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ASSESSMENT OF ACUTE TOXICITY OF CYPERMETHRIN AND ITS MITIGATION BY GREEN TEA EXTRACT IN FRESH WATER FISHES, C*HANNA PUNCTATUS.*

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Abstract:

Synthetic pyrethroids are used preferably due to high effectiveness and low toxicity to mammals but these are highly toxic to fishes. Green tea is the most popular beverage containing polyphenols and caffeine which are of considerable pharmacological significance. The protective effects of flavonoids have been attributed to a wide variety of mechanism by modulating enzyme activities resulting inhibitory effects of xenobiotics and drugs. The objective of this study was to determine the toxicity of cypermethrin alone and the protective effects of flavonoids on toxicity induced by pesticide. Two sets of experiments were performed. In first set acute toxicity bioassay of cypermethrin (Anukill 25%EC) was carried out by two exploratory and one definitive test. LC_{50} values estimated by plotting a graph between % mortality and concentration of definitive test were 0.00087ml/l, 0.00079ml/l, 0.00065ml/l and 0.00050ml/l after 24, 48, 72 and 96 hr exposure respectively in fresh water teleosts, Channa Punctatus . Secondly, to examine the effect of green tea extract(GTE), 50 fishes were divided into 5 groups of ten fishes each. All the groups received 0.00087ml/l of Anukill whereas groups 2^{nd},3^{rd}, 4^{th} & 5^{th} also received 10ml/l, 2ml/l, 1ml/l & 0.5ml/l GTE respectively. After 24 hrs the mortality rate reduced from 50% to 0% in group 1^{st} to 5^{th}. The same scenario was obtained after treating the fishes by 96 hr LC_{50} concentration of Anukill along with 4 concentrations of GTE describe above. The results of present study revealed that GTE suppresses the effect of pesticides by improving oxidative damage.

Key words: *Green tea, xenobiotics, antioxidant.*

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INTRODUCTION:

Flavonoids are polyphenolic compounds present in fruits, vegetables and beverages derive from plants like wine and in many dietary suppliments [1,2]. Flavonoids have been described as health promoting, disease preventing and inhibiting the effects of xenobiotics, drugs and carcinogens [3,4,5]. Green tea is most popular beverage obtained from natural plant *camellia sinensis*. Polyphenols found in green tea include catechins, theaflavins, tannins, caffeine and flavonoids. The highest contents of flavonoids are found in green tea among common food and other beverage products [6,7].

Pesticides have been one of the most effective weapons synthesized by man to protect the crops from the attack of pest. Recently large amount of pesticides have been developed and are extensively used in agricultural fields, mosquito control and household pest control. Cypermethrin is used in India over the past three decades for agricultural and domestic use [8,9]. These are continuously added to the water bodies such as lakes, ponds, rivers and oceans and cause toxicity not only to fishes but other aquatic organisms [10,11]. Fishes are highly sensitive to very low concentration of cypermethrin [12]. The toxic effects of cypermethrin on biochemical, haematological and histological characteristics have been studied by several workers [13,14,15,16]. Different studies have also shown the protective role of flavonoids against pesticides [17]. Although several studies have been carried out to investigate the effect of flavonoids on pesticides induced toxicity in rats but data concerning the assessment of toxicological stress of pesticides and mitigating behavior of flavonoids are scarce especially in fishes. Thus, we have choosen to study the acute toxicity bioassay of cypermethrin alone and in combination with green tea extract in fresh water fishes *Channa punctatus* of Bundelkhand region.

MATERIALS AND METHODS:

Collection and maintenance of fishes:

The fishes *channa punctatus* were collected from local fish market of Jhansi district in BundelKhand region and transferred to laboratory condition. Before acclimatization fishes were treated with 0.2% $KMnO_4$ to check the dermal infection for 2-4 minutes. After treatment fishes were acclimatized in laboratory conditions for 6-10 days at 26°C ± 5°C temp & pH 7.2. They were fed standard commercial diet and egg albumin. Feeding was stopped 24hrs before experiment.

Preparation of Cypermethrin and green tea extract:

Cypermethrin 25% EC trade name Anukill and Green tea bags were purchased from market. Stock solution was prepared by dissolving 0.5 ml of Anukill in 1 litre distilled water. Multiple concentration were prepared from stock solution and used for estimation of LC_{50}. 0.05gm/ml stock solution of green tea extract (GTE) was prepared by boiling in distilled water for 30 minutes.

Exprimental Design:

Two sets of experiment were performed, one to determine the LC_{50} value of anukill 25%EC and other to analyze the effect of green tea on lethal concentration of insecticide. Every day fresh solutions were prepared from stock solution.

