

## Some Blood Parameters of Albino and Normal Pigmented Rainbow Trout with Short Term Exposure to a Bath of Malachite Green

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### Abstract

The purpose of this research was to determine some haematological parameters of adult albino and normally pigmented rainbow trout (*Oncorhynchus mykiss*, Salmonidae) individuals after short-term exposure to a bath of malachite green. At the conclusion of the study, significant differences were observed in haemoglobin (Hb), erythrocyte (RBC), leukocyte (WBC) levels as well as mean cell volume (MCV) and mean cell haemoglobin (MCH) ( $p < 0.05$ ) between control and experiments groups. However no significant differences were found between the two groups in the levels of pH and Mean cell haemoglobin concentration (MCHC) ( $p > 0.05$ ) during exposure to the malachite green. It was also determined that the short term immersion in the bath of malachite green caused a significant increase in mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), a slight decrease in haemoglobin (Hb) and haematocrit (Ht); and a pronounced decrease in red blood cell (RBC) and white blood cell (WBC) levels..

**Keywords:** *Oncorhynchus mykiss*, Malachite Green, Haematological Parameters, Turkey.

## INTRODUCTION

The aquaculture industries have been employing malachite green ( $C_{23}H_5N_2$ ) extensively as a topical treatment through the use of bath or flush methods without paying any attention to the fact that topically applied therapeutants might also be absorbed systemically and produce significant internal effects. It is also used as a food colouring agent, food additive, a medical disinfectant and anti-helminthic as well as a dye in silk, wool, jute, leather, cotton, paper and the acrylic industries [1].

Short-term malachite green baths are treatments which last 1 to 1.5 h in duration at a bath concentration of 6.7 mg/l. If water temperature is below 10°C, the baths may be accommodated in different types of tanks. If water temperature exceeds that limit, baths should be conducted only in holding tanks that can be easily filled with water and emptied within 30 min. This type of bath has been used to control protozoan ectoparasites, particularly the ciliated protozoan *Ichthyophthirius multifiliis* [2]. Malachite green affects haematological parameters in fish: decreases in haematocrit values and anaemic responses have been reported in rainbow trout [3]. Decreases in monocyte count, haematocrit value and mean corpuscular volume and an increase in mean corpuscular haemoglobin concentration have also been noticed after exposure to malachite green [4]. However, elevated packed cell volume and haemoglobin values have been observed in rainbow trout exposed to malachite green [5].

Malachite green treatment has also been reported to result in severe haematological changes in juvenile salmonids, such as leucopenia (a decrease in the number of white cells) [6]. The effect of a general stress syndrome in the untreated control group apparently obscured any detectable changes [7].

In this research, it was aimed to determine some haematological parameters of adult albino and normally pigmented rainbow trout (*Oncorhynchus mykiss*, Salmonidae) individuals after a short-term therapeutic bath of malachite green.

## MATERIALS AND METHODS

### Experimental animals

For haematological testing, the mean length and weight of normally pigmented and albino rainbow trout were examined (Table 1). All fish were obtained from the fishery laboratory of the Kemaliye Hacı Ali Akın Vocational High School of Erzincan University in Turkey. They were fed with a commercial pellet food at 10°C, those in tanks were fed by hand at a rate of approximately 2% of fish body weight per day. In this study, the total number of fish was 60 (30+30) with 10 fish for each experimental group being placed in three different 1.5 m<sup>3</sup> aerated tanks. The groups consisted of one control and two experimental groups for both normal pigmented fish and albino fish. At a short therapeutic bath, the fish were exposed to 6.7 mg/L malachite green for 30 and 45 minutes, respectively. The malachite green was supplied from a commercial chemist. It is a triarylmethane dye ( $C_{23}H_5N_2O$ , CI 42,000), a dark green and crystalline solid prepared by condensing one part of benzaldehyde with two parts of diemethylaniline without zinc chloride. The physico-chemical parameters of the tank water used for the experiments were pH 7.2-7.5, dissolved oxygen 9.5-9.7 mg/L and temperature 10±1°C.

### Haematological analysis

After 30 and 45 minutes exposure, the fish under study and those in the control group were caught and blood was taken for the measurement of haematological parameters. Blood samples were collected by making an incision in the caudal blood vessels. A blood tube with heparin sodium salt was used for stabilization of the fish blood. Haemoglobin concentration was measured with Sahli-Hellige (Hb, g/dl), pH with Merck pH indicator papers and erythrocyte count (RBC,  $\times 10^6/\text{mm}^3$ ), leukocyte count (WBC,  $\times 10^4/\text{mm}^3$ ), haematocrit level (Ht, %), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were determined using routine methods [8].

### Statistical analysis

The statistical analysis of results was performed with SPSS 16.0 software and the significance of the data was determined by using one-way ANOVA and the Post Hoc Duncan test at 0.05 levels.

