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RESEARCH ARTICLE

Analysis of Protein Alteration in Liver of *Channa punctatus* after Intoxication of Cypermathrin

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ABSTRACT

Worldwide pesticide usage has increased dramatically during the past two decades, coinciding with changes in farming practices and increasingly intensive agriculture. Environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. Present study deals with the harmful effects of pyrethroids which is cypermethrin in the investigation, on fresh water fish Channa punctatus showing its deleterious effects. The experiment conducted on Channa punctatus results in certain valuable information with regard to effect of cypermethrin on biochemistry. The fishes constitute one of the major sources of nutritional food value for human beings. Despite high fish population densities, histology of liver indicates unfavorable environmental conditions for individual fish under stress of effluents. In the present study, a significant decrease in liver proteins in Channa punctatus after acute (4days), sub-chronic (20days) and chronic (45days) treatment to doses 8.124, 1.624 and 0.722µg/l of cypermethrin has been observed respectively. This decline in albumin, globulin and A/G ratio correlated with decrease in protein content as these are the integral contents of protein itself. In conclusion it can be stated that cypermethrin is highly toxic to aquatic fauna specially fishes and affect their biochemistry.

Key Words: *Channa punctatus, Cypermathrin, Liver, Protein*

INTRODUCTION

The population of the world is increasing continuously since time immemorial and utilization of natural resources is also on increase. The rise of human population has crossed all strides in the beginning of 20th century with the result scientists had to employ all tactics to feed the rising population. During this course some of the synthesized chemicals not only helped the mankind but also at the same time became reasons for his agony. A good number of chemicals in the form of pesticides reigned for quite some time, however left many problems, which were related to welfare of human beings. Pesticides are modern chemicals, which are used to kill the harmful insect, weed etc. of the crops, when they are applied on crop they also leached into soil and reach to water bodies. In water bodies they affect the aquatic life including fishes. The edible fishes are consumed by human and other livings. So they reached to next trophic level by biotransformation and causing adverse effects to living.

Cypermethrin [(RS)-α-cyano-3-phenoxy benzyl (1RS)-cis,trans-3-(2,2-dichl orovinyl)-2,2-dimethy lcyclopropane-carbo xylate] acts as a stomach poison and contact insecticide. It has wide uses in cotton, cereals, vegetables and fruit, for food storage, in public health and in animal husbandry. Its structure is based on pyrethrum, a natural insecticide which is contained in chrysanthemum flowers, but it has a higher biological activity and is more stable than its natural model. It was synthesised in 1974 and first marketed in 1977. In 1988, pyrethroids amounted to 40% of the sales for insecticides for cotton treatment in the world (cypermethrin 8%) and cypermethrin is one of the most important insecticides for cereals and vegetables in the UK. It is also used for impregnation of mosquito bed nets to prevent malaria, and extensively for indoor pests.

The fish, which sub serves the growing demands of food and is also a best source of protein and mineral salts has also been facing the havocs caused by environmental contamination.

Although ample data are available on the toxicity of synthetic pyrethroid to animals, the available literature refers to some of the important information's related to haematobiochemical, cytogenetic and general toxicity in animals. Channa is a genus of the Channidae family of snakehead fishes. This genus contains about 29 species, but the most well known are probably northern snakehead (*Channa argus*) and the giant snakehead (*Channa micropeltes*). The diets of various species of Channa include fish, frogs, snakes, rodents, birds, and insects. Some can move on land like snakes and breathe air.
The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemical's necessary for digestion. The liver is necessary for survival; there is currently no way to compensate for the absence of liver function long term, although liver dialysis can be used short term. The liver is the site of detoxification where as blood maintained the homeostasis mechanism of the body so any change in the biochemistry of the liver and blood reflect the overall function of the body due to this reason liver and blood are selected for proposed work.

