International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Chelating properties of *Cardiospermum halicacabum* against Cadmium toxicity on antioxidant enzyme activities in the Fresh water Crab, *Paratelphusa hydrodromaus*

K. Pugazhendy[1*], A. Revathi [1], Kadarkarai Murugan[2], Jiang-Shiou Hwang[3]

[1]Department of Zoology, Annamalai University, Annamalainagar-608 002, India,
[2]Department of Zoology, Bharathiar University, Coimbatore- 641 029, India,
[3]Institute of Marine Biology, National Taiwan Ocean University, Keelung 202-24, Taiwan
*Corresponding author

Abstract

The fresh water field crab, *Paratelphusa hydrodromous* is an important human food source in parts of South India and the crab is constantly exposed to heavy metal, which are used extensively to toxic elements in aquatic systems. Evaluation of the toxic effect of cadmium on the experimental crab for the LC_{50} value was carried out. Effect of cadmium on the antioxidant enzymes in the hepatopancreas was observed. Quantitative study of antioxidant enzymes of Super oxide dismutase, Catalyze and Lipid peroxidation were undertaken.

Keywords: Hepatopancreas, *C. halicacabum*, Cadmium, SOD, CAT, LPO. *Paratelphusa hydrodromaus* and Toxicity.

1.Introduction

Cadmium (Cd), a widely used heavy metal in modern industry, is one of the most abundant, ubiquitously distributed toxic elements in aquatic systems (Novelli *et al*., 2000). It has been implicated in oxidative injury involving the initial formation of reactive oxygen species (ROS). Cadmium promotes oxidative damage by increasing the cellular concentration of ROS and by reducing the cellular antioxidant capacity (Corticeiro *et al*., 2006). Freshwater crab has the capability of accumulating heavy metals (Reinecke *et al*., 2003) and is thus a suitable bio indicator for environmental contamination with these agents (Schuwerack *et al*., 2001). Hepatopancreas, the key site of Cd accumulation in Crustacea (Wang *et al*., 2001), is one of the most important organs that play important roles in metal detoxification (Gibson and Barker, 1979).
Cadmium (Cd) is one of the most toxic heavy metals for humans; the main source of non-occupational exposure to Cd includes smoking, air, and food and water contaminated by Cd (Nagata *et al*., 2005). In addition, herbal medicine is another source of Cd.

The World Health Organization (WHO) estimates that 4 billion people or 80 percent of the world population presently use herbal medicine (Naithani *et al*., 2010). Several articles have reported of adverse effects of these herbal preparations due to the presence of high level of heavy metals such as Cd, lead, chromium, nickel, etc. (Naithani *et al*., 2010). Saeed *et al*. (2010) investigated twenty five herbal products.

Aerobic organisms have developed antioxidant defense mechanisms that scavenge ROS or prevent ROS-mediated cellular damage (Valavanidis, *et al*., 2006), including enzymes sensitive to free radical proliferation such as superoxide dismutase (SOD), catalase (CAT), Among the main ROS-inflicted damages is lipid membrane oxidation, known as lipid peroxidation (LPO), a process that follows exposure to a wide variety of environmental pollutants (Livingstone *et al*., 2001). According to earlier reports by the authors, CPF is a major pesticide residue in fishery product in Taiwan (Feei and Shan, 2008).

A number of reports on the toxicity of CPF on different aquatic animal models indicated that it is a potent neurotoxic agent (Rao *et al*., 2005).

In recent years many medicinal plants have been used for pharmacological and toxicological studies. *Cardiospermum halicacabum*, commonly known as Mudakkathan in Tamil language, India. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific; its leaves and stalks are used in the treatment of diarrhoea, dysentery and headache and as a poultice for swellings (Chopra, *et al., 1986*). The aim of the present study was to evaluate effect of *C. halicacabum* on SOD and some antioxidant enzymes of fresh water crab *paratelphusa hydrodromous*.

2. Materials and Methods

The fresh water field crabs were collected from, in and around the irrigating channels and paddy fields, Cuddalore district, Tamil Nadu, India. The crabs were maintained in normal daylight illumination in the laboratory thereby providing normal acclimatization. The crabs were fed with uncooked oats. For all experiments, the crabs were used with carapace length ranging from 3 cm to 4.5 cm and breadth ranging from 5 cm to 6.5 cm. The water level was maintained carefully so that the crabs were partially immersed.

