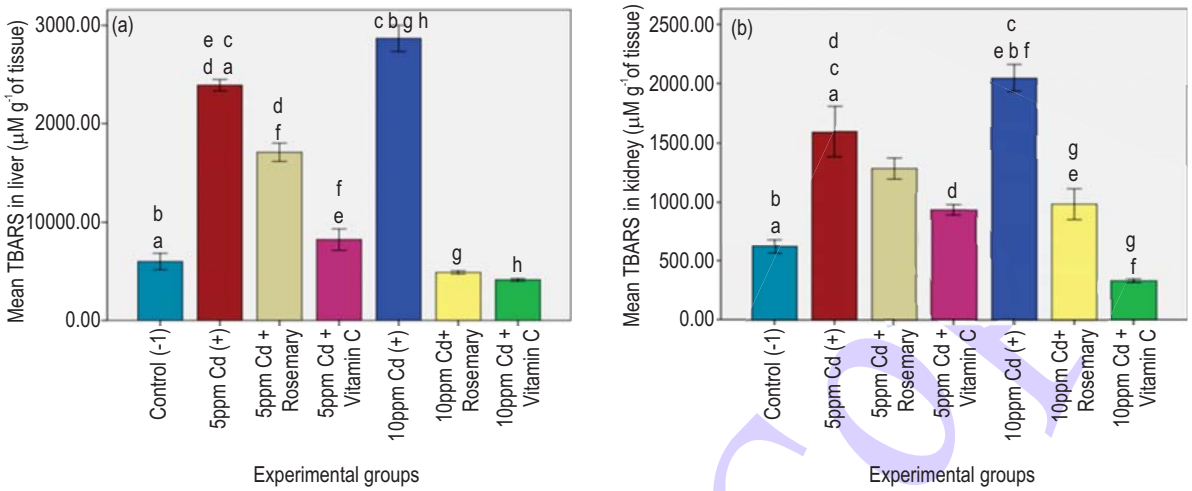

Fig. 2 : Lipid peroxides level (µM TBARS g^{-1} of tissue) in (a) liver; (b) kidneys of fishes exposed to 5ppm and 10ppm Cd treated with Vitamin C and Rosemary leaf extract. Values are expressed as mean of four replicates ± SE. Same letters indicate significant differences between groups ($p \geq 0.001$)

presence of C=O and –OH groups as side chain of sulfur, which further explains its role in reducing intestinal uptake and accumulation of Cd in mammals (Kumar *et al.,* 2009) possibly by competing for the sulphadryl binding sites on metallothioneins (Erdogan *et al.,* 2005). Carnosic-rich rosemary leaf extract has been reported to have potent antioxidative activity *in vitro* by oxygen radical absorbance capacity and ferric-reducing/ antioxidant power assays as well as *in vivo* (Ibarra *et al.,* 2010). Reduced cadmium accumulation in tissues, on treatment with rosemary leaf extract containing 10% carnosic acid used in the present study,is primarily due to carnosicacid, a phenolic diterpene compound which along with carnosol contributes to 90% of the antioxidative activity of the phytoconstituents in rosemary leaf. However, cadmium concentration in the muscle of all cadmium exposed groups was comparable to control after 21days of exposure. Further, treatment with Rosemary leaf extract and Vitamin C did not show any significant change in Cd concentration (Fig. 1d).

Toxicity of heavy metals like Cd results in generation of reactive oxygen species which elicits oxidative stress. Our findings showed that Cd treatment for 21 days on Nile tilapia induced oxidative stress which was manifested as increased lipid peroxidation in cells. Lipid peroxidation was significantly ($p \leq 0.001$) higher in groups exposed to 10ppm Cd in comparison to the group exposed to 5ppm Cd. The level of lipid peroxides (LPO) assessed as malondialdehyde (MDA) level in liver and kidney was significantly ($p \leq 0.001$) higher in Cd treated groups. Groups exposed to Cd showed significant reduction in LPO level in liver ($p \leq 0.001$) on treatment with rosemary leaf extract and Vitamin C in comparison to non-treated group. Further, LPO level in groups exposed to 5ppm Cd did not show significant change on treatment with rosemary leaf extract but did show a significant ($p \leq 0.01$) decrease on treatment with Vitamin C. However, groups exposed to higher dose of Cd (10ppm) showed significant ($p \leq 0.001$) decrease in LPO levels on treatment with both rosemary leaf extract and Vitamin C (Fig. 2 a, b).

