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Haematological alterations in a fresh water fish *Anabas testudineus* under cypermethrin exposure

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Abstract
Cypermethrin, a synthetic pyrethroid is a broad spectrum insecticide used extensively in households and agriculture fields to control the pests. Use of insecticides in agriculture to prevent the crop damage from pests come with a significant cost as they reach to the aquatic systems through irrigation and rain and deteriorates the water quality. It adversely affects the non-target organisms including fishes of the fresh water ecosystem. Pyrethroid insecticide cypermethrin was used for this investigation to study the alterations in blood biochemical of a fresh water fish *Anabas testudineus*. Acute toxicity of cypermethrin to the fish was determined for 24, 48, 72 and 96 hours. The LC_{50} value was measured 0.15 ppm, 0.20 ppm, 0.2 ppm and 0.30 ppm respectively by the regression equation. The sublethal concentration determined was 0.106 ppm. After exposure period of 30 days significant changes in the blood biochemical level was reported during the present study. Blood glucose, serum protein and serum cholesterol of cypermethrin treated fish exhibited hyperglycaemic, hypoproteinemic and hypercholesterolemic response. The present investigation come to a conclusion that cypermethrin alters the blood biochemistry of *Anabas testudineus* and hence, affects the fish health.

Keywords: *Anabas testudineus*, blood biochemical, cypermethrin, LC_{50}, serum protein

1. Introduction
Ample food supply to the increasing population is a challenge in underdeveloped and developing countries. To increase the crop yields use of agrochemicals become a compulsion. Pesticides are used to prevent the crop damage due to pests. However their use is hazardous to the environment and can affect the non-target organisms also [1]. Unfortunately, aquatic ecosystems like ponds and rivers are more vulnerable to the pesticide pollution as they are located close to or inside the agriculture areas. The pesticides liberated directly into the aquatic environment or due to surface run off have adverse effects on fish and subsequently on the human beings [2]. Fishes provide food and livelihood for millions of the people globally. Fishes represent the largest and most diverse group of vertebrates and are excellent experimental models for toxicological research [3]. Fishes are important component of food chain, so, any effect of toxicant may have adverse effect on the nutritive values of fish and on the human beings due to their consumption [4]. The organisms in aquatic environment respond to pesticide pollutants by changing the biochemical composition and by altering physiological phenomenon [5, 6].
Blood forms a unique compartment between the external and internal environment in fishes and therefore, any physical and chemical change in the environment induces changes in the nature of blood [7]. Blood parameters reflect the health conditions of animals and the serum biochemistry is an important tool for clinical assessment of wildlife and livestock [8, 9, 10]. Haematological and biochemical parameters in different species have their own suitable range and any change in these parameters reflect their health status and provides information about the surrounding environment also. Some of the notable experiments on biochemical alteration in fishes under pesticide exposure are those of lipid kinetics in relation to the toxicity of three pesticides in climbing perch *Anabas testudineus* [11], effects of cypermethrin and carbofuran on haematological indices of *Labeo rohita* [12], effects of sewage on the haematological parameters of *C. mrighala* [13], alterations in blood biochemical levels in *Heteropneustes fossilis* under nuvan exposure [14].

In the present study an attempt was made to examine the toxic effects of cypermethrin on the blood biochemical levels of a fresh water fish *Anabas testudineus* as this fish is available throughout the year in wetlands and consumed by the people of this region as their staple diet.

2. Materials and Method

Healthy specimens of test fish *Anabas testudineus* were collected locally and were transported to the laboratory in earthen pots half filled with natural water and covered by a piece of mosquito net. During transportation maximum effort was taken to minimize the stress. The fishes were washed with 0.1% $KMnO_4$ solution to remove dermal infections if any. Fishes of average length 12-16 cm and weight 80-100 gm. were selected for the experiments and transferred into 40 litre rectangular glass aquaria one by one by using hand net. Fish were kept for a period of two weeks for acclimatization. Dechlorinated tap water was used during entire process and aquaria were cleaned routinely. Fish were fed with chopped goat liver once every day. Static bioassay was conducted for the determination of LC_{50} values of cypermethrin for a period of 24, 48, 72 and 96 hours following the methods of APHA, AWWA and WPCF [15]. The sub-lethal dose determined was 0.106 ppm by the formula of Hart *et al.* [16]. At the end of exposure period of 30 days, control and cypermethrin exposed fishes were first weighed individually and then blood samples were collected in heparinized vials with the help of 1 ml disposable syringe equipped with 2- gauge hypodermic needle by puncturing the ventral aorta. Blood obtained from the individual fish was centrifuged at 3500rpm for 10 minutes and serum was collected in different vials and further stored at 20 °C until analysed [17, 18]. After the completion of sampling procedures blood glucose, serum protein and serum cholesterol of control and cypermethrin exposed fish were estimated [19, 20, 21].

3. Results

The physico-chemical characteristics of the normal water and cypermethrin dissolved water is shown in Table-1. In the present study elevation in blood glucose and serum cholesterol level while decrease in serum protein level was reported in cypermethrin exposed fish. In control fish blood glucose, serum cholesterol and serum protein content was estimated 72.66±0.83 mg/100ml of blood, 210±2.08 mg/100ml of blood and 6.01±0.37 gm/100 ml of blood respectively while in cypermethrin exposed fish these parameters were 93.82±1.41 mg/100 ml of blood, 226.8±1.96 mg/ 100ml of blood and 4.73±0.15 gm/100ml of blood respectively (Table-2 and Fig.1). The increase in blood glucose level and decrease in serum protein level in test chemical exposed fish was highly significant (p<0.001) while increase in serum cholesterol level was significant (p<0.05).

