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Malachite Green Induced Hepatotoxicity in *Channa punctata* (Bloch, 1793)

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Abstract

Malachite green is an N-methylated diaminotriphenyl methane dye widely used in the silk, wool, jute and leather industries and has also been applied in aquaculture to control fungal, protozoan, and helminth infections. Despite its use, the dye has raised serious concerns due to its unethical use in food adulteration and its documented toxic and carcinogenic effects. In the present study, the toxic impact of malachite green on hepatic tissue and liver enzyme profiles in the freshwater fish *Channa punctata* was evaluated. At first, fish were exposed to different sub-lethal concentrations of malachite green for a period of seven days under controlled laboratory conditions. Results of biochemical analyses revealed a significant change in serum liver marker enzymes, including Alkaline Phosphatase (ALP), Serum Glutamic Pyruvic Transaminase (SGPT), and Serum Glutamic Oxaloacetic Transaminase (SGOT), in treated fish compared to the control group, which indicates hepatocellular damage and impaired liver function. Exposure to malachite green has also been shown to induce oxidative stress, which is evidenced by a statistically significant increase in antioxidant enzyme activities such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione-S-Transferase (GST). Histopathological examination of hepatic tissues further confirmed toxic injury, showing pronounced cellular degeneration, distorted cellular architecture, cytoplasmic vacuolation, and disruption of intercellular connective tissue. The study showed that sub-lethal exposure to malachite green causes marked biochemical, oxidative, and histopathological alterations in *Channa punctata*, clearly demonstrating its hepatotoxic potential. Histopathological studies of the lesions in the liver tissues revealed several morphological alterations, and mild to moderate severity of the lesions was observed.

Major Findings: The findings highlight the ecological risk associated with the indiscriminate use of malachite green in aquaculture and emphasise the need for strict regulatory control to safeguard aquatic organisms and public health.

Keywords: Biochemical Parameters, *Channa punctata*, Enzyme Profile, Histopathology, Malachite Green, Oxidative Stress

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1. Introduction

Fish is regarded as one of the most affordable forms of animal protein, and it is widely cultivated in inland waterways. The Indian economy benefits greatly from freshwater aquaculture since it raises the nutritional status of rural areas. For aquaculture and capture fisheries in southern and Southeast Asia, murrels and catfish are economically significant species with enormous potential[1]. A chemical substance known as Malachite Green (MG) or N-methylated triphenylmethane, has long been used as an industrial dye for textiles like cotton, silk, wool, jute and leather. Additionally, it is widely used as an illegal food colouring agent, in many nations[2,3]. It has also been used as an efficient fungicide, particularly as general fish hatchery disinfection, and as a veterinary medication where it is administered topically as an antiseptic or to treat parasites, fungal infections, and bacterial infections in fish and fish eggs[4]. MG may enter fish through their gills and settle in edible tissues, and it has also been shown to cause Reactive Oxygen Species (ROS) to develop, which can damage DNA and create DNA adducts in the liver[5]. Liver cells are a suitable choice for toxicological and

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pharmacological testing because the liver is the primary site of metabolism and drug biotransformation[6]. ALP is a glycoprotein whose activity is a useful bioindicator to assess the buffer and major defender against oxidative stress[7]. Alpha-ketoglutarate and aspartate are converted to oxaloacetate and glutamate by the transaminase enzyme AST. All tissues except bones have the AST enzyme, which was originally known as SGOT. The liver and skeletal muscle contain the largest concentrations of this enzyme. Following bruising, trauma, necrosis, infection, or liver or muscle neoplasia, the concentration of AST is increased[8].

For the clinical detection of liver disease, especially hepatobiliary damage in humans and animals, ALP activity is a widely utilised blood biochemical marker. Cells include the crucial enzyme family known as superoxide dismutase, which helps them cope with stress and maintain normal physiological parameters. The most significant thiol-reducing agent and Cellular Indicator of Liver Disease (AASLD) is Glutathione (GSH), a tripeptide that is especially concentrated in the liver. An increase in the SGPT levels of greater than three times the upper limit of normal is the criterion used to identify whether liver damage is present or not. Elevated liver enzyme levels, additionally, SGOT is thought to be a sign of liver injury[9]. The liver is one of the most intensively investigated organs among vertebrate toxicological studies[10]. Because biotransformation reactions can increase the toxicity and carcinogenicity of metabolites, the liver is one of the most often targeted organs for cytotoxicity and carcinogenesis in fish and other vertebrates[11-13]. Therefore, to examine the toxicological consequences, the liver of *Channa punctatus* was chosen for the current investigation.

