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The Effects of a Disinfectant (Malachite Green) on Blood Biochemistry of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: The effects of sublethal doses of malachite green on blood parameters of rainbow trout (*Oncorhynchus mykiss*) were examined. Fish were exposed two different sublethal doses of disinfectant dye. After 14 days exposure; lactate dehydrogenase (LDH), phosphor (P) increased, total protein (TP), calcium (Ca) decreased, alkaline phosphates (ALP) and sodium (Na) parameters took various values depending on the doses of malachite green.

Key words: Malachite green, rainbow trout, blood biochemistry, disinfectant

INTRODUCTION

Malachite green (MG) has the chemical name 4-(p-(dimethylamino)-α-phenylbenzylidene)-2, 5-cyclohexadien-1-ylidene dimethylammonium chloride and is used to treat fungi on eggs and fish and certain external protozoan and bacterial infections on fish (Boyd, 1992).

MG, originally used as a dyeing agent of textiles, was introduced as an ectoparasiticide, fungicide and antiseptic in aquaculture in 1933. The broad fungicidal and anti-parasitical spectrum and its efficacy in treating trout suffering from proliferative kidney disease have made the drug very popular among fish culturists (Bergwerff and Scherpenisse, 2003).

Malachite green is a synthetic dye used to colour silk, wool, jute, leather, cotton and paper. The name comes from the similarity of colour of malachite green to the mineral, malachite. Malachite green has traditionally been used to treat fungal infections on fish eggs. Leucomalachite, produced through the transformation of malachite green, may persist in fish tissues for long periods (Anonymous, 2005).

Malachite green is extremely toxic to fish and 0.1 mg L^{-1} may kill fish (Boyd, 1992). After administration, MG is prevalently reduced into leuco-malachite green in channel catfish and rainbow trout and deposited in fatty tissue of fish (Bergwerff and Scherpenisse, 2003).

Hematological values are widely used to determine systematic relationships and physiological adaptations including the assessment of the general health condition of animals and are more quickly reflected in the poor condition of fish than in other commonly measured variables (Reddy and Bashamohideen, 1989).

The aim of this study is to determine the effects of malachite green (widespread disinfectant in aquaculture) on blood biochemistry of rainbow trout.

MATERIALS AND METHODS

Fish Source, Maintenance and Water

Rainbow trout (*Oncorhynchus mykiss*) were obtained from Atatürk University, The Faculty of Agriculture, Fisheries Section, Trout Breeding And Research Center. Fish (both sexes weighing 135±25 g) were acclimated to laboratory conditions for two weeks. They were maintained in fiberglass tanks (600 L volume), which have 0.6 L min kg^{-1} fish, fresh water flow and wastewater discharge.

Water temperature was 9.5±0.5°C during the experiment. There were three groups (2 tanks with sublethal doses of malachite green and a control tank) and each group has eight fish. Experiment doses were regulated as 0.1 mg L^{-1} (1/10 of lethal concentration) and 0.05 mg L^{-1} (1/20 of lethal concentration) (Srivastava *et al*., 1995). During acclimatization and experiment, fish were fed with trade trout pellets. After 14 days exposure blood samples were collected and analyzed.

Blood Collection and Biochemical Analyses

Blood was collected from the caudal vein of fish and set to vacuum-operated biochemical tubes (Blaxhall and Daisley, 1973; Bridges *et al*., 1976; Pottinger and Carrick, 1999). Blood samples centrifuged at 4.000 rpm for 10 min (Bricknell *et al*., 1999), then analyzed in autoanalyser (Merck-Mega/Toshiba).

Statistical Analyses

Differences among the groups were statistical tested with variance analyses and the averages of groups analyzed with Duncan's test.