Table 1: Physico-Chemical characteristics of Normal and Cypermethrin Dissolved water

Parameters	Normal water	Cypermethrin dissolved water
Odour	Odourless	Unpleasant
Temperature (°C)	28	29
P^H	7.3	6.2
DO (mg/L)	7.6	6.9
Total hardness (mg/L)	225	210

Table 2: Changes in Blood/Serum metabolite level in Anabas testudineus exposed to cypermethrin for 30 days. Values are Mean ± SE of 5 observations.

Parameters	Control Fish	Cypermethrin Exposed Fish	Percent increase (+) and Decrease(-)
Blood glucose (mg/100 ml of Blood)	72.66±0.83	93.82±1.41	+ 29.12
Serum Cholesterol (mg/100 ml of Blood)	210±2.08	226.8±1.96	+7.6
Serum protein (gm/100 ml of Blood)	6.01±0.37	4.73±0.15	-21.29

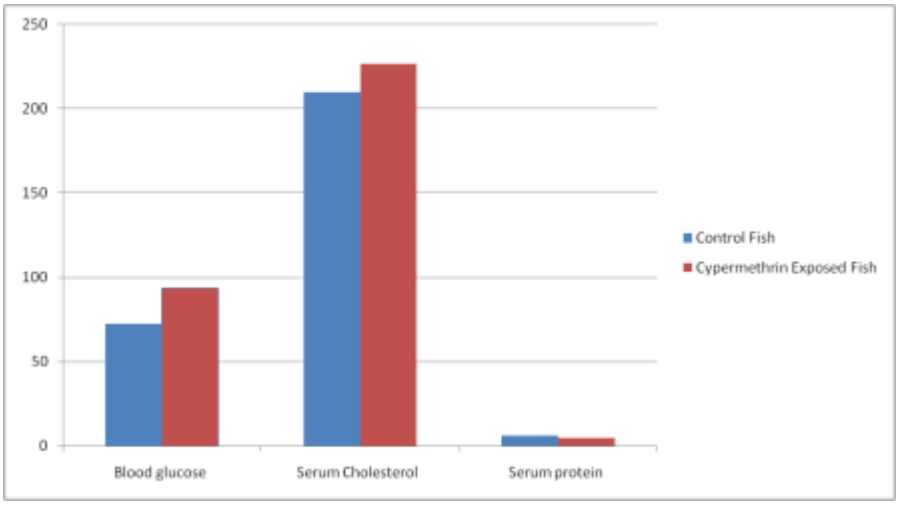

Fig 1: Changes in Blood/Serum metabolite level in Anabas testudineus exposed to cypermethrin for 30 days. Values are Mean ± SE of 5 observations

4. Discussion

4.1 Blood Glucose

Haematological parameters are considered as an indicator of fish health. Physiological responses are good sign because they provide information about the internal environment of organisms [22]. Among the biochemical profiles plasma glucose has been extensively used as a sensitive indicator of environmental stress in fish because the carbohydrates are the primary and immediate source of energy [23]. Marked significant increase in glucose level has been reported in

present study that clearly exhibit the effect of cypermethrin as the stressful manifestation to the test fish. In present study increase in blood glucose level was reported 29.12% in the cypermethrin exposed fish as compared to the control. Several reports demonstrated the similar results in various fish species under different pesticide exposure [24, 25, 26]. Stress is an energy demanding process and the mobilization of energy substrate to cope with the adverse condition is common in animals. The stress hormone cortisol under pesticide exposure increases glucose production in fish by both glycogenolysis and gluconeogenesis [27].

4.2 Serum cholesterol

Serum cholesterol level exhibited hypercholesterolemic response under cypermethrin exposure. In the cypermethrin exposed fish the increase in serum cholesterol level was 7.6% as compared to the control fish. This response was possibly due to accumulation of pesticides in the liver that caused disturbance of lipid metabolism and caused increase in the cholesterol level in serum. Various studies have demonstrated similar pattern of alteration in haematological parameters in fish under different pesticide exposure [28, 29, 30].

4.3 Serum Protein

In the present study serum protein level indicated declining tendency in the fish under the test chemical exposure. The decrease in protein level was reported 21.29% in test fish as compared to the control fish. Pesticide exposure affects the liver adversely thereby reduces the protein synthesis and causes protein deficiency. Hypoproteinemic response in the present investigation is in conformity with the several reports on fish species under different toxicants [31, 32].

5. Conclusion

Stress induced haematological alterations in fish was reported in the present investigation. The outcomes of haematological assessment in the cypermethrin exposed fish clearly indicates the hazardous impact of pesticides on aquatic fauna especially the fish. From the results of present study it can be concluded that exposure of *Anabas testudineus* to the sub-lethal concentrations for 30 days caused significant alterations in haematological parameters that is mirror of fish health and their poor nutritional value. Prevention of natural water resources from pesticide contamination is essential to provide a safe and unclouded habitat to the fish diversity.

6. Competing Interests

Authors have declared that no competing interests exist.

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