Most of the previous research has concentrated on residue persistence, acute toxicity or individual biochemical endpoints in fish, even though MG is widely used in aquaculture and has well-established hazardous, carcinogenic, and mutagenic qualities. Integrated studies assessing the combined effects of sub-lethal malachite green exposure on liver histo-architecture, antioxidant defence mechanisms, and hepatic enzyme biomarkers are still conspicuously lacking, especially in the economically and ecologically significant freshwater fish *C. punctata*. By evaluating oxidative stress using important SOD, GST, and Catalase, assessing changes in serum liver enzymes (ALP, SGOT, and SGPT), and analysing corresponding histopathological changes in the liver tissue of *C. punctata*, the current study aims to fill this knowledge gap by methodically investigating the hepatotoxic potential of malachite green at sub-lethal concentrations. This will provide a thorough understanding of malachite green-induced hepatic dysfunction in freshwater teleosts.

2. Materials and Methods

2.1 Experimental Animal

Channa punctata was selected as the experimental species for this study. Fish were collected from the local market of Nagaon, Assam, with an average length of 16-18cm and a weight of 45- 50gm. Before the experiment, the fish were acclimatised in the laboratory for three days. Fishes were fed commercial dry fish food pellets, and the water was changed daily to remove excretory materials. Optimal conditions were provided during the acclimatisation period, and no fish mortality occurred.

2.2 Experimental Chemical

Malachite green (N-methylated triphenylmethane) is an organic substance that is utilised in aquaculture as a contentious antibacterial agent and also as a pigment to dye silk, leather and paper. Contrary to its name, the dye is not made from the mineral malachite; The term is merely a result of the colour's likeness.

2.3 Acute Toxicity Experiment

The acute toxicity experiment was conducted in a static system to determine the 96-hour LC_{50} value of malachite green. The 96-hour LC_{50} value of malachite green was found to be 0.45 mg/l. In the present study, 1⁄10th (0.045mg⁄l) and 1⁄5th (0.09mg⁄l) of the 96 hr LC_{50} value were taken as the sub-lethal concentration of malachite green for further observation.

2.4 Experimental Design

Thirty healthy acclimatised fishes were randomly divided into 3 experimental groups of 10 fishes each and treated as follows: Group 1 treated as control, Group 2 treated with sub-lethal concentration 0.045mg/l and Group 3 treated with sub-lethal concentration 0.09mg/l. The experiment was conducted for 7 days to observe and study the effects of malachite green on the fish, which represents acute/

sub-acute toxicity. Therefore, the findings reflect short-term biochemical and histological responses and may not fully represent chronic or long-term effects. Extended exposure studies are necessary to evaluate cumulative toxicity, adaptive responses, or potential recovery patterns under environmentally relevant conditions.

2.5 Methods to Study Biochemical Aspects of Liver

2.5.1 Estimation of Alkaline Phosphatase (ALP)

ALP activity was estimated by the disodium phenyl phosphate method by the Kind and King method (1954)[14]. First, blood was drawn from both the control and treated groups of fish, and then centrifuged (Remi R-8C Plus) at 4000 rpm, and the supernatant was taken for the test. In this method, disodium phenyl phosphate is used as the substrate for Alkaline phosphatase enzyme. 1.0ml substrate was added to 1.0ml buffer (pH 10) and pre-warmed at 37°C for 3 minutes. Then 0.1ml of plasma was added and incubated at 37°C for 15 minutes. To that, 0.8ml N/2 NaOH was added, followed by 1.2ml M/2 $NaHCO_3$. Then 1.0ml of 0.6% 4-Aminoantipyrine (AAP) and 1.0mL of 2.4% potassium ferricyanide were added. Finally, absorbances were measured after the development of colour. Control is prepared similarly, but plasma is added after NaOH. Standards and blanks were prepared using a phenol standard and distilled water, respectively.

2.5.2 Estimation of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase Activity (AST)

Estimation of AST and ALT was done using a separate kit (C-MS/CX400) procured from Q-line Biotech Private Limited. Blood was drawn from both the control and the treated group of fish. The blood was then centrifuged at 4000rpm and 100µl of the supernatant was taken for each test. Reagent 1 and Reagent 2 were added in a 4:1 ratio, and absorbance was measured at 340nm in a spectrophotometer. Reagents (1+2) are stable for 21 days at 2-8^0 C and 72 hours at room temperature. The test results are presented as a reference range of values measured in enzyme units per litre (U/L).

