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## Effects of malachite green (Triarylmethane dye) and Pyceze (Bronopol) on the hematological parameters of a freshwater catfish *Heteropneustes fossilis* (Bloch)

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### Abstract

Malachite green is an extensively used biocide in the aquaculture industry world-wide. It has been widely used due to lack of a proper alternative. Pyceze, however, is a pharmaceutical alternative to malachite green, with bronopol as its active ingredient. The catfish *Heteropneustes fossilis* were exposed for four days to 1/5<sup>th</sup> of LC50 values of each of malachite green (0.24mg/l) and Pyceze (0.42mg/l). Different hematological parameters were evaluated. After four days total red blood corpuscles (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscles hemoglobin concentration (MCHC) and mean corpuscles hemoglobin (MCH) values exhibited significant decrease whereas total white blood corpuscles (WBC), clotting time (CT) and mean corpuscles volume (MCV) values were increased in fish of both groups when compared with controls. The present study clearly indicates the toxic effects of these chemicals on hematological parameters of fish. However, exposure to malachite green was found to be more toxic than to Pyceze.

**Keywords:** Malachite green, Pyceze, *Heteropneustes fossilis*, hematological parameters.

### 1. Introduction

Dyes have been used in a wide variety of areas, viz. pisciculture, textile, paper, rubber, plastic, paints, drugs and cosmetic industries. With expanding demands and utilization of dye stuffs, health hazards to workers coming in their contact are steadily increasing. Malachite green is widely used in aquaculture as a parasiticide and in food, health, textile and other industries for one or the other purposes. The dye has generated much concern regarding its use, due to its reported toxic effects. The toxicity of this dye increases with exposure time, temperature and concentration. Malachite green is generally used as disinfectant in aquaculture industries for the control of fungal infections and other external parasites such as protozoans, trematodes and larvae of parasitic crustaceans of fish and fish eggs<sup>[1-4]</sup>. Malachite green is reported to cause carcinogenesis, mutagenesis, chromosomal fracture, teratogenicity, reduced fertility, developmental abnormality and other physiological changes in animals<sup>[5-7]</sup>. Despite being banned in several countries and by European commission, malachite green is still being used in many parts of the world due to lack of a proper alternative. Recently, a pharmaceutical alternative to malachite green, Pyceze with bronopol as its active ingredient has been developed in U. K. It is being used for the treatment of fish and their ova and appears to be a safe and effective replacement for malachite green in prevention of fungal infections<sup>[8-11]</sup>.

The LC0, LC50 and LC100 values of malachite green and Pyceze were estimated by Chaturvedi, *et al.*, 2012<sup>[12]</sup>. The behavioural responses to these toxicants were also observed by Srivastav and Chaturvedi 2012<sup>[13]</sup>, for malachite green and Pyceze. However there is not enough data on the effects of these chemicals on hematological parameters of fish. Therefore, the aim of the present study is to determine the effects of malachite green and Pyceze on some hematological parameters in a catfish, *Heteropneustes fossilis*.

### 2. Materials and Methods

The study was done during February, 2014 to April, 2014. Live specimens of adult *Heteropneustes fossilis* (Weight  $30.25 \pm 1.25$  gm, length  $12.75 \pm 1.50$  cm) were collected from local fish farm and acclimated to the laboratory conditions for ten days. They were fed daily with a mixture of wheat flour and ground dried shrimps (1:1). They were fed daily with a

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mixture of oil cake and rice bran (1:1). Food was withheld 24h prior to sampling of the fish and no feeding occurred 96h following exposure. The water used during experiment was analyzed as per the standard method [14]. The physico-chemical characteristics of test water were- pH 7.8 ± 1.2, dissolved oxygen 8.40 ± 1.60 mg/L<sup>-1</sup>, BOD 16.20 ± 1.85mg/L<sup>-1</sup>, hardness 136.60 ± 4.70 mg/L<sup>-1</sup> as CaCO<sub>3</sub>, chloride 6.90 ± 0.95 mM/l. A stock solution of both malachite green and Pyceze (Sigma chemical company, Mumbai) were prepared in distilled water. A group of 15 fish (5 fish/10 liter glass jar) were exposed to each of tested chemical concentration. One fifth of LC50 of both the chemicals were directly added into the water for four days. Parallel group of control fish were also used for control hematological data.

