

**PROTECTIVE ROLE OF *CARDIOSPERMUM HALICACABUM* AGAINST THE  
CYPERMETHRIN TOXICITY IN THE REDUCED GLUTATHIONE (GSH) IN THE FRESH  
WATER FISH *CYPRINUS CARPIO* (HAMILTON)**

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**ABSTRACT**

The aim of study was to assess the effect of cypermethrin sublethal concentration of (52.82 mg/kg) for 120 hours on various antioxidant enzymes was carried out in the freshwater fish *Cyprinus carpio*. The activity of antioxidant enzymes, such as reduced glutathione (GSH) were increased was decreased. This observation clearly indicates the defensive nature and the adaptive mechanism of cells against free radical induced toxicity, *Cardiospermum halicacabum* in plant extracts may afford protection from pesticide toxicity.

**Keywords:** Cypermethrin, *Cardiospermum halicacabum*, *Cyprinus carpio*, Reduced glutathione (GSH).

**1.INTRODUCTION**

Pesticides are one of the most potentially harmful chemical introduced into the environment, though they have contributed considerably to human welfare, their adverse effects on non target organisms are significant, John (2007). Pesticides are highly toxic to the fish leading to serious changes in the gill epithelium, which impairs gaseous exchange (Caliskan et al., 2003), these compounds are also toxic to the fish liver, kidney, brain and muscle (Das and Mukherjee, 2003). Cypermethrin can be found in trace amount or at higher concentrations in soil and air. In mammals, cypermethrin can accumulate in body fat, skin, liver, kidney, adrenal glands, ovaries, lung, blood and heart (Wielgomas and Krechniak, 2007). The contamination of surface water by pesticide used in agriculture is a problem of worldwide importance. A major part of the world food is being supplied from fish source, so it is essential to secure the health of fishes (Tripathi et al., 2002). Increased use of chemical pesticides results in the excess of toxic chemicals. Mainly ensuring the aquatic ecosystem. Many enzymatical 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). The antioxidant GSH is tripeptide- $\gamma$ -glutamyl-cystenine-glycine or reduced glutathione. In living organisms GSH plays an important role in normal cell function. Extensive studies have shown that the GSH is involved in various biological reactions such as the

detoxification of hydrogen peroxide, amino acid transport and scavenging of free radicals (Kumar et al., 2000). Reduced glutathione (GSH) plays an important role in the regulation of blood pressure by improving the endothelial function by increasing the bioavailability of nitric oxide which acts as an antioxidant (Nosratola and Vaziri, 2000). Hence an attempt has been made to investigate the protective effect of *C. halicacabum* against the toxic impact of cypermethrin in the fresh water fish *cyprinus carpio*.

**2.MATERIALS AND METHODS**

The freshwater fish *cyprinus carpio* were collected from the Navarathna fish form at Pinnalur, Cuddalore district. The fish were brought to the laboratory and transferred to the rectangular cement tanks (100 x175) of 500 liters capacity containing chlorine free aerated well water. The fishes measuring 14-16 cm in length and 70-80 g in weight were selected irrespective of their sex for the experiments. During this time they were fed every 24 hour with a commercial diet. The physico-chemical parameters of the water were monitored throughout the acclimation period and remained constant (pH:  $7.18 \pm 0.5$ , conductivity:  $118.25 \pm 8.7 \mu\text{S cm}^{-1}$ , dissolved oxygen:  $8.49 \pm 0.9 \text{ mg O}_2 \text{ L}^{-1}$ , temperature:  $21.96 \pm 2.7 \text{ }^\circ\text{C}$ ).

Fishes were exposed in 4 groups.

**Group-1** fish exposed to tap water

**Group- 2** fish exposed to cypermethrin

**Group-3** Fish exposed to cypermethrin along with *Cardiospermum halicacabum*

**Group-4** Fish exposed to *Cardiospermum halicacabum* alone

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## PLANT PREPARATION

Healthy disease free leaves of *Cardiospermum halicacabum* were collected from Cuddalore district in and around Vadalore Village, plant was identified. The leaves were washed in running tap water for 10 minutes leaves were dried, aerial parts (1kg) of *Cardiospermum halicacabum* were macerated thrice at room temperature and prepared in powdered condition and equal amount of rice brane mixed well and small amount water added and prepared small pellet for used in treated fish.

## EXPERIMENTAL ANIMALS

Healthy *cyprinus carpio* were procured from the freshwater farm located in Navarathina fish form Pinnalur, Cuddalore district. They were acclimatized for a maximum period of 15 days in the laboratory condition. The fish each measuring 8.0 to 10.0 cm in length and weighing 10 to 15 g were used for the experimental studies. *cyprinus carpio* fingerlings were exposed to sublethal concentration of cadmium 51.82 mg/l for a period of 120 hrs.

