Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

Int. J. Modn. Res. Revs.
Volume 4, Issue 6, pp 1168-1171,June, 2016

ORIGINAL ARTICLE

HEPATOPROTECTIVE EFFECT OF *CARDIOSPERMUM HALICACABUM* IN CYPERMETHRIN TOXICITY ON LPO AND SOME ANTIOXIDANT ACTIVITIES IN THE LIVER TISSUE OF FRESH WATER FISH *CYPRINUS CARPIO (HAMILTON)*

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Article History: *Received 6th May,2016, Accepted 31st May,2016, Published 1st June,2016*

ABSTRACT

The present study was undertaken to evaluate the hepatoprotective effect of *Cardiospermum halicacabum* against the toxicity effects of herbicide cypermethrin on lipid peroxidation and some antioxidant enzyme system in the freshwater fish *cyprinus carpio*. In the present experimental study, *cyprinus carpio* were exposed to sublethal concentration of cypermethrin (21 mg/L of cypermethrin for 120 hours. The oxidative stress in the liver was evidence by increased lipid peroxidation levels. The antioxidants superoxide dismutase (SOD) and catalase (CAT) levels were decreased compared to control. During the treatment of *C. halicacabum* against cypermethrin exposed fish were restored near normal level (Group III and IV). The observed results were discussed in detail. Our findings clearly evidenced that the *C. halicacabum* supplementation is very effective in reducing the cypermethrin toxicity to the freshwater fish *cyprinus carpio*

Keywords: Hepatoprotective, Cypermethrin, *Cyprinus carpio*, *C. halicacabum*, LPO, SOD, CAT.

1.INTRODUCTION

Environmental studies provide an approach towards understanding the environment of our planet and the impact of human life upon the environment (Orun, *et al*., 2010). Herbicides eliminate wild vegetables and herbs thus reducing the amount of food for organisms and changing physicochemical parameters. Reductions in producer biomass can lead to lower dissolved oxygen, reduced pH, increased alkalinity and increased conductivity (Dorval, *et al*., 2003). Liver plays an important role in several vital functions of basic metabolism and it is also the major organ of accumulation, biotransformation and excretion of contaminants in fish, including degradation and bioactivation of pesticides (Triebskorn *et al*., 1994; Triebskorn *et al*., 1997). The liver is a very important organ performing vital functions such as detoxification, synthesis of several components of blood plasma, glycogen storage and release of glucose to the blood.

Antioxidant enzymes are important in coping oxidative stress caused by the metabolism itself and environmental factors (Jorgensen, 2010). 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), (Droge, 2002). SOD is a group of metalloenzymes that plays a crucial antioxidant role and constitutes the primary defense against the toxic effects of superoxide radical in aerobic organism (Switzer, 1980).

The medicinal properties of the plants have been investigated in the recent scientific developments throughout the world, due to their potential antioxidant activity, no side effects and economic viability (Auudy *et al*., 2003). *Cardiospermum halicacabum* L. of the family *Sapindaceae* has been used in Ayurveda and folk medicine for a long time in the treatment of rheumatism, lumbago, cough, hyperthermia, nervous diseases, as a demulcent in orchitis and in dropsy (Neuwinger, 2000). The anti-inflammatory, analgesic and vasodepressant activities of this plant have been established (Sadique *et al*., 1987). *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). Hepatoprotective effect of *C. halicacabum* on LPO and some antioxidant enzymes in liver tissue of *cyprinus carpio*.

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2.MATERIALS AND METHODS

Experimental animal collection and maintenance

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 ± 0.5, conductivity: 118.25 ± 8.7 µS cm-1, dissolved oxygen: 8.49 ± 0.9 mg O2 L-1, temperature: 21.96 ± 2.7 0C).

Supplementary feed

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 leafs 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.

Enzymatic assay

Superoxide dismutase (SOD) activity was determined by method of [12], the in absorbance was recorded at 560 nm. The activity of catalase (CAT) was determined by the method of(Sinha, 1972) was recorded at Spectrophotometically read at 620 nm. Lipid peroxides in liver tissue were estimated by the method of (Niehaus and Samuelson, 1968) which recorded at spectrophotometrically at 540 nm.

