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HAEMATOLOGICAL AND GENOTOXIC EFFECTS INDUCED BY ORGANOPHOSPHATE INSECTICIDE "ROGOR" IN FRESHWATER CATFISH *CLARIAS MAGUR* (HAMILTON, 1822)

Biological Science

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ABSTRACT

Rogor is one of the organophosphate insecticides, widely used in agriculture. In the present study, the effect of rogor on some haematological parameters of the freshwater catfish *clarias magur* was investigated. Fishes were exposed to 6.5mg/L and 13mg/L of rogor for 7 consecutive days. After 7 days of exposure, a decreased number of RBC (2.34, 1.83×10^6 mm^{-3}) and decreased Haemoglobin (Hb) concentration (7.18, 5.58 g dl^{-1}) was observed in both the groups in comparison with the control (with 2.83×10^6 mm^{-3} RBC and 7.76 g dl^{-1} Hb respectively). Increased number of WBC (78.27, 87.73 ×10^3 mm^{-3} in comparison with the control having 64.35±0.18×10^3 mm^{-3}) was also observed in the treated groups. The results were statistically significant at $p<0.05$ level. Again micronuclei were observed in the erythrocytes of peripheral blood of both the treated groups.

KEYWORDS

Clarias, Rogor, RBC, WBCs, Haemoglobin, Micronuclei

INTRODUCTION

Pesticides from agricultural runoff affect all the aquatic organisms. Many studies have established the harmful effects of various pesticides on aquatic environments and organisms[1-5]. Study of fish haematology is one of the most recent branches of physiology to assess the status of general health of fishes. Changes occurring in the haematological characters of fishes provide a sensitive measure to assess the health of fish fauna[6]. Hematological study is important in toxicological research because a hematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound[7]. Haematological techniques including measurements of Haematocrit, White Blood Corpuscles (WBC), Red Blood Corpuscles (RBC) and Haemoglobin (Hb) have proved valuable study for fishery biologists in assessing the health status of fish and monitoring stress response[8].

Micronuclei (MN) are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that are not incorporated into the nucleus after cell division. MN can be induced by defects in the cell repair machinery. A variety of genotoxic agents may induce MN formation leading to cell death, genomic instability, or cancer development[7,9]. Micronucleus assay is widely used to detect effect of many classes of chemicals, e.g., pharmaceutical, agricultural, food additives etc.[10].

Our health and environment have become almost synonymous today, and a large number of investigators have undertaken studies aiming monitoring of environmental quality. It is equally true for all biotic organisms, and fishes are thus no exception. Catfishes particularly the air breathing species are attracting attention of the pisciculturists owing to their high production potential from paddy fields and stagnant shallow ponds[11]. *Clarias magur* (Hamilton, 1822) is generally considered to be one of the most important catfish species for aquaculture as well as for its economic value as food in almost all over India. Therefore, the present studies were undertaken to study the effects of rogor on haematology and genotoxicity in terms of micronucleus assay on *C. magur*.

MATERIALS AND METHODS

a. Experimental chemical

Dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) is popularly known as Rogor. It is an organophosphate insecticide widely used for controlling insect pests of fruits, vegetables and crop plants.

b. Experimental animal

Healthy fresh water catfish *Clarias magur* weighing about 83±5 g were collected from the local market. Collected fishes were maintained in 3 earthen pots containing 25L of de-chlorinated tap water for 1 week to acclimatize for our laboratory conditions. The water was aerated properly and changed every day. Fishes were fed daily with commercial fish food and also with chironomus larva. The physiochemical characteristics of water were measured according to the standard methods[12]. These were found as P^H: 7.4± 0.5; Temperature: 24°±3; Dissolved oxygen: 8.4±0.2mg/L.

c. Haematological study

The LC_{50} value (lethal concentration) of Rogor to *clarias magur* is 65mg/liter for 96 hrs.[13]. Based on this value we took two sub-lethal doses of rogor; which are 1/5th and 1/10th part of the LC_{50} value to carry out our experiment (13 mg/L and 6.5 mg/L respectively). Fishes were randomly divided into 3 experimental groups having 6 fishes in each group. Group I was treated as control, whereas Group II and Group III were treated with the experimental doses 6.5 mg/L and 13 mg/L of rogor respectively. Treatment was carried out for 7 consecutive days. After 24 hrs of the last day of treatment, blood from all the fishes was collected separately in Eppendorf tubes containing EDTA anticoagulant, by severance of caudal peduncle[14] and used for haematological study. Total red blood cells were counted using an improved Neubaur haemocytometer[15] and total numbers were reported[16] as x10^6 mm^{-3}. Total white blood cells were counted using an improved Neubaur haemocytometer[17] and total numbers were calculated[16] in x10^3 mm^{-3}. Haemoglobin (Hb) was determined by using Sahli's haemoglobinometer. For micronuclei study, the peripheral blood smear was prepared and fixed with methanol for 5 min, air dried and stained with 2% Giemsa. Slides were then examined for micronuclei[18].

d. Statistical analysis

Results were expressed as mean ± SD of six replicates and differences between means were considered to be significant when $p < 0.05$

RESULTS

In both the treated groups, the total number of RBC and the amount of haemoglobin were decreased significantly in comparison with the controls ($P<0.05$). Whereas, the total number of WBC was increased significantly in both the treated groups in comparison with the controls. The results showed a dose dependent effect (Table 1).

