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Metabolic Effects of Malachite Green on *Clarias gariepinus* Juveniles (Burchell, 1822)

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Abstract: *Clarias gariepinus* juveniles were exposed to sub-lethal malachite green concentration of 0.15 mg L^{-1} at different exposure period of 6, 24, 48, 72 and 96 h to assess the metabolic response of fish to malachite green. Biochemical parameters of the fish revealed that glucose, cholesterol, triglycerides, sodium (Na^+), potassium (K^+) and urea showed significant differences ($p<0.05$) between the fish exposed to malachite green and the control fish. After 6 h of exposure, Na^+, K^+, glucose and creatinine increased significantly ($p<0.05$). There was a slight increase in uric acid content at 6, 24, 72 and 96 h, respectively but was not significantly different from the control. The observed metabolic response of *Clarias gariepinus* juveniles to malachite green suggest that fish encountered toxic stress and metabolic crisis when exposed to sub-lethal dose of malachite green. It is therefore recommended that treatment of *C. gariepinus* juveniles with 0.15 mg-L sub-lethal dose of malachite green should not exceed 24 h.

Key words: Malachite green, *Clarias gariepinus*, metabolism, biochemical parameters, toxicity

INTRODUCTION

Aquaculture is fast developing in Nigeria as a major source of animal protein (Obasa and Faturoti, 2001; Omitoyin, 2005). The safety consumption of aquaculture products is therefore an issue of public health concern. However, water pollution problem associated with toxic materials rank highest and has aroused global concern as sources of environmental threat to both human and other animal populations which interact with the aquatic environments (Biney *et al*., 1987; Svensson *et al*., 1995; Abdelmeguid *et al*., 2002).

FAO/WHO experts' committee on food additives includes dyes in the list of non-permitted (inedible) dyes and classified it in the group C II according to toxicological categorization which reflects that no long term effects of the dye are known (Goel and Sharma, 1988; Joint FAO/WHO, 2005, 2006).

There are various researches on toxic responses of animals to azodyes (Prasad and Rostogi, 1983; Goel and Sharma, 1988). Malachite green is one of the pond management tool used in aquaculture as disinfectant for prophylactic treatment of fry, fingerlings and adult fish and for control of fungal infection (Brown, 1993; Omitoyin, 2005); they are not totally safe to the fish, fish farmer and their environment. In some cases, this is because of their tetratogenic effects (Meyer and Jorgenson, 1983; Schreier *et al*., 1996) on fish.

Several researchers agreed on the need for reliable methods to assess the impact of chemical pollution on aquatic habitats which has generated an increasing interest as biochemical indicators of toxicant exposure and their effects in fish (Abdelmeguid *et al*., 2002). Protein biosynthesis and serum, lipids, cholesterol are to provide an early, warning of toxicity (Casarett and Doull, 1975; Abdelmeguid *et al*., 2002).

Hilmy *et al.* (1987) and Abdelmeguid *et al.* (2002) found an increase in cholesterol content of serum and liver in fish following toxicant toxicity. Elevated levels of creatinine in serum were reported in fishes where dissolved oxygen was depleted due to the presence of pollutants in water body (Saad *et al.*, 1973; Abdelmeguid *et al.*, 2002).

A few reports limited to aquatic animals have been published on dyes (Sharma *et al.*, 1982; Goel *et al.*, 1982; Goel and Sharma, 1988). There is scanty information on metabolic response of *Clarias gariepinus* to malachite green which is often use in hatchery management. This study therefore investigates the metabolic response of *Clarias gariepinus* juveniles exposed to sub lethal dose of malachite green for 96 h.

MATERIALS AND METHODS

Collection and Acclimatization of Experimental Fish

This study was carried out in the Research laboratory of the Department of Wildlife and Fisheries Management, University of Ibadan, Nigeria.

One hundred and eighty juveniles of *C. gariepinus* of the same parent stock (mean weight 12.5 g ±0.15, mean length 10.34 cm ±0.16) were obtained from a commercial fish farm in Ibadan, Nigeria. The farm has no history of pollution and the fish were collected in the morning between 7.00 and 9.00 am through seining. They were immediately transported to the laboratory in oxygenated plastic bag filled with the water of the pond from which the fish were collected.