## RESULTS AND DISCUSSION

In this study, Group A (normal pigmented adult rainbow trout) and Group B (albino adult rainbow trout) were exposed to malachite green for 30 and 45 minutes. The weight of fish (Control, Group A and B) is presented in Table 1. Typical clinical symptoms include restlessness and uncoordinated movements among the fish in the tank. The fish move in the upper half of the tank and; leap above the water surface. The gills display rapid breathing, with excessive amounts of mucous matter and are discoloured by the agent. However, no fish was detected to be dead during exposure. The results of this study show that malachite green had some effect on the haematological parameters of each pigment of rainbow trout. It was determined that the short time bath of malachite green caused a significant increase in mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) as well as; a slight decrease in haemoglobin (Hb) and haematocrit (Ht) and a pronounced decrease in red blood cell (RBC) and white blood cell (WBC) levels (Table 3, 4).

Between the groups, significant differences were observed in haemoglobin (Hb), erythrocyte (RBC) and leukocyte (WBC) levels, as well as in mean cell volume (MCV) and mean cell haemoglobin (MCH) ( $P < 0.05$ ). No significant differences were detected in the levels of pH and MCHC ( $P > 0.05$ ) during exposure to the malachite green (Table 2, 3, 4).

According to our results, after short term exposure to malachite green (30 and 45 minutes), the occurrence of leucopenia (low count of WBC), anaemia (low count of RBC) and low haemoglobin and haematocrit levels could be detected in fish blood (Figure 1, 2).

Similar reductions of these parameters have been reported by some authors [9, 10, 11, 12, 13, 14] and some changes in the biochemical parameters of rainbow trout under exposure to high and low concentrations of malachite green. As was the case with this study, malachite green has been shown to cause an increase in lactate dehydrogenase and phosphor and a decrease in total protein and calcium

[15]. The significant reduction in these parameters is an indication of severe anaemia caused by malachite green on the exposed fish. The anaemic response could be a result of destruction of RBC or the inhibition of RBC production and it could be a result of the destruction of intestinal cells [16, 17, 18].

The use of malachite green has been banned in several countries and it is not approved by the US Food and Drug Administration [19]. Finally, as the results of this study reveal the deleterious consequences on the health of fish subjected to exposure of malachite green by short term bath, therefore we recommend that the substance should not be used for any purpose in aquaculture.

**Table 1.** Overall average weights and total lengths of fish in groups

Groups N=10		Weight (g) X $\pm$ SD	Total Length (cm) X $\pm$ SD
Normal Pigmented Rainbow trout (Group A)	Control	167.50 $\pm$ 21.67	24.60 $\pm$ 1.15
	30 minutes	152.70 $\pm$ 10.53	23.35 $\pm$ 0.47
	45 minutes	152.80 $\pm$ 8.73	23.00 $\pm$ 0.24
Albino Pigmented Rainbow trout (Group B)	Control	173.70 $\pm$ 19.75	23.45 $\pm$ 1.21
	30 minutes	138.60 $\pm$ 17.04	21.50 $\pm$ 0.53
	45 minutes	144.50 $\pm$ 20.58	21.40 $\pm$ 0.97

**Table 2.** ANOVAs test results between groups

Parameters	Df	F	Sig.
Haemoglobin (Hb, g/dl)	5	2.649	0.036*
Haematocrit (Ht,%)	5	0.270	0.927
pH	5	2.226	0.070
Erythrocyte (RBC, $\times 10^6/\text{mm}^3$ )	5	34.899	0.000*
Leukocyte (WBC, $\times 10^4/\text{mm}^3$ )	5	5.679	0.001*
Mean cell volume (MCV)	5	6.670	0.001*
Mean cell haemoglobin (MCH)	5	6.912	0.000*
Mean cell haemoglobin concentration (MCHC)	5	0.812	0.553

\*In the same row indicate significant differences between groups, ( $p < 0.05$ )

