K. Pugazhendy et al. / Journal of Pharmacy Research 2015,9(5),337-342

Research Article
ISSN: 0974-6943

Available online through
www.jprsolutions.info

Protective effect of *Cardiospermum halicacabum* against Cadmium toxicity induced oxidative damage and biomarker enzymes alterations in the Fresh water Crab, *Paratelphusa hydrodromaus*

K. Pugazhendy[1*], A. Revathi[1], Kadarkarai Murugan[2] and 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

Received on:25-03-2015; Revised on: 12-04-2015 ; Accepted on:02-06-2015

ABSTRACT

Cadmium is a heavy metal widely used in industrial processes, has been recognized to be a highly toxic and dangerous environmental pollutant. These excess amounts in addition to naturally occurring levels gradually build up to toxic levels causing damages to the biota of the aquatic ecosystem. The fresh water field crab, *Paratelphusa hydrodromous* is an important human food source in parts of South India. Evaluation of the toxic effect of cadmium on the experimental crab for the LC_{50} value was carried out. Effect of cadmium on the oxidative damage and biomarkers antioxidant enzymes in the hepatopancreas was observed. Quantitative study of antioxidant enzymes GSH, GPx, ACP and ALP were undertaken, result and discussed in detail.

KEY WORDS: Hepatopancreas, *C.halicacabum*, Cadmium, GSH, GPx, ACP and ALP *Paratelphusa hydrodromaus*, Toxicity.

INTRODUCTION

Cadmium is an extremely toxic heavy metal commonly found in industrial workplaces. Due to its low permissible exposure limit, overexposures may occur even in situations where trace quantities of cadmium are found. Environmental pollution by toxicants has become one of the most important problems in the World[1] **(Chandran,*et al.*,2005)** Cadmium is a nonessential heavy metal but it has accumulative polluting effect, and causes toxicity to aquatic organisms even in minute concentrations. Therefore, it is regarded as one of the most toxic elements in the environment. The occurrence of cadmium in considerably toxic amounts was reported by earlier workers in various aquatic ecosystems[2-3] (**Arno Kaschl et al., 2002 and Chrastny, 2006)**. The toxicity are known to affect the crustaceans viz; monocrotophos affected the neuroendocrine regulation in freshwater crab, *Barytelphusa guerini*[4] (Patil *et al*., 2008), chlorpyrifos effects survival and growth of *Palaemonetes argentines*[5] (**Montagna and Collins, 2007**), and also oxygen consumption and ammonia excretion of the freshwater crab *Trichodactylus borellianus*[6] (**Montagna and Collins, 2008**).

***Corresponding author.**
Dr. K. Pugazhendy
Assistant Professor,
Department of Zoology,
Annamalai University,
Tamilnadu, India.

The sensitivity of the cell oxidants is attenuated by antioxidants enzymes such as glutathione peroxide (GPx), Acid phosphatase, (ACP) and alkaline phosphatase (ALP). The antioxidant enzymes maintain a relatively low rate of the reactive and harmful OH. Oxidative stress occurs as a result of the xenobiotics causing the disturbances in the antioxidant enzymes systems.[7] (**Smith and Litwac, 1980**).

Cardiospermum halicacabum, commonly known as Mudakkathan in Tamil. 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 [7] (**Chopra, *et al* 1986**). Hence, an attempt has been made to investigate the following enzymological parameters like GSH, GPx, ACP, and ALP freshwater crab *paratelphusa hydrodromous* exposed to sublethal concentration of cadmium (Group 2), cadmium along with *Cardiospermum halicacabum*, (group 3) and *C.helicacabum* supplemented alone (Group 4) for the period of 1, 7,14, 21 and 28 days.

MATERIALS AND METHODS

Healthy and active crabs *Paratelphusa hydrodromous* were collected

from the river bed, canals, paddy fields, etc., 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[8] (Abbott, 1995).

Enzymatic assay

The GSH level was determined as described by (Ellman, 1959) and expressed as mg per gram of protein (mg/g). The method utilized metaphosphoric acid for protein precipitation and 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) for color development and its density was measured at 412 nm. Glutathione peroxidase (GPx) activity was assayed according to the method described by (Rotruck, 1973)[9].

