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Research Article

ANTIOXIDANT EFFECTS OF GREEN TEA EXTRACT ON BIOCHEMICAL PARAMETERS OF *HETEROPNEUSTES FOSSILIS* POISONED BY CYPERMETHRIN

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Abstract

The biochemical response in Heteropneustes fossilis to cypermethrin and mitigating effect of Green tea extract (GTE) was assessed during this study. The LC₅₀ of cypermethrin evaluated in previous study was found to be 0.00066 ml/l, 0.00044 ml/l, 0.00033 ml/l and 0.00022 ml/l after 24, 48, 72 and 96 h exposure. To assess the effect of GTE on some biochemical parameters fishes were randomly divided into 4 groups of 10 fishes each. Ist group served as control, the fishes of IInd group were treated with 0.00066 ml/l of cypermethrin, IIIrd group received 0.5ml/l GTE whereas IVth group received cypermethrin (0.00066ml/l) +GTE (0.5ml/l). Same protocol was employed by taking LC₅₀ concentration of cypermethrin at 48, 72 & 96 h exposure periods and 0.5ml/l GTE was applied at all exposure periods. After completion of 24, 48, 72 & 96 h blood was collected to separate the serum and estimation of SGOT, SGPT, ALP, ACP, Total protein, Creatinine, Uric acid and Blood glucose, was carried out. The level of SGOT, SGPT, Creatinine, Uric acid and Blood glucose was elevated when treated with cypermethrin after 24, 48, 72 & 96 h, while decreased activity was observed in total protein, ALP and ACP after all exposure periods. After addition of GTE all the parameters became normalize showing the antioxidant effect of green tea.

Keywords: Cypermethrin, Green tea extract, *Heteropneustes fossilis*, biochemical parameters, Acute-toxicity.

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INTRODUCTION

Cypermethrin, a synthetic pyrethroid is widely used all over the world against pests in households, industries and agriculture fields to increase the production of food grains and other agricultural products [1]. Due to excessive use, cypermethrin enters into natural water bodies and affects several non-target aquatic organisms specially fishes. Fishes are unable to metabolize pyrethroids efficiently which disturbs fish metabolism and fish population. Cypermethrin produces many harmful effects on fishes and is also known to reduce the survival, growth and reproduction of fishes [2,3]. Olalekan, 2014 reported that pesticides accumulation in fish organs such as liver and muscle, cause organ dysfunction and death of fishes [4].

All the vital process in the body are indicated by the nature of blood and any changes in blood chemistry are useful to analyze the toxicity in fishes. Enzymes essential for metabolic functions are early indicators of toxic effects as activities of many enzymes are altered due to toxicity [5]. The alterations in the activity of liver enzymes following cypermethrin exposure were shown by many bioresearches [6-8]. Several workers also reported the poor function of kidney during stressed condition [9,10]. Further cypermethrin insecticides exert their biological effect by inducing oxidative stress leading to the generation of free radicals such as ROS [11,12].

More attention has been paid towards the protective effects of natural antioxidants against drug induced toxicities [13]. Flavonoids are polyphenolic compounds present in natural food supplements which provide stress protection, acting as scavengers of free radicals such as reactive oxygen species (ROS) [14,15]. The highest contents of flavonoids are found in green tea among common food and beverage products and have different pharmacological properties due to the presence of catechins (epigallocatechingallate and epicatechin), theoflavin, tannin and caffeine [16-18]. It was reported earlier that green tea inhibits oxidative stress and prevent toxicity [19,20]. Although several studies have been carried out to investigate the effect of green tea on hepato and nephro toxicity induced by pesticides on rats but scarce data are available on fishes. Therefore we found it appealing to investigate the attenuating behaviour of GTE on acute-toxicity caused by cypermethrin in *Heteropneustes fossilis*.

MATERIALS AND METHODS:

In toxicity studies Jackpot 25 (Cypermethrin 25% EC) insecticide manufactured by Crystal Crop Protection Private Limited, Delhi and Lipton green

tea bags were used. Different concentrations obtained from LC₅₀ estimation were prepared from stock solution. The stock solution of Green tea extract (GTE) was also prepared and 0.5ml/l was used in all the experiments [19].

- **Collection and Maintenance of Fishes -** Healthy and live fishes (wt 80-90g) were collected from different water bodies in Jhansi district of Bundelkhand region U.P. India. Fishes were acclimatized for 6-10 days in laboratory conditions and fed with commercial diet. The fishes were checked against injury or infection by keeping in 0.2% of potassium permanganate solution for 2-4 minutes. Feeding was stopped 24 h before commencement of test experiments.
- **Experimental procedure-** According to Finney's probit analysis previously estimated LC₅₀ were 0.00066 ml/l, 0.00044 ml/l, 0.00033 ml/l and 0.00022 ml/l after 24, 48, 72 and 96 h cypermethrin intoxication respectively [21] and 0.5 ml/l GTE from stock solution was found to be effective at which no mortality occurs [19]. To assess the effect of flavonoid on some biochemical parameters fishes were randomly divided into 4 groups of 10 fishes each, Ist group served as control. The fishes of IInd group were treated with 0.00066 ml/l of cypermethrin, IIIrd group received 0.5ml/l GTE whereas IVth group received cypermethrin(0.00066ml/l) + GTE (0.5ml/l). Same protocol was employed by taking LC₅₀ concentration of cypermethrin at 48, 72 & 96 h exposure periods and 0.5 ml/l GTE was applied at all exposure periods. After completion of 24, 48, 72 & 96 h exposure periods blood samples from all the fishes were collected, centrifuged at 3000rpm for 20 min and serum was separated. Serum total protein, SGOT, SGPT, Alkaline phosphatase, Acid phosphatase, Creatinine, Uric acid and Blood glucose were analyzed using colorimetric diagnostic kit through biochemical auto-analyzer.
- **Statistical analysis-** Student's 't' test was employed to assess the statistical significance of various biochemical parameters. The results were expressed as mean ± standard deviation (SD). Differences with a P-value <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION:

