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## Full Length Research Article

# EFFECT OF AN AZO DYE METHYL RED ON LIPID PROFILES IN MUSCLE TISSUE OF FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS*

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**ABSTRACT**

The present study was determine the effect of preferably used textile dye, methyl red on fingerlings of *Oreochromis mossambicus* as much report is unavailable on juvenile toxicity. Muscle tissue of fish were analysed for the effects of the azo dye methyl red, since it is a major target tissue for nutrient storage and is the main edible part of the fish. They affect the biologically active molecules like lipids. The fishes were maintained for a period of 40 days at two sublethal concentrations of the dye. The present study revealed a decline in total lipids, cholesterol, phospholipids, triglycerides and free fatty acid (FFA).



## INTRODUCTION

There are more than 100,000 different synthetic dyes available in the market, produced over 700,000 tons annually worldwide (Adedayo *et al*., 2004). Approximately 10 to 15 percent of the dye is disposed off during dyeing processes through effluents (Hussain *et al*., 2004). They are used in the textile, paper, cosmetics, food and pharmaceutical industries due to their ease of production, fastness and variety in colour compared to natural dyes. Some of them are dangerous to living organisms due to their possible toxicity and carcinogenicity. Methyl red is an anionic azo dye (Sahoo *et al*., 2005). The IUPAC name 2-[4 (dimethylamino) phenyl] diazonyl benzoic acid. It is also refered to as C1 Acid red 2. It is odourless and dark red in colour. pH for various colour ranges differently. It is red in colour under pH 4.4, yellow in pH 6.2 and orange with pH 5.1. (Material Data Sheet, 2010). Azo dyes, containing one or more azo bond (-N=N-), account for 60-70% of all textile dyestuffs used (Carliell *et al*., 1995). Azo dyes have diversity in structure but their most important structural feature is presence of azo linkage (ie) N=N-. This linkage may be present more than one time and thus mono azo dyes have one azo linkage while two in diazo and three in triazo respectively.

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These azo groups are connected on both sides with aromatics like benzene and naphthalene moiety. Sometimes aromatic heterocyclic units are also present being connected with azo groups (Zollinger, 1991). Among all the chemical classes of dyes, azo dyes are considered to be recalcitrant, non-biodegradable and persistent (Saratale *et al*., 2011). Moreover, azo dyes as well as their breakdown products are cytotoxic or carcinogenic (Khehra *et al*., 2006). Various azo dyes have been shown to produce positive toxic results for different parameters. It is in this perspective, the present investigation was undertaken to evaluate the effect of chronic methyl red intoxication on muscle lipid contents of *Oreochromis mossambicus*

## MATERIALS AND METHODS

### Maintenance of experimental animals

Freshwater fish *Oreochromis mossambicus* was used as the experimental model to evaluate the toxicity of methyl red. The fish used in this experiment were transferred from natural ponds around Tiruvallur district and brought to the laboratory and acclimatized for 15 days to laboratory conditions in tub aquaria each measuring (60 cm × 30 cm × 30 cm) filled with 25 litres of dechlorinated tap water with aerator fitted to the aquaria for continuous oxygen supply.

The aquaria were disinfected with potassium permanganate solution and washed thoroughly prior to introduction to prevent any fungal infection. Feeding was stopped 24 hours before the commencement of the toxicity test to keep the animals more or less in the same metabolic state. Initial mean weight and length of the fish were 20-28 gm and 8-12 cm respectively. The fishes were maintained in normal light dark period and optimal temperature. The fishes were fed twice (5% of body weight) a day with artificially prepared imported fish feed available in the market. The protein content of the feed was around 40%.

Methyl red dye toxicity and Determination of lethal concentration (LC50) Acute toxicity experiments were conducted for 96 hours using a static bioassay technique. Five groups of 10 fishes each were set for the LC50 bioassay method. The fishes were exposed to a range of six different concentrations (1.10, 0.9, 0.7, 0.5, 0.3, 0.1, 0.0 mg/L) of methyl red. The fishes were maintained in a narrow range concentration. The 96 hour LC50 was determined by Probit analysis method (Finney, 1971). The Safe Application Factor Equation (SAFE) was calculated from the LC 50 values. The concentration at which 50% mortality occurred after 96 hours was taken as the median lethal concentration. The dead fishes were removed immediately from the aquaria to avoid oxygen depletion. Mortality, behavioral and morphological changes were recorded during the 96 hr LC50 observation. LC50 was found to be 0.5 mg/L. The chosen mean concentration for methyl red was, one tenth (0.05 mg/L) and one fifth (0.10 mg/L) taken as lower and higher sub lethal concentration respectively to study the dose dependent effect. Experiments were conducted with sub-lethal and toxicologically safe concentrations of methyl red for a period of 40 days.

Group III: Fishes maintained at 0.10 mg/L of methyl red for a period of 40 days (Higher concentration)- Experimental group-II

The control and the experimental animals were fed with normal fish feed. Commercial food pellets with ingredients consisting of fish meal, wheat flour, soybean meal, yeast, vitamins and minerals were fed. Water was changed daily at 8.00 hours which facilitated the removal of unconsumed food. After renewal of water the required quantity of methyl red was added to maintain the concentration of the toxicant in water. At the end of 40th day five fishes were sacrificed by cervical dislocation. Muscle tissues were dissected out and washed thoroughly with 0.9N saline solution. Tissues were weighed and homogenized in Tris 0.1M HCL buffer using a homogenizer. The homogenate of the tissue were centrifuged at 2500 rpm for 15 minutes in a high speed centrifuged and clear supernatant was used for biochemical analysis.