After 96h, fish from both the control and treated groups were sacrificed for hematological studies. On completion of fixed exposure periods, caudal peduncle of fish was cut off. The free flowing blood from the caudal artery was directly collected for the estimation of hematological parameters. Hematological parameters were estimated by standard methods [15]. The RBC (Red Blood Corpuscles) and WBC (White Blood Corpuscles) counts were made by improved Neubauer hemocytometer. Hemoglobin (Hb) content (g/100ml) determination was performed by Sahli's Hellige instrument (Sahli hemoglobinometer) using 0.1 N HCL. Packed Cell Volume (PCV), was measured by the method of Snieszko 1960 [16], using 75x1.00-1.25 mm capillary tubes. Clotting time of the blood was determined by the capillary tube method as used in clinical hematology [17]. MCV (Mean Corpuscles Volume), MCHC (Mean Corpuscles Haemoglobin Concentration) and

MCH (Mean Corpuscles Haemoglobin) values were calculated from Hb (Haemoglobin), PCV (Packed Cell Volume) and RBC (Red Blood Corpuscles) values by employing standard formula [18]. The data were analysed for statistical significance between the controls and dye exposed fish by student 't' test. Significance differences were established at the 0.05, 0.02, 0.01 levels.

### 3. Results and Discussion

When exposed for short term (4 days) to 1/5<sup>th</sup> of LC50 values of both malachite green and Pyceze, fish showed noticeable differences in hematological parameters (Table-1). Total RBC, Hb, PCV, MCHC and MCH values exhibited significant decreases whereas increase in total WBC, clotting time and MCV values were recorded. In controls, levels of RBC, WBC, Hb, PCV, clotting time, MCV, MCHC, MCH were recorded to be 3.70 ± 0.10 (x10<sup>6</sup>/mm<sup>3</sup>), 5.20 ± 0.07 (x10<sup>3</sup>/mm<sup>3</sup>), 11.20 ± 0.33(g%), 46 ± 2.58(%), 40 ± 0.82 (sec.), 124.32 ± 2.30 (cuμ), 24.34 ± 0.65 (%) and 30.27 ± 0.72(pico. gm) respectively. On the other hand, the levels of RBC, Hb, PCV, MCHC and MCH in malachite green exposed fish were 2.90 ± 0.12 (x10<sup>6</sup>/mm<sup>3</sup>), 8.4 ± 0.21 (%), 40 ± 1.82 (%), 21 ± 0.50 (%) and 28.96 ± 0.94 (pico. gm) respectively and in Pyceze exposed fish were 3.20 ± 0.19 (x10<sup>6</sup>/mm<sup>3</sup>), 9 ± 0.47 (%), 42 ± 1.83 (%), 21.42 ± 0.21 (%) and 28.12 ± 0.53 (pico.gm) respectively. The levels of WBC, clotting time and MCV in malachite green exposed fish were 6.30 ± 0.26 (x10<sup>3</sup>/mm<sup>3</sup>), 48 ± 1.73 (sec.) and 137.93 ± 3.53 (cuμ) respectively and in Pyceze exposed fish were 5.80 ± 0.14 (x10<sup>3</sup>/mm<sup>3</sup>), 44 ± 1.82 (sec.) and 131.25 ± 3.02 (cuμ) respectively.

**Table 1:** Hematological changes in *Heteropneustes fossilis* on acute level of malachite green 0.24 mg/l and Pyceze 0.42 mg/l, (1/5<sup>th</sup> of 96h LC50 values) for 96h.

Parameters	Control	Experimental	
		Malachite green	Pyceze
Total WBC (x10 <sup>3</sup> /mm <sup>3</sup> )	5.20±0.07	6.30±0.26p*	5.80±0.14*
CT (sec.)	40±0.82	48±1.73*	44±1.82
MCV (cuμ)	124.32±2.30	137.93±3.53*	131.25±3.02
Total RBC (x10 <sup>6</sup> /mm <sup>3</sup> )	3.70±0.10	2.90±0.12**	3.20±0.19
Hb (%)	11.20±0.33	8.4±0.21***	9±0.47*
PCV (%)	46±2.58	40±1.82	42±1.83
MCHC (%)	24.34±0.65	21±0.50**	21.42±0.21*
MCH (pico. gm)	30.27±0.72	28.96±0.94	28.12±0.53

