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ORIGINAL RESEARCH ARTICLE

Acute Toxicity of Cypermethrin and its Impact on Biochemical Alteration in the Fresh Water Fish *Cirrhinus mrigala* (Hamilton) and Protective Effect of *Cardiospermum helicacabum* (Linn)

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ABSTRACT

The cypermethrin (synthetic pyrethroids) has shown strong piscicidal activity in fresh water fish *Cirrhinus mrigala* for all exposure periods of 24, 48, 72, 96 and 120 hours. The acute toxicity value was found to be 150µg/l and 1/5 as used for of LC_{50} (30µg/l) was selected for sub lethal concentration. The biochemical parameters of Gill, Liver and Kidney tissue of *Cirrhinus mrigala* was studied, after 120 hrs, the dose depend alteration in the level of total protein, free amino acid and glucose. During recovery period, the level of biochemical components progressively increased indicating a problem recovered by *Cardiospermum helicacabum.* Hence, the pesticide intoxication has made a disturbance in normal functioning of cells with significant alteration in the fundamental biochemical mechanisms of fish.

Key words: *Cirrhinus mrigala, Cardiospermum helicacabum*, Cypermethrin, LC_{50} value, Protein, Amino acid and Glucose.

1. INTRODUCTION

Cypermethrin (416:30 C_{22} H_{19} Cl_2 No_3) is a synthetic pyrothroid pesticide that has been widely used over the past 30 year in India and other countries against pests, particularly Lepidoptera, cockroaches and termites. In animals, cypermethrin has been used as chemotherapeutic agent against ectoparasite infestations [1]. Cypermethrin (CYP), alpha-cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylic acid, is the most widely used Type II pyrethroid pesticide. It is used to control many pests, including moths, and pests of cotton and soya bean [2].

The physiological and biochemical alterations observed in an animal under any physiological stress can be correlated with the structural and functional changes of cellular proteins. Proteins occupy a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways [3]. Biochemical indices of stress have been proposed to assess the health of non target organisms exposed to toxic chemical in aquatic ecosystem [4]. Proteins are important organic substance required by organisms in tissue building. They are intimately related with almost physiological processes, which maintain a simple biochemical system in living condition.

The amino acid is the building blocks of proteins which are synthesized in the body must be supplemented through diet. Since, the food value of fish is directly dependent on their protein content, the contamination by the toxic substance will reduce their nutritive value [5]. Glucose serves as an immediate and major metabolic fuel. Increased glucose concentrations in white muscle have been reported in *Oreochromis niloticus* after cadmium exposed to sub-lethal concentrations [6].

The plant *Cardiospermum halicacabum* Linn. (Sapindaceae) is an annual or sometimes perennial climber, commonly found as a weed throughout India. The tender, young shoots are used as a vegetable, fodder, diuretic, stomachic, and rubefacient. It is used in rheumatism, lumbago, nervous diseases, and as a demulcent in orchitis and in dropsy. In Sri Lanka, it is used for the treatment of skeletal fractures. The juice of the herb is used to cure ear-ache and to reduce hardened tumours. It exhibits significant analgesic, anti-inflammatory and vaso-depressant activity, which is transient in nature. *In vitro* studies have revealed its antispasmodic and

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curative actions confirming the use of the herb in Ayurvedic medicine [7]. Hence, an attempt has been made to investigate the effect of sub-lethal concentration of cypermethrin on the biochemical parameters of the fresh water fish *C. mrigala* and chelating property of *C. helicacabum*.

2. MATERIALS AND METHODS

The fish *Cirrhinus mrigala* of size 8 to 12 cm and 50 to 70g weight were brought from a local fish farm at Pinnaloor, in Navarathna form. Fish collected and acclimatized at 28°C in the large sized aquarium tank disinfected with potassium permanganate and washed thoroughly prior to conduction of fish to prevent the fungal disease for acclimatization in the laboratory condition for 15 days. During laboratory condition fishes were feed with artificial feed, water was renewed on every day to maintain water quality. The LC_{50} concentration of cypermethrin was noted at 120 hrs. Fishes exposure, the tissues such as Gill, Liver and Kidney were collected by dissected the animal and stored at -20^{0}C for biochemical parameters studies. Fishes were exposed to 4 groups.

