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Sub-Lethal Effects of Herbicide Paraquat on Hematological Parameters of Benny Fish *Mesopotamichthys Sharpeyi*

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Abstract. Pesticides may appear as environmental contaminants once they enter aquatic ecosystem after using in agriculture. Less degradable compounds may even enter aquatic food chains and cause health problems for aquatic organisms and sometimes for human. Paraquat is a chlorinated herbicide which usually is used in agriculture fields in the northern part of Iran. In this area is the main habitat for benny fish in Iran and fish culture (particularly benny fish) is practiced there. To study the toxicity of paraquat on some hematological parameters of benny fish, 40 individuals were devided into 4 groups and each groups was acutely exposed to paraquat concentrations of 0.37, 0.74 and 1.11 mg/l (equal to 25, 50 and 75 percent LC_{50} 96h respectively). After 96 h exposure fish were taken from aquariums, the blood samples were prepared and their hematological parameters including erythrocyte (RBCs) and leukocyte (WBCs) counts, hemoglobin concentration (Hb), hematocrit (Hct) and blood indices (MCV and MCH) were studied. Results showed that paraquat significantly reduced all studied parameters ($P<0.05$), while the percentage of reduction was proportional with paraquat concentrations. It is suggested that paraquat exposure might produces adverse effect on blood parameters of fish which results in anemia. This condition may affect normal growth, reproduction, immunity and survival of fish in both natural and culture conditions. It is recommended that farmers replace paraquat by less harmful and more degradable herbicides.

Keywords: Paraquat, Toxicity, Heamatology, *Mesopotamichthys sharpeyi*.

1. Introduction

Population increase together with limitation in agriculture lands have significantly increased the need for using of herbicides in agriculture [1]. Though agriculture products have been greatly depended on using of herbicides to control unwanted plant and weeds, however they may endanger other organisms specially when they rich to aquatic environment [2]. Floods and run off could dissolve applied herbicides and shift them into the rivers or seas. Aquatic animals may uptake herbicide from surrounding medium by different routs as non-target organisms. Non biodegradable compounds even can enter food chains and affect people who consume contaminated foods. Producing toxic effects for animals like fish is another impact of herbicides in aquatic environment. Paraquat is an organochlorine herbicide which is widely used in southern part of Iran where the largest fresh water system is located [3]. Among local fishes, Benny *Mesopotamichthys sharpeyi* (Günther, 1874) is dominant and occupies an important position in people diet. It is successfully cultured in aquaculture [4]. Previously some studies have been conducted to investigate the toxic effects of some pesticides on hematological parameters of fishes particularly on cold water species [5, 6]. However the effects of paraquat on benny fish are not well understood. This study was carried out to investigate toxicity of paraquat on hematological parameters of *Mesopotamichthys sharpeyi*.

2. Materials and Methods

Benny fish fingerlings (9.36 + 0.56g) were obtained from Shahid Maleki warm fish propagation and culture center of Ahwaz and transferred to the laboratoty. The fingerlings were acclimatized in 300 L tanks for one week prior to the toxicity test. During acclimatization the fish were fed with commercial pellets for 2% of their body weight.

Commercial paraquat (20% emulsion) was purchased from Patonar Co. and used to prepare test solutions. The stock solution of 1000 mg/l was prepared in a volumetric flask using distilled water. In order to prepare test solutions, appropriate amounts of the stock solution were added the aquariums.

The test procedure was set according to the standard method proposed by OECD [7]. A number of 40 fish were divided into 4 groups, 10 fish in each group. Based upon 96h median lethal concentration (96h LC50) of paraquat on Benny fish (1.49 mg/l), three groups were exposed to 25%, 50% and 75% of LC50 (0.37, 0.74 and 1/11 mg/l) in 25 L aquariums containing 20 L of test solution. A group of fish was kept away from the toxicant as control. The aquariums were continuously aerated with air stones. The test was carried out according to static method with no water change during experiment. The fish were not fed during the experiment. The exposure duration was 96 h and physicochemical parameters of test water were monitored regularly. During the experiment, the average water temperature was 24.1+ C, pH was 7.38 and Dissloved oxygen was 7.42 mg/l.

After 96 h the fish were taken from aquariums, their caudal peduncles were cut off and their blood was collected using 1ml syringes. The blood samples were transferred into heparinized eppendorf tubes and immediately analyzed for erythrocyte count (RBCs, × 106 µl-1), total leukocytes (WBCs, × 103 dl-1) and hematocrit (volume of the packed cells %). The blood cells counts were determined by hemocytometry method. The blood hematocrit was measured by the aid of microhematocrit method. Determination of hemoglobin was achieved by cyanomethemoglobin method [8]. For this propose 20 µl of blood was mixed with 5 ml Drabkins solution and the mixture was placed in a dark environment for 10 minutes . Then the absorption of mixtures were read in 540 nm wavelength and multiplied in 3.68 to obtain actual concentration of hemoglobin. A part of each blood sample was transferred into microhematocrit tubes. After centrifuging of the tubes (3000 rpm for 5minutes), their hematocrit were determined using hematocrit ruler. The blood indices were computed using following equations [9].