Our results on enhanced LPO level in tissues is in consensus with the previous findings Cd –induced oxidative stress in rats (Ognjanović *et al.,* 2008) in poultry (Bharavi *et al.,* 2010; Kant *et al.,* 2011) and in different fish species like *O.niloticus* (Almeida *et al.,* 2009), *C. batrachus* (Kumar *et al.,* 2009), *P.olivaceus* (Cao *et al.,* 2010) and *R.quelen* (Preto *et al.*, 2011). Cd does not directly generate free radicals like other heavy metals but does generate non-radical hydrogen peroxide that eventually is a source of free radicals through Fenton chemistry (Almeida *et al.,* 2009). Thus, increased MDA level, an end-product of lipid peroxidation, in liver and kidney of Cd exposed fish is mainly due to degeneration of lipids caused by hydrogen peroxide formed. Lipoperoxidation is a free radical-mediated chain reaction, since it is self-perpetuating. The extent of propagation depends on antioxidants that arrest chain reaction. In the present study, treatment with vitamin C and rosemary leaf extract did overall mitigate lipid peroxidation in liver and kidneys as compared to non-treated Cd exposed groups. A similar trend was observed earlier in an experiment on Cd-induced oxidative stress in freshwater catfish, *C. batrachus,* where elevated levels of LPO in liver and kidneys were reduced on treatment with antioxidants such as, Vitamin C, garlic extract and taurine (Kumar *et al.,* 2009).

SOD and CAT activities were comparable and did not show a dose dependent effect of Cd. The profile of CAT activity in the present study showed a decrease with Cd exposure both in liver and kidney at 5ppm and 10ppm which on treatment with the antioxidants, vitamin C and rosemary leaf extract was enhanced thus alleviating Cd induced oxidative stress. CAT activity was