In the laboratory, the fish were acclimatized for three weeks before the experiment. During these periods, the fish were kept in three circular fiber glass tanks of 1.0×0.5×0.5 m dimensions. The test fish were fed in the laboratory with a commercial floating pellets (Coppens feed) containing 45% crude protein. They were fed twice daily at 3% of their body weight. The tanks were supplied with a continuous flow of dechlorinated tap water (mean temp. 27.0°C ±0.24, dissolved oxygen 7.6±0.04.0 mg L^{-1}, pH 7.0±0.03 and mean total hardness 96.7 mg L^{-1} ±1.89) at the flow rate of 1 L min^{-1}. The fish were starved for 24 h before the commencement of the bioassay. Only fish of similar size were selected from acclimatization tanks into pre-experiment holding tanks for bioassays.

Bioassay Techniques

Sub-lethal test concentration of 0.15 mg L^{-1} of malachite green (Zinc free) was used for this study. This was based on 96 h LC_{50} of 3.0 mg L^{-1} of malachite green recorded for most fishes as reported by Svobodová *et al.* (1993). Static renewal bioassay technique was adopted in which the test media (toxicant and dilution water) was renewed at the same concentration once every 48 h (Solbe, 1995). For this study, the fish were kept in 80×40×40 cm plastic tanks. The toxicant was first dissolved in 0.5 L of water part of the total water per tank (16 L of water), before being introduced to the whole. To evaluate the effects of sub lethal concentration of this toxicant on the test fish, fifteen test fish were stocked per tank. Each treatment was replicated thrice while fish in tank without malachite green serve as control. The fish were subjected to 12 h light and 12 h darkness.

Blood samples from test fish were collected at 0, 6, 24, 48, 72 and 96 h into heparinised bottles according to the method of Morgan and Iwama (1997). Plasma sodium and potassium were analyzed in the blood samples by using flame emission photometry. Glucose was determined after enzymatic oxidation in the presence of glucose oxidase (Morgan and Iwama, 1997). The plasma urea, uric acid, triglycerides and creatinine by the standard methods described by Coles (1986), while cholesterol was determined by the direct method described by Hrubec *et al.* (1996) and Abdelmeguid *et al.* (2002).

Statistical Analysis

The data from the treatments were subjected to one-way analysis of variance (ANOVA) test to determine the level of interaction among the treatments. All the tests were carried out by using STATISTICA for windows XP 2000 on PC (Linea version).

RESULTS AND DISCUSSION

Plasma glucose level, cholesterol and triglyceride increased significantly in fish exposed to 0.15 mg L^{-1} of malachite green compared to control. Increased plasma urea was observed in all treatments compared to control, although only fish exposed to malachite green for 72 and 96 h has values which were significantly different from others. There were no significant differences in uric acid concentration in all the treatment. Plasma creatinine values shows no significant differences in fish exposed to 0.15 mg L^{-1} of malachite green at 6, 24 and 48 h however those at 72 and 96 h has values which were significantly higher. The values of sodium increased significantly ($p<0.05$) from 136.6±0.90 14 mmol L^{-1} in control fish to 145.0±7.02 mmol L^{-1} in fish exposed for 96 h. The mean values of potassium also significantly increased during the 96 h of exposure from 35.5±1.62 mmol L^{-1} in the control fish to 45.20±2.14 mmol L^{-1} in fish exposed to 96 h of 0.15 mg L^{-1} of malachite green (Table 1).

Quality of water used in the present study are within the optimal range reported by Viveen *et al.* (1985) and Omitoyin *et al.* (2006) as optimal requirement for *C. gariepinus*, thus suggesting that parameters seems not to affect the toxicity of the malachite green to the test fish.

A constant sub-lethal concentration of 0.15 mg L^{-1} of malachite green in the surrounding water for 96 h appears to be physiologically stressful to the fish. Blood sugar level was elevated in fish during the exposure to various pollutants including fungicide used in aquaculture to control outbreaks of water borne fungal infections (saprolegniasis) on fish and fish eggs (Schreier *et al.*, 1996).