The ALP enzyme activity was estimated by the method of Moss *et al.* (1971)[10], ACP enzyme activity was estimated by the method of Jabeen (1984)[11] and expressed as mg *p*-nitrophenol g–1 protein h–1. The detail protocols are given in detail Narra *et al.* (2011b)[12].

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)[13] 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.

Observation

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 freshwater crab *Paratelphusa hydrodromous*, exposed to (**group 2**), cadmium the GSH content was increased when compared with group 1. The percent changes are, 11.99, 48.33, 119.18, 140.44, and 172.00 for 1 to 28 days, respectively. The cadmium along with *C. helicacabum* exposure (**group 3**) gradually recovered, when compared with group 2. The recovery percent changes are -64.77, -7.29, 38.06, 49.23 and 53.80 for the period of 1 to 28 days, respectively. The *C.helicacabum* supplemented feed exposure fish (**group 4**) are increased in all other 3 groups. The percent changes are 10.91, 11.87, 11.32, 7.90, and 10.10 for the period of 1 to 28 days, respectively. The GSH activities in hepatopancreas tissue for four groups are statistically significant at 1% and 5% levels (**Table 1**).

Table 1. Variation of GSH (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	8.34	8.42	8.44	8.48	8.48
Control	± 0. 58	± 0.58	± 0.58	± 0.59	± 0.59
II	9.34 *	12. 48**	18.37**	20.35**	20.35**
Cadmium	± 0.46	± 0.62	± 0.91	± 1.01	± 1.01
	11.99	48.33	119.18	140.44	140.44
III	15.40**	13.39**	11.36 **	10.35**	10.35**
Cadmium +	± 0.92	± 0.80	± 0.68	± 0.65	± 0.65
C. helicacabum	84.53	59.02	35.75	22.05	22.05
	-64.77	-7.29	38.06	49.23	49.23
IV	9.25**	9.42**	9.34 **	9.15**	9.15**
C. helicacabum	± 0.64	± 0.65	± 0.65	± 0.64	± 0.64
	10.91	11.87	11.32	7.90	7.90

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

The present investigation cadmium treatment (**group 2**) has resulted in an increased GPx content in liver tissue when compared to control. The percent changes are 0.68, 2.70, 1.32, 5.36, and 9.74 for the period of 1, 7, 14, 21 and 28 days, respectively. Administration of cadmium along with *C.helicacabum* (**group 3**) the GPx response gradually recovered when compared to group 2. The recovery percent changes are -2.05, -8.45, -4.57, -7.00 and -8.28 for the period 1 to 28 days respectively. In the *C.helicacabum* supplemented feed exposed (**group 4**) was slightly increased when compared with control. The percent changes are 2.06, 6.75, 4.63, 4.45 and 3.89 for 1, 7, 14, 21 and 28 days, respectively. The increased and decreased values in GPx activity in hepatopancreas tissue are statistically significant at 1% and 5% levels (**Table 2**).

Table 2. Variation of GPx (U min / mg protein) activity in the fresh water crab *Paratelphusa hydrodromous* exposed to cadmium and *Cardiospermum helicacabum* for 28 days

Groups	Periods of exposure (days) 1	7	14	21	28
I	0.145	0.148	0.151	0.149	0.154
Control	± 0.01	± 0.01	± 0.01	± 0.01	± 0.01
II	0.144**	0.152*	0.153 NS	0.157 NS	0.169 NS
Cadmium	± 0.01	± 0.02	± 0.01	± 0.01	± 0.01
	0.68	2.70	1.32	5.36	9.74
III	0.149 NS	0.154*	0.160 NS	0.168*	0.183*
Cadmium +	± 0.02	± 0.01	± 0.01	± 0.01	± 0.01
C. helicacabum	2.75	8.10	5.96	1.9	18.83
	2.05	-8.45	-4.57	-7.00	-8.28
IV	0.148**	0.155*	0.158 NS	0.156 NS	0.160 NS
C. helicacabum	± 0.01	± 0.02	± 0.02	± 0.01	± 0.01
	2.06	6.75	-4.63	4.45	3.89

