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Impact of Malachite Green Dye on Freshwater Bivalve *Parreysia corrugate*

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Abstract

The acute toxicity effects of malachite green dye on the freshwater bivalve Parreysia corrugata are examined in this study, with an emphasis on changes in protein content and lipid peroxidation in the tissues of the gills and hepatopancreas following a 96-hour exposure. Two malachite green concentrations (0.2 ppm and 0.8 ppm) were applied to the experimental organisms. There was a noticeable decrease in protein content and an increase in lipid peroxidation, which suggests significant cellular damage and biochemical stress. These findings underline the need for additional biochemical and molecular research as well as the negative effects of textile dye effluents on aquatic bivalves.

Keywords: *Malachite green, Parreysia corrugata, protein content, lipid peroxidation, freshwater bivalves, aquatic toxicity, dye pollution.*

1. Introduction

Synthetic dyes are extensively used in numerous industrial sectors, including textiles, leather processing, paper manufacturing, printing, paints, cosmetics, pharmaceuticals, food processing, photography, and biological staining, owing to their excellent color fastness, stability, and wide range of shades [1]. Among these industries, the textile sector is one of the largest consumers of synthetic dyes and colorants. The global textile industry plays a vital role in economic development, contributing significantly to international trade, industrial production, and employment. However, its rapid expansion has also resulted in considerable environmental challenges, particularly due to the large volume of wastewater generated during dyeing and finishing processes [2]. Textile wastewater is characterized by high concentrations of synthetic dyes, salts, surfactants, heavy metals, and other hazardous chemicals, many of which are resistant to biodegradation and conventional wastewater treatment methods [3]. It is estimated that a substantial proportion of dyes used during textile processing is discharged into aquatic environments through untreated or inadequately treated industrial effluents. The release of these colored effluents reduces light penetration in water bodies, disrupts photosynthetic activity, decreases dissolved oxygen levels, and adversely affects aquatic ecosystems [4].

Many synthetic dyes possess complex aromatic molecular structures that confer high chemical stability but also make them persistent environmental pollutants. Numerous azo, anthraquinone, and triphenylmethane dyes have been reported to exhibit mutagenic, carcinogenic, teratogenic, and cytotoxic properties, posing serious risks to both environmental and human health [5]. Continuous exposure to dye-contaminated water has been associated with skin irritation, respiratory disorders, allergic reactions, liver and kidney dysfunction, and increased cancer risk in humans, while also causing toxicity and reproductive impairment in aquatic organisms [6]. The growing concern regarding dye pollution has intensified research efforts toward developing efficient, environmentally friendly, and sustainable wastewater treatment technologies. Advanced oxidation processes, adsorption, membrane filtration, photocatalysis, electrochemical methods, and biological degradation have emerged as promising approaches for the effective removal of textile dyes from industrial wastewater. Among these, biological treatment has gained considerable attention because of its cost-effectiveness, eco-friendliness, and ability to achieve complete mineralization of dye molecules under appropriate conditions [7].

Malachite Green (MG)

Malachite Green (Basic Green 4) is used in dyeing wool, silk, leather, jute, cotton, and acrylic industries; it is also used as a food additive, food disinfectant, food colouring agent, medical disinfectant, and antihelminthic [8]. In aquaculture industries, it is used as an antiprotozoan, antibacterial, antifungal, and antihelminthic [9-12]. MG is reported as carcinogenic, mutagenic, teratogenic, and causing chromosomal fracture, reduced fertility, developmental abnormality, and other physiological changes in animals [13-15] reported the presence of malachite green (0.5 µg/l) in potable and drinking water.

