



Biochemical Stress Response in *Parresia corrugata* Exposed to Malachite Green

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Malachite green (Basic Dye) is more used in dyeing and aquaculture industries is reported as toxicant. MG is acutely toxic to wide range of aquatic and terrestrial animals. It is environmentally persistent and causes serious health hazards. At very low concentration of malachite green showed changes in biochemical profile of *Parresia corrugata*. By examining the biochemical profile against control in tissues like gill and hepatopancreas, the effects of 0.2 ppm and 0.8 ppm concentrations were evaluated. There was highly significant ($p < 0.01$) in both the protein and lipid content in tissues at 0.8 ppm concentration of malachite green. At 0.2 ppm concentration, Protein content was moderately depleted ($p < 0.1$) in both the tissues after exposure to 96 hours.

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1. Introduction

The most important compound for the survival of human beings, animals and plants is water. But due to various anthropogenic activities like urbanization and industryization there is continuous discharge of pollutant in water bodies (Mckay & Allen, 1980; Saminathan *et al.*, 2016). The aquatic bodies are contaminated by dyeing process carried out in paper, leather, textiles, printing, cosmetics and food industries (Cao *et al.*, 1999). The aqueous streams are contaminated by dyeing process carried out in leather, paper, textile, printing, cosmetics and food industries.

Malachite green is a basic dye, used in dyeing wool, silk, leather, jute, cotton and acrylic industries and it is readily soluble in water. It is also used as food additive, food disinfectant, food coloring agent, medical disinfectant and antihelmenthic (Culp and Beland., 1996). In aquaculture industries it is used as antiprotozoal, antibacterial, antifungal and antihelmenthic (Hussein *et al.*, 1999; Cheng *et al.*, 2002; Sudova *et al.*, 2007). Malachite green (0.5µg/l) in clean and drinking water. Due to its extreme toxicity and ecotoxicity hazards associated with MG, restriction has been implemented in many countries from 2000. It is still being used in many parts of the world due to its low cost, ready availability and efficacy. A considerable amount of research is being devoted to work out the wide spectrum of biological effects which exerts on different animals and mankind.

In fish breeding, technical grade malachite green was used due to its property. The exact composition was not given prior to use therefore necessary to take into account its different toxicological and therapeutic properties. The malachite green bath treatment without a prior test of fish and other aquatic animals' tolerance could result in the death of all the animals thus treated. For that reason, each new production batch of malachite green had to be tested for toxicity to fish and for its antiparasitic action.

Molluscs are very important food stuff. Some fresh water mussels are used for pearl culture. In bivalves, energy storage is in the form of glycogen (carbohydrates), lipid and protein. Study of biochemical compositions are concerned with energy metabolism. The biochemical compositions of mollusc are influenced by its size, growth and reproductive status. Change in biochemical contents are depend upon the environmental conditions and utilization of these reserves during gametogenic cycle and maturation of the animals (Gabbott and Bayne, 1973) and they play a major role as energy precursors for aquatic organisms exposed to stress conditions (Ramlingam *et al.*, 1980; Janakiram 2003).

Molluscs are used as reliable bioindicator of the pollutants for earliest responses enabling to indicate the presence and predict the consequences of undesirable anthropogenic effects (Salanki *et al.*, 2003). Many species of molluscs from marine, fresh water and terrestrial ecosystems are the key species to determine the impact of pollutant, which will affect the molluscan population and results in negative impact on entire ecosystem (Markett *et al.*, 2004). In comparison to other aquatic groups like fishes, and crustaceans, bivalves are having low level of enzyme activity to metabolize persistent organic pollutants. So that, the concentration of contaminants in the tissues of bivalves can more accurately reflects the magnitude of environmental contaminants (Phillips *et al.*, 1990).