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

The present investigation, crab *Paratelphusa hydrodromous* exposed to group 2 showed an increased in the level of ACP activity in the hepatopancreas tissue compared to group 1. The percent increases are, 58.13, 40.00, 31.91, 20.83 and 13.24, for the period of 1 to 28 days, respectively. The group 3 (cadmium along with *C. helicacabum* exposure) ACP activities was slightly differ (regained) from control, and group 2. The percent changes are 10.29, 6.34, 9.67, 5.17 and 3.63 for the period of 1 to 28 days, respectively. The *C. helicacabum* supplemented feed expose (**group 4**) ACP value near to control. The percent changes are -11.62, -13.95, -19.14, -14.58 and -14.28 for the period of 1, 7, 14, 21 and 28 days, respectively (**Table 3**).

Table 3. Variation of ALP(U min / mg protein) activity in the fresh water crab *Paratelphusa hydrodromous* exposed to cadmium and *Cardiospermum helicacabum* for 28 days

Groups	Periods of exposure (days) 1	7	14	21	28
I	0.635	0.639	0.642	0.653	0.679
Control	± 0.04	± 0.04	± 0.04	± 0.04	± 0.04
II	0.412 **	0. 404**	0.385 **	0.636 **	0.342 **
Cadmium	± 0.02	± 0.02	±0.01	± 0.01	±0.01
	35.11	36.77	40.62	44.41	49.63
III	0.529**	0.535 **	0.551**	0.576 *	0.612*
Cadmium +	± 0.03	± 0.03	± 0.03	±0.03	± 0. 05
C. helicacabum	16.69	16.27	14.17	11.79	9.86
	-28.39	-32.45	-43.11	-49.43	-78.94
IV	0.642**	0.653 NS	0.672 NS	0.712 NS	0.739 *
C. helicacabum	± 0.04	± 0.04	± 0.04	± 0.04	± 0.05
	1.10	2.19	4.67	9.03	8.83

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

In the present investigation, *Paratelphusa hydrodromous* exposed to cadmium group 2 showed significant decreases in ALP activity in hepatopancreas tissue when compared to control. The increased percent changes are 35.11, 36.77, 40.62, 44.41, and 49.63 for the period of 1 to 28 days, respectively. The group 3 the ALP activity gradually increased when compared to group 2. The percent changes are -28.39, -32.45, -43.11, -49.43 and -78.94 for the period of 1 to 28 days, respectively. The *C. helicacabum* supplemented feed exposed (group 4) increased when compared with all other 3 groups. The (regained) percent changes are 1.10, 2.19, 4.67, 9.03 and 8.83 for the period for 1 to 28 days, respectively. The observed levels of ALP activity are statistically significant at 1% and 5% levels (**Table 4**).

Table 4. Variation of ACP (U min / mg protein) activity in the fresh water crab *Paratelphusa hydrodromous* exposed to cadmium and *Cardiospermum helicacabum* for 28 days

Groups	Periods of exposure (days) 1	7	14	21	28
I	0.43	0.45	0.47	0.48	0.49
Control	± 0.03	± 0.03	± 0.03	± 0.03	± 0.03
II	0.68**	0.63**	0.62**	0.58**	0.55 NS
Cadmium	± 0.03	± 0.03	± 0.03	± 0.02	± 0.02
	58.13	40.00	31.91	20.83	13.24
III	0.61**	0.59 **	0.56 **	0.55*	0.53*
Cadmium +	± 0.03	± 0.03	± 0.03	± 0.03	± 0.03
C. helicacabum	41.86	31.11	19.14	14.58	8.11
	10.29	6.34	9.67	5.17	3.63
IV	0.48 NS	0.51 NS	0.56 NS	0.55 NS	0.56 NS
C. helicacabum	± 0.02	± 0.02	± 0.03	± 0.03	± 0.03
	-11.62	-13.95	-19.14	-14.58	-14.28