All values are mean ± SE (n=4), \*P<0.05, \*\*P<0.02, \*\*\*P<0.01

Malachite green affects various hematological parameters and elicits anaemic responses in rainbow trout and *Clarias gariepinus* [19, 20]. Significant increase was observed in the total WBC count after exposure to malachite green and Pyceze. WBC plays a major role in the defence mechanism of fish. An immediate activation of the fish immune system is proved by increase in leucocytes [21]. Leucocytosis was also observed in other teleost fish after exposure to other toxicants [22-24]. An initial leucocytosis, which may be directly proportional to the severity of the causative stress conditions, may be attributed to an increase in leucocyte mobilization [25]. Srivastava and Singh have observed rise in WBC count following exposure to various insecticides [26]. In the present study also, WBC count increased following exposure to both malachite green and Pyceze.

In the present study a significant delay in blood clotting time was observed. Similar results have also been reported in other fish exposed to other toxicants [22, 23, 26-29]. An inverse relationship between thrombocyte count and blood clotting time has been reported [26]. The present study also shows a

significance increase in clotting time. Perhaps malachite green and Pyceze trigger a rapid mobilization of the haemostatic system and the fish normally appears to deal with it by adjusting blood clotting and thrombocyte concentration.

A significant increase in MCV values was observed in malachite green, exposed fish also reported by other workers [24, 30]. Significant rise in MCV and decline in erythrocyte count of the stress exposed fish have been reported by Varadraj, *et al.*, [31]. Verma and Panigrahi are of the opinion that increase in MCV value may be due to swelling of RBC and/or production of large number of lymphocytes and reduction in erythrocytes [32]. Svobodova, *et al.*, noticed decrease in MCV values [33]. In the present study, rise in MCV values seems to be correlated with decline in RBC count. A significant increase in MCV values was observed in both the chemicals.

In our experiment a significant decrease was observed in total RBC count after exposure to malachite green and Pyceze, and similar observation were reported for exposure to malachite green also reported by other workers [22, 24, 25, 27, 30, 32, 34]. Some

workers believe that reduction in total RBC count may be due to microcytic or normocytic anaemia<sup>[19-21, 24]</sup>.

A significant decrease was observed in hemoglobin after exposure to malachite green also reported by other workers<sup>[21, 22, 24, 30, 32]</sup>. Anemia can be caused by a number of pathological conditions such as lysis of erythrocytes, erythropenia<sup>[35]</sup> and increased sedimentation of erythrocytes<sup>[36]</sup>. In the present study, a significant decrease was observed in hemoglobin after exposure to malachite green and Pyceze, anaemic state of fish may be attributed to inhibition of erythropoiesis, disturbed hemoglobin synthesis and hemodilution as also reported by other workers<sup>[5, 19, 20, 22, 34]</sup>.

In the present study, we found a decrease in PCV value exposure to malachite green and Pyceze, were also similar with other workers, observed after exposure to malachite green<sup>[23, 24, 29, 30, 32, 33]</sup>. Similar reduced hemoglobin content and PCV have also been noticed by other workers<sup>[24, 34]</sup>. Change in PCV value have been associated with stress<sup>[30]</sup>, impaired osmoregulation<sup>[37]</sup>, impairment of gas exchange by gills<sup>[38]</sup> and increased sensitivity to hypoxia<sup>[33]</sup>.

In the present study a significant decrease was observed in MCHC values after exposure to malachite green and Pyceze were found similar by other workers also with malachite green<sup>[23, 24, 30-33]</sup>. It has been suggested that decrease in MCHC values was due to greater loss in hemoglobin<sup>[23, 24, 30, 32]</sup>.

The present study also shows a significant decrease in MCV values for both the chemicals also reported by Ramalingam, *et al.*, 2000<sup>[23]</sup>, after exposure to malachite green.

#### 4. Conclusion

The present study clearly indicates that the detrimental effects on hematological parameters are much less pronounced in case of Pyceze exposure than in case of malachite green exposure. Thus, Pyceze can be considered as a relatively safe alternative of malachite green in aquaculture production.

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