Acute toxicity study was carried out to determine the potency of cadmium for static but renewal type of bioassay was adopted in the present investigation to estimate the LC_{50}values (Table 1). The cadmium was used as commercial preparation. The experiment was carried out to find the range of concentrations for confirmatory evaluation. The mortality was recorded for the crab at 1, 7, 14, 21 and 28 days exposure to cadmium was corrected for natural response by Abbott's formula (Abbott, 1995).

2.2. Statistical analysis

The data obtained in the present work were expressed as means ± SE, percentage changes and were statistically analyzed using student t-test (Milton, *et al*., 1983) to compare means of treated data against their control ones and the result were considered significant at ($P<0.05$) and ($P<0.01$) level.

3. Results

In the present investigation *Paratelphusa hydrodromous* administered with cadmium (Group 2) showed an increased the activities when compared to control. The overall increased percent changes are 4.34, 9.47, 14.96, 22.94, and 26.07 for the period of 1 to 28 days, respectively. The cadmium along with *C.helicacabum* exposure group 3, the recorded value of SOD content in the liver tissue was decreased, the percent decrease are 0.11, 4.90, 8.74, 13.17 and 14.37 for the period of 1 to 28 days, respectively. The *C.helicacabum* supplemented (Group 4), the SOD levels are decreased, when compared with group 2 and 3, which are near to control. The decreased percent changes are 0.27, 0.96, o.47, 2.24 and 1.68 for the period of 1 to 28 days, respectively. The observed values SOD content in the liver tissues are statistically significant at 1% and 5% level (Table - 1).

The CAT activity of *Paratelphusa hydrodromous* under the sub-lethal concentration of cadmium exposure group 2 showed decreases when compared to control. The decreased percent changes are 9.09, 12.83, 22.23, 30.43 and 40.69 for the period of 1 to 28 days respectively. Cadmium along with *C. helicacabum* supplemented feed exposed (Group - 3) are increased when compared with Group - 2. The increased percent changes are 20.77, 15.28, 3.29, -18.23, and -34.97 for the period of 1 to 28 days respectively. The supplemented feed exposed Group - 4 the CAT activity are near to control. The percent changes are 0.51, -3.54, 2.01, 0.33 and 2.29 for the period of 1 to 28 days, respectively. The increase and decreased CAT activity in liver tissues are statistically significant at 1% and 5% levels (Table - 2).

The observation of LPO activity in cadmium exposure Group - 2, are increased when compared with control. The increased percent changes are 10.14, 15.54, 46.99, 28.14, and 48.61 for the period of 1to 28 days, respectively. The cadmium along with *C.helicacabum* (group 3), the LPO levels are increased when compared with group 2. The increased percent changes are -50.44, -26.49, 13.06, -37.58 and -47.55 for 21 to 28 days, respectively (Table 3).

Table – 1: Variation of Superoxide dismutase (U min / mg protein) activity in the fresh water crab *Paratelphusa hydrodromous* exposed to cadmium and *Cardiospermum helicacabum* for 28 days

Periods of exposure (days)					
Groups	**1**	**7**	**14**	**21**	**28**
I Control	48.12 ± 2.40	48.65 ± 2.43	48.92 ± 2.44	49.02 ± 2.45	49.32 ± 2.46
II Cadmium	50.21 ^{NS} ± 2.51 4.34	53.26 ^{NS} ± 2.66 9.47	56.24 ^{NS} ± 3.37 14.96	60.27 ^{NS} ± 3.01 22.94	62.18 ^{NS} ± 3.73 26.07
III Cadmium + *C. helicacabum*	50.15 ^{NS} ± 2.50 4.21 0.11	50.65 ^{NS} ± 2.53 4.11 4.90	51.32 ^{NS} ± 2.56 4.90 8.74	52.33 ^{NS} ± 3.13 6.75 13.17	53 24 ^{NS} ± 2.66 7.94 14.37
IV *C. helicacabum*	48.25 ^{NS} ± 2.41 0.27	49.12 ^{NS} ± 2.45 0.96	49.15 ^{NS} ± 2.45 0.47	50.12 ^{NS} ± 3.00 2.24	50.15 ^{NS} ± 3.00 1.68

Values are mean ± SE of six replicates parentage changes and student "t" test, Significant at * P > 0.05; ** P < 0.01 levels, NS - Non-Significant

Table – 2: Variation of Catalase (µ mole / min / mg protein) activity in the fresh water crab, *Paratelphusa hydrodromous* exposed to cadmium and *Cardiospermum helicacabum* for 28 days