$MCV = Hct / RBCs \times 10$

$MCH = Hct / Hb \times 100$

The normality of data was determined using Shapiro-wilk normality test. The difference between various treatments was determined by One Way Analysis Of Variance (ANOVA). If there was significant difference, Tukey's Post Hoc was employed to recognize different groups. The significant level of the tests was set at 5% (α=0.05)

3. Results

Following acute exposure to sub lethal concentrations of paraquate the number of red blood cells (RBCs count) was significantly decreased in the experimental fish ($P<0.05$). While RBCs counts were not considerably different between those exposed to a , and b, the third group showed the minimum RBCs count (Fig1). The number of white blood cells (WBCs) was less sensitive to paraquate exposure so that the WBCs count did not significantly changed in fish exposed to a paraquat while the two other groups exhibited significant decrease in their WBCS count ($P<0.05$).

The exposure of fish to paraquat caused clear decrease in hematocrit and hemoglobin levels as well (Fig 2). While the mentioned decreases were not statistically significant for the first treatment, the second and third treatments experienced significant reduction in both hematocrit and blood hemoglobin ($P<0.05$).

The results also revealed that after paraquat exposure, other hematological indices including MCV, MCH and MCHC experienced considerable decrease (Fig 3). For MCV and MCHC the mentioned change was only significant for those fish which exposed to b and c mg/l of paraquate while, MCH values significantly decreased in all exposed groups ($P<0.05$).

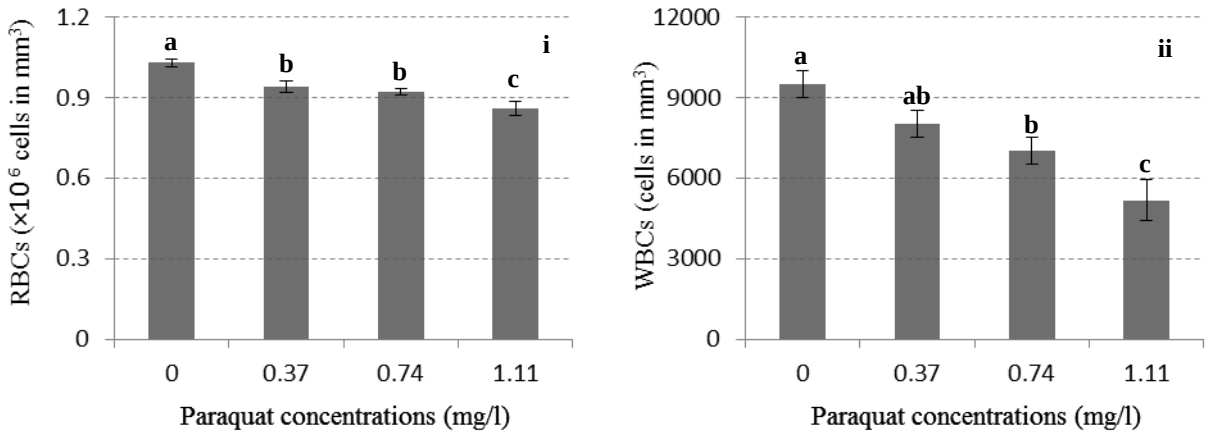

Fig. 1: Effect of paraquat on erythrocyte (i) and leukocyte (ii) counts of benny fish

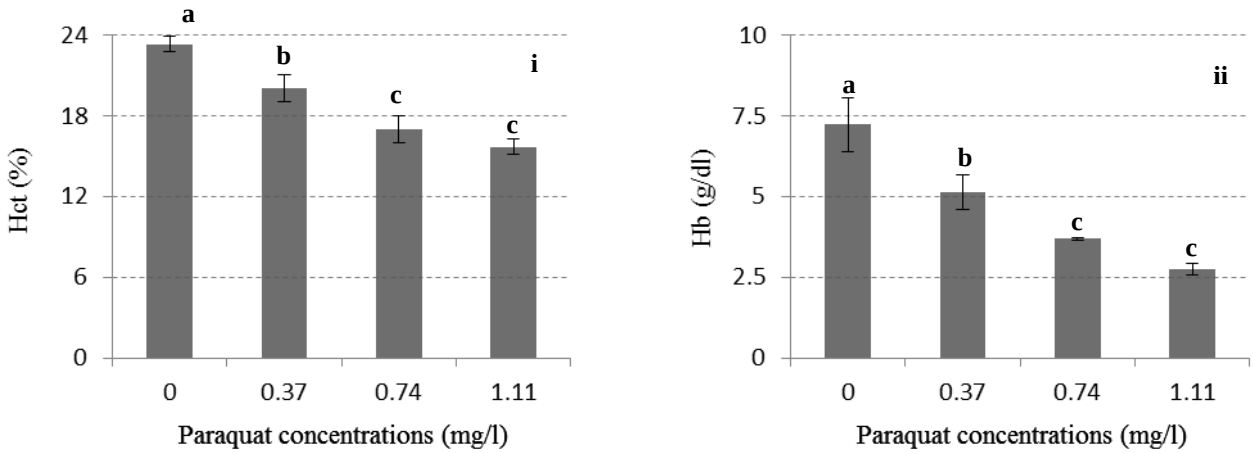

Fig. 2: Effect of paraquat on blood hematocrit (i) and hemoglobin (ii) of benny fish

