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SEM Studies on Erythrocyte Alterations in *Ctenopharyngodon idellus* (Cuvier and Valenciennes) induced by Fenvalerate

Jindal, R. and Batoye S.
Aquatic Biology Lab, Department of Zoology, Panjab University, Chandigarh, 160014, INDIA

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Abstract

Pesticides used in agriculture may ultimately enter the aquatic environment, get biomagnified and cause severe environmental and health problems. Present investigation have been made to assess the detrimental effects of fenvalerate on the structural alterations of erythrocytes of a freshwater fish, Ctenopharyngodon idellus (Cuvier and Valenciennes) after exposure of the fish to two sublethal concentrations of the pesticide (1.2 ppm and 2 ppm) for 15, 30 and 60 days. The erythrocytes of the exposed fish exhibited various morphological alterations such as appearance of echinocytes, spherocytes and acanthocytes; contraction and serration of cell membrane, hole-like depressions on the surface of erythrocytes, lobopodial projections, etc. which were analysed by scanning electron microscopy (SEM). These alterations increased with the increase in concentration of toxicant and duration of exposure.

Keywords: Ctenopharyngodon idellus, pyrethroid, fenvalerate, erythrocytes, SEM, toxicity.

Introduction

The contamination of aquatic ecosystem with pesticides is one of the important consequences of industrial and agricultural revolution. Indiscriminate use of such chemicals may cause environmental pollution and toxicity risk to non-target organisms, which is a matter of concern at present worldwide. Pyrethroid is analog of naturally occurring pesticide pyrethrum which is produced by the flowers of pyrethrums (*Chrysanthemum cinerariaefolium* and *C. coccineum*[1]. These pesticides have been widely used over the past two decades in agricultural, veterinary, medical and home use; also accounts for about one quarter of the world pesticides market[2]. Pyrethroids are among the most potent and effective insecticides available. In recent years, active ingredients of pyrethroids have been widely detected in soil, urban and agricultural streams, as well as indoor dust which cause potential threat to wildlife and humans[3]. These are less poisonous to mammals and humans as compared to the other insecticides. On the other hand, because of their high toxicity to the aquatic organisms like fish and invertebrates, emphasis has been paid to pyrethroids residues in the runoff and river water[4]. Pyrethroids toxicity is 1000-times greater to fish than to birds and mammals at comparable concentrations[5]. Fish are more affected by pyrethroid due to slower rate of hydrolytic detoxification and hypersensitivity of the nervous system[6]. The voltage-gated sodium channels (VGSCs) are the prime target sites of pyrethroids[7]. They bind to sodium channels and delay their inactivation, thus results in neurotoxicity[4].

Pyrethroids are categorized into two types i.e. type I and type II on the basis of their chemical structure[8]. Fenvalerate, (RS)-alpha-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methyl butyrate, is a type II synthetic pyrethroid with superior insecticidal properties[9]. Limited information is available regarding the harmful effects of fenvalerate on non-target organisms[10]. As a consequence of lipophilicity, fenvalerate is rapidly absorbed through the gills of fish. Moreover, fishes have poor capacity to metabolise and excrete fenvalerate[11] and thus are prone to even minute concentrations of the pesticide.

The blood cell profile of fish has been considered as an important indicator of diseases and other toxicants[12]. In the fish respiration, erythrocytes play an important role and acts as a model to study the damage induced in different cellular compartments by xenobiotics[13]. SEM is considered as a useful tool for analysing the effects of contaminants on fish structures essential to their fitness or survival[14]. Light or transmission electron microscopy cannot identify the surface ultrastructural damage; therefore, SEM is used for the changes and damage that may occur on tissues[15]. There are limited studies on fish blood treated with pesticides using SEM[16,17]. Therefore, in the present investigation, morphological changes induced on erythrocytes of fish exposed to sublethal concentrations of fenvalerate by SEM have been undertaken.

Material and Methods

The live fingerlings of *Ctenopharyngodon idellus* (Cuvier and Valenciennes) with an average weight: 12±2 gm, length: 10±2 cm were collected from Nanoke Fish Seed Farm and acclimated for 2 weeks in aquarium provided with aerators, filters and heaters. They were fed by artificial feed, 'Gold Tokyo', manufactured by Tairopet Product Pvt. Ltd., Chennai, India which contains crude protein 32%, crude fat 4%, fiber 6%, crude ash 10% and moisture 10%. The fish were starved for 24

hours before the start of the experiment. Fenvalerate (EC 20%) manufactured by Shivalik Agrochemicals, JandK, India, was used during the present study.