Periods of exposure (days)					
Groups	**1**	**7**	**14**	**21**	**28**
I Control	11.66 ± 0.79	11.84 ± 0.71	11.92 ± 0.59	12.06 ± 0.60	12.63 ± 0.63
II Cadmium	11.31 ^{NS} ± 0.68 9.09	10.32 ^{NS} ± 0.61 12.83	9.27 ** ± 0.46 22.23	8.39 ** ± 0.50 30.43	7.49 ** ± 0.44 40.69
III Cadmium + *C. helicacabum*	8.39 ** ± 0.50 26.33 20.77	8.74 ** ± 0.52 26.18 15.28	9.15 ** ± 0.54 23.23 3.29	9.92 * ± 0.59 17.74 -18.23	10.11 ** ± 0.50 19.95 -34.97
IV *C. helicacabum*	10.36 * ± 9.04 0.51	11.42 ^{NS} ± 0.68 -3.54	11.68 ^{NS} ± 0.70 2.01	12.02 ^{NS} ± 0.72 0.33	12.34 ^{NS} ± 0.86 2.29

Values are mean ± SE of six replicates parentage changes and student "t" test, Significant at * P > 0.05; ** P < 0.01 levels, NS - Non-Significant

Table 3. Variation of Lipid peroxidation (n mole / mg protein) activity in the fresh water crab, *Paratelphusa hydrodromous* exposed to cadmium and *Cardiospermum helicacabum* for 28 days

Periods of exposure (days)					
Groups	**1**	**7**	**14**	**21**	**28**
I Control	**12.13 ± 0.60**	**12.48 ± 0.74**	**12.32 ± 0.61**	**12.65 ± 0.75**	**12.96 ± 0.90**
Ii Cadmium	**13.36** NS ± 0.80 10.14	14.42 NS ± 0.84 15.54	18.11 * ± 1.80 46.99	16.21 ** ± 0.81 28.14	19.26 NS ± 0.71 -48.61
III Cadmium + ***C.helicacabum***	20.13 ** ± 1.20 65.95 -50.44	18.24 ** ± 0.91 46.15 -26.49	20.48 ** ± 1.04 41.88 13.06	22.34 ** ± 1.11 81.50 -37.58	28.42 ** ± 1.42 119.29 -47.55
IV ***C.helicacabum***	13.10 NS ± 0.78 7.99	13.48 NS ± 0.94 8.01	13.76 NS ± 0.82 11.68	14.08 NS ± 0.84 11.30	14.12 NS ± 0.98 8.95

Values are mean ± SE of six replicates parentage changes and student "t" test, Significant at * P > 0.05; ** P < 0.01 levels , NS - Non-Significant

4.Discussion

The results obtained in the present study of the effect of cadmium , a pyrethroid compound on a fresh water crab, *Paratelphusa hydrodromous* at two different sub lethal concentrations and two different exposure periods showed interesting results. Enzymatic investigations of Superoxide dismutase and Catalyzes and Lipid peroxidation at lower and higher sub lethal concentrations of cadmium on the liver revealed highly fascinating information's. Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden (Hansen et *al.,* 1992)

Cells have a wide array of enzymatic and non-enzymatic antioxidant defence system. Defence systems that tend to inhibit oxygen radicals formation include the antioxidant enzyme such as SOD, CAT, and (Torres *et al.,* 1993). These enzymes play an important role in countering the oxidative stress induced by the formation of ROS. Superoxide dismutase is an enzyme that catalyzes the dismutation of superoxide to hydrogen peroxide which is detoxified to hydrogen peroxide which is detoxified CAT (Li *et al.,* 2003).

SOD provides the first line of defence against oxygen derived free radicals. SOD activity decreases oxidative stress by disputation of O_2. The increase in the activity of SOD in our study reflects compensatory mechanism to increased oxidative stress (Jhon *et al.,* 2001). SOD induction could occur owing to increased production of the O_2 radical. Therefore, an increase in SOD activity indicates an increase in O_2 production similar to other results of pollution (Wendelaar Bonga, 1997). The relation between SOD activities and pollution levels, and the possible use of SOD levels as biomarkers have often been discussed.