2.5.3 Measuring Antioxidant Enzyme Activity

Preparation of the sample: Tissues were weighed, homogenised (10%) in 1M Tris-HCl buffer, pH=7.4, centrifuged for 10 minutes at 12000rpm and the supernatant was removed for analysis of biochemical parameters**.**

2.5.4 Estimation of SOD Activity

The method given by Flohe and Otting, 1984 was used to calculate the SOD activity[15]. This method is based on measuring the inhibition of the decreased rate of cytochrome-c oxidation at 550nm by the superoxide radical. The enzyme assay mixture comprises buffer (200µl), Nitro Blue Tetrazolium (NBT) (200µl), Nicotinamide Adenine Dinucleotide plus Hydrogen (NADH) (200µl), Sample (µl), Distilled water (190µl) and Phenazine Methosulfate (PMS) (200µl). Then the reaction mixture was incubated for 5 min in the light, and after that, the Optical Density (OD) was recorded at 550nm. The expression of the enzyme activity was expressed in Units/mg of protein.

2.5.5 Estimation of Glutathione-S-Transferase (GST)

The activity of GST was determined spectrophotometrically at 25^{o}C following the formation of GSH conjugate with 1-chloro- 2, 4-dinitrobenzene (CDNB) at 340nm using the extinction coefficient of 96 $Mm^{-1}cm^{-1}$ [16]. The enzyme assay mixture comprises buffer (2ml), CDNB (100 µl), distilled water (2ml), Sample (100µl), and glutathione (50µl).

2.5.6 Estimation of Catalase (CAT) Activity

Catalase activity is determined by monitoring the H_2O_2 decomposition from the decrease in absorbance at 240nm. The mixture comprising Buffer (1ml), sample (10µl and H_2O_2 (500µL).

2.6 Histopathological Aspects

Histopathological studies of the liver of both control and treated groups of fish were conducted following the standard histological procedure[17]. The sections were cut at a 4-micron thickness. Double staining was done by using haematoxylin and eosin. The histopathological changes were observed under a microscope.

The tissue lesions were evaluated qualitatively and further graded using a semi-quantitative scoring system based on the severity and extent of damage. Histological alterations were categorised as: Normal histology (0); Mild, where less than 10% of the tissue was affected (1); Moderate, where more than 10% but less than 50% of the

tissue was affected (2); and severe, where more than 50% of the tissue was affected (3). The scoring criteria were developed according to the microscopic characteristics of lesions observed in the liver tissues following the methodological approaches described by Al-Ghanim *et al.* and Zulkipli *et al.*[18,19].

2.7 Statistical Analysis

The results were expressed as means and Standard Deviation (SD). The data were studied and subjected to One-Way Analysis of Variance (ANOVA) followed by a post-hoc test to determine the significant differences.

3. Results

3.1 Hepatic Enzyme Profile

3.1.1 Estimation of ALP

The control fishes showed a mean value of 47.78±0.5 U/l of ALP. The fish exposed to sub-lethal concentrations of 0.045mg/l and 0.09 mg/l of malachite green showed the mean values of alkaline phosphate activity as 55.84±0.3 U/l and 63.75±0.23 U/l, respectively (Table 1). The values showed a significant increase when compared to the control (P<0.05).

3.1.2 Estimation of SGOT (AST)

The control fishes showed a mean value of 62.14±1.63 U/l of an enzyme activity. The fish exposed to sub-lethal concentrations of 0.045mg/l and 0.09mg/l of malachite green showed the mean values of enzymes as 75.26±0.23 U/l and 87.29±1.40 U/l, respectively (Table 2). The values showed a significant increase when compared to the control (p<0.05).

3.1.3 Estimation of SGPT (ALT)

The control fish showed a mean value of 79.08±1.61 U/l of serum determination. The fish exposed to sub-lethal concentrations of 0.045mg/l and 0.09mg/l of malachite green showed the mean values of serum determination as 85.92±3.48 U/l and 92.09±0.55 U/l, respectively (Table 3). The values showed a significant increase when compared to the control.