Renewal toxic test methods[18] were performed to determine the 96-h LC_{50} of fenvalerate for *C. idellus.* It was calculated by Probit analysis[19] and was found to be 5.9 ppm. The fish were divided into three groups (20 fish each) and kept in separate plastic (Syntax) tanks. In order to ensure the reproducibility of the results, all tests were performed in three replicates. Group I and II were exposed to two sublethal concentrations of fenvalerate i.e., 1.2 ppm and 2 ppm for 15, 30 and 60 days while group III was maintained as control in dechlorinated water. Water containing the toxicant was renewed daily to maintain the required concentration of the pesticide and to minimise the changes due to fish metabolites.

Blood sample collection and processing: Fish were sacrificed by cervical dislocation according to the guidelines of institutional animal ethics committee of Panjab University Chandigarh and blood was collected from heart using a 23 guage needle. Few drops were fixed in glutaraldehyde (1% in 0.2M phosphate buffer, pH 7.2) for 10-15 min and then centrifuged for 5-10 min at 1500 rpm. Supernatant was discarded and the erythrocyte pellet was washed thrice with 0.1 M phosphate buffer (pH 7.2). The pellet was then washed twice with triple distilled water. A drop of the suspended erythrocytes was placed over silver tape attached to the aluminium stub and air-dried. These were then sputter coated with gold (100A°) and finally viewed under JEOL, JSM-6100 scanning electron microscope (at RSIC, CIL, Panjab University, Chandigarh).

Semiquantitative scoring: The morphological changes in the erythrocytes were analysed in 3 fish (randomly selected) from each group per replicate. The mean occurrence of each structural deformity was categorized as none (−), mild (+, <25% of sections), moderate (++, 25–50% of sections) and severe (+++, >50% of sections)[1].

Results and Discussion

Erythrocytes exhibit few stereotypic responses to a variety of environmental pollutants, which are sometimes considered as physiologically significant. Moreover, the changes in the shape and size of the erythrocytes are considered to represent the most common morphological deformities occurring in pathological conditions[20]. In fish blood, erythrocytes are the dominant, nucleated, biconvex shaped cells, through which O_2 andCO_2 swiftly diffuse in or out of the cell[21]. Hence, any morphological modification will quickly affects the gaseous exchange resulting in hypoxaemia, hypercapnia, blood acidosis, etc.

In the present studies, SEM of erythrocytes showed normal morphology in control group (figure-1a, b). They were regularly elliptical with the usual smooth surface.

