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Research Paper

MALACHITE GREEN INDUCED ACUTE TOXICITY IN *Cyprinus carpio*

Sinha, Reshma and Rajinder Jindal

Aquatic Biology Laboratory,
Department of Zoology,
Panjab University, Chandigarh, 160014,
India.

Abstract

Colours are essence of Indian tradition. Diverse use of colours in textile, paper, plastic, wool and confectionaries industry leads to high production of dyes. More than 20,000 dyes have been reported worldwide. About 16 lakh tons of dyes are produced annually. India with annual production of 2 lakh tons, accounts for 12.5% of world share, being second in position after China. These dyes and effluents containing dyes from industries have also emerged as aquatic pollutants. These affect the pH, DO, BOD and aesthetic sense of water. Malachite green is a widely used, easily available and cheap dye. It has wide uses, as besides colouring agent can be used as therapeutant in aquaculture industry which leads to its increased use. Inspite of all these it can cause genotoxicity, histopathological and biochemical alterations in aquatic organisms. Present study was aimed to evaluate the acute toxicity after exposure of malachite green for 96 h in Cyprinus carpio and evaluating behavioural response.

Key words: *Malachite green, dye, LC_{50}, behavioural, chronic toxicity*.

INTRODUCTION

India is colourful, colours have become an integral part of our tradition. Dyes are used as colouring agent in textile, leather, paper, wool and confectionary [1] industry. Dyes are used in idol making, toy making and in aesthetics. With the chemical advancement, new synthetic colours are being regularly developed and added. Dyes and dye intermediates consist of basic dyes; azo acid and direct dyes; disperse dyes; fast colour bases; reactive dyes; sulphur dyes; vat dyes; organic pigments; naphthols; and optical brighteners. Dyestuff sector is among major chemical industries in India. It is also the second highest export segment in chemical industry. The Indian dyestuff industry is made up of about 1,000 small scale units and 50 large organized units, who produce around 1,30,000 tonnes of dyestuff. Maharashtra and Gujarat account for 90% of dyestuff production in India due to the availability of raw materials and dominance of textile industry in these regions.

Dyes while developing a new bright world have also started leaving remarkable negative impact on ecosystem. These dyes and effluents containing dyes from industries have emerged as aquatic pollutants. These affect the pH, DO, BOD and aesthetic sense of water which not only affects the water quality, but also raised concern as health hazard.

Malachite green is a tri-aryl methane dye is widely used, easily available and cheap dye. It is being regularly used in textile, wool, paper and confectionary industry. Besides colouring agent, is an effective disinfectant against bacterial and protozoan diseases which leads to its increased use [2]. Some of the common fish diseases such as "wools" disease (*Icthyophthirius*) and "white spot" (*Saprolegniasis*) can be effectively cured after giving curative bath [3]. Inspite of all this it is classified as class III hazardous dye (W.H.O.) and is even banned in European countries [4] but due to its effective colour and price, is being regularly used in India [5]. It can cause genotoxicity, histopathological and biochemical alterations in aquatic organisms [5]. Malachite green being cationic in nature can directly bind to DNA forming DNA adduct. Also on being metabolised get converted to non-green, colourless compound, leucoform *i.e.* leucomalachite green. This leucoform has higher half time than malachite green and being lipophilic get easily attached to lipid molecules and retained for longer duration. On metabolism it produces reactive oxygen species. Thereby inducing oxidative stress, altering metabolic system in malachite green exposed organisms.

Cyprinus carpio is a hardy, exotic fish. It is widely cultivable fish, can tolerate wide range of temperature and physico-chemical parameters of water. In the present experiment, acute toxicity of malachite green was evaluated in the *Cyprinus carpio.*

MATERIALS AND METHODS

Procurement and acclimatization of fish Healthy fingerlings of *Cyprinus carpio*. (length, 10±2 cm; weight, 10±5gm) were procured from Sultan Singh Seed Farm, Karnal (Haryana). These were acclimatized for 15 days in a glass aquarium at temperature, 23±1˚C; pH 7.2± 0.2; and DO, 8±2 mg/l under laboratory conditions and were fed by pelletized feed (Gold Tokyo), manufactured by Tairopet Products Pvt. Ltd., Chennai, India.

Toxicant

Malachite green oxalate ($C_{48}H_{50}N_4O_4.2HC_2O_4$), 90% pure of analytical grade was purchased from CDH Laboratories, New Delhi, India. Stock solution of dye was prepared in distilled water for exposure to fish.

Toxicity test

Acute toxicity test: APHA [6] guidelines were followed to study the toxic effect of malachite green on the fish. Bioassay toxicity tests have been carried out in plastic syntax tank of 60 L capacity. Fish were exposed to varying concentrations (0.4-0.5 mg/l) of malachite green oxalate for 96 h at 23±1˚C temp. and 8±2 mg/l DO. Experiment was carried out in triplicates and mortality was observed for calculation of LC_{50} malachite green. LC_{50} was determined by Probit analysis [7]. Maximum allowable toxicant concentration (MATC), was also calculated [8].