Effect on Mollusca

Molluscs serve as excellent bioindicators due to their sedentary nature, long lifespan, filter-feeding habits, and ability to accumulate contaminants. Bivalves such as *Parreysia corrugata* are sensitive to pollutants and are widely used to assess aquatic toxicity. Previous research reports structural and biochemical damage in bivalves exposed to pesticides, heavy metals, and dyes. The freshwater mussel *Parreysia corrugata* is widely distributed across India and plays ecological, nutritional, and economic roles. This study evaluates the biochemical alterations induced by acute exposure to malachite green dye. [16] proposed the definition for bioindicators; he defined as bioindicators are the species or group of species that readily reflect the biotic and abiotic state of environment, which represents the environmental change on habitat, community or ecosystems. Mollusca are used as reliable bioindicator of the pollutants for early responses enabling to indicate the presence and prediction of the consequences of undesirable anthropogenic effects [17]. In metal monitoring programme, molluscan has become a popular choice for several reasons [2-4]. Molluscs inhabiting marine, freshwater, and terrestrial ecosystems play an important ecological role and are widely recognized as effective bioindicators for monitoring environmental pollution. Because many molluscan species are relatively sedentary, long-lived, regionally abundant, and possess a high capacity to accumulate contaminants from water, food, and suspended particulate matter, they provide reliable information on the bioavailability and long-term effects of environmental pollutants [8–10]. Exposure to toxic contaminants can adversely affect molluscan growth, reproduction, survival, and physiological functions, ultimately disrupting population dynamics and ecosystem stability [11]. In molluscs exhibit distinct behavioral, biochemical, and physiological alterations, making them valuable sentinel organisms for assessing the ecological health of aquatic ecosystems [12]. Lipid peroxidation is one of the most important biomarkers of oxidative stress in living organisms. It is a free radical-mediated process that results in the oxidative degradation of polyunsaturated fatty acids within cellular membranes. This process involves the generation of reactive oxygen species (ROS), oxygen uptake, and structural modifications of membrane lipids, ultimately leading to the formation of secondary oxidation products such as malondialdehyde, aldehydes, ketones, alcohols, alkanes, and ethers [13]. Excessive lipid peroxidation compromises membrane integrity, alters cellular permeability, disrupts enzyme activity, and may ultimately result in cell dysfunction or death. Consequently, lipid peroxidation is widely employed as a sensitive biomarker for evaluating oxidative damage induced by environmental pollutants. Proteins are essential macromolecules that perform diverse structural, metabolic, and regulatory functions in all living organisms. They are involved in enzymatic catalysis, DNA replication, cellular signaling, immune responses, transport of molecules, tissue repair, and maintenance of cellular architecture. Environmental contaminants can significantly alter protein metabolism by affecting protein synthesis, degradation, and enzymatic activity. Therefore, changes in total protein content are frequently used as biochemical indicators of physiological stress and toxicant-induced metabolic disturbances in aquatic organisms.

The freshwater mussel *Parreysia corrugata* is a widely distributed unionid bivalve inhabiting rivers, streams, lakes, ponds, canals, and other freshwater habitats throughout the Indian subcontinent. The species occurs in several Indian states, including Punjab, Bihar, Madhya Pradesh, Karnataka, and Maharashtra, where it contributes to freshwater ecosystem functioning through filter feeding and nutrient cycling. In recent years, *P. corrugata* has gained considerable attention as a promising candidate for freshwater pearl culture because of its favorable shell characteristics and adaptability to culture conditions. Additionally, the species possesses socioeconomic and medicinal importance, serving as a source of food for several indigenous communities in India, Nepal, and Bangladesh. Owing to its ecological significance, contaminant bioaccumulation capacity, and sensitivity to environmental stressors, *P. corrugata* is increasingly utilized as a model organism in ecotoxicological studies for monitoring freshwater pollution and assessing the biological effects of chemical contaminants.

2. Material and Methods

2.1 Systematic Position of *Parreysia corrugata*

Kingdom: Animalia
Phylum: Mollusca
Class: Bivalvia
Order: Unionida
Family: Unionidae
Genus: *Parreysia*
Species: *corrugata*

2.2 Collection and Maintenance

Freshwater bivalves (*Parreysia corrugata*) measuring 4.5–5.5 cm was collected from the Krishna River (Karad region). The shells were cleaned and acclimatized for 7 days in glass aquaria with regularly renewed tap water.

2.3 Experimental Design

Based on previous literature, sublethal concentrations of malachite green were determined. Bivalves were divided into three groups: - Control - 0.2 ppm malachite green - 0.8 ppm malachite green.

After 96 hours of exposure, gill and hepatopancreas tissues were dissected for biochemical analysis.

2.4 Biochemical Estimations

2.4.1 Protein Estimation (Lowry method adopted)

Protein content was measured spectrophotometrically at 624 nm using the Lowry method.

2.4.2 Lipid Peroxidation (Will's Method)

Malondialdehyde (MDA) levels were quantified using the thiobarbituric acid reaction, measured at 532 nm.

Amount of MDA per mg tissue was calculated using the following formula,

$$\text{Lipid Peroxidation (X)} = \frac{\text{O.D. of Sample} \times 3 \times 6}{0.156 \times 0.2}$$

Where,

X = Amount of MDA in homogenate nM MDA/mg tissue.

3 = Volume or sample taken for photometric observation.

6 = Scaling factor for conversion to per hour.