Group-1 fish exposed to tap water

Group- 2 fish exposed to cypermethrin

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

Group-4 Fish exposed to *Cardiospermum helicabum* alone

Toxicity experiment was performed according to Bayne *et al*. (1977)[8]. The fishes were exposed to 24, 48, 72, 96 and 120 hrs at five different concentrations of tap water, five tubs were set up for each concentration 300.11, 250.02, 200.81, 150.02 and 100.04 and each tub contains 10 fishes in 10 liters water de-chlorinated tap water, control animals were kept in similar condition without any treatment. Mortality was recorded at every 24 hrs up to 120 hrs exposure period. Fish were considered dead if any failed to respond to stimulus provided with glass rod.

Plant preparation

Healthy disease free leaves of *Cardiospermum helicacabum* were collected from Villupuram district, Nallavur Village in January-2011 and plant was identified. The leaves were washed in running tap water for 10 minutes leaves were dried, aerial parts (1kg) of *Cardiospermum helicacabum* were macerated thrice at room temperature and prepared in powdered condition and equal amount of rice bran mixed well and small amount water added and prepared small pellet for used in treated fish.

LC_{50} and biochemical assay

The LC_{50} values were evaluated by Finney (1971)[9] method. The formula used for assessing the mortality of the fish was recommended by the WHO and FAO. Protein levels were estimated according to Lowry *et al*. (1957)[10], using bovine serum albumin as a standard. Homogenates (2ml w/v) cold distilled water was prepared in 10% TCA as such, values have been expressed as mg/100mg wet, wt of tissue. Free amino acid were estimated using the method of Moor and Stein (1954)[11] (5% w/v) were prepared in 10% TCA and centrifuged at 3000 rpm, whereas supernatant was used for amino acid estimation. FAA has been expressed as mg/100 mg wet, wt of the tissue. Glucose were estimated using the method of Kemp and Kits (1954)[12].

Statistically analyses

The data obtained in the present work were expressed as means ± SE, percentage changes and were statistically analyzed using student t-test [13], to compare means of treated data against their control ones and the result were considered significant at ($P < 0.05$), ($P < 0.01$) level.

3. RESULTS AND DISCUSSION

The median lethal concentration (LC_{50}) of cypermethrin for 24, 48, 72, 96 and 120 hrs and its 95 percent confidence limits were determined from the data of the toxicity limits were determined from the data of the toxicity tests to get a basis of reference analysis and determination on the mode of action for cypermethrin toxicity on the test fish *C. mrigala*.

The acute toxicity of the pesticide cypermethrin on *C. mrigala* were assessed and presented in the form of median lethal concentration (LC_{50}) for 120 hours of exposure. The mortality rates of *C. mrigala* in different concentration of cypermethrin were studied. The selected concentration to determine LC_{50} for cypermethrin was ranging from 24, 48, 72, 96 and 120 hrs. LC_{50}values were 300.11 to 100.04µg/l. In this studies used 1/5 of value 96 hours LC_{50} value used for sub-lethal concentration on 30µg/l in this concentration to mortality were observed during the experimental period. For cypermethrin the toxicity test revealed that rate of mortality increased with increased concentrations (**Table 1& Fig 1**).

Sha and Kaviraj (2003)[14] has observed the Lethal concentration of cypermethrin, in cat fish *Heteropneustes fossilis* who reported that, up to 48 hours, there was no difference between LC_{50} values of aqueous acetone solublized cypermethrin. 72 hours LC_{50} values of aqueous

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cypermethrin and acetone solublized cypermethrin to *Heteropneustes fossilis* were 0.76 and 1.27 micro g/l, respectively; lethal values remained unchanged beyond 72 hours. The fish exposed to even lower concentration of cypermethrin (0.5 micro g/l showed hyperactivity).

For the sake of comparison with the different pesticides on the common carp, it was found that Diazinon, an organophosphate, has a LC_{50} value of 1530µg/l to the larvae of common carp [15]; Dichlorvos, a synthetic chemical pesticide, has LC_{50} value of 9410µg/l to fingerling mirror carp, *Cyprinus carpio* [16] and 2,4-D (2,4-dichlorophenoxyacetic acid), a herbicide, has an acute toxic LC_{50} of 63240µg/l to common carp (Sarikaya and Yilmaz, 2003 [17]. These studies revealed that the cypermethrin is more toxic to common carp than the other pesticides.

