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Original Article

Influence of Cypermethrin on DNA and RNA Content in Different Organs of Fresh Water Fish *Cyprinus carpio*

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Abstract

In this study, freshwater fish *Cyprinus carpio* was exposed to sub lethal concentration of synthetic pyrethroids insecticide cypermethrin to evaluate the impact on nucleic acids in different organs. Fishes were exposed to sub lethal concentration ($1/10^{th}$ of LC_{50} value) of cypermethrin for three different durations, 7, 14 and 21 days. In seven day exposed fishes, DNA and RNA contents were not altered by cypermethrin. Whereas in 14 day and 21 day exposed fishes the DNA and RNA content decreased significantly in brain, gills and liver. However, the decrease was more in 21 day exposed fishes. The reduction in the DNA and RNA content in cypermethrin exposed fishes is comparatively less in liver when compared to the brain and gills. Cypermethrin treatment diminished RNA/DNA ratio in all the fish organs tested. In all the three organs studied, the influence of cypermethrin was found to be exposure time dependent.

Key words: Brain, Cypermethrin, *Cyprinus carpio*, DNA, Gills, Liver, RNA.

1. Introduction

Pesticides are of an important class of chemical compounds, which cause a serious threat to the aquatic ecosystem. In whatever form they are applied, pesticides finally contaminate and accumulate in the ecosystem. Many workers have reviewed the effects of have reviewed the effects of pesticides on aquatic ecosystem. Synthetic pyrethroids have been introduced over the past two decades for agricultural and domestic use as replacements for more toxic pesticides, such as chlorinated hydrocarbons, organophosphates and carbamates [1].

Several studies have reported that pyrethroids are highly toxic to a number of non- target organisms [2, 3] and these pyrethroids are readily absorbed by the gills of fish even at very low concentration [4, 5]. Among the synthetic pyrethroids, cypermethrin is one of the top ranked and widely used pesticides in annual

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usage [6]. The widespread use of the cypermethrin in agricultural and public health applications are considered as the most effective pyrethroids and extensively used in agricultural fields. In India, cypermethrin is registered for use on a wide array of crops including cotton, cabbage, brinjal, sugarcane, wheat and sunflower. Almost 70% of all sprays used on cotton fields of Andhra Pradesh in India are pyrethroids, which consists of mostly the cypermethrin [7].

Toxicants impair the metabolic, physiological and histological analysis in vital tissues. In our previous study we have reported the time dependant variation of antioxidant enzymes in different organs of cypermethrin exposed *Cyprinus carpio* [8]. Toxic impact can be evaluated by genotoxicity studies to assess the damage caused to fish at molecular level. The maintenance of DNA integrity is vital to the protection of genetic diversity in natural populations [9]. The detection of structural/functional disturbances in the DNA enables the assessment of organism's health and can assist in the prevention of DNA damage [10]. Cypermethrin exhibits the potential to induce DNA damage in different organs of mouse, with maximum damage taking place in brain [11].

The objective of the present investigation was to determine the sub lethal effects of cypermethrin on nucleic acid content of brain, gills and liver in *Cyprinus carpio* and to establish toxicity risk of cypermethrin exposure in this test species.

2. Materials and Methods

2.1. Maintenance of Fishes

Healthy specimens of adult *Cyprinus carpio* of both sexes, with uniform weight of 95 ± 5 g were procured from Government fish pond, Puducherry. While collection, care was taken to avoid stress and injury to fishes, then they were carefully transported to the laboratory in oxygen pack. The active and healthy *Cyprinus carpio* were selected for acclimatization during which they were kept in glass aquaria for 10 days. During acclimatization, the fish were fed with commercial food pellets. The water was changed daily, the remaining food and faecal matters were removed and water quality is also monitored periodically.

Physico-chemical characteristics of the experimental medium such as temperature, pH, salinity, dissolved oxygen and total hardness were analyzed following standard procedure [12]. The healthy fishes were subsequently used for the present study. The fishes were examined carefully for any pathological symptoms and placed in water containing 0.1 mg/L of potassium permanganate solution to avoid the possibility of any dermal infection.

