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ORIGINAL ARTICLE

AMELIORATIVE EFFECT OF *CARDIOSPERMUM HALICACABUM* AGAINST CADMIUM TOXICITY ON ACETYLCHOLINESTREASE ENZYMES ACTIVITY IN THE FRESH WATER CRAB, *PARATELPHUSA HYDRODROMAUS*

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ABSTRACT

Cadmium, 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 chloride on the experimental crab for the LC_{50} value was carried out. The present study evaluates toxicity of cadmium impact on acetylcholinestrease enzymes changes in the fresh water field crab *Paratelphusa hydrodromous*.

Keywords: Hepatopancreas, *C.halicacabum*, Cadmium, Acetylcholinestrease, Enzymes changes, *Paratelphusa hydrodromaus*, Toxicity.

1.INDRODUCTION

As a major pollutant, cadmium can penetrate into organisms and create adverse effects (Thurberg *et al*., 1973; Bjerregaard and Vislie, 1985; Zyadah and Abdel-Baky, 2000). Although some studies have focused on its uptake and accumulation in aquatic organisms (Rainbow, 1995, 1997). Besides these stimulating factors, growing evidence indicates that heavy metals such as cadmium (Cd) are also crucial ones in inducing apoptosis (Kim *et al*., 2008). Cd is one of the most toxic environmental and industrial pollutants (Templeton and Liu, 2010). Some GST isozymes in organism also display peroxidase activity (Ahmad, 1992).

Glutathione S- transferase (GST) is a group of multifunctional enzyme involved in biotransformation and detoxification of xenobiotics (Smith and Litwack, 1980). Many enzymatic markers find application to be used to determine pollutional exposure in animals. Several specific enzymes have been proposed for monitoring purposes of water pollution (Agradi *et al*., 2000). Glutathione S-transferase (GST) activity involved in defence against oxidative stress could reveal effective biochemical markers of toxic effect (Petrivalsky *et al*., 1997). AChE is a key enzyme in cholinergic transmission in the nervous system. The broad function of this enzyme is to catalyze the hydrolysis of acetylcholine into acetate and choline in the synaptic cleft (Yi *et al*., 2006). In the present study, we used the freshwater crab *Paratelphusa hydrodromous* that is widely distributed at interfaces of water and sediments in South India as an experimental model to investigate the effects of cadmium level in the hepatopancreas and the enzymatic activities of cadmium.

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 (Chopra *et al*. 1986). Hence, an attempt has been made to investigate the following enzymological parameters like GST, AChE, and ACh 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.

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

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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 studies

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).

Assay method

The activity of GST was measured by the method of Habig (1974). Activity of glutathione – s- transferase was measured in tissues homogenate by following the increase in absorbance at 340 nm using 1-chloro-2, 4 dinitro benzene as substrate. The enzyme Acetylcholine and Acetylcholinesterase are assayed by Matcalf (1951), 2 m hydroxylamine hydrochloride and 3 m sodium hydroxide. Ach content was determined by the method of Hestrin, Augustinson (1957).

Statistical analysis

The statistical analysis of data was done by using SPSS 11.5 statistical software, Mean ± SEM standard error and T value was calculated. The significance of the test result was observed at<0.05%level. Percentage changes were calculated.

3.OBSERVATIONS

Glutothion-s-transferase(GST)

In the present investigation *Paratelphusa hydrodromaus* when exposed to cadmium along with *C.halicacabum* group 3, the hepatopancreas GST activity was recovered as to cadmium group 2. The percent changes of GST activity are 6.05, 4.55, 7.02, 15.61 and 26.19 for 1, 7, 14, 21 and 28 days, respectively. While in the crab exposed to group 4, the GST activity in hepatopancreas was noticed without any changes when compared with control. The levels of GST content exposure for four groups are statistically significant at 1% and 5% levels (Table 1)

Acetylcholine (ACH)

Paratelphusa hydrodromaus were exposed to group 2, increase in the activity of Ach is found compared to control (group1). The percent changes for group 2 are 18.39, 29.89, 41.92, 54.48 and 63.23 for 1, 7, 14, 21 and 28 days, respectively. When the crabs are exposed to group 3, the activity of Ach was recovered. The percent recoveries are -12.28, -9.01, -8.45, -13.56 and -29.04 for 1, 7, 14, 21 and 28 days. In the crab exposed to group 4, the Ach activity was decreased. Slight variation was noticed the percent changes are -0.60, -1.22, -1.88, -2.18 and -2.97 for 1, 7, 14, 21 and 28 days, respectively. The activity of Ach in liver tissues was statistically significant at 1% and 5% levels (Table 2).