CAT activation is the second main antioxidant enzyme, CAT, occurs due to high pollutant levels and without relation to SOD response (Uner *et al.,* 2005; Moraes *et al.,* 2007). The depletion of CAT or its tolerance along with activation of SOD may be observed (Pandy *et al.,* 2003; Stanic *et al.,* 2006 and Huang *et al.,* 2007), although CAT mRNA levels were higher in the exposed fish (Hansan *et al.,* 2006). In our study, CAT activity was not connecting to other markers of the oxidative stress. The low levels of CAT could be attributed to high production of O_2. This has been reported to inhibit CAT activity increase of excess production (Kono and Fridovich 1982). CAT enzymes located in peroxisomes and facilitated the

removal of H_2O_2, which is metabolized to molecular oxygen and water. (Aebi *et al.*, 1974; Oruc *et al.*, 2002; Yilmaz *et al.*, 2006). (Deduve *et al.*, 1985) Increased activity of acid phosphatase was attributed to the activation of the enzyme which was kept in a latent state inside the membrane of lysosomes, due to the disruption of the membrane. Similar reports observed in fresh water crab, *Spiralothelphusa hydrodroma* treated with the pesticides, cypermethrin by Sreenivasan *et al.* (2011). Similar observations were noted in the *scylla serrata* crab in response to naphthalene (Elumalai *et al.*, 1998). In the present investigation, the activity of SOD was found to decrease near to control in the test crabs when compared to the control crabs. The maximum decrease was seen in the crabs exposed to higher sub-lethal concentration of cadmium for 28 days.

5.Conclusion

This study has shown that cadmium as a free radical causes while feeding on Oats and *C. halicacabum* reversed the hepatotoxicity. The present results offer information about the deleterious effects of heavy metal, cadmium on fresh field crab *paratelphusa hydrodromous*. From the results it was clear that the effects were dose and time dependent. This kind of information could be beneficial to take preventive measure to protect the aquatic animals from the polluted areas.

Acknowledgments

The author is grateful to University Grant Commission, New Delhi, for providing financial assistance by granting major research project.

References

Abbott WS. A Method of Computing the Effectiveness of an Insecticide. J. Econ. Entomo. 1925; 18: 265-267.

Aebi, H, 1974. Catalse In: Bergmeyar, H.U. (Ed). *Meth. Enzy. Anal. Acad. Press, London, pp*. 671-684.

Chopra, R.N and Nayar, I.C and Chopra, S.L.R. 1986. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research.

Corticeiro, S.C., Lima, A.I.G., Figueira, E.M.d.A.P., 2006. The importance of glutathione in oxidative status of *Rhizobium leguminosarum* biovar viciae under Cd exposure. Enzyme Microb. Technol. 40, 132–137.

Deduve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. 1985. Intracellular Distribution Patterns of Enzymes in Rat Liver Tissue. Biochem. J. ; 60: 604-617.

Elumalai, M., Balasubramanian, S.E., Balasubramanian, M.P., 1998, Influence of naphthalene on protein, carbohydrate and phosphatases system during the vitellogenesis marine edible crab, *Scylla serrata*, Bulletin of Environmental Contamination Toxicology, 60, pp 25-29.

Feei Sun., Hui-Shan Chen, 2008. Monitoring of pesticide chlorpyrifos residue in farmed fish: Investigation of possible sources, Chemosphere, 71, pp 1866-1869.

Gibson, R., Barker, P.L., 1979. The Decapoda hepatopancreas. Oceanogr. Mar. Biol. Annu. Rev. 17, 285–346.

Hansen. B. H., S. Romma., L. I. Softeland., P. A. Olsvilk and R. A. Anderson, 2006a. Antioxidative stress proteins and their gene expression in brown trout (*Salmo turutta)* from three rivers with different heavy metal levels. *Comp. Biochem. Physiol*., 143:263-274.

Haung, D.J., Zhang, Y.M., Song, G., Long, J., Liu, J.H., W.H. Ji, 2007. Contaminants induced oxidative damage on the carp *Cyprinus capio* collected from the upper Yellow River, China. *Environ. Monitoring Assessment*. 128, 483- 488.

Jhon, S., M. Kale., N. Rathore and D. Bhatnagar, 2001. Protective effect of vitamin E in dimethoate and Malathion induced oxidative stress in rat erythrocytes. *J. Nutri. Biochem*., 12:500-4.

Kono, Y and I. Fridovich, 1982. Superoxide radical inhibits catalase. *J. Biol. Chem*. 257:51-57.

Li, X., Y.Liu., L. Song and J.S.H.Liu, 2003. Responses of antioxidant systems in the hepatocytes of common carp (*Cyprinus carpio* L.) to the toxicity of microcystin-LR. *Toxicon.,* 42: 85-89.

Livingstone, D.R. 2001. Contaminant stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656–666.

Milton, T.S and Tsokos, J.O. 1983. Statistical methods in the biological and health science. McGraw – will. Internet Book comp. 381-405.