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

DISCUSSION

The results obtained in the present study of the effect of cadmium, on a fresh water crab, *Paratelphusa hydrodromous* at with sub-lethal concentrations a different exposure periods showed interesting results. The GSH plays an important role in the detoxifying of electrophilis and prevention of cellular oxidative stress[14] (Benova *et al.*, 1990). The considerable decline in the GSH tissue content during exposure to atrazine may be due to an increased utilization of GSH, which can be converted into oxidized glutathione and an inefficient GSH regeneration. GSH catalyses the reduction of H_2O_2 and lipid hydroperoxides at expense of GSH. Reduced glutathione is the main nonoprotein thiol and one of the primary resultants found in cells (Moreno 2005). Decrease in GSH levels after administration of various pesticides is well documented in literature[15,16](Hazarika *et al.*,

2001; Prasanthi *et al*., 2005)[16]. Singh *et al*. (2006)[17] showed that, the reduced levels of GSH in the cadmium could be the results of either utilization of GSH for conjugation and /or participation of GSH as an antioxidant in terminating toxicity.

GSH is the major thiol, which binds electrophile molecules spices and free radical intermediates. It plays a central role in the antioxidant defence system, metabolism and detoxification of exogenous and endogenous substances[18] (Ketterer *et al.*, 1998). GSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. Because of the low activity of antioxidant enzymes in the liver and decreased content of GSH, the hepatopancreas is hypothesized to be highly susceptible to oxidative stress one of GSH to near to control value. Monteria *et al.* (2006) have pointed out that, the enzyme activity can decrease by negative feedback from excess substrate or by damage induced by oxidative modification[19]. However, Elia *et al.* (2002) have reported significant increase in the activity of hepatopancreas GPx but no change in the activity[20]. Sharma *et al.* (2005) have reported an increased peroxide activity in the liver of rats after treatment with dimethote[21]. Fatima *et al.* (2000) also reported a low activity of GPx in different fish tissue after exposure to paper mill effluent, indicating an in efficiency of this organ to neutralizing the peroxide impacts[22]. The increase in GPx activity was observed, predominantly in liver and kidney similar to the results reported by Li *et al.* (2003) who have studied the responses of the antioxidant systems in the hepatocyte of common carp (*Cyprinus carpio* L) to microcystin[23].

ACP is microsomal enzyme implicated in membrane transport because of its high level in vertebrates in kidney and its hydrolytic action on a number of phosphomonoesters of organic materials like glucose. The reason for this decrease is probably due to inhibition by the toxicant. ALP is known to occur into the cell membrane and may be involved in metabolic transport (Edquist *et al.*, 1992)[24].

The phosphatases (ALP and ACP) are important biomarkers because they are involved in adaptive cellular response to the potential cytotoxicity and genotoxicity of pollutants (Lohner *et al.*, 2001)[25] Decline in ALP activity may results from fall in the rate of synthesis of glycogen caused by lowered metabolic demands and electrolytic imbalance due to tissue over hydration (Shaffi, 1979)[26].

O'Connor and Gilbert (1968) reported the increase in acid phosphatase activity due to accumulation of mercury in the lysosome and blockage in the release of enzymes and carbohydrate forms the major reserve of many crustaceans accumulated in the hepatopancreas[27]. In edible crab, *Scylla seratta* were of the opinion that degradation and necrosis induced by toxicants in hepatopancreas causes release of acid phosphates (Ahmed et al., 1997)[28]. The alkaline phosphatase activity was decrease in the hepatopancreas throughout exposure period. Goldfisher, (1964) studied the effect of the pollutants in aquatic animals and stated that alkaline phosphatase is a brush border enzyme, which splits various phosphorous esters at an alkaline pH and mediated transport[29]. ALP is involved in carbohydrate metabolism, growth and differentiation, protein synthesis, synthesis of certain enzymes, secretion activity, and transport to phosphorylated intermediates across the cell membranes (Vijayavel and Balasubramanian, 2006)[30]. Thus, any alteration in the activity of ALP affects the organisms.

Ahmed et al. (1997) reported the effect of copper on oxygen consumption and phosphatase in *scylla serrata* and concluded that there was decrease in alkaline phosphatase activity in muscle, hepatopancreas and haemolymph[28]. The decrease in ALP activity has been reported in the 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*., 1997)[31]. In the present investigation, the activity of ALP was found to decrease in the tissues of the test crabs when compared to the control crabs.