RESULTS

Acute toxicity bioassay: 96 h LC_{50} was determined considering different toxicant concentration and percentage of mortality using SPSS software. Value obtained after probit analysis was 0.438 ppm (Fig. 1& 2). This concentration is lethal for the 50% of the fish population. MATC, is harmless (safe) concentration of malachite green with no

adverse effects on organisms. Harmless concentration (MATC) of malachite green came out to be 0.0043 mg/l.

S.No.	Dye conc. (mg/l)	Total no. of Fish	Mortality %
1	0.40	6	16.7
2	0.42	6	33.3
3	0.44	6	50.0
4	0.46	6	66.7
5	0.48	6	83.3
6	0.50	6	100

Fig.1. Table showing percentage mortality of fish on exposure to different concentrations of malachite green.

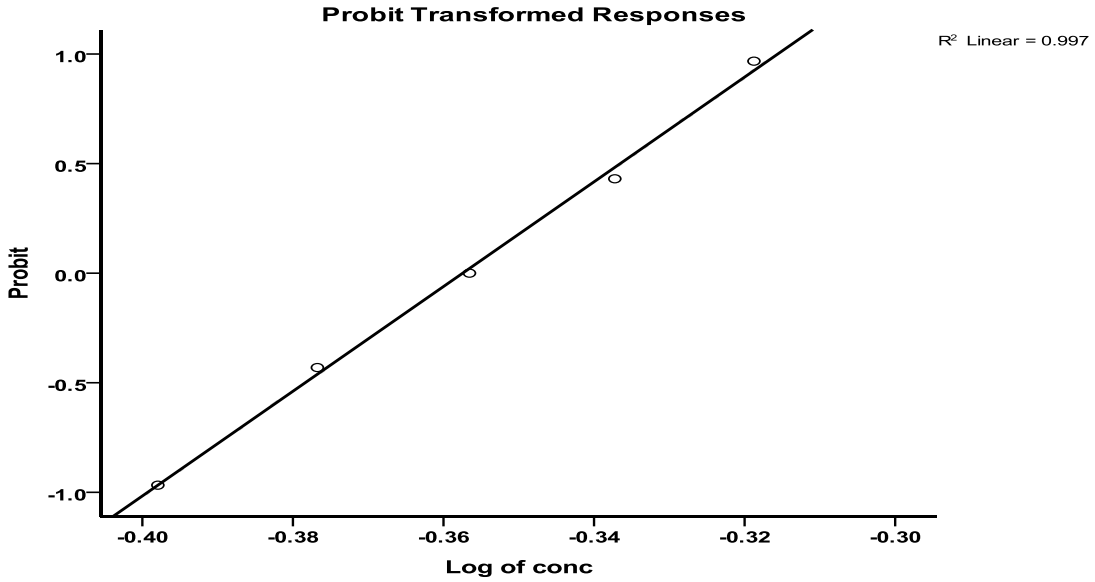

Fig.2. Graph showing probit transformed response in malachite green induced mortality in *Cyprinus carpio.*

Morphological changes observed were increased mucous secretion, oedema of epithelial layer of cornea, blood deposition at the base of fins and near cephalic region along with shedding of scales (Fig. a and b). Another significant change observed was greenish tinge of fish skin color at higher concentrations of dye. Behavioural observations comprised of jerking, increased opercular movement, jumping out of tank and loss of balance.