Moreas, B.S., Laro., V.L.., Glusczak, L., Pretto, A., Menezes, C., Marchezan, E., de Oliveria

Machado, S., 2007. Effect of four rice herbicides on some metabolic and toxicology parameters of teleost fish (*Leporinus obtusidens*). *Chemosphere* 68, 1597-1601.

Nagata, C., Nagao, Y., Shibuya, C., et al. 2005. Urinary Cadmium and Serum Levels of Estrogens and Androgens in Postmenopausal Japanese Women. *Cancer Epidemiology, Biomarkers & Prevention*, Vol.14, (March 2005), pp.705-708, ISSN 1055- 9965.

Naithani, V., Pathak, N., Chaudhary, M. 2010. Evaluation of Heavy Metals in Two Major Ingredients of Ampucare. *International Journal of Pharmaceutical Sciences and Drug Research*, Vol.2, No.2, (April-June, 2010), pp.137-141, ISSN 0975-248X

Novelli, F., Novelli, E., Manzano, M.A., Lopes, A.M., Cataneo, A.C., Barbosa, L.L., Ribas, B.O., 2000. Effect of tocopherol on superoxide radical and toxicity of cadmium exposure. Int. J. Environ. Health Res. 10, 125–134.

Ortiz, J.B., M.L. Gonzalez and C. Sarasquete, 2002. Histological alterations in different tissues of fishes under the impact of persistent chemical pollution. *Ecotoxicol*. *Environ. Restor*.,54: 45-52.

Pandey, S., S. Parvez., I. Sayeed., R.Haque., B. Bin-Hafeez and S.Raisuddin, 2003. Biomarkers o oxidative stress: a comparative study of river Yamuna fish *Wallago Attu* (Bl&Schn). *Sci. Total Environ*.,309:105-115.

Rao, J.V., Begum, G., Pallela, R., Usman, P.K., Rao, R.N., 2005. Changes in behavior and brain acetylcholinesterase activity in mosquito fish, *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos, International Journal of Environmental Research & Public Health, 2, pp 478-83.

Rao, J.V., G. Begum., R. Palela., P.K. Usman and R.N. Rao., 2005. Changes in behavior and brain acetylcholinesterase activity in mosquito fish *Gambusia affinis* in reference to sublethal exposure of chlopyriphos, *Int. J. Envrion. Res Public Hlth*. 2:478-483.

Reinecke, A.J., Snyman, R.G., Nel, J.A.J., 2003. Uptake and distribution of lead (Pb) and cadmium (Cd) in the freshwater crab, *Potamonautes perlatus* (Crustacea) in the Eerste River, South Africa. Water Air Soil Pollut. 145, 395–408.

Saeed, M., Muhammad, N., Khan, H., et al. 2010. Analysis of toxic heavy metals in branded Pakistani herbal products. *Journal of the Chemical Society of Pakistan*, Vol.32, No.4, pp. 471-475, ISSN 0253-5106.

Schuwerack, P.M.M., Lewis, J.W., Jones, P., 2001. The potential use of the South African river crab, *Potamonautes warreni*, as a bioindicator species for heavy metal contamination. Ecotoxicology 10, 159–166.

Sreenivasan, R.S., Krishna Moorthy, P., Deecaraman, M., 2011. Cypermethrin Induced Toxicity to phosphatases and dehydrogenases in gills and hemolymph of fresh water crab, *Spiralothelphusa hydrodroma* (Herbst), International Journal of Biology & Medical Research, 2(3), pp 784-788.

Stanic, B., Andric, N., Zoric, S., Grubor-Lajsic, G., R., Kovacevic, 2006. Assessing pollution in the Danube River near Novi Sad (Serbia) using several biomarkers in starlet (*Acipenser ruthenus L.) Ecotoxicol*. *Environ. Saf*. 65, 395- 402.

Valavanidis, A., Vlahogianni, T., Dassenakis, M and Scoullos, M. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol Environ Saf*. 64, 178–179.

Wang, L., Yang, X.Q., Wang, Q., Wang, D.X., 2001. The accumulation of Cd and the effect of EST in five tissues and organs of Eriocheir sinensis. Acta Zool. Sin. 47 (Suppl.), 96–100.

Wendelaar Bonga, S.E., 1997. The stress response in fish, *Physiol. Rev*. 77, 592- 625.

Yilmaz, R., A. Atessahin., E. Sahna., I. Karahan and S. Ozer, 2006. Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. *Toxicol*., 218:164-171.