The protein contents observed in the tissue of gill, liver and kidney tissue of *C. mrigala* during sub-lethal concentration of cypermethrin for 24, 48, 72, 96 and 120 hrs of exposed periods. The protein activity significantly decreased in compared to control group-1 in all tissue during the toxic exposure periods. The fish was exposed to group-3 the protein content was recovered when compared to group-2 while in the fish exposed to group-4 when compared with group-1 the slightly increased. The recorded protein content were statistically significant at 5%, 1% levels (**Table 2**).

The decrease in total protein content in tissues of *C. mrigala* indicates active degradation of proteins under cypermethrin stress. Protein depletion in tissues constitutes a physiological mechanism with an important role in providing energy to cope with the stress situation. Depletion of proteins might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery as suggested by David *et al*. (2004)[18], reduction of proteins might be due to the blocking of protein synthesis, protein denaturation, or interruption in the amino acid synthesis.

Decrease in protein content of *Clarias batrachus* exposed to fenvalerate was reported by Tripathi *et al*. (2002)[19]. A reduction in protein content was also observed in *C. carpio* and *Labeo rohita* exposed to cypermethrin [20]. These results are in agreement with report of Ravider *et al*. (1988)[21] had demonstrated a similar situation in *C. batrachus* exposed to deltamethrin. A reduction in protein was also observed in *C. batrachus* exposed to cypermethrin. When the fish were transferred to freshwater, recuperation in protein content in liver and gill tissues was noted [22]. However, increased of consumer concentration of pesticide decrease the protein in tissues of the fish *Labeo rohita*. The present study is coincides with the reported data that the protein content was decreased in liver, by Tilak *et al*. (2005)[23].

A significant decreased in proteins was observed in all the tissues under sub-lethal concentration of the technical grade of cypermetrin. The variation in distribution suggests difference in metabolic calibers of various tissues, Pandi Bhaskaran (1991)[24] reported depletion in the protein content in muscle and liver of *Tilapia mossambica*, *Mystus vittatus* and *Channa striatus* exposed to fenvalerates. Jeba kumar *et al*. (1990)[25] reported decrease in protein content of *Lipidocephalichthys thermalis* exposed to sub-lethal concentration of fenvalerate [26].

The activity of amino acids is observed in the tissue of gill, liver and kidney tissue of *C. mrigala* during sub-lethal concentration of cypermethrin for 24, 48, 72, 96 and 120 hrs of exposure periods. The amino acid activity significantly increased when compared to group-1 in all tissue during the exposure period, the cypermethrin along with *C. helicacabum* group-3 the amino acid content being recovered when compared to group-2 while in the fish exposed to group-4 when compared to their control group-1. The slightly decreased of amino acid (**Table 3**). Increased of total free amino acids is an induced of stepped up proteolysis or fixation of ammonia into keto acid resulting in amino acid synthesis. Generally, these two processes contribute to the amino acid pool [27].

The free amino acid (FAA) pool was increased in the tissues of the fish during exposure to cypermethrin, while the elevated FAA levels were utilized for energy production by supplying them as keto acids into TCA cycle through aminotransferases to contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis [28]. It is also attributed to lesser use of amino acids [29] and their involvement in the maintenance of an acid-base balance [30]. Various researches suggested that stress conditions induce elevation in the transanimation pathway. The increase in FAA levels of tissues indicates stepped up proteases activities and fixation of ammonia into keto acids [31].

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The activity of glucose observed in the tissue of gill, liver and kidney tissue of *C. mrigala* during sub-lethal concentration of cypermethrin for 24, 48, 72, 96 and 120 hrs of exposed periods. The glucose activity significantly increased in compared to control group-1 in all tissue during the toxic exposure periods. The fish was exposed to group-3 the glucose content was recovered when compared to group-2 while in the fish exposed to group-4 when compared with group-1 the slightly decreased. The recorded glucose content were statistically significant at 5%, 1% levels (**Table 4**).