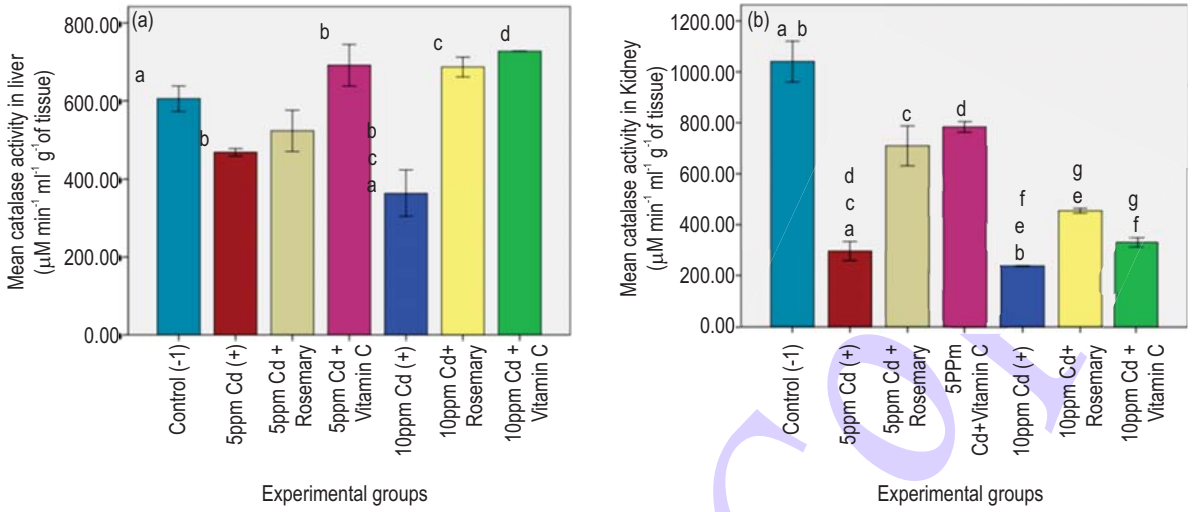

Fig. 3 : Catalase activity (U g^{-1} of tissue) in a) liver b) kidneys of fish exposed to 5ppm and 10ppm Cd treated with Vitamin C and Rosemary leaf extract. Values are expressed as mean of four replicates ± SE. Some letters indicate significant differences between groups ($p \geq 0.001$)

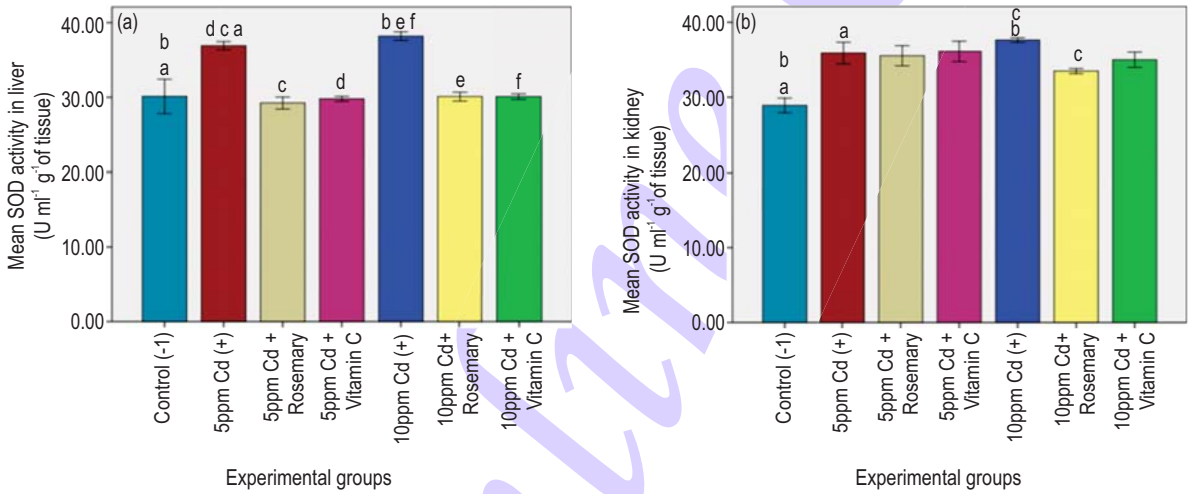

Fig. 4 : Superoxide dismutase activity (U g^{-1} tissue) in a) liver b) kidneys of fishes exposed to 5 ppm and 10ppm Cd treated with Vitamin C and Rosemary leaf extract. Values are expressed as mean of four replicates ± SE. Some letters indicate significant differences between groups ($p \geq 0.001$)

reduced in liver of fish from Cd exposed groups, however a significant ($p \leq 0.05$) decrease was observed in the groups exposed to higher level (10ppm) of Cd in comparison to control. Within the lower Cd dose groups (5ppm) treatment with rosemary leaf extract did not show a significant change in CAT activity, treatment with Vitamin C did significantly ($p \leq 0.05$) increase CAT activity in comparison to non-treated cadmium exposed group.