In the present study biochemical alternations caused by cypermethrin and protective effect of green tea extract on fishes were carried out and results are given in Table 1 & 2. The significantly (<0.05) elevated levels of SGOT, SGPT, Uric acid and Blood glucose were observed in IInd group as compared to control group, while in IVth group a remarkable reduction was seen after all exposure periods. The level of SGOT, SGPT and Blood glucose in

cypermethrin exposed group was increased gradually along with the exposure periods. The slight increase was observed in creatinine level when compared to untreated control group. A significant (<0.05) decrease was seen in total protein level in IInd group whereas in group IIIrd & IVth it was enhanced. The activity of both ALP and ACP was found to decrease slightly but insignificantly increased after treatment of GTE.

Table-1.Effect of Green tea extract (GTE) on cypermethrin induced toxicity in *Heteropneustes fossilis*

		24h Exposure period				48h Exposure period			
		C	T	GTE	T+GTE	C	T	GTE	T+GTE
1	S.G.O.T	37.46 ± 3.65	91.76 ^a ± 1.77	40.01 ^b ± 2.21	75.90 ^{a,b} ± 4.53	37.60 ± 1.70	96.23 ^a ± 1.30	39.88 ^b ± 1.34	70.46 ^{a,b} ± 2.07
2	S.G.P.T	38.33 ± 5.15	63.83 ^a ± 5.15	42.7 ^b ± 1.26	54.33 ^{a,b} ± 0.55	36.39 ± 3.10	65.16 ^a ± 5	40.56 ^b ± 4.59	49.63 ^{a,b} ± 8.15
3	T.PROTEIN	6.94 ± 0.08	5.77 ^a ± 0.20	6.78 ^b ± 0.25	6.69 ± 0.63	6.95 ± 0.24	6.41 ± 0.87	6.85 ± 0.18	6.42 ± 0.46
4	ALP	474.97 ± 9.85	466.16 ± 9.50	473.26 ± 4.48	472.16 ± 7.72	485.26 ± 6.25	483.66 ± 2.44	484.16 ± 4.59	485.46 ± 5.05
5	ACP	3.92 ± 0.04	3.63 ^a ± 0.46	3.82 ± 0.10	3.77 ± 0.14	3.92 ± 0.31	3.63 ± 0.56	3.88 ± 0.08	3.86 ± 0.07
6	CREATININE	1.25 ± 0.13	1.38 ± 0.07	1.26 ± 0.41	1.07 ± 0.27	1.27 ± 0.12	1.53 ± 0.17	1.30 ± 0.18	1.32 ± 0.23
7	URIC ACID	5.92 ± 0.32	7.19 ^a ± 0.15	5.61 ^b ± 0.82	6.25 ± 1.51	6.09 ± 0.19	6.70 ± 3.88	5.71 ± 0.50	5.13 ^a ± 0.19
8	BLOOD GLUCOSE	88.66 ± 2.51	95.00 ^a ± 2.64	87.33 ^b ± 2.51	84.00 ^b ± 6.80	95.66 ± 3.05	103.33 ^a ± 2.51	93.66 ^b ± 3.21	92.66 ^b ± 4.16

Table -2.Effect of Green tea extract (GTE) on cypermethrin induced toxicity in *Heteropneustes fossilis*