**Biochemical analysis:** Biochemical constituents were estimated by using standard procedures. Tissue lipids were extracted by the method of Folch *et al*., (1957). Quantification of lipid classes were analysed by the following methods. Total lipids (Frings *et al*., 1972), cholesterol (Zlatkis *et al*., 1953), phospholipid (Marinetti, 1962), triglycerides (Foster and Dunn 1973) and free fatty acids (Itaya, 1981).

**Statistical Analysis:** The data collected on the different parameters of the control and experimental study were subjected to statistical analysis by using statistical software SPSS version 6.0. The statistical significance was tested at 1% and 5% levels using Paired Sample 't' test.

**Table 1. Effect of methyl red dye on muscle total lipids, cholesterol and phospholipids in freshwater fish *Oreochromis mossambicus***

| Biochemical parameters | Control | Lower concentration (0.05mg/l) | Higher concentration (0.10mg/l) | t -value | p -value |
|---|---|---|---|---|---|
| Total lipid | 12.23 ± .24 | 10.21 ± 0.01 | 9.22 ± 0.01 | 19.99 | < 0.001** |
| Cholesterol | 8.04 ± .03 | 8.17 ± 0.02 | 6.41 ± 0.12 | 392.80 | < 0.001** |
| Phospholipid | 11.31 ± 0.11 | 10.52 ± 0.27 | 9.07 ± 0.48 | 59.52 | < 0.001** |

Values are expressed as mg/gm wet tissue; Values are Mean ± SD (n=5) observations.** denotes significance at 1% level,.
* denotes significance at 5% level

**Table 2. Effect of methyl red dye on muscle triglycerides and free fatty acids in freshwater fish *Oreochromis mossambicus***

| Biochemical parameters | Control | Lower concentration (0.05mg/l) | Higher concentration (0.10mg/l) | t - | p -value |
|---|---|---|---|---|---|
| Triglycerides | 4.81 ± .39 | 2.24 ± 0.74 | 1.11 ± 0.08 | 75.039 | < 0.001** |
| Free fatty acids | 5.09 ± .05 | 2.09 ± 0.02 | 2.14 ± 0.19 | 1.020 | < 0.001** |

Values are expressed as mg/gm wet tissue; Values are Mean ± SD (n=5) observations.** denotes significance at 1% level

### Experimental Design

Group I served as the control while Group II was exposed to methyl red at sub-lethal concentration.

Group I: Control fishes maintained in toxicant free water.

Group II: Fishes maintained at 0.05 mg/L of methyl red for a period of 40 days (Lower concentration)- Experimental group-I

## RESULTS

The lipid constituents showed variation in their contents in fishes exposed to methyl red when compared to control fishes. Muscle cholesterol declined in higher concentration ($P < 0.001$) whereas no significant changes were noticed in lower concentration. Total lipid and phospholipid content showed a significant decrease ($P < 0.001$) after 40 days of exposure to methyl red, when compared with the control animals (Table 1).

The contents of triglycerides and free fatty acid in the muscle was declined significantly ($P < 0.001$) in both the concentrations of the dye after 40 days exposure (Table 2).

## DISCUSSION

Lipids are an important fuel reserve of aquatic organisms during stress. It provides a reliable and sensitive index for assessing the magnitude of physiological stress (Kaushik and Srivatsava, 2003). Lipids provide much more energy than carbohydrates and they also supply fatty acids which are used for the building up energy reserves in fishes (Lidman *et al*., 1979; Sheridan, 1994). In the present study the lipid content of fish species exposed to the both sub lethal concentration of methyl red showed decline in lipid contents. It can be suggested that the accelerated hydrolysis of lipid might be to cope up with the increased energy demand occurring due to the methyl red toxicity. Decline in lipid contents may be either due to oxidation or hydrolysis of lipids (Levesque *et al*., 2002). *Cyprinus carpio* exposed to anthroquinone dyes exhibited a decrease in their lipid contents of muscle tissue (Olaganathan and Patterson, 2013).

Cholesterol and phospholipids are generally considered to be structural or functional lipid being incorporated to a large extent in the membrane structure of cell and subcellular organelles (Afaq, 2010; Padmapriya and Avasn Maruthi, 2013). The decrease in the cholesterol and phospholipids may have a drastic effect on the structural integrity and permeability of the cellular and subcellular membranes in fishes exposed to sublethal concentrations of methyl red. It may be due to the utilisation of these lipid constituents to meet the energy demands caused by azo dye, methyl red (Vasanthi *et al*., 2013). The present study also showed a decrease in triglycerides in muscle, in sublethal concentration of methyl red. The breakdown of triglycerides could have increased fatty acid contents.

This study suggests that energetic cost of detoxification process have an impact through altered intermediary metabolism (Javed and Usmani, 2015). The glycerol moiety from the breakdown of triglycerides may enter gluconeogenic pathway and converted to glucose providing a small fraction of energy in the stressed animals. Free fatty acid has been shown to affect several metabolic activities in various tissues. It has profound effects on mitochondrial metabolism and inhibition of oxidative phosphorylation and ATPase activity (Price, 1976). Furthermore, fatty acids are known to induce "swelling" in whole cells and cell organelles (Mehendale, 1987). Due to the effect azo dye, methyl red and its toxicity fatty acid may have been oxidised for energy supplies, since FFA are the primary energy depot in animals during periods of extensive movements and during inadequate energy supply.

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