Groups exposed to 10ppm Cd, treatment with both rosemary leaf extract and Vitamin C significantly ($p \leq 0.01$, $p \leq 0.001$) increased CAT activity (Figs.3 a,b). A significant ($p \leq 0.001$) decrease observed in CAT activity in kidney as well from the groups exposed to both Cd level, in comparison to control. Treatment with rosemary leaf extract and Vitamin C significantly ($p \leq 0.001$) enhanced CAT activity in Cd exposed groups (5ppm and 10ppm) in comparison to non-treated groups. In general, inhibition of CAT activity might be due to direct effect of formation of oxyradicals by metals (Radhakrishnan, 2008). Thus, findings of the present study and a previous experimental study on Cd exposure to fish by Kumar *et al.* (2009) are in consensus with this report. A similar trend of reduced CAT activity in response to water borne contaminants was also observed in liver of grey mullets *Mugilcephalus* collected from a contaminated estuary in India (Padmini *et al.,* 2009). Decreased CAT activity could also be

attributed to direct binding of metal to active site of the enzymes or due to their increased usage in scavenging free radicals induced by metal, thus causing irreversible inhibition in their activities (Waisberg *et al.,* 2003). In comparison to control, non-treated groups exposed to 5ppm and 10ppm Cd showed invariably significant increase in SOD activity both in liver ($p \leq 0.01$) and kidney ($p \leq 0.05$). A similar trend was reported in various experimental Cd exposure studies on fish, tilapia *O. niloticus* (Almeida *et al.,* 2009), *O. mossambicus* (Basha and Rani, 2003) and *C. batrachus* (Kumar *et al.,* 2009). As suggested by Basha and Rani (2003) upregulation of enzyme activity might be a defense mechanism, providing first line of defense against Cd toxicity before induction of metallothionein synthesis. SOD catalyzes the destruction of superoxide radical by dismutation and H_2O_2 formation which explains a parallel increase in lipid peroxidation with increase in SOD activity in liver and kidney of Cd-exposed non-treated fish. Fish exposed to 5 and 10 ppm Cd and treated with Rosemary leaf extract and Vitamin C showed a significant ($p \leq 0.001$) decrease in SOD activity in liver in comparison to non-treated Cd exposed groups. While, in kidney there was no significant effect of antioxidant treatments on SOD activity, except for the group exposed to 10ppm Cd and treated with Rosemary leaf extract which showed a significant($p \leq 0.01$) reduction in SOD activity (Figs.4 a,b). Treatment with vitamin C and rosemary leaf extract for a period of 21 days did bring about a detectable decrease in SOD activity in liver.

Our findings showed a distinct ameliorative effect of antioxidants on cadmium induced oxidative stress in terms of decreased lipid peroxidation, decreased SOD activity and enhanced CAT activity, in support to the previous reports that stated that protection against cadmium toxicity can be achieved through supplementation of antioxidants (Karbownik *et al.,* 2001). Vitamin C is a natural antioxidant that inhibits increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues. It reacts directly with superoxide hydroxyl radicals and singlet oxygen (Bodannes and Chan,1999).The antioxidant potential of vitamin C has been well established over the years.

There are a couple of studies which have reported the antioxidant properties of rosemary leaf extract. Rosemary plant extracts have been shown to inhibit lipid peroxidation and free radicals generation *in-vitro* (Munne-Bosch *et al.,*1999) and *in vivo* (Amin and Hamza, 2005), in addition to their abilities to scavenge peroxy radicals (Jindal *et al.,* 2006). Previous studies on rosemary leaf extract have reported a marked decrease in lipid peroxidation and modulation of antioxidant enzyme activities, in brain tissue of aged –rats (Posadas *et al.,* 2009) and in liver, kidney and brain of experimental rats exposed to doxorubicin (Ahmed and Abdella, 2010). A recent study by Ozogul *et al.,* (2010)showed that rosemary extract can be used in fish preservation as a natural preservative agent to prevent lipid oxidation of fish flesh.

Rosemary leaf extract owing to its potent antioxidative ability can be potentially used in phytoremediation to mitigate the toxic effects of metals and other pollutants in contaminated water or soil.

Acknowledgment

The authors extend their appreciation to King Abdulaziz City for Science and Technology (KACST),Riyadh, Saudi Arabia for providing necessary funds for this project under the registration no: A-S-12-0840.

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