(a) **Estimation of LC_{50}:-** For the estimation of LC_{50}, two exploratory (5 fishes each) and one definitive (10 fishes) were conducted. In 1st exploratory test two concentrations, a lower and higher were taken to estimate supposed mortality between 0%and 100% in two separate aquaria. To get a narrow range of applied toxicant 2nd exploratory test was conducted in which four concentrations were applied in separate aquaria of 5 fishes each. After that 7 concentrations were selected in definitive bioassay test and 10 fishes were exposed to each concentration. Mortality was recorded at each test after a period of 24, 48, 72 and 96 hrs and LC_{50} was calculated by plotting a graph between % mortality and concentration of cypermethrin.

(b) **Effect of Green Tea Extract (GTE):** The exposure protocol employed in the present study was to analyze the effect of GTE after 24 and 96hrs intoxication with cypermethrin. The concentration of toxicant remained constant in all groups. To find out of the impact of flavonoid, fishes were divided into 5 groups of10 fishes each. Group –I received only toxicant T (LC_{50} concentration of 24 hrs). Group-II received toxicant and GTE(10ml/l). Group-III was subjected to T and GTE (2ml/l) while the group – IV and V were exposed to T and GTE(1ml/l) and GTE(0.5ml/l) respectively. Mortality was recorded after 24 hrs of exposure period. Same procedure was repeated as describe above but only the toxicant concentration was 0.00050ml/l(LC_{50} conc. of 96 hrs). Mortality was recorded after 96hr intoxication.

RESULTS:

Results of LC_{50} determination were presented in table 1-3. In 1st exploratory test 100% mortality occurred after 48hrs at 0.001 ml/ltr. where as no mortality was observed at 0.0001ml/l. In 2nd exploratory test 4 range finding concentrations viz. 0.00010ml/ltr, 0.00040ml/l 0.00070ml/l and 0.001ml/l were taken and mortality rate was observed after 24, 48, 72 and 96 hr exposure (Table-2). On the basis of 2nd exploratory test 7 concentrations 0.00035ml/l, 0.00045ml/l, 0.00055ml/l, 0.00065ml/l, 0.00075ml/l, 0.00085ml/l and 0.00095ml/l were choose for definitive test and mortality rate was presented in table-3. To determine the LC_{50} value graphs were plotted between % mortality and concentrations of

toxicant. The concentration was estimated by drawing a perpendicular against 50% mortality which were 0.00087 ml/l, 0.00079ml/l, 0.00065ml/l and 0.00050ml/l after 24,48,72,and 96hrs cypermethrin intoxication respectively (fig 1).

Table 1: 1st Exploratory Test

Conc. Of toxicant(ml/lt)	No.of fishes	24hrs		48hrs		72hrs		96hrs	
		M	% M	M	% M	M	% M	M	% M
0.0001	05	0	0	0	0	0	0	0	0
0.001	05	04	80	01	100	-	-	-	-

Table 2: 2nd Exploratory Tests

Conc. Of toxicant(ml/lt)	No.of fishes	24hrs		48hrs		72hrs		96hrs	
		M	% M	M	% M	M	% M	M	% M
0.0001	05	0	0	0	0	0	0	0	0
0.00040	05	0	0	1	20	1	40	0	40
0.00070	05	1	20	1	40	2	80	0	80
0.001	05	4	80	1	100	-	-	0	-

Table 3: Definitive Test

Conc. Of toxicant(ml/lt)	No. of fishes	24hrs		48hrs		72hrs		96hrs	
		M	% M	M	% M	M	% M	M	% M
0.00035	10	0	0	0	0	0	0	1	10
0.00045	10	1	10	1	20	1	30	1	40
0.00055	10	1	10	2	30	1	40	2	60
0.00065	10	2	20	1	30	2	50	3	80
0.00075	10	2	20	2	40	3	70	2	90
0.00085	10	4	40	3	70	2	90	1	100
0.00095	10	7	70	2	90	1	100	-	-