		72h Exposure period				96h Exposure period			
		C	T	GTE	T+GTE	C	T	GTE	T+GTE
1	S.G.O.T	34.63 ± 5.37	99.3 ^a ± 1.9	37.4 ^b ± 3.88	68.6 ^{a,b} ± 2.35	37.73 ± 2.25	101.76 ^a ± 3.45	39.05 ^b ± 5.52	67.26 ^{a,b} ± 7.55
2	S.G.P.T	34 ± 7.53	68.26 ^a ± 5.47	39.12 ^b ± 5.45	58.13 ^{a,b} ± 1.70	36.63 ± 5.22	69.86 ^a ± 6.43	41.2 ^b ± 4.16	53.7 ^{a,b} ± 6.1
3	T.PROTEIN	7.17 ± 0.07	5.10 ^a ± 0.01	6.54 ^{a,b} ± 0.23	6.01 ^{a,b} ± 0.19	7.16 ± 0.62	6.52 ^a ± 1.32	7.04 ± 0.37	6.79 ± 0.57
4	ALP	502.06 ± 6.80	496.06 ± 7.0	501.36 ± 7.25	500.86 ± 1.46	509.5 ± 5.15	504.3 ± 7.39	505.06 ± 6.10	508.7 ± 6.33
5	ACP	3.96 ± 1.04	3.69 ± 0.54	3.95 ± 0.15	3.79 ± 0.93	4.11 u ± 0.75	3.69 ± 0.90	4.05 ± 0.37	3.80 ± 0.73
6	CREATININE	1.24 ± 0.22	1.36 ± 0.17	1.18 ± 0.09	1.13 ± 0.18	1.23 ± 0.28	1.37 ± 0.15	1.26 ± 0.04	1.19 ± 0.11
7	URIC ACID	6.43 ± 0.04	6.85 ^a ± 0.01	5.61 ^{a,b} ± 0.14	6.63 ^b ± 0.02	6.42 ± 0.40	7.55 ± 0.61	6.47 ± 0.64	6.15 ^b ± 0.12
8	BLOOD GLUCOSE	100 ± 5.29	107 ± 2.64	105.66 ± 2.08	102 ± 7.93	92.67 ± 4.50	110.33 ^a ± 1.52	88.33 ^b ± 2.51	105 ^a ± 7.81

(^a): Significant when compared to control group ($p < 0.05$).

(^b): Significant when compared to cypermethrin treated group ($p < 0.05$).

Pesticide exposure causes several alterations in enzyme activities in blood of fishes indicating major pathological disorders. The liver faces the threat of maximum exposure to pesticides and its metabolites as detoxication process occurs mainly in liver. Several parameters viz. SGOT, SGPT, ALP, ACP, Creatinine, Total protein, Uric acid and Blood glucose are mainly used for liver and renal dysfunction [22-24].

Oxidative damage during stressed condition primarily occurs through production of ROS which initiate lipid peroxidation altering phospholipid membrane leading to cellular damage [25,26]. Previous reports showed that free radicals (ROS) are generated after cypermethrin poisoning [11,27]. The use of natural antioxidants having free radical scavenging ability is the common protecting strategies during stressed condition caused by exposure of pesticides. Several studies showed the protective effects of natural substances having antioxidant activity [28,29]. Green tea contains polyphenols which increase the removal of detoxified metabolites. Its administration improves the oxidative injuries and acts as scavengers of free radicals [30]. Catechins found in green tea reduced the formation of peroxides more effectively. Shimizu *et al.*, (2015) reported the anti-oxidative and anti-inflammatory activities of green tea catechins [31].

In the present study SGOT and SGPT levels were increased significantly after cypermethrin exposure. These enzymes are considered as sensitive indicators of stress, elevated levels may indicate hepatic damage as shown by several researchers [6,23]. In order to cope with the energy demand during stressed condition amino acids input in kreb's cycle occurs which increases the activities of transaminases [3]. Further the present work demonstrated significant decrease in SGOT and SGPT levels after treating with GTE highlighting the mechanism that green tea being an antioxidant have hepato-protective effect. Such findings are concomitant with the findings of others work [32,33].

Treatment of cypermethrin in the present observation reduced the level of total protein content in the serum of fishes. The reduced level suggests the impaired incorporation of amino acids in protein synthesis in liver due to hepatocyte damage [34]. A significant decrease in protein content was also observed in liver and muscles tissues by several other workers [4,35]. Bhanu and Deepak (2015) reported increased production of protease enzyme which cause decrease in protein content in fish tissue [9]. In group treated with cypermethrin + GTE, there was a significant

increase in protein content. It may be due to the effect of green tea flavonoids which might protect liver toxicity by inhibition of oxidative damage of hepatocytes.

The alterations in the activities of Alkaline phosphatase and Acid phosphatase are the biomarkers of hepatic injury. In our experiment the ACP and ALP levels were reduced by cypermethrin exposure. Tiwari *et al.*, 2012, mentioned the reduced activity of ACP and ALP in fingerlings of *Labeo rohita* [34]. These two enzymes act to modulate the activities of proteins in a cell in response to external stimuli, the low level of protein may cause the reduction in these enzymes. Co-administration of GTE along with cypermethrin normalized at least partially the level of ALP and ACP.

The rise in uric acid and serum creatinine levels are indicators of renal dysfunctions, as they are not properly excreted by the kidneys. The uric acid and creatinine were elevated in the blood of fishes under the impact of cypermethrin in present investigation. These findings are also supported by other workers [5,9]. Table 1 & 2 showed the gradual increase of blood glucose level in serum of fishes following cypermethrin exposure. Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically [36]. Hyperglycaemic response is an evidence of stress due to cypermethrin exposure [37]. The increase in blood glucose by pesticide treatment might indicate disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen [9]. Addition of GTE restored the damage caused by cypermethrin.

CONCLUSION:

Acute toxicity effects of cypermethrin to fishes showed changes in some biochemical parameters. The administration of green tea extract exerted a protective role against pesticide toxicity.

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