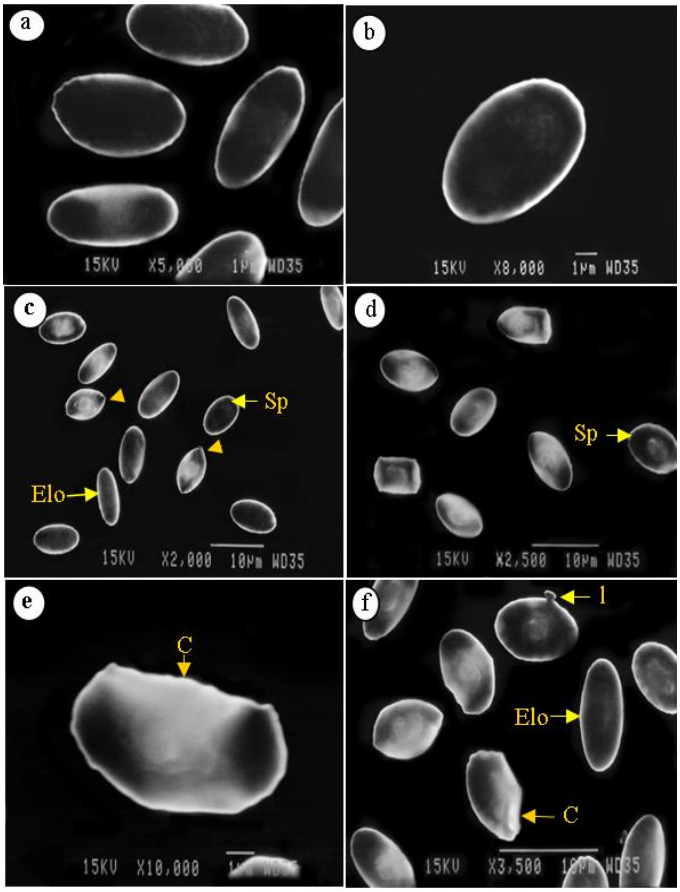

Figure-1
Scanning electron micrographs of erythrocytes of *C. idellus*. (a), (b) control: normal elliptical cells; (c), (d) irregularly shaped cells like spherocytes, elongated cells, cells with tapering ends (arrow head) upon 1.2 ppm of fenvalerate exposure (15th day); (e), (f) contraction of erythrocytes from one side and small lobopodial projections upon 2 ppm of fenvalerate exposure (15th days). Abbreviations: Sp – spherocytes, Elo – elongated cells, C – contracted cells, l – lobopodial projections

On exposure of fish to 1.2 ppm fenvalerate for 15 days, swollen (spherical) erythrocytes identified as 'spherocytes' were observed (figure-1c, d). Upon exposure for 30 and 60 days, marked increase in the number of these spherical erythrocytes and a notable change in size and shape of cells like elongated cells, cells with tapered ends was reported (figure-2,3). At higher concentration of the toxicant (2 ppm fenvalerate), numerous spherocytes, erythrocytes showing contraction from one side and with small lobopodial projections were observed (figure-1e, f). The disrupted lipid membrane and increased lipid peroxidation might be the proposed reason for the above altered shapes of red blood cells[22]. Lipid peroxidation produces malondialdehyde which interact with free amino groups of proteins and phospholipids and causing structural and functional alterations in biomembranes[23]. It has also been recognized that

malathion induced abnormalities in red blood cells of *Channa punctatus* occurred due to depressed ATP under hypoxic condition[16].

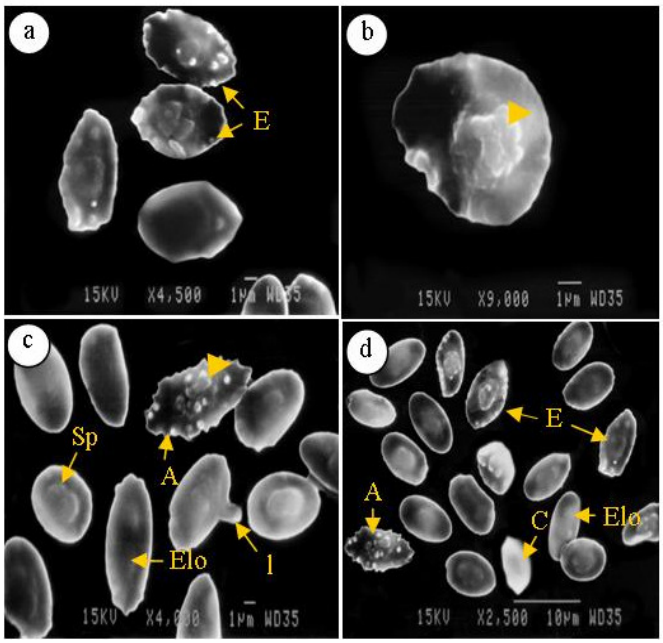

Figure-2

Scanning electron micrographs of erythrocytes of *C. idellus* on 30th Days. (a), (b) drastic damage with hole like depressions on the surface of erythrocytes (arrow head), crenated cells with numerous projections i.e. echinocytes, spherocyte, contracted cells, lobopodial projection upon 1.2 ppm exposure of fenvalerate; (c), (d) echinocytes or irregularly crenated cells, tapering end (arrow head), elongated cells and spherocytes upon 2 ppm exposure of fenvalerate. Abbreviations: Sp – spherocytes, Sh – shrinkage, Elo – elongated cells, C – contracted cells, l – lobopodial projections, E – echinocytes

On 30th day, at lower concentration of the toxicant, a drastic damage with hole-like depressions on the surface of erythrocytes was observed; spherocytes, contracted cells and cells with lobopodial projections were also observed (figure-2a,b). At higher concentration of the toxicant, formation of crenated cells with numerous projections was found due to oozing out of cytoplasmic content (figure-2c,d). These may be referred as "echinocytes". With the increase in the exposure period, the increase in occurrence of these echinocytes with disrupted overlying plasma membrane was reported. Conversion of erythrocytes into echinocytes may be attributed to increased cholesterol level caused by liver dysfunction[24]. It has been illustrated that change in membrane lipid composition could be the key reason for such deformities in shape of blood cells in response to various chemical treatments[25]. Alteration in forms of blood cell could be illustrated as induced stress caused by toxicant; as reported by other workers on chloropyrifos[26,27].