RESULTS AND DISCUSSION

Alkaline phosphates (ALP) level of control group was found as 125.33±19.39. The high concentration (HC) of malachite green decreased ALP (83.83±13.71) but low concentration (LC) increased (131.33±13.71) (Table 1). The difference between control and HC was important. Ahmad *et al*. (1995) and Atamanalp *et al*. (2002a) reported that pesticide exposure had increased fish ALP. Lactate dehydrogens was increased in two sublethal doses too. This result was found parallel with Asztalos *et al*. (1990), Shakoori *et al*. (1991) and Shakoori *et al*. (1994). According to these researchers, the exposure of different chemicals increased the LDH value of different fish species. But Mughal *et al*. (1993) and Shakoori *et al*. (1996) reported that fenvalarate decreased LDH of *Ctenopharyngodon idella*. This difference between these reports and the result of this experiment may be originated from the fenvalarate pesticide or fish species.

Total protein was found as 5.20±0.52 and decreased according to doses of MG (4.35±0.37 in LC and 4.08±0.37 in HC). Similarly, calcium level felt from 16.85±0.60 (control group) to 10.68±0.42 (LC group) and 10.95±0.42 (HC group). The differences in Ca parameter were very important but not the TP differences. Acute toxicity of MG decreased significantly total protein and calcium level of catfish (Srivastava *et al*., 1995). Besides, some researchers reported that different chemicals decreased total protein levels of different fish species: Malathion-*Cyprinus carpio* (Reddy and Bashamohideen, 1989); mercury chloride-*Ctenopharyngodon idella* (Shakoori *et al*., 1991); Fenvalarate-*Ctenopharyngodon idella* (Mughal *et al*., 1993) and mercury-*Ctenopharyngodon idella* (Shakoori *et al*., 1994). Atamanalp *et al*. (2002) reported that cypermethrin (a synthetic pyrethroid) decreased calcium of rainbow trout. So, our experiment's result is harmonic with all these reports.

Serum phosphor level of rainbow trout increased with MG exposure. This was an opposite outcome with Atamanalp *et al*. (2002b) who reported P decreased with cypermethrin. Sodium became

Table 1: General results of experiment

Parameters	ALP	LDH	TP	Ca	P	Na
	$\bar{X}$ -S $\bar{x}$	$\bar{X}$ -S $\bar{x}$	$\bar{X}$ -S $\bar{x}$	$\bar{X}$ -S $\bar{x}$	$\bar{X}$ -S $\bar{x}$	$\bar{X}$ -S $\bar{x}$
LC	131.33±13.71^{a}	3529.17±796.95^{a}	4.35±0.37	10.68±0.42^{b}	17.17±3.59	151.33±4.77
HC	83.83±13.71^{b}	3865.50±796.95^{a}	4.08±0.37	10.95±0.42^{b}	17.57±2.38	160.67±4.77
Control	125.33±19.39^{a}	1434.00±1127.05^{b}	5.20±0.52	16.85±0.60^{a}	14.95±1.00	156.00±6.75
Importance	*	*	NI	**	NI	NI

LC: Low concentration HC: High concentration, NI: Not important *: Important **: Very important less with LC but increased with HC. According the conclusion of Atamanalp *et al.* (2002b), ½ lethal dose of pesticide increased Na but 1/8 lethal dose decreased. Therefore, the result of this experiment is appropriate with this report.

Serum phosphor level of rainbow trout increased with MG exposure. This was an opposite outcome with Atamanalp *et al.* (2002b) who reported that cypermethrin decreased P. Sodium became less with LC but increased with HC. According the conclusion of Atamanalp *et al.* (2002b), ½ lethal dose of pesticide increased Na but 1/8 lethal dose decreased. Therefore, the result of this experiment is appropriate with this report.

Research has indicated that malachite green can be toxic to human cells and promotes liver tumor formation in rodents. Due to potentially harmful human health effects, the U.S. Food and Drug Administration nominated malachite green as a priority chemical for toxicity and carcinogenicity testing in 1993. The results of the rodent studies found liver toxicity, anemia and thyroid abnormalities. The significance of these results to human health is unknown at this time. It is very unlikely that adverse effects will result by eating fish with the very low levels of malachite green and leucomalachite green detected. Other countries have established minimum performance levels for sampling (Anonymous, 2005).

Malachite green is toxic to fish but there is less known or expected human health effects from consuming fish products at the low levels detected in farmed salmon and trout. So it is important to bring up new researches with this chemical.

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