Stressful behaviour elicits rapid secretion of glucoseticoids catediolamines from the adrenal tissue of fish; both hormones produce a rapid hyperglycemia. The hyperglycemia observed in *C. gariepinus* juveniles exposed to malachite green at sub-lethal concentration might be as a result of glycogenolysis in muscle and liver causing a significant increase in blood glucose level. This agreed with Goel *et al.* (1982) and Sharma *et al.* (1982) that observed biochemical changes in blood glucose of *Heteropnestes fossilis* under the stress of Congo dye and enzymological changes in the liver and kidney of two teleost fishes under toxication with diphenyl disazobinaphthionic acid (dye).

After exposure to sub-lethal of malachite green fishes were stressed. During stress, fish need more energy to detoxify the toxicants and try to minimize their toxic effect (Tiwari and Singh, 2005). Carbohydrate represents the principal and immediate energy source while protein is the energy source to spare during chronic periods of stress (Umminger, 1977; Tiwari and Singh, 2005).

The level of lactic acid content is acting as an index of anaerobiosis, which might be beneficial for animal to tolerate hypoxic condition during stress (Tiwari and Singh, 2005). Although the reduction in the glycogen level supposes to be the result of greater stress the organs experienced during the processes to detoxification of active moieties and their metabolites. This agreed with Yadav *et al.* (2003) that observed metabolic changes in freshwater fish *Channa punctatus* due to stem-bark extract of *Croton tiglium*.

Exposure of *C. gariepinus* to malachite green in this study suggests that lipid metabolism was impaired, similar results were described by Yadav *et al.* (2003) and Tiwari and Singh (2004, 2005), respectively.

Table 1: Plasma biochemical values of *C. gariepinus* juveniles exposed to sub-lethal concentration of malachite green

Parameters	Control	6 h	24 h	48 h	72 h	96 h
Glucose (mg dL^{-1})	22.17±1.50a	24.33±2.14a	25.93±2.25a	29.47±3.31b	31.17±4.74bc	32.33±6.18bc
Cholesterol (mg dL^{-1})	78.13±11.20a	83.90±4.43b	88.90±8.47c	93.57±9.94d	100.70±14.88e	94.30±9.98d
Triglycerides (mg dL^{-1})	66.67±0.58a	68.97±0.84a	73.33±4.92b	79.87±7.82c	85.27±13.31d	82.57±13.78d
Urea (mg dL^{-1})	11.17±0.55a	11.27±1.55a	12.43±1.72a	13.43±1.40a	15.33±2.29b	15.60±2.62b
Uric Acid (mg dL^{-1})	3.10±0.53a	3.27±0.65a	3.20±0.17a	3.43±0.33a	3.63±0.67a	3.73±1.27a
Creatinine (mg dL^{-1})	0.57±0.12a	0.50±0.1a	0.68±0.08b	0.53±0.06a	0.73±0.11b	0.75±0.09b
Sodium Na^{+} (mmol dL^{-1})	136.60±0.90a	137.50±0.61a	144.50±3.7b	145.10±2.73b	147.70±2.77b	145.00±7.02b
Potassium K^{+} (mmol dL^{-1})	35.50±1.62a	45.94±3.05b	42.90±2.46b	43.07±0.32b	42.67±2.93b	45.20±2.14b

Means with the same alphabets along the horizontal row are not significantly different ($p>0.05$) from each other

An increase in blood urea and uric acid level as observed in *C. gariepinus* after exposure to malachite green in this study may suggests that probably proteins are being used to meet the increased energy demands during pesticide intoxication as described by Tiwari and Singh (2004). An accelerated rate of protein catabolism would result in an increase of amino groups released from amino acids which was later converted to uric acid and eventually to urea in detoxification process that takes place in the liver. This biochemical process occurs in mud catfish as well as in other animals containing the enzymes uricase.

The present study suggests that exposure of *C. gariepinus* juveniles to 0.15 mg L^{-1} sub-lethal dose of malachite green caused the fish to encountered metabolic crisis as a result of changes in blood plasma levels of various metabolites which indicates manifestation of hyper metabolic state. It is therefore recommended that treatment of *C. gariepinus* juveniles with 0.15 mg-L sub-lethal dose of malachite green should not exceed 24 h.

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