According Gabriel *et al.* (2007) enzyme activities under toxicant exposure could be influence by concentration and mode of action by toxicant mode of exposure, duration of exposure and specific response[32]. At the same the C. *halicacabum* having the certain important medicinal properties but it compared to the *oats* was less. These bioactive compounds present in *C. halicacabum* and *oats which* may give recovery to crab in the presence of oxidative damage.

CONCLUSION

This study has shown that cadmium as a free radical causes while feeding on Oats and *C. halicacabum* reversed from the oxidative damage. 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.

ACKNOWLEDGEMENTS

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

Conflicts of interest

The Authors declare no conflicts of interest.

Compliance with ethical standards

All applicable International and National guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Institution or practice at which the studies were conducted.

Informed consent

Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Chandran. R, A.A. Sivakumar, S. Mohandass, M. Aruchami, (2005). Effect of cadmium and zinc on antioxidant enzyme activity in the gastropod, *Achatina fulica. Comp. Biochem. Physiol.* 140, 422–426,
2. Arno Kaschl, Volker Römheld and Yona Chen, (2002) Cadmium binding by fractions of dissolved organic matter & humic substances from municipal solid waste compost. *Journal of Environment Quality*, 31, 1885-1892.
3. Chrastny, M. Komarek, P. Tlustos, J, Svehla, (2006). Effects of flooding on lead and cadmium speciation in sediments from a drinking water reservoir." *Environmental Monitoring and Assessment*, 118,13, 113-123.
4. Montagna, M.C., Collins, P.A., (2007), Survival and growth of *Palaemonetes argentinus* Decapods: Caridae) exposed to insecticides with chlorpyrifos and endosulfan as active element, Archives of Environmental Contamination Toxicology, 53, pp 371-378.
5. Montagna, M.C., Collins, P.A., (2008), Oxygen consumption and ammonia excretion of the fresh water crab *Trichodactylusborellianus* exposed to chlorpyrifos and endosulfan insecticides, Pesticide Biochemistry & Physiology, 92, pp 150-155.
6. Smith, G.J and G.Litwack, (1980). Roles of ligandin and the glutathione S-transferase in binding steroid metabolites, carcinogens and other compounds.*Rev. Biochem. Toxicol.*,**2**:1-47.
7. 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.
8. Abbott WS. A (1925) Method of Computing the Effectiveness of an Insecticide. J. Econ. Entomo.;18: 265-267.
9. Rotruck, J.T., A.L. Pope and H.E. Gantex, (1973). Biochemical role as a component of glutathione peroxidase. Science 179:588-590.
10. Moss. D.W., Baron. D.N., Walker, P.G., Wilkinson, J.H., (1971), Standardization of clinical enzyme assays, Journal of Clinical Pathology, 24, pp 740-748.
11. Jabeen, K., (1984), Toxicological evaluations of some pesticides against chicken (*Gallus gallus domesticus*) and Rat (*Rattus rattus noregius*) with special reference to haematology, blood and urine biochemistry. Ph.D thesis, Osmania University, HYD., India.
12. Narra Madhusudan Reddy, Ghousia, B., Rajender, K., Rao, J. V., (2011). Sub-lethal effect of chlorpyrifos on protein metabolism of the food fish *Clarias batrachus* and monitoring of recovery, Toxicology & Environmental Chemistry, 93(8), pp 1650-1658.
13. Milton, T.S and Tsokos, J.O. (1983). Statistical methods in the biological and health science. McGraw – will. Internet Book comp. 381-405.
14. Benovo, L. V., I. C. Benchev and O.H. Monovich, (1990). Thiol antidotes effect of lipid peroxidation in mercury-poisoned rats. *Chem. Biolog. Interaction.*, **76:** 321.
15. Hazarika A, Sarkar S.N., M. Kateria, (2001). Subacute toxicity of anilofos, a new organophosphorus herbicide in male rats: effect on lipid peroxidation and ATPase activity. *Indian J Exp Biol*, 39,1113-7.
16. Prasamthi, K., Muralidhara, R.S. Rajini., (2005). Fenvelarate induced oxidative damage in rat tissue and its attenuation by dietary sesame oil. *Food Chem Toxicol*, **43**, 299-306.
17. Singh, M., R. Sandhir, R. Kiran, (2006). Erythrocyte antioxidant enzymes in toxicological evaluation of commonly used organophosphate pesticides. *Indian J Exp Biol*, **44**, 580-3.
18. Ketterer, B, (1998).Glutathione-S-transferase and prevention of cellular free radical damage. *Free Radical Research* 28:647-658.
19. Monterio, M., C. M. Quintanerio., M.L.Pastorinho., F.Pereira., L.Morgado., A.M.Guilhermino and V.M. Soares, (2006). Acute effects of 3, 4-dichloroaniline on biomarkers and spleen histology of the common goby *Pomatoschistus microps*. *Chemosphere* **62**:1333-1339.
20. Elia, A. C., W. T. Waller and S.J. Norton, (2002). Biochemical responses of Bluegill sunfish (*Lepomis macrochirus*, Rafinesque) to atrazine induced oxidative stress. *Bull. Environ. Contam.Toxicol.*,**68**: 809-816.
21. Sharma, S and S. Sharma, (2005). Protective role of *Spirulina* feed in a freshwater fish (*Poecilia reticulata* peters) exposed to an azo dye – methyl red. *Indian J Exp Biol*, **43**:1165.
22. Fatima, M., I. Ahmad., I. Sayeed., M. Athar and S. Raisuddin, (2000). Pollutant induced over activation of phagocytes is concomitantly associated with peroxidative damage in fish tissue. *Aquat.Toxicol.*,**49**:243-250. 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.