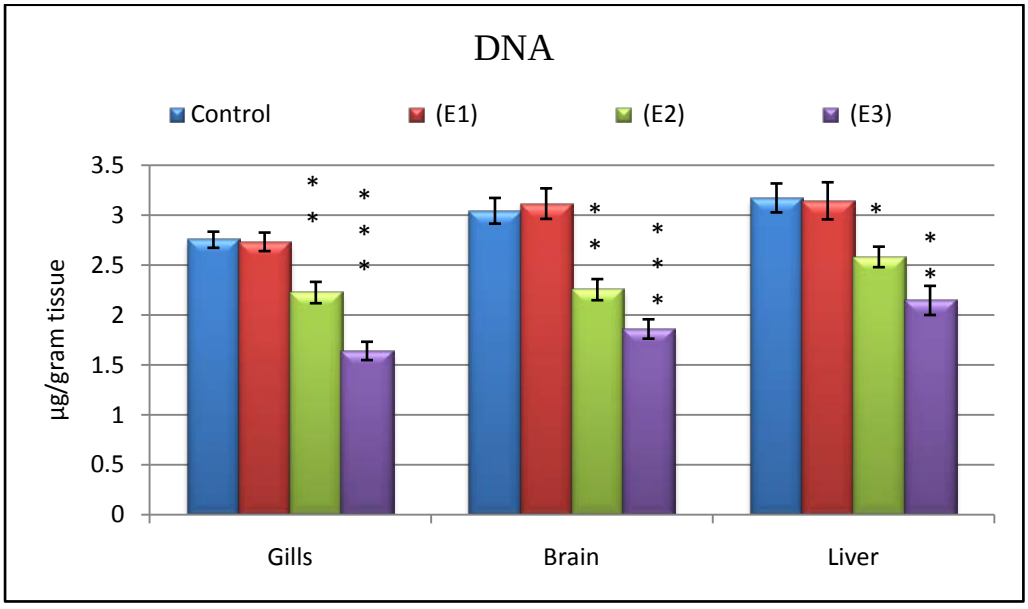

Figure 1. Influence of cypermethrin on DNA content in gills, brain and liver of fresh water fish, *Cyprinus carpio*.

2.2. Experimental Design

Healthy and same sized *Cyprinus carpio* were chosen and sorted into 4 groups of 15 fishes each.

Group I: Control fishes

Group II: Fishes exposed to 1/10 of Lc_{50} value of cypermethrin (0.6mg/L), for 7 days) E1

Group III: Fishes exposed to 1/10 of Lc_{50} value of cypermethrin (0.6mg/L), for 14 days) E2

Group IV: Fishes exposed to 1/10 of Lc_{50} value of cypermethrin (0.6mg/L), for 21 days) E3

The dose was selected based on 96 hours LC_{50} value.

Test solution was renewed daily, which facilitated the removal of nitrogenous waste excreted by the test fishes and for the removal of unconsumed food. The fishes were fed during the experiment at least twice (morning and evening) a day. Feeding was stopped 24 hours prior to sacrifice. The stock and test solution was prepared by dissolving the pesticide in acetone. Fishes kept in a pesticide free medium served as control. The same volume of acetone used in the dissolution of pesticide was maintained in the control.

24 hours after the respective experimental period the fishes were sacrificed and the key organs such as gills, brain and liver were surgically removed. Tissues were thoroughly washed in normal cold saline (4-6°C), blotted dry, weighed, and processed immediately for nucleic acid analysis.

2.3. Estimation of Nucleic acids

Nucleic acids were extracted from the tissue following the method of Schneider [13]. The