MATERIAL AND METHODS

1. COLLECTION OF MATERIAL AND TREATMENTS FOR LABORATORY EXPERIMENTS

The selection of *Channa punctatus* (soli) as experimental fish went in for reason of its easy availability, its hardy nature in terms of survival despite pollutant treatments proposed which might indicate an advantage of long stay of toxic effects in soft tissues of liver. The fishes were washed in 0.1% $KMnO_4$ solution to smear dermal infection if any. Then they were washed with ordinary water and smeared to aquaria filled with water.
The latter was already equipped with sand and *Hydrilla* plants, overcrowding was avoided. The fishes were fed with readymade fish food after every 24 hrs. The water was changed to smear the faecal matter and excess food after every 24 hrs. If any mortality occurred the fish was removed immediately to avoid depletion of oxygen. Normally, the fish to be used for experiments were left for fifteen days. So they might acclimatize to the prevailing ecological conditions. For the analysis of insecticide toxicity, insecticide was used in commonly occurring chemical compound cypermethrin 25%EC is a synthetic pyrethroid insecticide.

2. EXPERIMENTATION

For each the concentration duplicates were runs as control. The water was, however changed every alternate day to maintain the constant concentration of the cypermethrin during the period of exposure. The fishes were not provided any food for 24 hrs, prior to experiments in order to avoid any change in toxicity of the chemical likely to be caused by excretory materials. The concentrations were selected on the basis of LC_{50} value. The selected concentrations were- $1/10^{th}$ of LC_{50} for acute (4days) treatment, $1/20^{th}$ of acute for sub-chronic (20days) treatment and $1/45^{th}$ of acute for chronic (45days) treatment respectively. Recovery studies were also done at 45 days.

i. ESTIMATION OF TOTAL PROTEIN

Total protein was estimated by Biuret method described by Henry *et al*. (1974)-

$$\text{Total protein (mg/dl)} = \frac{\text{O.D. of Test}}{\text{O.D. of standard}} \times 5.7$$

ii. ESTIMATION OF ALBUMIN

The serum albumin was calculated by the following formula-

$$\text{Serum Albumin (gm/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of Standard}} \times 4.0$$

iii. ESTIMATION OF GLOBULIN

The globulin was calculated by the following formula from the protein and albumin:-

Globulin = Protein – Albumin

OBSERVATION

The effect of cypermethrin on acclimated snakehead fishes (*Channa punctatus*) has been observed in the present study. From the collected data mean ($\overline{X}$) and standard deviation (S.D.) have been calculated and significance of difference has been tested by student's't' test.

1. LIVER TOTAL PROTEIN

I. Control Set:
The value of Liver total protein ranges from 5.10-6.20 with mean 5.65±0.06mg/dl in control set (Table 1).

ii. Acute treatment (4days):
In acute treatment (4days), the value of Liver total protein ranges from 3.59-4.59 with mean 4.15±0.08mg/dl after cypermethrin treatment. The decrease was non-significant after cypermethrin treatment as compared to control set.

iii. Sub-chronic treatment (20days):
In sub-chronic treatment (20days), the value of Liver total protein ranges from 3.15-3.89 with mean 3.55±0.09mg/dl after cypermethrin treatment. The decrease was significant after cypermethrin treatment as compared to control set.

iv. Chronic treatment (45days):
In chronic treatment (45days), the value of Liver total protein ranges from 2.45-3.18 with mean 2.88±0.08mg/dl after cypermethrin treatment. The decrease was highly significant after cypermethrin treatment as compared to control set.

2. LIVER ALBUMIN

i. Control set:
The value of Liver albumin ranges from 3.20-4.18 with mean 3.85±0.19mg/dl in control set (Table 2).

ii. Acute treatment (4days):
In acute treatment (4days), the value of Liver albumin ranges from 1.81-2.19 with mean 2.12±0.18mg/dl after cypermethrin treatment. The decrease was significant after cypermethrin treatment as compared to control set.

iii. Sub-chronic treatment (20days):
In sub-chronic treatment (20days), the value of Liver albumin ranges from 1.32-1.79 with mean 1.59±0.13mg/dl after cypermethrin treatment. The decrease was highly significant after cypermethrin treatment as compared to control set.

iv. Chronic treatment (45days):
In chronic treatment (45days), the value of Liver albumin ranges from 1.10-1.51 with mean 1.22±0.12mg/dl after cypermethrin treatment. The decrease was very highly significant after cypermethrin treatment as compared to control set.