Table 1. ALP activity in the control and treated fishes

Concentration of Malachite Green	ALP (U/l)
Control (0mg/L)	47.78±0.5[a]
0.045mg/l	55.84±0.3[b]
0.09mg/l	63.75±0.23[c]

*Results are represented as mean±SD. Different superscript along the mean values differs significantly

3.2 Antioxidant Enzyme Profile

3.2.1 Estimation of SOD Activity

The control fishes showed a mean value of 56.43±0.06 U/l of an enzyme activity. The fish exposed to sub-lethal concentrations of malachite green (0.045 mg/l and 0.09 mg/l) showed mean enzyme values of 64.6 ± 0.08 U/l and 73.53 ± 0.05 U/l, respectively (Table 4). The values showed a significant increase when compared to the control (p<0.05)

3.2.2 Estimation of GST Activity

The control fishes showed a mean value of 48.43±0.12 U/l of an enzyme activity. The fish exposed to sub-lethal concentrations of 0.045mg/l and 0.09mg/l of MG showed the mean values of enzymes as 51.86 ±0.43 U/l and 56.83±0.36 U/l, respectively (Table 5). The values showed a significant increase when compared to the control (p<0.05).

3.2.3 Estimation of Catalase Activity

The control fishes showed a mean value of 32.41±0.12 U/l of an enzyme activity. The fish exposed to sub-lethal concentrations of 0.045mg/l and 0.09mg/l of malachite green showed the mean values of enzymes as 35.11±0.53 U/l and 41.26±0.47 U/l, respectively (Table 6). The values showed a significant increase when compared to the control (p<0.05).

3.3 Histopathological Aspects

Pathological research of malachite green has been shown by histopathology to have negative effects on the liver. It induces necrosis and sinusoidal congestion

Table 2. SGOT activity in the control and treated fishes

Concentration of SGOT	Enzyme Activity(U/L)
Control (0mg/L)	62.14±1.63[a]
0.045mg/l	75.26±0.23[b]
0.09mg/l	87.29±1.40[c]

*Results are represented as mean±SD. Different superscript along the mean values differs significantly

Table 3. SGPT activity in the control and treated fishes

Concentration of Malachite Green	Determination of SGPT
Control (0mg/L)	79.08±1.61[a]
0.045mg/l	85.92±3.48[b]
0.09mg/l	92.09±0.55[c]

*Results are represented as mean±SD. Different superscript along the mean values differs significantly

Table 4. SOD activity in the control and treated fishes

Concentration of Malachite Green	Enzyme Activity(U/L)
Control (0mg/L)	56.43±0.06[a]
0.045mg/l	64.6±0.08[b]
0.09mg/l	73.53±0.05[c]

*Results are represented as mean±SD. Different superscript along the mean values differs significantly

Table 5. GST activity in the control and treated fishes

Concentration of Malachite Green	Enzyme Activity(U/L)
Control (0mg/L)	48.43±0.12[a]
0.045mg/l	51.86±0.43[b]
0.09mg/l	56.83±0.36[c]

*Results are represented as mean±SD. Different superscript along the mean values differs significantly

Table 6. Catalase activity in the control and treated fishes

Concentration of Malachite Green	Enzyme Activity(u/l)
Control (0mg/L)	32.41±0.12[a]
0.045mg/l	35.11±0.53[b]
0.09mg/l	41.26±0.47[c]

*Results are represented as mean±SD. Different superscript along the mean values differs significantly