**Table 3.** Some haematological parameters of fish in groups

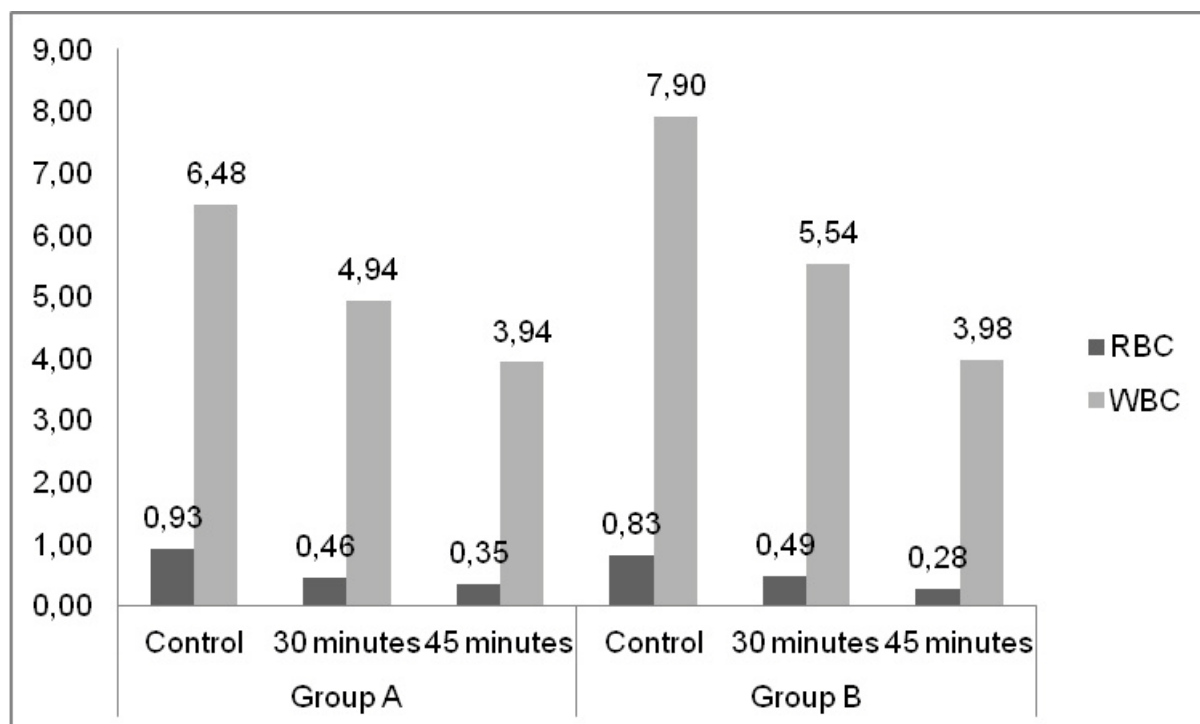
N=5			X±SD	Min.	Max.
Haemoglobin (Hb) (g/dl)	Group A	Control	8.03±1.38 <sup>ab</sup>	6.40	10.70
		30 minutes	8.13±1.65 <sup>ab</sup>	5.20	10.00
		45 minutes	9.15±2.12 <sup>b</sup>	6.90	14.20
	Group B	Control	7.16±0.79 <sup>a</sup>	6.10	8.80
		30 minutes	7.75±0.60 <sup>ab</sup>	6.90	8.40
		45 minutes	6.98±0.84 <sup>a</sup>	5.90	8.20
Haematocrit (Ht) (%)	Group A	Control	32.86±4.41 <sup>a</sup>	29.00	42.00
		30 minutes	30.30±12.02 <sup>a</sup>	10.00	49.00
		45 minutes	29.90±7.19 <sup>a</sup>	22.00	44.00
	Group B	Control	28.90±13.24 <sup>a</sup>	10.00	52.00
		30 minutes	29.20±7.26 <sup>a</sup>	20.00	37.00
		45 minutes	26.25±6.70 <sup>a</sup>	19.00	35.00
pH	Group A	Control	6.67±0.29 <sup>b</sup>	6.50	7.10
		30 minutes	6.35±0.24 <sup>ab</sup>	6.00	6.50
		45 minutes	6.35±0.24 <sup>ab</sup>	6.00	6.50
	Group B	Control	6.55±0.28 <sup>ab</sup>	6.00	7.00
		30 minutes	6.30±0.27 <sup>a</sup>	6.00	6.50
		45 minutes	6.38±0.25 <sup>ab</sup>	6.00	6.50
Erythrocyte (RBC) (x10 <sup>6</sup> /mm <sup>3</sup> )	Group A	Control	0.93±0.05 <sup>c</sup>	0.88	0.99
		30 minutes	0.46±0.08 <sup>b</sup>	0.35	0.54
		45 minutes	0.35±0.02 <sup>ab</sup>	0.33	0.38
	Group B	Control	0.83±0.09 <sup>c</sup>	0.71	0.92
		30 minutes	0.49±0.19 <sup>b</sup>	0.25	0.69
		45 minutes	0.28±0.09 <sup>a</sup>	0.16	0.36
Leukocyte (WBC) (x10 <sup>4</sup> /mm <sup>3</sup> )	Group A	Control	6.48±1.07 <sup>bc</sup>	5.30	8.20
		30 minutes	4.94±0.43 <sup>ab</sup>	4.50	5.40
		45 minutes	3.94±0.43 <sup>a</sup>	3.50	4.40
	Group B	Control	7.90±3.04 <sup>c</sup>	5.50	12.80
		30 minutes	5.54±0.87 <sup>ab</sup>	4.50	6.70
		45 minutes	3.98±0.96 <sup>a</sup>	2.90	5.00