Table1: Changes in total number of RBCs, WBCs and Haemoglobin in rogor treated *C. magur*.

Concentration of Rogor	Total no. of RBC (10^6 mm^{-3})	Total no. of WBC (10^3 mm^{-3})	Haemoglobin (g dl^{-1})
Control (group I)	2.83±0.25	64.35±0.18	7.76±0.07
6.5mg/L (group II)	2.34±0.32	78.27±0.25	7.18±0.06
13mg/L (group III)	1.83±0.48	87.73±0.21	5.58±0.09

Results are significant at 0.05 level ($P<0.05$)

The erythrocytes of control fishes showed well defined boundary, centrally placed rounded nuclei and clear cytoplasm (Fig. 1 with magnification × 40), whereas, the peripheral blood erythrocytes of the treated fishes showed the induction of micronuclei; and their structures were more prominent in high dose exposed fishes (Fig. 2 and 3 with magnification × 40).

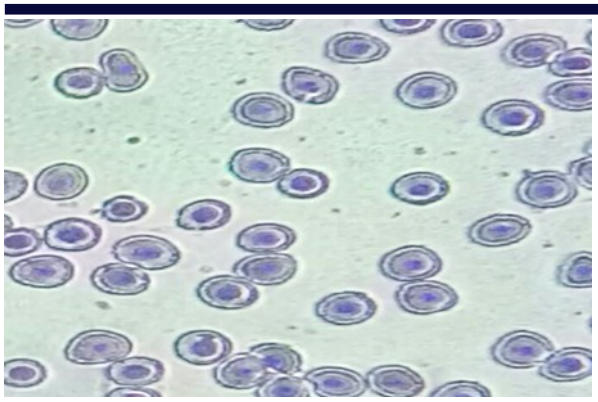

Fig. 1. Normal peripheral erythrocytes of Clarias magur from control group

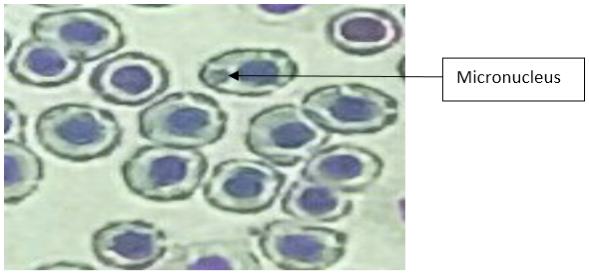

Fig. 2. Erythrocytes having micronuclei (arrow) at the dose level of 6.5mg/l of rogor

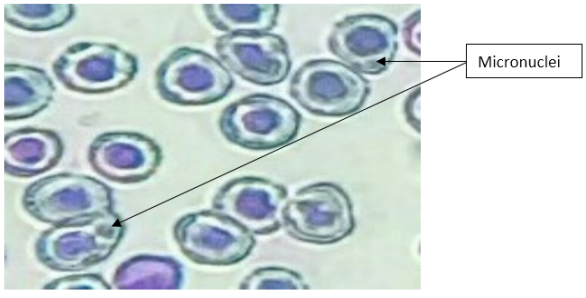

Fig.3. Erythrocytes having micronuclei (arrow) at the dose level of 13mg/l of rogor

Discussion

The significant decrease in RBC count may be due to haemolysis, decrease in erythropoietic activity or increased destruction of blood cells and decreased Hb concentration[7,4]. The significant increase in WBC count may be attributed to a stimulation of the immune system in response to tissue damage caused by rogor. An increased number of total WBC counts in fresh water fish, *Clarias batrachus* was observed after mercuric chloride exposure[19]. A significant decrease in Hb concentration may also be due to a decrease in the rate of Hb synthesis that may led to poor oxygen transport by blood. A number of workers have earlier reported similar haematelogical alterations produced by different toxicants[3,4,7,19]. Induction of micronuclei was observed in the RBCs of the treated fishes. Similar structure of micronuclei was observed[20] in *C. batrachus* when treated with fungicide (Propiconazole), Formation of these anomalies clearly indicates that rogor has high genotoxic potency that is very harmful to aquatic organism.

Conclusion

Pesticides are an integral part of modern agriculture and for the eradication of household pest especially in developing countries. Pesticide poisoning has become a global problem around the world that linked to a wide range of human health hazards. Haematological and genotoxic results of the present study indicate that Rogor (organophosphate) is toxic to fishes and thus such type of contaminants in edible fish body may affect our environment including health of human beings consuming these fishes. Therefore, we should try to avoid the use of this type of chemical in our agricultural field.

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