The glucose content of gill, liver, and kidney showed an increased but the increased was not uniform in all the tissue. In case of control fish, biomolecules like glucose, glycogen, total protein, free amino acids and lipids of the five tissues. The increased in the glucose level of the tissue while decrement in tissue glycogen in exposed fish makes it clear that the glycogen reserves are being used to meet the stress caused. Increased in serum glucose level in fish under stress was reported by Bedii and Kenan (2005)[32]. This can be attributed to several factors and one them is the decreases in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decreased the capacity of glycolysis [33].

The chemical profile of *Cardiospermum halicacabum* L. is relatively complete; there is some variability in the content of specific chemicals profile: specified fatty acids 98.8 % of lipids; Oil content 31.60% by weight; Iodine value 71% by weight. However, Barclay and Earle (1974)[34] noticed that leaves contain considerable amounts of saponins, alkaloids, (+)-pinitol, apigenium, luteolin and chrysoeriol. The major cyano lipid (49%) is a diester having two fatty acid moieties esterfied with 1-cyano-2-hydroxymethyl-prop-2ene-l-ol followed by a diester derived from 1-cyano-2-hydroxymethyl-prop-2-ene-3-ol (6%). Of the fatty acids, 11-eicosenoic acid is the major component (42%), other chief components of the oil include oleic acid (22%), arachidic acid (10%), linolenic acid (8%), palmitic acid (3%) and stearic acid (2%) including small proportions (1-2%) of a low-molecular weight acid, and several C22 acids [35]. Other minerals such as Ca (1.30%), K (4.01%), Mg (0.43%), P (0.83%), Organic-N (5.19%), Total-N (7.16%), and C (48.1%) were recorded by Broadley *et al*. (2004)[36].

The plant *C. hilicacabum* has been used as anti-inflammatory [37,38], an antipyretic [39]. Extracts of this plant have been reported to contain different triterpenoids, glycosides, and a range of fatty acids, [40] investigated the antioxidant potency. The multiple antioxidant activity of this plant was evident, as it also possessed reducing power, superoxide scavenging ability, nitric oxide scavenging activity, and also ferrous ion chelating potency. Further research is needed to substantiate these medicinal claims.

Fig 1: Median lethal concentration (LC_{50}) values for the fish *Cirrhinus mrigala* exposed to different period of cypermethrin

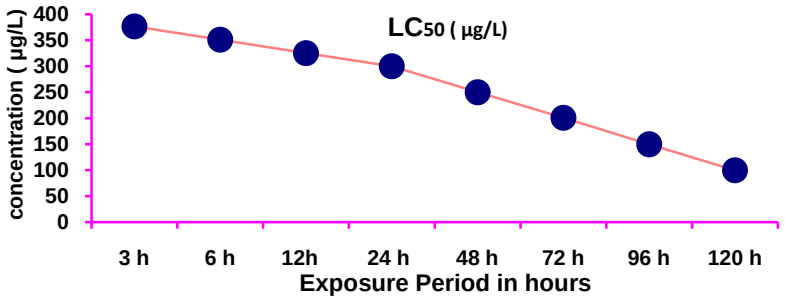

Table 1: Acute median lethal concentration for 120-h LC_{50} slope and 95% confidence limits of cypermethrin on *Cirrhinus mrigala*

Hours	LC_{50} (µg/l)	LC_{90} (µg/l)	Regression	95 % confidence limit		χ^2
				LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	
3h	376.60	388.30	Y= -1269.495 ± 3.503X	375.52 (386.60)	377.66 (390.46)	3.157
6h	351.17	363.38	Y= -1142.486 ± 3.394X	350.05 (361.60)	352.27 (365.64)	1.198
12h	325.25	337.45	Y= -1043.733 ± 3.360X	324.11 (335.72)	326.35 (339.67)	1.440
24h	300.11	328.49	Y= -407.638 ± 1.523X	293.97 (320.64)	305.44 (342.73)	9.067
48h	250.02	274.76	Y= -364.676 ± 1.654X	244.90 (268.16)	254.65 (285.87)	8.469
72h	200.81	225.50	Y= -285.305 ± 1.666X	198.50 (221.95)	203.02 (230.03)	3.015
96h	150.02	177.63	Y= -180.981 ± 1.534X	147.46 (173.58)	152.44 (182.92)	5.633
120h	100.04	123.64	Y= -118.019 ± 1.691X	97.89 (119.80)	102.28 (128.59)	5.080

LC-lethal concentration, LCL- lower confidence limit, UCL- upper confidence limit and χ^2 chisquares test.