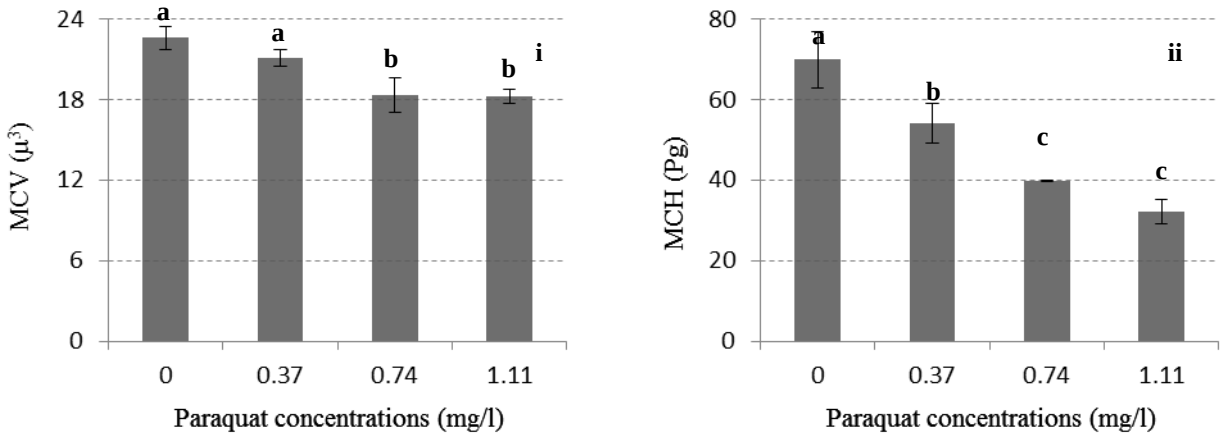

Fig. 3: Effect of paraquat on hematological indices: MCV (i) and MCH (ii) of benny fish

4. Discussion

Paraquat is a chlorinated herbicide which is mainly used to control weeds in agriculture, however once it enters surface waters it may affect other organisms such as fish as a non-target organism either in natural and culture conditions. Results of the present study clearly showed that paraquate exposure results in significant hematological alterations in benny fish. Following exposure to various concentrations of paraquat, the RBCs

count decreased up to 16.5 % (Table 1). Meanwhile Hct reduced to about 67 % of its natural level. The count of WBCs was also diminished by 45.6%. All mentioned alterations indicate that exposed fish suffered from anemia induced by the pesticide. This is an indication of disruptive effects of paraquat on erythropoietic tissues as well as cells viability [10]. It is also possible that paraquate adversely suppressed fish osmoregulation. The disturbed osmoregulation may finally results in dilution of blood [11]. Similar kind of actions has previously considered by other researchers for some pesticides such as diazinon [5, 12], Dichlorvos [13], malathion [14], and trichlorphon [15]. Erythrocytes and their hemoglobin contents are responsible for oxygen transportation within the body. Low number of red blood cells or insufficient amount of their hemoglobin content could influence energy balance of the body. In this case fish may suffer from oxygen deficiency [16], which ultimately prohibits its normal growth. Moreover it seems that reduction in red blood cells is a key factor which could be responsible for productivity reduction [17].

Table 1: The percentage of reduction in hematological parameters following 96h exposure of benny fish to paraquat.

Parameter	Paraquat concentration (in proportion to 96h LC_{50})			
	control	25%	50%	75%
RBCs	0	8.7	10. 7	16.5
Hb	0	29.1	48. 9	61.8
WBCs	0	15.8	26.3	45.6
Hct	0	14.3	27.1	32.9
MCV	0	6.5	18.7	19.4
MCH	0	22.5	43.0	54.0

Another adverse effect of paraquat on fish rises from decrease of leukocyte count. This study showed that following 96 h of paraquat exposure all exposed fish experienced lower leukocyte count. The percentage of such decrease (compared to controls) varied from 15.8 to 46.8% (Table 1). The white cells play a vital role in fish immunology. Generally fishes with low WBCs count are more susceptible for bacterial or fungal infections. It has been demonstrated that after acute exposure of *Cyprinus carpio* to pesticide diazinon, non-specific immunity of fish was significantly decreased due to leukocyte count reduction [12].

5. Conclusion

The present study showed that paraquat is clearly toxic to benny fish *Mesopotamichthys sharpeyi*. The exposure of fish to this pesticide resulted in significant reduction in the studied haematological parameters. These alterations may negatively suppress normal growth, reproduction, immunity and even survival of fish in natural environment as well as culture conditions. This should be considered when farmers use paraquat to control weeds in their fields. Replacing of paraquat with less harmful and more biodegradable pesticide is recommended.

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