## ANALYSED OF REDUCED GLUTATHIONE (GSH)

The level of Reduced glutathione in tissues (gill, liver and kidney) was estimated by the method of Ellman, (1959).

## STATISTICALLY ANALYSES

The data obtained in the present work were expressed as means  $\pm$  SE, percentage changes and were statistically analyzed using student t-test by the method of Trivedy and Goel, (1984), to compare means of treated for the various haematological parameters studies data against their control ones and the result were considered significant at ( $P < 0.05$ ), ( $P < 0.01$ ) level.

## 3.RESULTS

In the present investigation, fresh water fish *cyprinus carpio* exposed to group 2 has resulted in gill tissue at all period causing a significant ( $p < 0.05$ ) changes in the values which are -8.22, -12.16, -17.45, -21.10, -26.10 and -26.40 for 24, 48, 72, 96 and 120 hours, respectively. The cypermethrin along with *Cardiospermum halicacabum* (Group 3), the GSH response gradually recovers when compared to group 2. In the *Cardiospermum halicacabum* fish (group 4), there were no makeable changes occurring in the GSH content. The increased and decreased values of GSH activity in gill tissue were statistically significant in all groups (Table 1). The GSH level of tissue exhibit remarkable changes. The increased GSH content is observed in group 2. The per cent changes were -9.42, -14.91, -90.90, -25.14 and -29.97 for 24, 48, 72, 96 and 120 hours, respectively. In the administration of group 3, the GSH response was gradually recovered. The decreased per cent changes were -6.88, -8.98, -10.54, -11.33 and -12.75 for 24, 48, 72, 96 and 120 hours, respectively. The increased and decreased activities of GSH in liver are statistically significant in all groups (Table 1).

The per cent increase in GSH activities for 24, 48, 72, 96 and 120 hours of sub lethal concentration of cypermethrin and the per cent changes were -7.19, 12.98, -18.54, -22.23 and -27.23 for 24, 48, 72, 96 and 120 hours, respectively. When exposed to group 3, the GSH content is recovered when compared to group 2. The per cent decreased where -4.08, -7.82, -10.33, -11.74, and -13.92 for 24, 48, 72, 96 and 120 hours, respectively. While in the fish exposed group 4, the GSH response in kidney is recovered without any changes. The percentage recoveries were 0.64, 0.81, 0.99, 1.21 and 1.69 for 24, 48, 72, 96 and 120 hours, respectively. The increased and decreased levels of GSH in kidney tissue were statistically significant in group 2, 3 and 4 at 1% and 5% levels (Table 1).

Table 1. Variations of reduced glutathione (GSH) ( $\mu\text{g}/\text{min}/\text{protein}$ ) activity in the freshwater fish *Cyprinus carpio* exposed to cypermethrin and *C. halicacabum* for 120 hours