Acetyl cholinesterase (AChE)

In the percent investigation, crab exposed to cadmium group 2 shows a decrease in the level of AChE activity in hepatopancreas tissue compared to control group 2. The percent changes over control are -7.52, -13.11, -21.88, -25.32 and -30.76 for 1, 7, 14, 21 and 28 days respectively. In the group3, the increased AChE activity was observed. The increased percent recoveries are 5.58, 8.97, 3.84, 8.21, and 30.25 for1, 7, 14, 21 and 28 days, respectively. In the crab exposed to group 4, the AChE recorded response recorded is similar to control. The recorded AChE content in liver tissue for all 4 groups are statistically significant at 1% and 5% levels (Table 3).

4.DISCUSSION

The results obtained in the present study of the effect of cadmium, on a fresh water crab, *Paratelphusa hydrodromous with* sub-lethal concentrations at different exposure periods showed interesting results. Fetoui *et al.* (2009) have demonstrated that the depletion of intracellular sulfhydryl groups (SH groups) by insecticides is the prerequisite for ROS generation. GST are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms; and also reduces lipid peroxides (Mosialou *et al.*, 1993; Mansour and Mossa, 2009). Kale *et al*. (1999) and El-Demerdash (2007) reported a significant decrease in GST activity.

The primary role of GST multifunctional enzyme system is to facilitate conjugation of endogenous glutathione with electrophiles, thus resulting in more polar compounds to be excreted or further metabolized (Ketterer *et al*., 1983). GST also functions as an antioxidant enzyme by conjugating breakdown products of lipid peroxides to glutathione (Ketterer *et al*., 1983; and Lee, 1991).

In the present study sublethal concentration of cadmium inhibiting acetylcholinesterase (AChE) activity in the group 2. Because cadmium is a neurotoxic substance, it interferes with neurotransmitter activity. Inhibition of acetylcholinesterase preventing enzyme substrate complex formation finally acetylcholine (Ach) not reduced into acetate and choline. Vittozzi and Angelis (1991) have been reported as inhibition of acetylcholinesterase (AChE) that is responsible for the degradation of acetylcholine will result in the excessive stimulation of cholinergic nerves. This will result in tumors, convulsions and finally the death of the aquatic organism.

The inhibition of acetylcholinesterase (AChE) activity in fish can be dangerous since it will affect feeding capability, swimming activity, identification, avoidance of predators and spatial orientation of the species (Adedeji *et al*., 2008). Acetylcholine (ACh) activity was increased in the treated group II, the inhibition of AChE consequently leads to excessive Ach accumulation at the synapses and neuromuscular junctions resulting in over stimulation of Ach receptors which could ultimately end in death due to respiratory failure Gupta (1994). Group III acetylcholinesterase (AChE) activity was considerably recovered and diminishing the acetylcholine (Ach) this may due to the supplementary feed. While in the group 4 observed slight changes in AChE and ACh activity. It is equal

Table 1. Changes in the level of glutathione- s-tranferase (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 Control	0.410 ± 0.01	0.416 ± 0.009	0.423 ± 0.02	0.425 ± 0.009	0.427 ± 0.007
II Cadmium	0.380 ± 0.009 - 7.31	0.373 ± 0.01* - 10.33	0.356 ± 0.02* - 15.83	0.333± 0.01** - 21.64	0.313 ± 0.01** - 91.98
III Cadmium + *C. helicacabum*	0.403 ± 0.02* - 1.70 + 6.05	0.390 ± 0.02* - 6.25 + 4.55	0.381 ± 0.01* - 9.92 + 7.02	0.385 ± 0.02** - 19.41 + 15.61	0.395± 0.02** - 7.49 + 26.19
IV *C. helicacabum*	0.413 ± 0.02 + 0.73	0.420 ± 0.01 + 0.96	0.426 ± 0.01 + 0.71	0.428 ± 0.01 + 0.47	0.429 ± 0.01 + 0.46

Mean± S.E - mean of six individual observation; *Significant at P<0.05 level. **Significant at P<0.01 level. (+,-) denotes increased and decreased values.