0.156 = Absorbance for a 1-hour mole solution of MDA measured in a thick cell at 532nm.

0.2 = Volume of sample.

3. Results

3.1 Effect on Protein Content

A significant decrease in protein levels was observed in both tissues at both concentrations. The hepatopancreas showed greater depletion compared to the gills.

Table 1: Changes in protein content after acute exposure of Parreysia corrugata to malachite green (values are in mg/100 mg wet tissue)

Tissues	Control (mg/100mg)	0.2ppm (mg/100mg)	% change over control	0.8ppm (mg/100mg)	% change over control
Gill	14.666±0.6	11.57±0.34**	-21.07	10.42±0.082***	-28.92
Hepatopancreas	41.296±2.1	31.156±0.5***	-24.55	27.966±0.9***	-32.28

*Values are mean ± S.D., **indicates significance level p<0.01, ***indicates significance level P<0.001, (n = 3)*

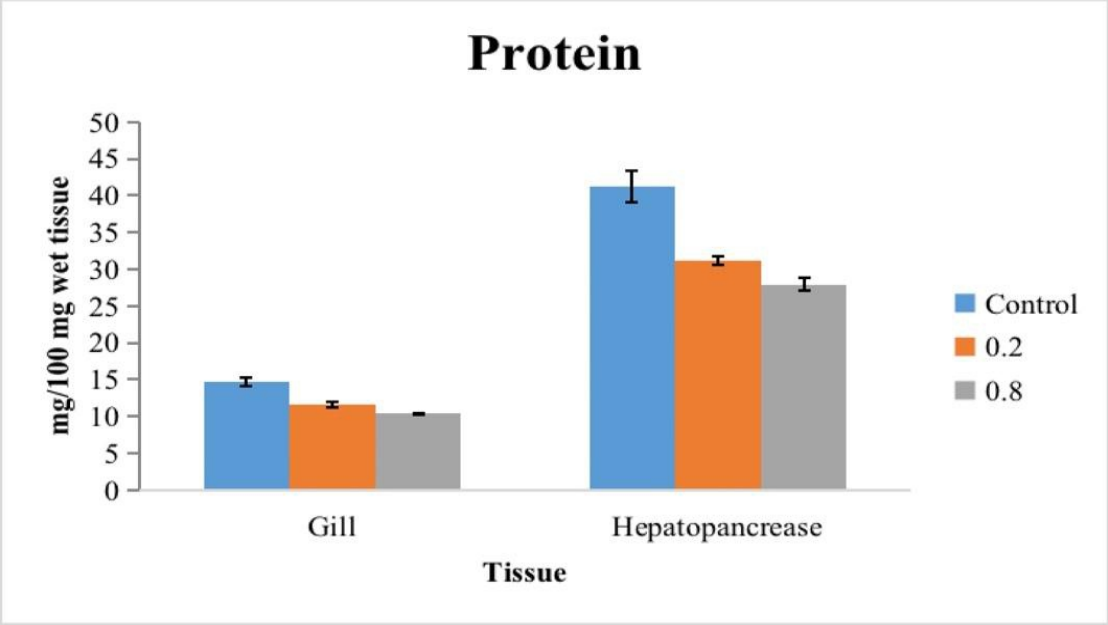

Figure No. 1: Protein content in different tissues of fresh water bivalve, Parreysia corrugata after acute exposure to malachite green (values are in mg/100 mg wet tissue)

3.2 Effect on Lipid Peroxidation

A significant increase in MDA levels was recorded, particularly in the gills.

Table 2: Changes in Lipid peroxidation content after acute exposure of Parreysia corrugata to malachite green (nM of MDA per mg wet tissue)

Tissues	Control (nM of MDA/mg)	0.2ppm (nM of MDA/mg)	% change over control	0.8ppm (nM of MDA/mg)	% change over control
Gill	14.613±0.3	38.456±1.4***	163.16	41.726±2.6***	185.54
Hepatopancreas	57.88±4.9	61.916±1*	4.03	70.96±1.4**	22.59

*Values are mean ± S.D., *indicates significance level p<0.1,**indicates significance level p<0.01, ***indicates significance level P<0.001, (n = 3)*