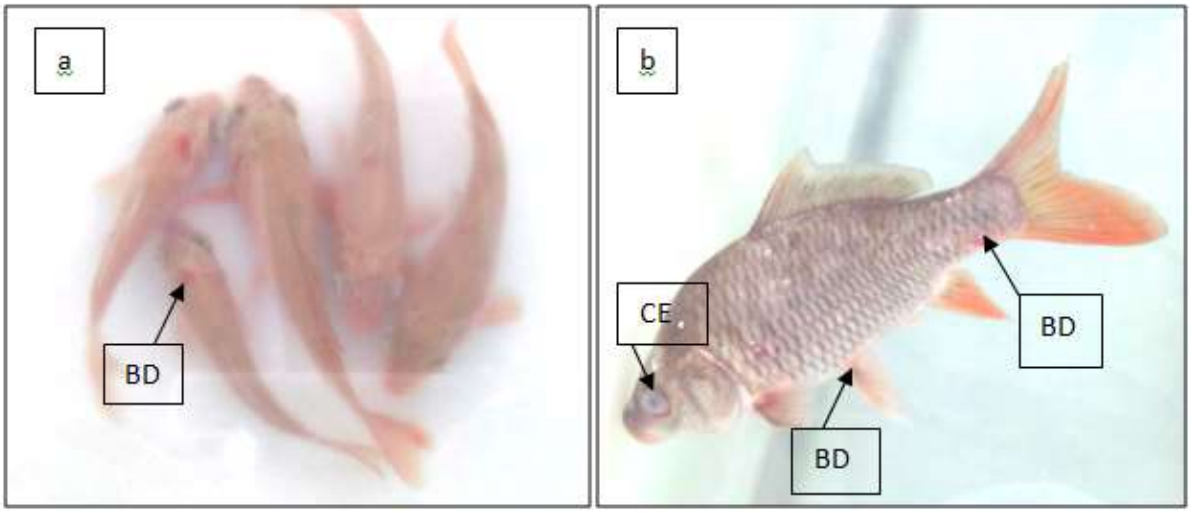

Fig.3. Depicting morphological changes after acute malachite green toxicity.
Abbre: BD-blood deposition, CE-corneal epithelial swelling.

DISCUSSION:

LC50 is lethal concentration of malachite green at which there is 50% mortality of organism. LC_{50} differ for organisms, depending on the species and size of fish; the pH and temperature of the medium. Toxicity of the malachite green observed to increase with increase in temperature and pH of the water [9]. Acute toxicity determines the short term toxic effect of malachite green in fish. Acute toxicity, 96 h LC_{50} for *C. carpio* came out to be 0.438 mg/l of malachite green, which was higher as compared to that (0.013 mg/l) of [10] in *C. carpio*, this increase can be inferred due to size of fish. The LC_{50} values for 24, 48, 72 and 96 h were 5.60, 1.40, 1.25 and 1.00 mg/l, respectively in *H. Fossilis* as per study of [11].

Walthall and Stark, [12] studied the acute toxicity of two xanthene dyes, Fluorescein and phloxine B to *Daphnia pulex* following 48 h exposure, LC_{50} values were (337 mg/l) and (0.423 mg/l) respectively. Phloxine B was found to be almost 800 times more toxic to *Daphnia pulex* than Fluorescein sodium salt.

Bae and Freeman [13] demonstrated the biological toxicity of the direct azo dyes used in the textile industry. The results indicated that C. I. Direct Blue 218, was very toxic to daphnids, with a 48 h LC_{50} between 1.0 and 10.0 mg/L. Epolito et al. [14] reported acute toxicity value of reactive blue 4 (anthraquinone dye) as 1500 mg/l in fish by EPI suite. Studies were made on toxicity of five azo dyes viz. mordant black 17, direct red 2, direct blue 14, reactive red 4 and reactive yellow 2 [15]. They noticed mortality of freshwater shrimps, *Desmocaris trispinosa* with the increase in exposure time and concentrations of dyes. By performing acute toxicity test using chemical dye effluent, Sponza [16] reported that the bacteria and fish (*Poecilia reticulata*) were the most sensitive organisms with the EC_{50} values of 0.02% and 0.2%, respectively. The relative toxicity of six xanthene dyes viz. rose bengal, phloxine B, erythrosin B, eosin yellow, rhodamine B and fluoroscein considering LC50 value in *Gambusia affinis* was studied [17]. All this indicates towards the initial invaluable indication towards the increasing toxicity of dyes in aquatic ecosystem.

Srivastava et al. [11] observed elicited hyperactivity characterized by rapid pectoral and opercular movement, erratic swimming and gradual loss of equilibrium associated with breathing difficulties in *Heteropneustes fossilis* after exposure to acute assay which is in accordance with the behavioural observations observed in present

study. Srivastava et al. [5] reported dye induced morphological and behavioural alteration in rainbow trout like restlessness and uncoordinated movements of the fish in the tank followed by the loss of balance, apathy, agony and death. Intoxication in fish also resulted in greenish tinge of fish skin, increased production of skin slime and oedematous gills with excessive amounts of mucous matter.

In regard to the presumably harmless (safe) concentration of malachite green, it was reported to be 0.025 mg/l for the catfish, *H. fossilis* [11] and was 0.0032 mg/l for the fingerlings of *C. carpio* [10] which was considered safe to common carp fingerlings and other Indian freshwater fingerlings. These are in close relation to the present findings (0.004 mg/l). Allen et al. [18] reported rapid absorption and high concentration of malachite green in the tissue during water borne exposure. Malachite green residues have also been detected in eggs, fry and adult muscle tissue of rainbow trout, *Oncorhynchus mykiss*.

Alderman [19] reported that malachite green was absorbed by fish and that all fish tissues accumulated significant and fairly persistent residues of the dye. The use of this dye on fish, destined for human consumption, is thus not suggested unless a long withdrawal period is employed. Yonar and Yonar [20] evaluated behavioural, biochemical and immunological parameters induced in *Oncorhynchus mykiss.* Behavioural changes compromised hyperactivity characterised by the rapid pectoral and opercula movement, erratic swimming, loss of reflexes and gasping for air. These all suggest towards induced acute toxicity by malachite green in *Cyprinus carpio*.

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