Table 2. Changes in the level of acetylcholine (µ mole / 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 Control	16.36±0.06	16.39±0.03	16.41±0.03	16.45±0.03	16.47±0.04
II Cadmium	19.37±0.07** +18.39	21.29±0.02** + 29.89	23.29±0.07** + 41.92	24.91±0.04** + 54.48	26.72±0.02** + 63.23
III Cadmium + *C. helicacabum*	16.99±0.02** + 3.85 - 12.28	19.37±0.03** +18.18 - 9.01	21.32±0.07** + 29.92 - 8.45	21.53±0.10** + 30.88 - 13.56	18.96±0.02** + 15.11 - 29.04
IV *C. helicacabum*	16.25±0.05 - 0.60	16.19±0.03 - 1.22	16.10±0.02 - 1.88	16.09±0.01 - 2.18	15.98±0.02 - 2.97

Mean± S.E - mean of six individual observation; *Significant at P<0.05 level. **Significant at P<0.01 level. (+,-) denotes increased and decreased values.

Table 3. Changes in the level of acetylcholine esterase (n mole / 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 Control	2.32 ± 0.01	2.32 ± 0.06	2.33 ± 0.09	2.33 ± 0.04	2.34 ± 0.04
II Cadmium	2.15 ± 0.07* - 7.52	2.02 ± 0.04* - 13.11	1.82 ± 0.04** - 21.88	1.74 ± 0.08** - 25.32	1.62± 0.06** - 30.76
III Cadmium + *C.helicacabum*	2.27 ± 0.15** - 2.36 + 5.58	2.20± 0.12** - 5.41 + 8.91	1.89 ± 0.01** - 18.88 + 3.84	1.97 ± 0.01** - 15.55 + 8.21	2.11 ± 0.03** - 9.65 + 30.25
IV *C.helicacabum*	2.33 ± 0.07 + 0.21	2.34 ± 0.02 + 0.60	2.35 ± 0.04 + 0.8	2.36 ± 0.02 +1.28	2.37 ± 0.04 + 1.28

Mean± S.E - mean of six individual observation; *Significant at P<0.05 level. **Significant at P<0.01 level. (+,-) denotes increased and decreased values.

to normal (group 1). The observed inhibition of AChE in the present investigation was in agreement with the findings of Bandyopadhyay (1982).

The enzymes activity alteration observed in the present study are regained gradually and recovered when the crab exposed to *C. halicacabum* to the fish. This may be due to presence of phyto-chemicals compounds are, Vitamin C, thiamine, riboflavin, nicotinic acid (Vitamin B3), alkaloids, proteins and fats. Vitamin C is one of the four dietary antioxidants, the others being Vitamin E, precursor β-carotene and Selenium alkaloids, flavonoids, tannins, phytosterols, vitamin A, Glycosides, linolenic acid, pinitol, apigenium, luteolin, rutin, chrysoeriol, palmitic acid and fatty acid etc.,

Phytochemicals with antioxidant capacity naturally present in food are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration (Scalbert and Williamson, 2000). The antioxidant activity of flavonoids can be explained through their chelating action. They bind with transition metal particularly iron and copper and thus inhibit of transition metal-catalysed free radical formation. Flavanoids inhibit lipid peroxidation, oxidation of linoleic acid and $Fe^{+}2$ catalyzed oxidation of glutamine synthase, through free radical scavenging and removal of metal ions from catalytic sites *via* chelation (Kondratyuk and Pezzuto, 2004; Andjekovic, 2006). The electron and H+ donating capacity of flavonoids seem to contribute in the termination of lipid peroxidation chain reaction based on their reducing power. Due to their reducing power these phytochemicals act as both antioxidant and pro-oxidant depending upon the exposed environment.

5.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.

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