23. Edqvist, L.E., A. Medej and M. Forsberg, (1992). Biochemical blood parameters in pregnant mink fed PCB and fractions of PCB. Ambio, 21: 577-581.
24. Lohner *et al*., 2001[25]
25. Shaffi S.A. (1979). Effects of starvation on tissue and serum gluconeogenic enzyme, alkaline phosphatase and tissue glycogen in the fresh water catfish, *Heteropneustes fossilis* (Bloch). Acta *Physiol. Acad. Sci*..**53(4)**: 50501-505.
26. O'Connor, J.D., Gilbert, L.I., (1968). Aspects of lipid metabolism in crustaceans, American Zoology, 8, pp 529-539.
27. Ahmed, M.R., Elumalai, M., Balasubramanian, S.E., Balasubramanian, M.P., (1997), Individual and combined effect of copper and chromium on oxygen consumption and phosphatases of a marine edible crab, *Scylla seratta*, Biomedical letters Letters, 55, pp 147-152.
28. Goldfisher, S.E., Esser, E., Novikoff, A.B., (1964), Use of histological and histochemical assessment in the prognosis of the effects of aquatic pollutants (ed.) D.E.Hinton, M.W.Kendall and B.B.Silver. Sect. 528, ASTM, Philadelphia, pp 194-208.
29. Vijayavel, K., Balasubramanian, M.P., (2006). Fluctuation of biochemical constituents and markers enzymes as a consequence of naphthalene toxicity in the edible estuarine crab *Scylla serrata*, Ecotoxicology Environmental Safe, 63, pp 141-7.
30. 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.
31. Gabriel, V. V., N.E. Amakiroi and G. N. O. Ezeri, 2007.*J. Anim. Vet Advances.*, **6 (3)**: 461- 465.
32. Moreno, S. Pichardo, L., Goomez-Amores, A. Mate, C.M. Vazquez, Camean. (2005). Antioxidant enzymes activity and lipid peroxidation in liver kidney of rats exposed to microcystin-LR administered intraoperation Toxicon, **45**, 395-402.
33. Patil, C., Paul, R., Malkanna, (2008), Neuroendocrine reglulation and pesticidal impact on freshwater crab, *Barytelphusa guerini*, Journal of Environmental Biology, 29, pp 887-892.
34. Sreenivasan, R.S., Krishna Moorthy, P., Deecaraman, M., (2011). Cypermethrin Induced Toxicity to phosphatases and dehydrogenases in gills and hemolymph of fresh water crab, *Spiralothelp husahydrodroma* (Herbst), International Journal of Biology & Medical Research, 2(3), pp 784-788.

Source of support: University Grant Commission, New Delhi,India, **Conflict of interest: None**