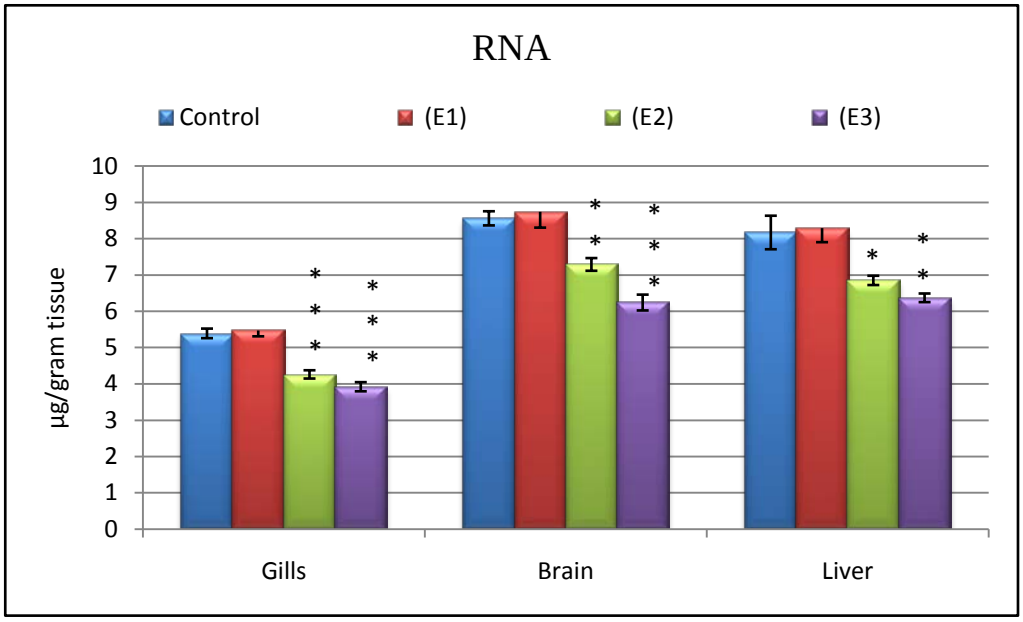

Figure 2. Influence of cypermethrin on RNA content in gills, brain and liver of fresh water fish, *Cyprinus carpio*.

The results are expressed as Mean ± SEM (n = 10) per treatment and respective control groups. Levels of significance values are *p<0.05, **p<0.01, ***p<0.001 compared with control group.

DNA content was estimated by the method of Burton (14). The RNA content was estimated by the method of Ceriotti [15].

2.4. Statistical Analysis

All the data were analyzed using Student's t-test and the data were expressed as mean± SEM. The p value of <0.05 was considered as significant against control.

3. Results and Discussion

Table 1 and Figure 1 show the DNA content in the gills, brain and liver of fishes exposed to cypermethrin and control fishes. The DNA content is comparatively low in the gills and more in brain followed by liver of the control fishes. In seven days exposed fishes, no significant changes were observed in DNA content in all the three organs studied. In 14 days and 21 days exposed fishes the DNA content decreased significantly in the brain, gills and liver. However, the decrease was higher in 21 days exposed fishes. The reduction in the DNA content in cypermethrin exposed fishes is comparatively less in liver when compared to the brain and gills. The maximum reduction of 40% was observed in DNA content in fishes exposed for 21 days (P<0.001). In this group, the decrease was comparatively more in gills

Table 1. Influence of cypermethrin on DNA content in gills, brain and liver of fresh water fish, C*yprinus carpio*.

Organs	Control	(E1)	(E2)	(E3)
Gills	2.756 ± 0.081	2.735 ± 0.093 - 1%	2.227 ± 0.107** - 19%	1.642 ± 0.092 *** - 40%
Brain	3.046 ± 0.129	3.118 ± 0.153 2%	2.256 ± 0.106** - 25%	1.862 ± 0.097*** - 38%
Liver	3.175 ± 0.145	3.146 ± 0.186 - 1%	2.584 ± 0.103* -18%	2.148 ± 0.146** - 32%

(40%) and followed by brain (38%) and liver (32%).

Table 2 and Figure 2 shows the RNA content in the control and experimental fishes after exposure to cypermethrin in common carp. In seven days exposed fishes, RNA content was not significantly altered by cypermethrin. However, in both 14 days and 21 days exposed fishes in all the three organs RNA concentration declined significantly. In 14 days exposed fishes the RNA content decreased significantly in gills (20%), brain (15%) and in liver (16%). The decrease was comparatively higher in 21 days exposed fishes in the brain and gills (27%) followed by liver (22%).

DNA and RNA dynamics during conditions of stress corresponds to degenerative changes in the tissues leading to decreased protein synthesis and cellular proliferation activity. The energy may be diverted for maintenance of metabolism during stress by the breakdown and utilization of stored glycogen, depletion of protein and lipid.

In the present study the decrease in the DNA and RNA content of the liver, gills and brain tissues of cypermethrin exposed carp was observed. A decline in the DNA content in the tissues of animals subjected to toxic stress may be due to inhibition of the enzymes in DNA synthesis. Ansari and Kumar [16], has reported a significant decline in the DNA and RNA content of the liver tissue of zebra fish, *Brachydani orerio* by the exposure to cypermethrin. Significant decline in the DNA and RNA ratio in *Labeo rohita* due to ammonia toxicity was observed by Acharya *et al* [17].

The synthesis of RNA plays an important role in protein synthesis. The inhibition of RNA synthesis at transcription level may affect the protein content [18]. In this study, a significant decrease of RNA was observed in different organs of cypermethrin exposed fishes. The decrease in RNA concentration might have caused protein depletion in these organs. Cypermethrin was also significantly diminished

Table 2. Influence of cypermethrin on RNA content in gills, brain and liver of fresh water fish, C*yprinus carpio*.

