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Acute toxicity of malachite green (Triarylmethane dye) and pyceze (Bronopol) on carbohydrate metabolism in the freshwater fish *Heteropneustes fossilis* (Bloch.)

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Abstract
Fish farmers have widely used malachite green, as an antifungal, antibacterial, antiparasitical and antiprotozoal agent. It is also used as dye on silk, wool, jute, leather, cotton and paper industries. Through the use of this dye has been banned in several countries, due to its low cost, and due to proper alternative, it is still being used in many parts of the world. Recently a pharmaceutical alternative to malachite green, pyceze as its active ingredient has been developed at U. K. It is being used for the treatment of fish fungal infection and their ova and appears to be a safe and effective replacement. Fish were exposed for four days to 1/5th of LC50 values of each of malachite green (0.24 mg/l) and (0.42 mg/l). After four days blood glucose level exhibited significantly increased whereas liver glycogen and muscle glycogen were decreased in fish in both groups when compared with controls. The present study evaluvated the effect of malachite green and pyceze on carbohydrate metabolism in *Heteropneustes fossilis*. However, exposure to malachite green was found to be more toxic than to pyceze.

Keywords: carbohydrate metabolism, malachite green, pyceze

1. Introduction
Malachite green is highly effective for the control of fungal infections and other external parasites such as protozoans, trematodes and larvae of parasitic crustaceans of fish and fish eggs [1-4]. It is also used as a food colouring agent, food additive, a medical disinfectant and antihelminthic product as well as a dye in silk, wool, jute, leather, cotton, paper and acrylic industries [5]. With expanding utilization of dye stuffs, health hazards to workers coming in their contact are steadily increasing dermatitis, hyperpigmentation, biochemical and hematological disturbances as well as carcinogenic effects caused by dyes have been reported in aquatic organisms [6]. Anliker (1977)[7] considers environmental problems posed by dyes to be rather moderate in variety in comparision to those caused by other chemicals such as pesticides, detergents, oils, heavy metals etc. Dyes are released into the aquatic environment from different sources [8] enter into the food chain and may produce carcinogenic, mutagenic and teratogenic effects on human health [9]. Though the use of this dye has been banned in several countries and not approved by U S Food and Drug Administration [10] it is still being used in many parts of the world due to its low cost, ready availability and efficacy [4] and due to lack of a proper alternative. A phamaceutical 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 the prevention of fungal infections [11-14].
The LC0, LC50 and LC100 values of malachite green and pyceze were estimated by Chaturvedi *et al*, (2012) [15]. The behavioural effects of both the chemicals were also observed by Srivastav and Chaturvedi, (2012) [16]. However, there is not enough data on the effects of these chemicals on carbohydrate metabolism of fish.
Therefore, the aim of the present study was to check the glucose, liver glycogen and muscle glycogen effects of malachite green and pyceze of a freshwater catfish *Heteropneustes fossilis*.

2. Materials and methods
The fish *Heteropneustes fossilis* (Weight 30.5±2.20 gm; length 16.50± 0.95 cm) were collected locally and maintained under laboratory conditions.

They were acclimatized for 15 days under a natural photoperiod and ambient temperature (23.8± 1.5^{O} C). The fish were fed daily with dried shrimp ground and flour pellets. Food was withheld on the day before and during acute i.e., 24 hr and 96 hrs exposure. The water used during the experiment was analyzed as per standard method [17].The physicochemical characteristics of the test water used were: dissolved oxygen (7.8± 1.6 mg dm^{-3}, hardness 135.50± 5.20 mg dm^{-3} (as CaCO3) and pH 8.3± 0.50). Stock solution of both malachite green and pyceze were prepared separately. A group of 30 fish (6 fish/15 l glass jar) was subjected to each of acute (0.24 mg/l) of malachite green and (0.42 mg/l) of pyceze of both were 1/5th of LC50 values respectively. Exposure period for acute doses of both were 24h and 96h. Six fish from both experimental as well as control were selected randomly at specific time intervals for analysis of carbohydrate metabolism parameters. At autopsy, the fish were anesthetized with 100 mg/l MS222 (tricaine me than esulfonate). The caudal peduncle was cut off with a sharp razor blade and free flowing blood was collected for the carbohydrate metabolism study. Blood glucose was measured by the method [18]. Liver and muscle glycogen contents were measured by the method of [19].The statistical significance between the treated and control groups was calculated by the t-test.

3. Results and Discussion

The effects of malachite green and pyceze on the carbohydrate metabolism content of *Heteropneustes fossilis* were observed in the acute [(0.24 mg/l) for malachite green and (0.42 mg/l) for pyceze], concentration for 24hr and 96hrs.