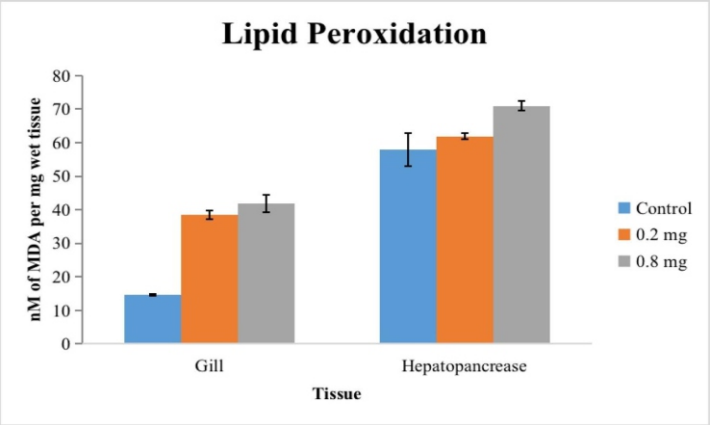

Figure No. 2: Changes in Lipid peroxidation content after acute exposure of Parreysia corrugata to malachite green (nM of MDA per mg wet tissue)

4. Discussion

Many studies show that malachite green has a toxic effect on different animals. The toxicity of this dye increases with exposure time, temperature, and concentration. This dye is mutagenic, carcinogenic, and teratogenic, and to cause chromosomal fractures. In certain fish species, it acts as a respiratory enzyme poison, damaging cells that are unable to create metabolic activities [12]. The dye exposure showed altered parameters like biochemical profile, antioxidant activity, histopathology, and DNA damage by commet assay after acute and chronic exposure to malachite green dye [4]. In the presence study, we have seen the toxic effect on freshwater bivalves, with acute exposure to 0.2 ppm and 0.8 ppm concentration of malachite green showing significant changes in lipid peroxidation and protein content in the target organs, the gills and hepatopancreas. Bivalves are high in protein content, and it's a major biochemical component that acts as a source of energy for various physiological functions, including reproduction. It also plays a major role in the metabolism of a cell because of the proteinaceous nature of all the enzymes. Several workers have reported the impact of various aquatic pollutants on protein metabolism of different species. Under stress conditions, energy supply from protein was less due to interference of protein metabolism pathways [2-5].

Dimedone showed toxic impact on biochemical composition, like protein, in the gill, muscle, and kidney of *Aris dessumudis* after 96 hours of exposure [7-9] observed the decrease in protein content in *Parresia cylindrica* when exposed to indoxacarb. *Lamellidens marginalis* also showed altered levels of protein after exposure to tannery effluent [10]. There was variation in protein content after exposure to 24, 48, 72, and 92 hours of copper chloride in the foot, gill, hepatopancreas, and mantle of the freshwater bivalve *Lamellidens corrianus* [11]. Lead chloride and nickel lowered the protein content in the freshwater bivalve *Lamellidens marginalis*. [12-15] studied the protein content after acute exposure to lead chloride in fresh freshwater bivalve *Indonaia coeruleus*. While chronic treatment also showed a decreasing trend in protein content up to 15 days to Folithion in *lamellidens marginalis* [13]. Bivalves are high in protein content.

There is a depletion of protein and an increase in lipid peroxidation that can be seen in bivalve tissue after exposure to malachite green dye. In the present study, we have seen depletion of protein in various tissues, like the gill and hepatopancreas, by exposure to 96 hours of malachite green dye. Lipid peroxidation is currently thought to be the primary molecular mechanism responsible for both the oxidative damage to cell structure and the toxicity process that results in cell death. Heavy metal promotes the formation of reactive oxygen species (ROS), such as hydrogen peroxide. The ROS enhances the peroxidases and reactive hydroxyl radicals [14-16]. These lipid peroxides and hydroxyl radicals may cause cell membrane damage and thus destroy the cell. Heavy metal increases the rate of formation of reactive oxygen species, including superoxide anion radical O_2 and hydroxyl radical (OH), through a chain reaction [17]. Referring above references, it is said that the toxic molecule changes the normal content of protein, i.e, a decrease in protein content. White, there was an increase in lipid peroxidation, i.e, damage in the cellular membrane.

5. Summary

A. Protein content decreased significantly in both tissues, with a maximum in hepatopancreas at 0.8 ppm (32.28%).

B. Lipid peroxidation increased significantly, with a maximum in gills at 0.8 ppm (185.54%).

C. Malachite green exerts severe biochemical stress on *P. corrugata*.

6. Conclusion

The present study demonstrates that malachite green causes significant toxic effects on the freshwater bivalve *Parreysia corrugata*, leading to biochemical disturbances such as protein depletion and increased lipid peroxidation. These results suggest that textile dye effluents pose serious threats to aquatic organisms.

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