Parreysia corrugata is fresh water bivalve widely distributed in Indian sub-continent. This bivalve is reported as medicinally important while some used to control blood pressure. The animal is reported to be medicinally important (Day *et al.*, 2008) and used by aboriginal people to control blood pressure (Prabhakar & Roy, 2009). It is also used in cement, lime, button, toys and cosmetic industries. In certain parts of the country, the animal is consumed as food by poor people. Recently, Pearl production has been successfully reported using this sepsis in Orissa (Jankiram *et al.*, 2003).

In present investigation, the effect of malachite green on the concentration of protein and lipid profile of *Parresia corrugata* was studied.

2. Materials and Methods

The fresh water bivalve *Parresia corrugata* were collected from Krishna river, Karad (46°41' 22.02" N and 17° 50' 38.112" E). The average size was about 4-5 cm. The bivalves were washed and transferred to aquarium for 1 month to acclimatize. Malachite green used in laboratory to treat aquarium fishes were chosen as toxicant. Three groups were done as control group, 0.2 ppm and 0.8 ppm. The 10 animals were categories in these groups and kept for 96 hours. After 96 hours, Gill and hepatopancreas were removed and protein (Lowry method., 1951) and lipid (Barnes and Blackstock., 1973) were estimated.

Estimation of Protein (Lowry et al, 1951): 100 mg fresh tissues from gill, hepatopancreas were homogenized in 10 % TCA and the homogenized material was centrifuged at 3000 rpm separately for 10 minutes. The residue was diluted in 0.1 N NaOH. 0.1 ml of this solution was made up to 1ml by adding distilled water. 3 ml Lowrys reagent was added, wait for 5 minutes and then 0.5 ml follin phenol reagent was added. Kept it for 30 minutes at room temperature in dark. Blue colour developed. The optical density was measured at 660 nm with UV spectrophotometer.

Estimation of Lipid (Barnes and Blackstock., 1973): Lipid content was estimated by using vanilline reagent. The chilled tissue (gill and hepatopancreas) was homogenized in Folch mixture (chloroform: methanol = 2: 1). A pinch of sodium sulphite was added and the solution was filtered. 1 ml of above sample was taken and boiled for 2 hours and dried it. 1 ml of concentrated H₂SO₄ was added and boiled for 5 minutes and cool it. In cooled solution 2 ml of Vanillin reagent was added. Pink colour developed. The optical density was measured at 530 nm with UV spectrophotometer.

2.1 Data Analysis

The Results were analyzed by using SPSS software.

3. Results

The bivalves were grouped in three groups control, 0.2 ppm and 0.8 ppm. The alterations in Protein and lipid were analyzed.

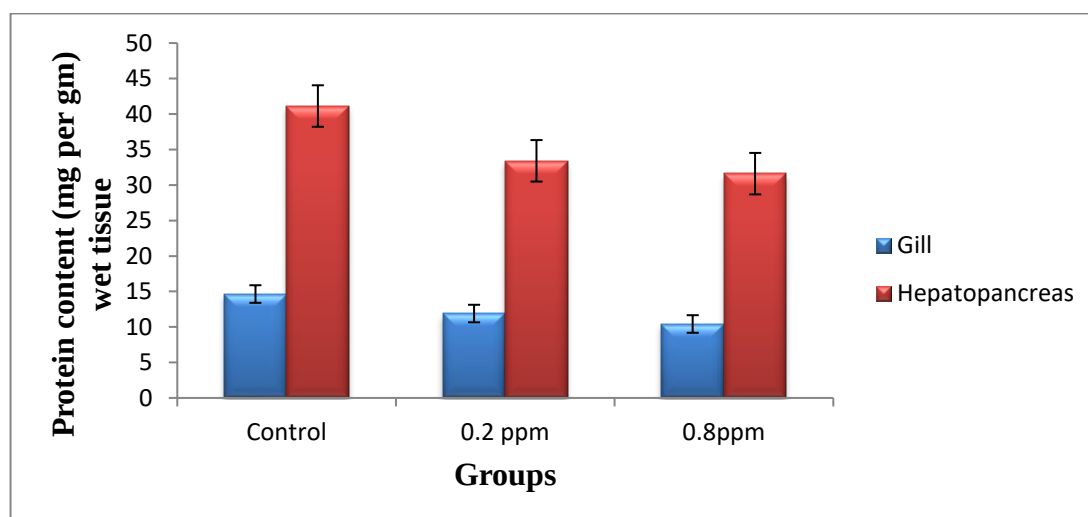


Fig. 1. Protein content (mg/Gm) in fresh water bivalve *Parresia corrugata* exposed to 0.2 ppm and 0.8 ppm concentration to Malachite green