The hepatic glycogen content in control fish was 11.23 mg per 100 mg ww of the tissue during experiments. The decrease level of hepatic glycogen was found in treated fish was 9.41 mg per 100 mg ww at 24 hr for malachite green and 9.85 mg per 100 mg ww at 24 hr for pyceze and 8.13 mg per 100 mg ww at 96hrs for malachite green and 9.95 mg per 100 ww at 96 hrs for pyceze.

The muscle glycogen level of control fish was 1.17 mg per 100 mg ww of tissue during experiments. The decrease level of muscle glycogen was found in treated fish was 1.06 mg per 100 mg ww at 24 hr for malachite green and 1.06 mg per 100 mg ww at 24 hr for pyceze and 0.80 mg per 100mg ww at 96hrs for malachite green and 1.00 mg per 100 ww at 96 hrs for pyceze.

The blood glucose level in the control fish was 43.85 mg per 100 cm ^{3}. The increase level of blood glucose was found in treated fish was 48.81 mg per 100 at 24 hr for malachite green and 45.73 mg per 100 cm ^{3} at 24 hr for pyceze and 53.40 mg per 100 cm ^{3} at 96hrs for malachite green and 49.73 mg per 100 cm ^{3} at 96 hrs for pyceze.

Glycogen in the form in which carbohydrate is stored in animals mainly in the liver and muscle. It may provide a reserve for acute demand occurring as a result of transient stress [20]. A stress situation, such as that caused by acute hypoxia [21], strong muscular exercise [22] or pesticides [23-26], has been reported to decrease the hepatic and muscle glycogen contents in fish. Srivastav, *et al*., 2014 [27] also reported to decrease the hepatic and muscle glycogen contents in fish *Cyprinus carpio.* Stress evokes circulating levels of both catecholamines [22] and glucocorticoids [28] in fish. Catecholamines deplete the liver and muscle stores of the fish [29]. Thus the marked glycogenolysis in the liver and muscle after acute exposure of malachite green and pyceze in this study was most likely due to the stress induced increase in circulating catecholamines.

Studies on the brown bullhead *Ictalurus nebulasus* indicated that both epinephrine and glucagon bring about a decline in hepatic glycogen concentrations and an increase in the specific activity of hepatic total glycogen phosphorylase assayed with AMP [30]. This provides substantial support regarding glycogenolysis in this study with the activation of the adrenal medullary hormones which are important mediators of stress-induced glycolytic response.

Alteration of the blood sugar level is the primary metabolic sign in vertabrates subjected to a stressful situation. Metal toxicity [31], exposure to pulp effluent [32] and pesticides [24-26, 33-35] produce hperglycaemia in fish. Srivastav, *et al*., 2014 [27] also reported to increase the blood glucose level in fish *Cyprinus carpio.* The occurrence of malachite green and pyceze- induced hyperglycaemia in the catfish in this study have been due to the mobilization of both hepatic and muscle glycogen stores perhaps brought about by the individual or combined effects of emzymatic, hormonal and respiratory disturbances. Bhatia *et al*. (1973) [36] reported an increase in blood glucose concentration due to the gluconeogenic effect of the steroid hormone. A similar impairment in calcium permeability at the level of the cell membrane of the pancreas may inhibit insulin release [37] resulting in hyperglycaemia.

4. Conclusion

Glucose is primary source of energy. In stress condition glycogen is converted into glucose and fulfills the requirement of energy. Normally, during stress condition feeding does not occurs in fishes so, glycogen is converted into glucose. When fishes are treated with malachite green it becomes in stress condition and stops feeding in response of which glycogen are converted into glucose. Although, in experiment it has been observed that there was change in level of glucose and glycogen of liver and muscle. Similar observations has been seen with pyceze treatment also. On comparison of both malachite green and pyceze detrimental effects of malachite green are more than effect of pyceze. So, it can be concluded that pyceze may be relatively more safe than malachite green for fishes.

Table 1: Carbohydrate metabolism changes in *Heteropneustes fossilis* on acute levels of malachite green (0.24 mg/l) and pyceze (0.42 mg/l), (1/5th of 96hr LC50 values) for 24 and 96 hrs

Parameters	Control	Experimental			
		Malachite green (24h)	Pyceze (24h)	Malachite green (96h)	Pyceze (96h)
Liver glycogen (mg/100 mg WW)	11.23±0.19	9.41±0.31***	9.85±0.26***	8.13±0.28****	9.95±0.36**
Muscle glycogen (mg/100 mg WW)	1.17±0.13	1.06±0.05	1.06±0.02	0.80±0.06*	1.00±0.03
Blood glucose (mg/100 cm^{3})	43.85±0.85	48.81±0.50***	45.73±0.51	53.40±0.87****	49.73±0.69***

All values are mean ± SE (n=6)

*P<0.05, **P<0.02, ***P<0.01, ****P<0.001

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