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Table 2: Variations of Proteins (µg/g wet wt. of tissue) contents in the *C.mrigala* exposed to cypermethrin and *C.helicacabum* for the period of 120 hours

Tissue		Hours of experiment				
		24	48	72	96	120
Gill	Group-1	37.254±0.831	36.444±0.676	36.226±0.633	35.808±0.519	34.462±0.763
	Group-2	33.374*±0.783 % -10.41	31.296**±0.720 % -14.12	29.376**±0.756 % -18.91	28.254**±0.296 % -21.09	27.876**±0.535 % -19.11
	Group-3	29.442**±0.653 % -20.97	31.558**±0.570 % -13.41	32.698*±0.912 % -9.74	33.142^{NS}±0.246 % -7.44	33.758^{NS}±0.644 % -12.56
	Group-4	38.556^{NS}±0.101 % 3.49	38.590*±0.131 % 6.99	36.575**±0.497 % 9.24	39.623**±0.117 % 10.65	39.927**±0.384 % 15.86
Liver	Group-1	76.144±0.418	75.618±0.535	75.096±0.340	74.584±0.659	74.246±0.663
	Group-2	72.156**±0.460 % -5.24	68.272**±0..410 % -9.71	66.752**±0.455 % -11.11	63.128**±0.432 % -15.36	62.604**±0.458 % -15.68
	Group-3	64.490**±0.884 % -15.30	65.172**±0.797 % -13.81	67.356**±0.528 % -10.31	68.532**±0.653 % -8.11	70.534**±0.634 % -5.00
	Group-4	77.336^{NS}±0.763 % 1.56	78.496*±0.557 % 3.80	78.498**±0.521 % 4.53	79.420**±0.572 % 6.48	79.566**±0.564 % 7.16
Kidney	Group-1	48.344±0.474	48.284±0.990	47.500±0.720	46.510±0.490	45.470±0.705
	Group-2	45.084**±0.391 % -6.74	43.624**±.572 % -9.65	414.120**±0.395 % -13.43	40.466**±0.391 % -12.99	39.504**±0.635 % -13.12
	Group-3	40.622**±0.837 % -15.97	41.442**±0.666 % -14.17	42.862**±0.617 % -9.76	43.684*±0.969 % -6.08	44.552^{NS}±0.661 % -2.02
	Group-4	49.378^{NS}±0.643 % 2.14	49.492^{NS}±0.524 % 2.50	50.222*±0.480 % 5.73	50.342**±0.715 % 8.24	50.632**±0.538 % 11.35

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

Table 3: Variations of Amino acid (mg/g wet wt. of tissue) contents in the *C.mrigala* exposed to cypermethrin and *C.helicacabum* for the period of 120 hours