Experimental design

Group- I: Fish exposed to tap water (control)
Group- II: Fish exposed to cypermethrin
Group-III: Fish exposed to cypermethrin along with *Cardiospermum halicacabum*
Group- IV: Fish exposed to *Cardiospermum halicacabum* alone

Statistically analyses

The data obtained in the present work were expressed as means ± SE, percentage changes and were statistically analyzed using student t-test (Milton and Tsokos, 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.OBSERVATION

In the present study, observed that liver tissue antioxidant such as SOD and CAT levels are decreased significantly at 5 % level (p<0.05) in the treated group II (Table – 1 and 2). At the end of 120 hours SOD and CAT levels are decreased when compared to control group I. In the group III and IV SOD and CAT levels are near to normal when compared to group II.

In the group III and IV SOD and CAT levels are increased significantly at 120 hours compared to group II and which was near to control group I. LPO levels were significantly increased in the group II when compared to control group I (Table - 1). In the group III and IV LPO level was regained compared to group II. In the group III and IV LPO level near to normal significantly at 120 hours compared to group II and which was near to normal when compared to control group I.

Table.1. Changes of superoxide dismutase (U/min/mg of protein) and catalase (µmol of H_2O_2 consumed/ min/mg of protein) activities in the liver tissue of freshwater fish *Cyprinus Carpio* exposed to cypermethrin followed by the supplementary feed of *Cardiospermum halicacabum* exposed to 120 hours

	Groups	Hours of exposure				
		24	48	72	96	120
SOD (superoxide dismutase (U/min/mg of protein)	Group-I Control	30.706 ± 0.780	30.644 ± 0.917	30.628 ± 0.922	30.607 ± 0.811	30.591 ± 0.762
	Group-II cypermethrin	28.543** ± 0.617	28.370** ±0.572	29.696** ±0.622	29.117** ±0.686	25.884** ±0.665
	% COC	-4.757	-6.410	-8.542	-13.712	-17.655
	Group-III atrazine +*C. halicacabum*	28.945 ^{NS} ± 0.428	28.437 ^{NS} ±0.584	27.809 ^{NS} ±0.581	27.379** ±0.708	27.005** ±0.719
	% COC	-2.455	-3.622	-5.452	-9.834	-11.202
	%COT	2.394	3.756	4.016	4.874	8.557
	Group- IV *C. halicacabum*	31.724^{NS} ± 0.924	32.740^{NS} ±0.877	32.761^{NS} ± 0.807	32.780^{NS} ± 0.609	32.792^{NS} ± 0.655
	% COC	0.058	0.313	0.434	0.565	0.657
CAT (µmol of H_2O_2 consumed/ min/mg of protein)	Group-I Control	6.744 ± 0.074	6.712 ± 0.065	6.685 ± 0.096	6.653 ± 0.090	6.650 ± 0.064
	Group-II cypermethrin	8.112** ± 0.054	7.870** ± 0.073	7.224** ± 0.074	6.765** ± 0.096	6.076** ± 0.088
	% COC	-8.042	-10.906	-19.246	-24.736	-33.720
	Group-III atrazine +*C. halicacabum*	8.502** ± 0.067	8.345** ± 0.053	7.079** ± 0.058	7.854** ± 0.08	7.357** ± 0.097
	% COC	-2.986	-4.759	-8.018	-10.804	-17.140
	%COT	5.497	6.899	13.904	18.510	25.014
	Group- IV *C. halicacabum*	8.742^{NS} ± 0.085	8.258^{NS} ± 0.078	8.771^{NS} ± 0.065	8.785^{NS} ± 0.079	8.797^{NS} ± 0.056
	% COC	0.116	0.622	0.974	1.446	1.775
LPO (nmol/mg of protein)	Group-I Control	4.225 ± 0.024	4.238 ± 0.038	4.249 ± 0.054	4.255 ± 0.044	4.268 ± 0.056
	Group-II cypermethrin	4.538** ± 0.033	4.835** ± 0.035	4.998** ± 0.047	4.271** ± 0.048	4.545** ± 0.053
	% COC	9.497	17.369	22.121	32.223	38.087
	Group-III cypermethrin +*C. halicacabum*	4.483** ± 0.052	4.597** ± 0.044	4.751** ± 0.038	4.920** ± 0.037	4.985** ± 0.038
	% COC	7.790	11.021	14.408	20.159	22.283
	%COT	-2.558	-7.207	-7.079	-8.430	-14.081
	Group- IV *C. halicacabum*	4.334** ± 0.028	4.425** ± 0.034	4.524** ± 0.038	4.625** ± 0.056	4.715** ± 0.042
	% COC	3.351	5.711	8.374	11.370	13.621
		-6.612	-11.693	-12.977	-16.128	-19.309

Values are mean ± 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), NS- Nonsignificant.