A. Protein Alterations

Protein content in hepatopancreas (41.13 mg/gm) was more than gill(14.64mg/gm) of *Parresia corrugata* in control group was observed. As the concentrations of malachite green were increased there was more depletion

in protein content. After 96 hours exposure to 0.2 ppm malachite green, both tissues showed significant decrease in protein content. There was highly significant depletion was recorded in gill (10.42mg/gm) and hepatopancreas (31.6mg/gm) of *Parresia corrugate* were recorded after exposure for 96 hours to 0.8ppm concentration of Malachite green.

Table 1. Changes in protein content (mg/gm) of *Parresia corrugate* after 96 hours exposure to Malachite green

Tissues	Control	0.2ppm	0.8ppm
Gill	14.64 ± 0.14	11.89 ± 0.89**	10.42 ± 0.11***
Hepatopancreas	41.13 ± 0.86	33.41 ± 1.49**	31.6 ± 0.1***

NS = Non significant, * = $p < 0.5$, ** = $p < 0.1$, *** = $p < 0.01$.

B. Lipid Alterations

The fresh water bivalve *Parresia corrugate* showed lipid content more in Hepatopancreas (57.14mg/gm) than gill (14.74mg/gm). After 96 hours exposure to 0.2 ppm and 0.8 ppm concentrations of malachite green, the gill (11.33mg/gm;10.33mg/gm) and hepatopancreas (51.25mg/gm;46.9mg/gm) showed highly significant decrease.

Table 2. Changes in protein content (mg/gm) of *Parresia corrugate* after 96 hours exposure to Malachite green

Tissues	Control	0.2ppm	0.8ppm
Gill	14.4±0.2	11.33±0.6***	10.33±1.2***
Hepatopancreas	57.14±0.79	51.25±0.8***	46.9±1.3***

NS = Non significant, * = $p < 0.5$, ** = $p < 0.1$, *** = $p < 0.01$.

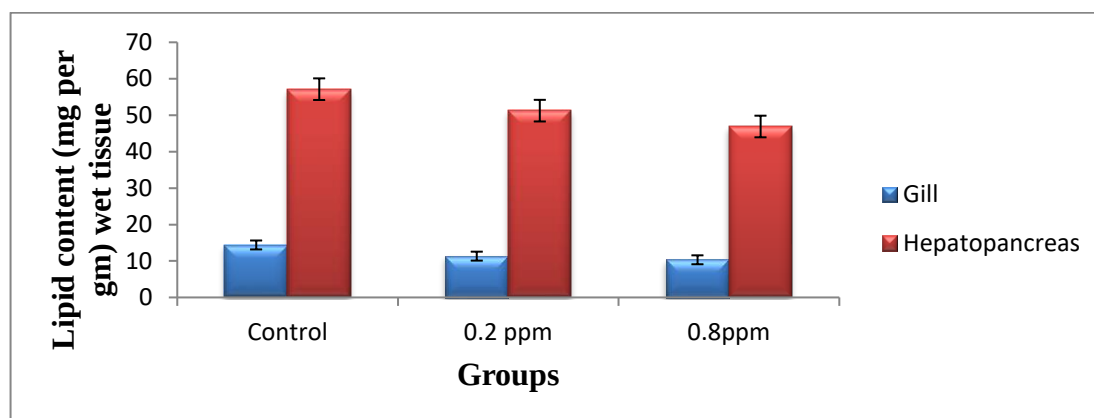


Fig. 2. Lipid content (mg/Gm) in fresh water bivalve *Parresia corrugate* exposed to 0.2 ppm and 0.8 ppm concentration to Malachite green