Tissue		Hours of experiment				
		24	48	72	96	120
Gill	Group-1	3.379±0.410	3.856±0.496	4.487±0.473	4.775±0.539	4.980±0.512
	Group-2	5.686*±0.761 % 68.27	6.973**±0.508 % 80.83	7.212*±0.489 % 60.73	7.933**±0.406 % 66.14	8.230**±0.377 % 65.26
	Group-3	7.880**±0.288 % 133.20	6.520**±0.320 % 69.09	6.023*±0.281 % 34.23	5.455^{NS}±0.514 % 14.23	5.303^{NS}±0.357 % 6.48
	Group-4	3.300^{NS}±0.414 % -2.34	3.437^{NS}±0.437 % -10.87	3.609^{NS}±0.392 % -19.57	3.877^{NS}±0.254 % -18.81	3.999^{NS}±0.330 % -19.70
Liver	Group-1	8.760±0.505	9.086±0.574	9.229±0.577	9.367±0.466	9.633±0.504
	Group-2	10.187*±0.210 % 16.29	11.336*±0.382 % 24.76	12.581**±0.538 % 36.32	13.986**±0.480 % 49.311	14.101**±0.446 % 46.38
	Group-3	13.123**±0.800 % 49.80	12.887**±0.114 % 41.83	11.076*±0.251 % 20.01	10.875^{NS}±0.383 % 16.10	10.276^{NS}±0.471 % 6.67
	Group-4	8.179^{NS}±0.351 % -6.63	8.287^{NS}±0.460 % -8.79	8.334^{NS}±0.287 % -9.70	8.440^{NS}±0.179 % -9.89	8.501±0.211 % -11.75
Kidney	Group-1	5.968±0.339	6.086±0.419	6.165±0.321	6.197±0.566	6.237±0.389
	Group-2	8.280*±0.489 % 38.74	9.190**±0.316 % 51.00	10.412**±0.477 % 68.89	11.182**±0.321 % 80.44	11.970**±0.358 % 90.82
	Group-3	10.716**±0.301 % 79.56	9.836**±0.516 % 61.62	8.186**±0.290 % 32.78	7.903*±0.334 % 27.53	7.064^{NS}±0.360 % 12.61
	Group-4	5.700^{NS}±0.250 % -4.490	5.776^{NS}±0.305 % -5.09	5.827^{NS}±0.220 % -5.48	5.866^{NS}±0.254 % -5.34	5.901^{NS}±0.279 % -5.93

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

Table 4: Variations of Glucose (mg/g wet wt. of tissue) contents in the *C.mrigala* exposed to cypermethrin and *C.helicacabum* for the period of 120 hours

Tissue		Hours of experiment				
		24	48	72	96	120
Gill	Group-1	2.461±0.183	2.577±0.244	2.689±0.564	2.726±0.406	2.828±0.466
	Group-2	4.865*±0.821 % 97.68	5.743*±0.764 % 122.86	6.187**±0.531 % 130.08	7.961**±0.700 % 192.04	8.344**±0.287 % 195.05
	Group-3	7.069**±0.281 % 187.24	6.693**±0.543 % 159.72	6.049*±0.867 % 124.95	5.751±0.417 % 110.97	4.861*±0.504 % 71.89
	Group-4	2.151^{NS}±0.467 % -12.60	2.181^{NS}±0.663 % -15.37	2.273^{NS}±0.361 % -15.47	2.306^{NS}±0.243 % -15.41	2.383^{NS}±0.401 % -15.73
Liver	Group-1	5.096±0.801	5.177±0.756	5.220±0.227	5.299±0.601	5.314±0.461
	Group-2	8.126*±0.400 % 59.46	10.767**±0.641 % 107.98	11.250**±0.464 % 115.52	12.905**±0.222 % 143.54	13.278**±0.501 % 149.87
	Group-3	12.116**±0.266 % 137.75	11.862**±0.707 % 129.13	10.541**±0.441 % 101.93	9.096±0.617 % 71.65	8.745±0.267 % 64.56
	Group-4	5.009^{NS}±0.591 % -1.71	5.080^{NS}±0.666 % -1.87	5.116^{NS}±0.409 % -1.99	5.186^{NS}±0.223 % -2.13	5.206^{NS}±0.496 % -2.03
Kidney	Group-1	3.901±0.401	3.955±0.445	3.997±0.708	4.096±0.406	4.224±0.281
	Group-2	5.743*±0.441 % 47.22	6.187*±0.409 % 56.43	7.961*±0.703 % 99.17	8.344**±0.566 % 103.71	9.770**±0.766 % 131.30
	Group-3	8.690**±0.961 % 122.76	7.253*±0.771 % 83.39	6.871*±0.456 % 71.90	5.866*±0.207 % 43.21	4.926^{NS}±0.287 % 16.524
	Group-4	3.883^{NS}±0.460 % -0.46	3.906^{NS}±0.201 % -1.24	3.946^{NS}±0.541 % -1.27	3.999^{NS}±0.384 % -2.59	4.069^{NS}±0.208 % -3.67

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

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4. CONCLUSION

Acute toxicity studies of cypermethrin on the fresh water fish *C. mrigala* revealed significant changes in the biochemical constituents of the fish like sub-lethal, total proteins, free amino acid and glucose to observe in selected tissue in fish, toxic control use to of plant *C. helicacabum* the similarly the control of toxic effect in experiment fish.

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