* M = Mortality
* %M = Percent mortality

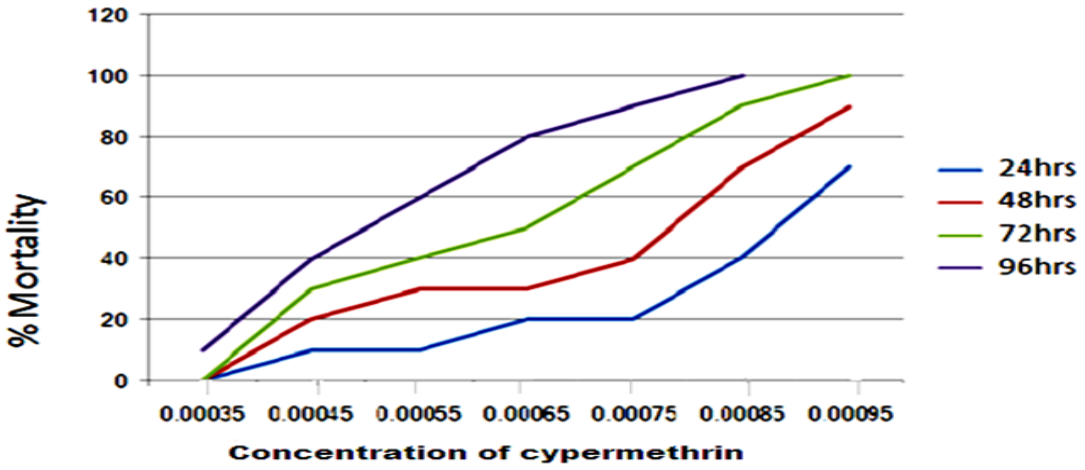

Fig-1- Estimation of LC_{50} at different exposure period

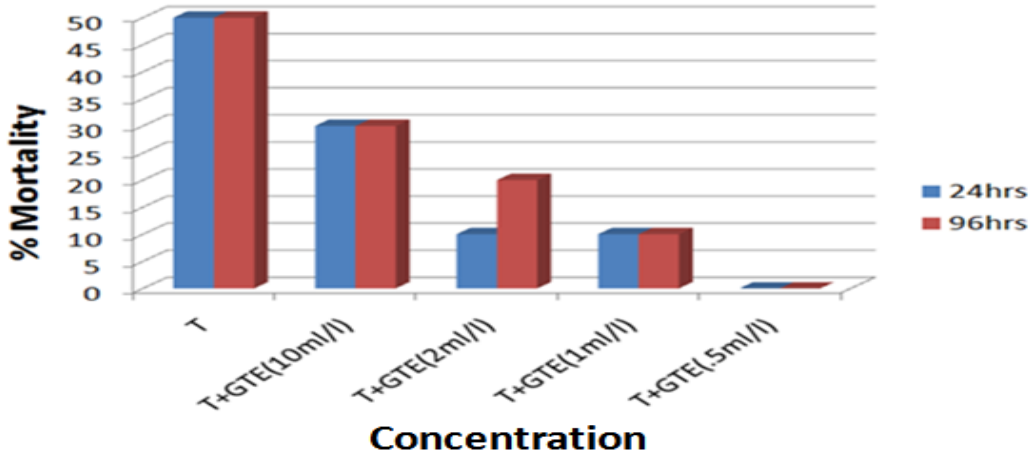

Fig-2 Impact of green tea extract on mortality

Fig-2 shows the reduction in mortality rate due to GTE. At 0 .00087ml/l & 0.00050ml/l toxicant conc. fifty percent fishes died after 24 & 96 hrs but the mortality rate gradually reduced by the combined treatment of toxicant &GTE. There was no mortality in group 5 which received 24 & 96 hrs LC_{50} concentration of cypermethrin & 0.5ml/l GTE.

DISCUSSION:

Pesticides may induce oxidative stress leading to the generation of free radicals & cause lipid peroxidation which affect the activities of antioxidative enzymes. Any alteration occurred in oxidative enzymes have been shown to be the sensitive indicator of increased oxidative stress[18]. Increase in LPO after cypermethrin exposure was also observed by many workers [19,20]. Several studies have been carried out to investigate the protective effects of natural substances having antioxidant biochemically as well as histologically [21]. In the present study different concentrations as 10ml/l, 2ml/l, 1ml/l & 0.5ml/l extract of green tea was added along with 96 hrs & 24 hrs LC_{50} concentration of cypermethrin. It was observed that toxicity reduces gradually and all the fishes survived after 96 &24hrs exposure in group 5. Co-administration with green tea normalizes the toxic effect of cypermethrin due to its antioxidant properties[22]. The toxicity of cypermethrin leads to changes in normal enzymatic activities and reduction in cellular antioxidant capacity[23]. Addition of GTE resulted in protective effects against the toxic influence of cypermethrin. It was shown earlies that high concentration of flavonoids can produce oxidative stress by auto oxidation and redox cycling but at low levels flavonoids provide anti oxidant protection [24]. The results of our study are correlated with those researches as after addition of GTE(500mg/l) 30% mortality occurred which reduces gradually and 10% & 0% mortality was observed in low concentration of GTE.

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