3. LIVER GLOBULIN

i. Control set:
The value of Liver globulin ranges from 2.70-2.96 with mean 2.80±0.08mg/dl in control set (Table 3).

ii. Acute treatment (4days):
In acute treatment (4days), the value of Liver globulin ranges from 2.37-2.69 with mean 2.57±0.09mg/dl after cypermethrin treatment. The decrease was non-significant after cypermethrin treatment as compared to control set.

iii. Sub-chronic treatment (20days):
In sub-chronic treatment (20days), the value of Liver globulin ranges from 2.12-2.58 with mean 2.39±0.10mg/dl after cypermethrin treatment. The decrease was significant after cypermethrin treatment as compared to control set.

iv. Chronic treatment (45days):
In chronic treatment (45days), the value of Liver globulin ranges from 1.84-1.99 with mean 1.91±0.09mg/dl after cypermethrin treatment. The decrease was significant after cypermethrin treatment as compared to control set.

4. LIVER A/G RATIO

i. Control set:
The value of Liver A/G ratio ranges from 1.20-1.39 with mean 1.31±0.05 in control set (Table 4).

ii. Acute treatment (4days):
In acute treatment (4days), the value of Liver A/G ratio ranges from 1.05-1.21 with mean 1.12±0.08 after cypermethrin treatment. The decrease was non-significant after cypermethrin treatment as compared to control set.

iii. Sub-chronic treatment (20days):
In sub-chronic treatment (20days), the value of Liver A/G ratio ranges from 0.82-1.09 with mean 0.98±0.08 after cypermethrin treatment. The decrease was significant after cypermethrin treatment as compared to control set.

iv. Chronic treatment (45days):
In chronic treatment (45days), the value of Liver A/G ratio ranges from 0.51-0.68 with mean 0.61±0.07 after cypermethrin treatment. The decrease was highly significant after cypermethrin treatment as compared to control set.

Table 1: Liver total protein (mg/dl) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S. No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean±S.Em.	Significance level
1.	Control	-	5	5.10-6.20	5.65±0.06	-
2.	Acute (4 days)	8.124	5	3.59-4.59	4.15±0.08	P>0.05
3.	Sub-chronic (20 days)	1.624	5	3.15-3.89	3.55±0.09	P<0.05
4.	Chronic (45 days)	0.722	5	2.45-3.18	2.88±0.08	P<0.01

Table 2: Liver albumin (mg/dl) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S. No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean±S.Em.	Significance level
1.	Control	-	5	3.20-4.18	3.85±0.19	-
2.	Acute (4 days)	8.124	5	1.81-2.19	2.12±0.18	P<0.05
3.	Sub-chronic (20 days)	1.624	5	1.32-1.79	1.59±0.13	P<0.01
4.	Chronic (45 days)	0.722	5	1.10-1.51	1.22±0.12	P<0.001

Table 3: Liver globulin (mg/dl) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S. No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean±S.Em.	Significance level
1.	Control	-	5	2.70-2.96	2.80±0.08	-
2.	Acute (4 days)	8.124	5	2.37-2.69	2.57±0.09	P>0.05
3.	Sub-chronic (20 days)	1.624	5	2.12-2.58	2.39±0.10	P<0.05
4.	Chronic (45 days)	0.722	5	1.84-1.99	1.91±0.09	P<0.05

Table 4: Liver A/G ratio in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S. No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean±S.Em.	Significance level
1.	Control	-	5	1.20-1.39	1.31±0.05	-
2.	Acute (4 days)	8.124	5	1.05-1.21	1.12±0.08	P>0.05
3.	Sub-chronic (20 days)	1.624	5	0.82-1.09	0.98±0.08	P<0.05
4.	Chronic (45 days)	0.722	5	0.51-0.68	0.61±0.07	P<0.01

PURPOSE OF STUDY

The present work is a contribution to the assessment of the toxicity and effects of a cypermethrin-based pesticide on fish. The aim was to assess the effects of cypermethrin on *Channa punctatus* through toxicity tests on liver protein.