	Control	(E1)	(E2)	(E3)
Gills	5.39 ± 0.132	5.47 ± 0.161 1%	4.26 ± 0. 114*** - 20%	3.92 ± 0.126*** - 27%
Brain	8.56 ± 0.195	8.74 ± 0.436 2%	7.29 ± 0.175** - 15%	6.24 ± 0.218*** - 27%
Liver	8.17 ± 0.462	8.29 ± 0.387 1%	6.85 ± 0.128* - 16%	6.37 ± 0.119** - 22%

The results are expressed as Mean ± SEM (n = 10) per treatment and respective control groups.Percentage change in each category is also mentioned

Levels of significance values are *p<0.05, **p<0.01, ***p<0.001 compared with control group. P <0.05 considered to be statistically significant.

the nucleic acid level in various tissues of fish [19].

The development and growth of the fishes depend upon the DNA and RNA which serve as biochemical indices [20]. Cellular enlargement and active protein synthesis are dependent on DNA and RNA content. Pesticides induce deoxyribonucleic acid damage [21, 22] and structural chromosomal changes [23]. Pesticides may attack DNA directly or modify other cellular process associated with the integrity of the genome. The physio-chemical interaction of the pesticides with the cellular DNA produces a variety of primary lesions such as single strand breaks, double strand breaks, DNA protein cross-link and damage to purine and pyrimidine bases [24]. The intactness of the DNA is the important part of the normal cellular process. The changes in DNA: RNA ratio results in eventual losses of cell structure, proliferation and formation of new tissue and tissue degradation [25] with a total loss of cellular control mechanism [26].

Similar results were also reported in the liver and muscle tissues of *Channa punctatus* exposed to sub lethal doses of methanol [27]. Aditya *et al* [28] studied the effect of mercury and methyl parathion on the ovarian tissues of *Labeo rohita* and observed a significant decline in the RNA: DNA ratio. RNA content of the liver and muscle tissues of freshwater catfish, *Clarias batracus* exposed to endosulfan [29] and pyrethroids [30] was declined.

Lepidocephalicthys thermalis exposed to cypermethrin also showed a decline in the RNA content of the liver and muscle tissues [31].

Similar results were reported in liver, brain and muscle tissue of *Oreochromis mossambicus* exposed to quinalophos [32] and in the liver tissue of *Tilapia mossambica* exposed to monocrotophos [33]. A decrease in the DNA content of whole chick embryos was observed due to high dose of cypermethrin, suggesting DNA damage inhibiting cell multiplication and decreasing energy supply [34]. Khurshid [35] also reported a decline in the nucleic acid content of the liver and muscle tissue of *Labeo rohita* exposed to cypermethrin.

A decline in the DNA, RNA content of the brain, muscle and liver tissues of *Clarias batrachus* was reported when the animals were subjected to starvation stress [30]. Tripati *et al* [36] reported a dose-dependent decrease in the nucleic acid content of the liver, muscle and gonad tissues of freshwater teleost fish, *Channa punctatus* subjected to the toxic effects of the organophosphate, dimethoate. Altered nucleic acid metabolism was observed in the ovary of arsenic exposed *Colisa fasciatus*, a freshwater fish [37]. The tissue specific change in the nucleic acid content observed in the present study may be due to the differential effect of cypermethrin on the synthesis of nucleic acids.

Ribonucleotidase reductase, an enzyme required for the deoxyribonucleotide synthesis might be inactivated by the free radicals generated due to exposure to the toxic compound which might have led to the decreased levels of deoxyribonucleotide leading to a decline in the DNA content. Oxidative damage to the nuclear material by reactive oxygen species could also have led to the decreased nucleic acid synthesis. Direct mechanism for damage involves reaction of hydroxyl radicals with DNA as a result of oxidative stress making DNA a suitable target for oxidative damage. Indirect mechanism of DNA damage involves Reactive Oxygen Species (ROS) mediated triggering of a series of metabolic events within the cell that may lead to inhibition or activation of a number of enzymes. Inhibition of enzymes that replicate or repair DNA might predispose DNA to damage [38].It is also possible that the disruption of DNA synthesis might have affected RNA synthesis as already suggested [39].

The reason for decreased nucleic acids levels in different organs of fish exposed to cypermethrin may be due to the genotoxicity action by decreased mitotic index and disturbed cell division or due to inhibitory action of pesticides on DNA and RNA synthesis. Cypermethrin toxicity indicates alteration in nucleic acid synthesis.

4. Conclusion

It may be concluded that the significant reduction in RNA and DNA content in different organs of cypermethrin exposed fishes may be due to decrease in protein synthesis, defective nucleic acid metabolism and also degradation of cells. The results of this study suggest that cypermethrin is a potent inhibitor of nucleic acid synthesis even at sub lethal concentrations,

which in turn may lead to altered cellular activity.

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