Differences between average values are shown by different letters (a-c) on the same line (p&lt;0.05)

**Table 4.** MCV, MCH and MCHC values of blood of fish in groups

N=5			X±SD	Min.	Max.
Mean cell volume (MCV)	Group A	Control	361.16±69.23 <sup>a</sup>	308.51	471.91
		30 minutes	620.93±327.25 <sup>b</sup>	222.22	954.55
		45 minutes	815.80±111.39 <sup>b</sup>	705.88	972.22
	Group B	Control	317.23±128.78 <sup>a</sup>	211.11	535.21
		30 minutes	652.33±192.16 <sup>b</sup>	378.79	804.35
		45 minutes	795.08±106.82 <sup>b</sup>	666.67	900.00
Mean cell haemoglobin (MCH)	Group A	Control	89.62±15.15 <sup>a</sup>	68.09	108.08
		30 minutes	170.83±63.26 <sup>ab</sup>	115.56	265.71
		45 minutes	249.14±37.90 <sup>bc</sup>	218.42	314.29
	Group B	Control	85.88±13.84 <sup>a</sup>	67.78	97.18
		30 minutes	184.31±84.10 <sup>b</sup>	120.29	324.00
		45 minutes	285.33±125.25 <sup>c</sup>	183.33	462.50
Mean cell haemoglobin concentration (MCHC)	Group A	Control	25.23±5.09 <sup>a</sup>	20.24	33.44
		30 minutes	32.16±13.71 <sup>a</sup>	19.68	52.00
		45 minutes	31.19±7.76 <sup>a</sup>	25.15	44.00
	Group B	Control	29.02±6.53 <sup>a</sup>	18.16	35.50
		30 minutes	28.45±8.67 <sup>a</sup>	18.65	40.50
		45 minutes	38.55±17.86 <sup>a</sup>	21.85	61.67

Differences between average values are shown by different letters (a-c) on the same line (p<0.05)

**Figure 1.** Erythrocyte (RBC) and leukocyte (WBC) counts of fish blood after exposure malachite green

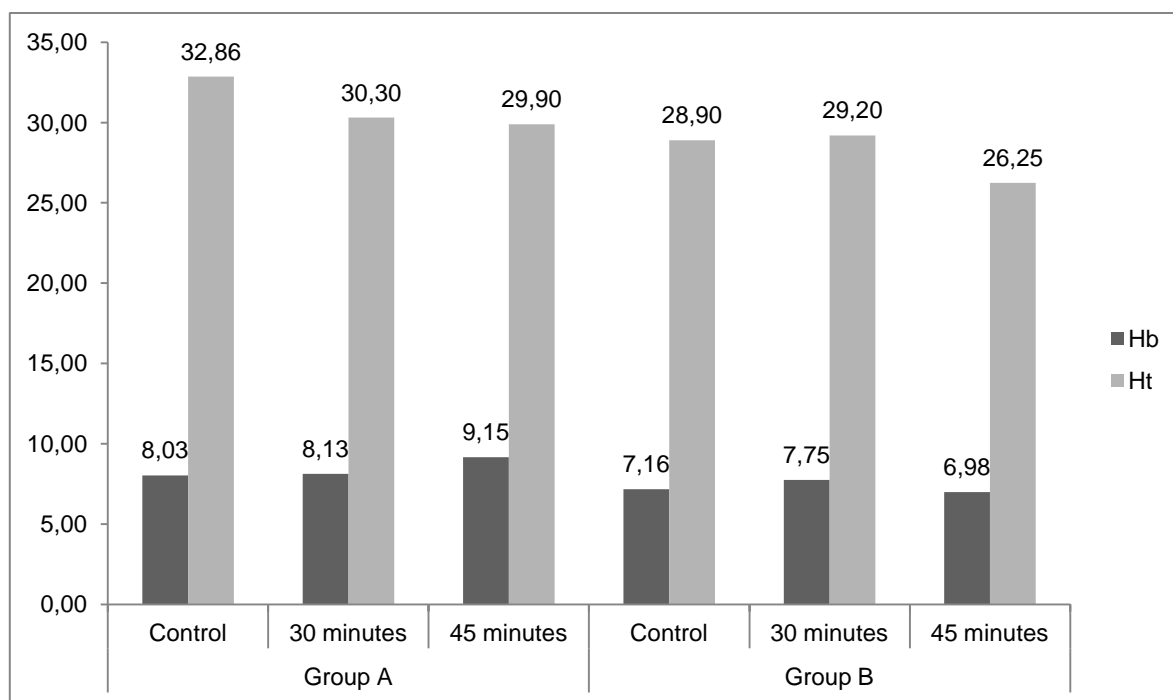


Figure 2. Haemoglobin (Hb) and Haematocrit (Ht) levels of fish blood after exposure malachite green

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