4. Discussion

In bivalve Mollusc, the biochemical compositions were stored in the form of glycogen, protein and lipid which was used for energy metabolism. Proteins are the major biochemical component, which act as source of energy for various physiological functions including reproduction. It also plays a major role in metabolism of cell because of proteinaceous nature of all the enzymes, which mediates various metabolic pathways. Several workers have reported the impact of various aquatic pollutants on protein metabolism of different species. The change in biochemical content was depended upon environmental conditions and utilization of this reserve during maturation i.e. gametogenic cycle (Gabott and Bayne et al., 1973). The altered biochemical content in bivalves was due to toxicological stress were reported by several workers. The biochemical composition was affected by toxicant stress. In present investigation, at 0.2 ppm and 0.8 ppm concentration of malachite green showed significant decrease in protein and lipid content in gill and hepatopancreas of *Parresia corrugate*. Under

stress condition energy supply from protein was less due to interference of protein metabolism pathway (Vincent et al., 1995).

In laboratory animals exposed to environmental chemicals/toxicants results in increase or decrease in biochemical content is an important tool in assessment of the risk and hazards to human and animal exposure (Krishna and Ramachandran.2009). Any kind of toxic stress leads to changes in biochemical and physiological mechanisms. Protein is molecular phenotype of cells which have a direct effect on organism physiology (Feder and Walser et al, 2005). The decrease amount of protein content was due to enhancement of proteolysis to cope up with high energy demands in toxic stress (Vincent *et al.*, 1995; Waykar and Lomte, 2001). The significant decrease in protein level indicates rapid initiation of protein breakdown to meet the energy demands during toxic stress (Vincent et al., 1995). Proteins represent the molecular phenotype of cells which have a direct effect on organismal physiology (Feder and Walser, 2005).

A marked fall in the protein level in all the tissues indicates a rapid initiation of breakdown of protein. To meet energy demands during toxic stress mobilization of protein might have taken place. The depletion of protein tissue was due to diversification of energy, to meet the impending energy demand under toxic stress (Vincent et al., 1995) and to prevent fatigue due to pesticide toxicity (Parate and Kulkarni, 2003). The decrease in amount of protein is due to proteolysis. After exposure to toxicant, to cope with high energy demands under toxic stress, the toxicants inhibit the synthesis of protein (Wayker and Lomte., 2001).

The lipids are the energetic reserve because of their high caloric value. These are the major sources of metabolic energy and essential compound for formation of cell and tissue membranes and also important in egg production. They also provide energy for growth during conditions of limited food supply, when carbohydrate levels are low. Lipid composition of molluscs is affected by external factors (environmental), or by internal factors such as metabolic and physiological activities.

Lipid is major constituent of mollusc. It plays role in energy conservation (Wenne and Styczynska- Jurewicz, 1987); in maturing gonadal tissues. Jadhav (2012) observed the seasonal changes in lipid content in *Lameliidens marginalis*. There was reduction in lipid content when *L. marginalis* was exposed totributyltin oxide (Humbe et al., 2016). The pesticide induces decrease in lipid and protein content in snail *Bellamya dissimilis* (Padmaja and Balaparameswara Rao, 1994). Khandekar (2018) observed the depletion in lipid content of *Lamellidens marginalis* after exposure to Basic blue 3 dye. The same observations were recorded in *Lamellidens marginalis* after exposure to Malachite green dye (Khandekar & Muley, 2019, Ramalingam, 1980).

5. Conclusion

Results obtained in this study indicate that the malachite green which was used for number of purposes affect the metabolism in fresh water bivalve *Parresia corrugata* by reducing biochemical contents due to stress condition. The malachite green dye showed toxicological effects on physiology of the bivalves at lethal concentration for short exposure.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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