Tissues	Groups	Hours of exposure				
		24	48	72	96	120
Gill	Group-I Control	4.138 $\pm$ 0.046	4.144 $\pm$ 0.035	4.149 $\pm$ 0.054	4.152 $\pm$ 0.066	4.148 $\pm$ 0.039
	Group-II CYP	3.798** $\pm$ 0.066	3.640** $\pm$ 0.058	3.425** $\pm$ 0.043	3.276** $\pm$ 0.025	3.053** $\pm$ 0.039
	% COC	% -8.22	% -12.16	% -17.45	% -21.10	% -26.40
	Group-III CYP+C. <i>halicacabum</i>	3.956* $\pm$ 0.027	3.895** $\pm$ 0.038	3.804** $\pm$ 0.033	3.753** $\pm$ 0.025	3.707** $\pm$ 0.048
	% COC	% -4.40	% -6.01	% -8.31	% -9.61	% -10.63
	% COT	% +4.16	% +7.00	% +11.06	% +14.56	% +21.42
	Group-IV <i>C. halicacabum</i>	4.156 <sup>NS</sup> $\pm$ 0.054	4.169 <sup>NS</sup> $\pm$ 0.061	4.181 <sup>NS</sup> $\pm$ 0.048	4.191 <sup>NS</sup> $\pm$ 0.039	4.202 <sup>NS</sup> $\pm$ 0.054
	% COC	% +0.43	% +0.60	% +0.77	% +0.93	% +1.30
	Group-I Control	4.580 $\pm$ 0.035	4.586 $\pm$ 0.044	4.593 $\pm$ 0.038	4.598 $\pm$ 0.056	4.595 $\pm$ 0.059
	Group-II CYP	4.123** $\pm$ 0.030	3.902** $\pm$ 0.028	3.679** $\pm$ 0.044	3.442** $\pm$ 0.023	3.218** $\pm$ 0.054
% COC	% -9.42	% -14.91	% -19.90	% -25.14	% -29.97	
Liver	Group-III CYP+C. <i>halicacabum</i>	4.265** $\pm$ 0.031	4.174** $\pm$ 0.042	4.109** $\pm$ 0.030	4.077** $\pm$ 0.029	4.009** $\pm$ 0.047
	% COC	% -6.88	% -8.98	% -10.54	% -11.33	% -12.75
	% COT	% +3.44	% +6.97	% +11.69	% +18.45	% +24.58
	Group-IV <i>C. halicacabum</i>	4.593 <sup>NS</sup> $\pm$ 0.026	4.608 <sup>NS</sup> $\pm$ 0.047	4.623 <sup>NS</sup> $\pm$ 0.056	4.635 <sup>NS</sup> $\pm$ 0.033	4.646 <sup>NS</sup> $\pm$ 0.069
	% COC	% +0.28	% +0.48	% +0.65	% +0.80	% +1.11
	Group-I Control	3.115 $\pm$ 0.026	3.121 $\pm$ 0.031	3.128 $\pm$ 0.049	3.135 $\pm$ 0.054	3.132 $\pm$ 0.037
	Group-II CYP	2.891** $\pm$ 0.025	2.716** $\pm$ 0.030	2.548** $\pm$ 0.028	2.438** $\pm$ 0.034	2.280** $\pm$ 0.038
	% COC	% -7.19	% -12.98	% -18.54	% -22.23	% -27.21
	Group-III CYP+C. <i>halicacabum</i>	2.988* $\pm$ 0.023	2.877** $\pm$ 0.021	2.805** $\pm$ 0.040	2.769** $\pm$ 0.038	2.696** $\pm$ 0.012
	% COC	% -4.08	% -7.82	% -10.33	% -11.74	% -13.92
% COT	% +3.35	% +5.93	% +10.09	% +13.58	% +18.24	
Kidney	Group-IV <i>C. halicacabum</i>	3.135 <sup>NS</sup> $\pm$ 0.024	3.146 <sup>NS</sup> $\pm$ 0.031	3.159 <sup>NS</sup> $\pm$ 0.029	3.173 <sup>NS</sup> $\pm$ 0.043	3.185 <sup>NS</sup> $\pm$ 0.040
	% COC	% +0.64	% +0.81	% +0.99	% +1.21	% +1.69
	% COT	% +0.64	% +0.81	% +0.99	% +1.21	% +1.69

Values are mean  $\pm$  S.E-Mean of six individual observations; and student t-test. Significant at \* $P < 0.05$ ; Significant at \*\* $P < 0.01$  levels. (+, -) denotes decreased and increased. % COC (change over control); % COT (change over treated).

#### 4.DISCUSSION

In the present investigation *cyprinus carpio* fish exposed to sub lethal concentration of cadmium and *Cardiospermum halicacabum* plant supplementary feed shows a significant change of reduced glutathione (GSH). The GSHenzymes play a critical role in the defence against oxidative stress. The activity of GSH can be induced by xenobiotic and detoxification of peroxides can be achieved by this induction (Hamed *et al.*, 1999). The biological function of GSH is to reduce H<sub>2</sub>O<sub>2</sub> and lipid hydro peroxides (Verma *et al.*, 2007). The decreased activities of antioxidant enzyme SOD, CAT and increasing of GSH in all tissues of cypermethrin treated fish, which indicated the failure of antioxidant defence system to overcome the influence of ROS induced by cypermethrin.

The GSH enzymes play a critical role in protecting the cell form free radical damage, particularly lipid peroxidation. The GSH enzymes catalyse the reduction of H<sub>2</sub>O<sub>2</sub> to water and organic peroxides (R-O-O-H) to the corresponding stable alcohols (R-OH) using glutathione (GSH) as a sources of reducing equivalents. Giray *et al.* (2000) observed that the liver can be accepted as sources of GSHPx and therefore a higher activity was found in this organ compared to the other organs. Uner *et al.* (2001) reported that cadmium caused an increase in GSH activity while it caused a decreased in CAT activity in the liver and kidney of some fresh water fish species. Because GSH-Px is found mainly in cytosol and mitochondria, it is widely affected by xenobiotic. The increase in GSH activity is observed, predominantly in liver and kidney similar to the resulted reported by Li *et al.*(2003) who have studied the responses of the antioxidant systems in the hepatocytes of common carp (*cyprinus carpio*) to microcystin- LR.

#### 5.CONCLUSION

In view of cypermethrin toxicity on *cyprinus carpio* fresh water fish has severely affected where as in supplemented feed *Cardiospermum halicacabum* exposed group gradually recovered from the toxic effect. Because of the presence of active compound which have rich antioxidant potential.

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