RESULT AND DISCUSSION

The change in total protein concentration after pyrethroid exposure demonstrated the response of exposed fish to metabolic stress. Cypermethrin caused an increase in plasma NH_3 levels, presumably due to an increase in amino acid catabolism and a failure of ammonia excretion mechanisms.

Dalela *et al*. (1981) reported that decrease in protein levels of pesticide treated *Mystus vittatus* may be due to the excretion of proteins by kidney due to kidney disorder or impaired protein synthesis as a result of liver disorder. Decrease in proteins also supports the view of Holbrook (1980), who reported that the decrease in amino acid incorporation and desegregation of polysomes lead to the decrease in protein synthesis. In the present investigation reduction in the serum total protein may also be attributed to intensive proteolysis which contributes to the increase in the free amino acids to be fed into TCA cycle as ketoacids. Das et al., 1999 and Satyapal and Singh 2006 find out the toxicity of cypermathrin bio chemical constituents of *C. panctatus*.

It is obvious that exposure of fish for certain period to most toxicants including pesticide interferes with protein metabolism. The present work has also got support from the observations of Sastry *et al*. (1983) who observed the depletion of total protein in the plasma of fish when exposed to quinalphos. Similarly organophosphate and organochlorine pesticides lead to the considerable loss of blood proteins by renal excretion further augmenting its depletion in the blood (Verma *et al*. 1979: Sastry and Sharma, 1980). It is, therefore, evident that in case of continuous exposure to the pesticides the deleterious effects of these substances on protein synthesis and kidney function accounts for the progressive reduction in the concentration of total serum protein.

Toxicological and environmental problems resulting from the widespread use of pesticides in agriculture have raised concerns, particularly with respect to the potential toxic effects in human beings and animals. The acute exposure of *Channa punctatus* to the pyrethroid cypermethrin, was associated with alterations in haematological and biochemical indices as well as in tissue enzymes, resulting in stress to the organism. Some changes were observed only with the higher exposures.

REFERENCES

1. Dalela R.C., Bhatnagar M.C. and Verma S.K. (1981): *In vivo* haematological alterations in the fresh water teleost, *Mystus vittatus* following sub-chronic exposure to pesticide and their combination. *J. Environ. Biol*., 2 (2): 79-86.

2. Das V.L, Jeewaprada P.N. and Veeraiah K. (1999): Toxicity and effect of cypermethrin on bio chemical constituents of freshwater teleost, *Channa punctata*. J. Ecotoxicol. Environ. Monitoring, 9(3-4): 197-203.

3. Henry R.J. and Henry M. (1974): Clinical Chemistry: Principles and techniques. Harper and Row, New York.

4. Holbrook Jr. D.J. (1980): Effect of toxicants on nucleic acid and protein metabolism In: Introduction to biochemical toxicology (eds.) Hodgson, E .and Guthrie, F.E. Blackwell Scientific Publication, Oxford, .216-284.

5. Sastry K.V. and Sharma K.S. (1980): Effect of mercuric chloride on the activities of brain enzymes in a fresh water teleost; *Channa punctatus* (Bloch.). *Arch. Environ. Contam. Toxicol*., 9: 425.

6. Sastry K.V., Siddiqui A. A. and Singh S.K. (1983): Alteration in some bio-chemical & enzymological parameters in the snakehead fish *Channa punctatus*, exposed chronically to quinalphos, Chemosphere; 11(12): 1211-1216.

7. Satyapal S. and Singh S. (2006): Toxicity of folidol on liver protein metabolism in a fish *Channa punctatus*. Bionotes, 8(4): 104.

8. Verma S.R., Bansal D.K., Gupta A.K. and Dalela R.C. (1979): Pesticide induced haematological alteration in a freshwater fish, *Saccobranchus fossilis*. *Bull. Environ. Contam. Toxicol.,* 22: 467-474.