At lower concentration of toxicant on 60th day of exposure period, echinocytes with oozed out cytoplasmic content were observed (figure-3a,b). Cytoplasmic disturbances and protuberances were also reported while studying the impact of chloropyrifos on erythrocytes of *Ctenopharyngodon idellus*[17]. At higher concentration of toxicant, acanthocytes with cytoplasmic blebbing and badly disrupted cell membrane were found (Fig.3 c,d). The Semiquantitative analysis, as represented in table-1, revealed that the number of spherocytes, irregular shaped cells, echinocytes, acanthocytes, etc was found to be increased with increase in exposure period and toxicant concentrations. Among the various abnormalities investigated, lobopodial projections and oozed out cytoplasmic content, echinocytes were more frequently observed. *Anabas testudineus* was exposed to sublethal concentrations of cypermethrin reported same changes[1]. It is speculated that oxidative stress in erythrocytes may lead to significant alterations in their structural conformation which effect blood flow, oxygen uptake and release[28]. Results of the present study indicate that fenvalerate caused significant modifications in the shape of the erythrocytes.

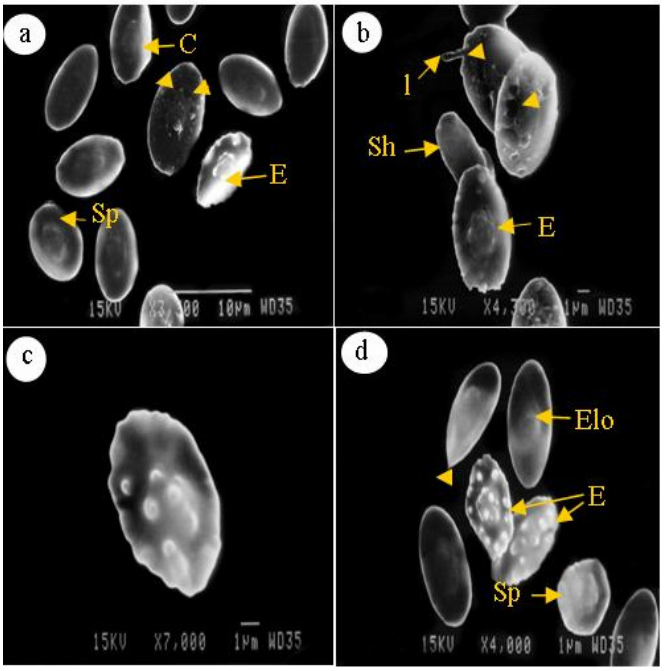

Figure-3

Scanning electron micrographs of erythrocytes of *C. idellus* on 60th Day (a), (b) crenated cells with oozed out cytoplasmic content (arrow head) upon 1.2 ppm of fenvalerate; (c), (d) spherocyte, lobopodial projection, elongated cell, contracted erythrocytes, acanthocytes with cytoplasmic blebbing , cytoplasmic oozing (arrow head) upon 2 ppm exposure of fenvalerate. Abbreviations: Sp – spherocytes, Elo – elongated cells, C – contracted cells, l – lobopodial projections; E – echinocytes, A – acanthocytes

Table-1
Semiquantitative analysis of alterations on the erythrocytes of *C. idellus* exposed to sublethal concentrations of fenvalerate

Alterations	Durations (Days)	Control		Fenvalerate	
			1.2 ppm		2 ppm
	15	-	-		+
Lobopodial projections	30	-	+		+
	60	-	+		++
	15	-	-		-
Echinocytes	30	-	+		++
	60	-	++		+++
	15	-	-		-
Acanthocytes	30	-	-		+
	60	-	++		+++
	15	-	-		-
Hole-like depressions	30	-	++		++
	60	-	+		+
	15	-	+		+
Irregular shaped cells	30	-	+		++
	60	-	++		+++
	15	-	-		-
Oozed out cytoplasmic content	30	-	+		++
	60	-	++		+++
	−, none; +, mild; ++, moderate; +++, severe				

Conclusion

The scanning electron microscopic observations revealed that there is morphological damage to the erythrocytes of *C. idellus*, frequency of which is directly proportional to the pesticide concentrations and duration period. Since blood is the most important body fluid, various physiological changes in the body due to the toxicant may be reflected in the blood. So, even slight variations in the aquatic environment are reflected in the morphology of fish erythrocytes, making it a sensitive pollution indicator.

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