4.DISCUSSION

SOD is vital for fish because the induction in the SOD enzyme activity is considered as a first defense mechanism against oxidative stress. SOD and CAT are reported as the key enzymes which condense ROS formed during bio-activation of xenobiotics in the hepatic tissue (Doyotte *et al*., 1997). In the present experimental result shows that the, levels of SOD was decreased when the fish exposed to cypermethrin. But the group IV (cypermethrin along with *Cardiospermum halicacabum*) the SOD level was gradually increases when compared to the group II. Moreover, the group III (cypermethrin along) also enhance the SOD level very slowly than the group IV. Pointed out that a decreased SOD activity response may accompany a first exposure to pollutants, which can be followed by an induction of antioxidant systems. They Suggested that the superoxide radicals by themselves or after their transformation to H2O2 cause an oxidation of the cysteine in the enzyme and decrease SOD activity. CAT is an enzyme located in peroxisomes and facilitates the removal of H_2O_2 (Markuland and Marklund, 1974). Fish develop the defense mechanism. Similar observation of decreased in SOD activity has been reported by Fatima and Ahmad (2005). In the present observation, the levels of CAT were decreased when the fish exposed to atrazine. But, the group IV (cypermethrin along with *Cardiospermum halicacabum*) the CAT level was gradually regained when compared to the group II. Moreover, the group III (cypermethrin along) also enhance the CAT level very slowly than the group IV. Carbaryl produced a simultaneous decrease in CAT at the end of the assay period. CATs are localized in the peroxisomes of most cells and are involved in fatty acid metabolism; changes in activities may often be difficult to interpret (Filho, 1996). These results are in agreement with Sayeed *et al*. (2013) demonstrated a decrease of 45% in hepatic catalase activity and high levels of TBARS formation in freshwater fish (*Channa punctatus*) exposed to deltamethrin insecticide.

Lipids play an important role as source of energy for fish. Since, most insecticides are lipophilic compounds, they can easy pass through biological barriers which content lipids and accumulate in fat tissue. Lipids molecules are highly susceptible to oxidative reactions. Due to cell membrane lipid peroxidation of unsaturated fatty acids, short chain fatty acids (Tejada *et al*., 2007).

In the present observation the level of LPO was increased when the fish exposed to atrazine. But the group IV (cypermethrin along with *Cardiospermum halicacabum*) the LPO level was gradually decreases when compared to the group II. Moreover, the group III (cypermethrin along with *Cardiospermum halicacabum*) also enhance the LPO level very slowly than the group IV. Vasantharaja, *et al.,* (2012) also point out the elevated level of lipid peroxidation in the liver of *C. mrigala* exposure to cypermethrin. The Increased LPO level may be due to the ROS production associated with the metabolism of cypermethrin leading to the peroxidation of membrane lipoid of the liver (Atli, *et al*., 2006). Crestani *et al*. (2007) analyzed the declined catalase activity in *Rhamdia quelen* exposed to the clomazone herbicide. These bioactive compounds present in *C. halicacabum* which may give recovery to fish in the presence of toxic stress.

5.CONCLUSION

It has been concluded from the evidence that the plants *C. halicacabum* produced significant hepatoprotective of cypermethrin induced toxicity on freshwater fish *cyperinus carpio*. When compared to both plant extracts have significantly preventing the hapatic damages in fishes. Besides, the recovery group (group III and IV) regain the SOD and LOP level. Because of the *Cardiospermum halicacabum* having potential protective effect against cypermethrin toxicity. At the *C. halicacabum* having the certain important medicinal properties and having the more valuable therapeutic properties of the *C. halicacabum* was less.

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