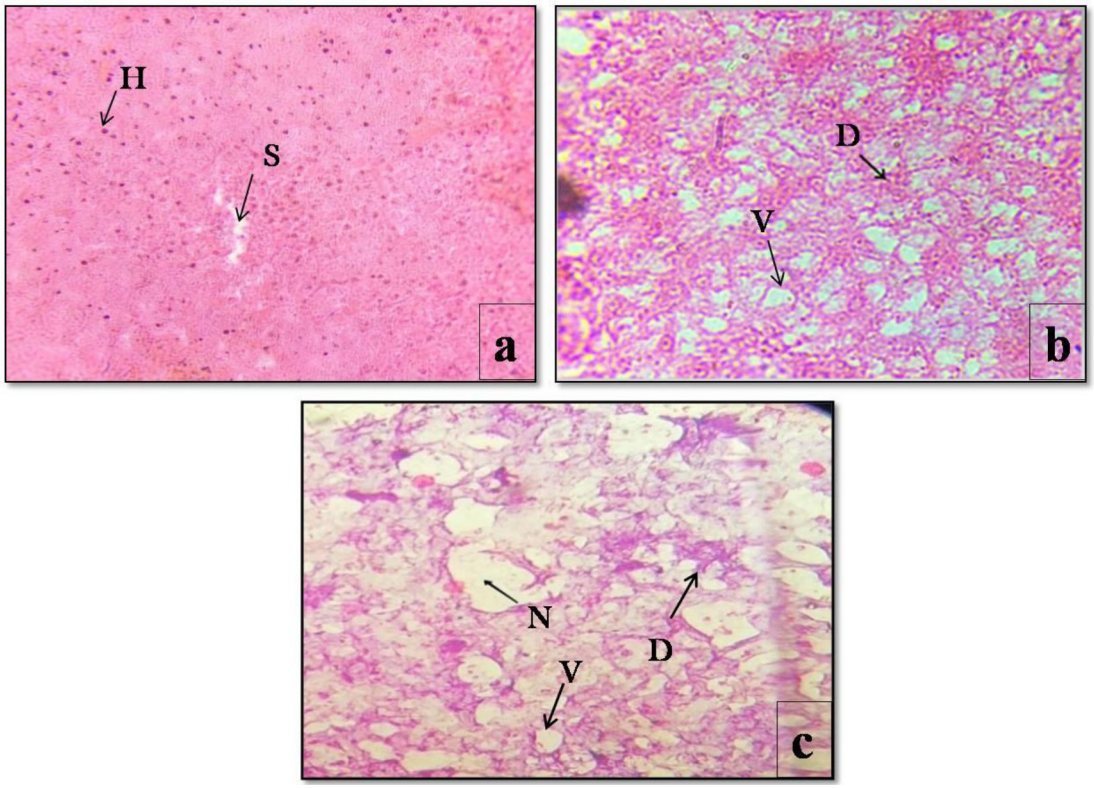


Figure 1. Photomicrograph of histology of liver of *C. punctata* of both control and treated groups under light microscopy. **(a)** Normal appearances of hepatocytes with distinct nucleus (H) and sinusoids (S), **(b)** Treated with 0.045mg/l showing distorted hepatocytes (D), vacuolation in hepatocytes (V), **(c)** Treated with 0.09 mg/l showing distorted hepatocytes (D), vacuolation in hepatocytes (V), necrosis in hepatic cell (N).

in the liver, which also results in nuclear damage and clear modification. The liver histology of the control fish revealed the presence of polygonal hepatocytes containing a centrally located nucleus, and Kupffer cells and sinusoids were observed. Vacuolation and damage of the membrane of the liver cell and marked loss of intracellular connective tissue led to massive degenerative changes in the histological architecture of the liver (Figure 1). Prominent changes in the histology of the liver were noticed. The polygonal shape of hepatocytes appeared to be distorted due to cellular degeneration. Progressive degenerative changes of hepatocytes led to complete distortion of the hepatic parenchymatous cells.

3.3.1 Histopathology Lesion Scores

In *C. punctata*, control fish liver tissue showed normal histology (0). However, 0.045mg/l sub-lethal concentration of malachite green showed a mild (1) score in liver tissues, and a concentration of 0.09 mg/l showed a moderate (2) score in the liver tissues.

4. Discussion

Fish are sensitive to any changes in the aquatic environment because they are in constant contact with it. Fish exposed to various synthetic chemicals show abnormalities in biochemical and histological parameters[20-23]. MG, which is used in aquaculture, has negative effects on fish. Every organism has a different antioxidative defence system, which is primarily made up of both enzymatic and non-enzymatic elements[24]. Many studies showed the effects of the toxic chemicals on the antioxidant defence system, which is composed of many enzymes like SOD, GSH and Catalase, etc. The SOD is a group of metalloenzymes that have a major antioxidant role and are the main line of defence against the harmful effects of superoxide radicals in living things[25,26]. This oxidative stress induced by toxic chemicals causes a significant increase in SOD activity[27]. Therefore, there is a significant impact of toxicants on various enzyme activities of the liver and the defence system. A similar result is reported from the present study, where a significant effect on SOD activity is observed in fish exposed to sub-lethal concentrations of MG. An increase in SOD activity was observed in bronchi and hepatic tissues of *Oreochromis mossambicus* after acute exposure to Endosulfan[28].

In the study, ALP activity in *C. punctata* after one week of exposure at sub-lethal concentrations was observed. In control fish, the sample showed 47.78± 0.5 U/l, and it was 55.84±0.3 and 63.75±0.23 in experimental fish. When the liver is not functioning properly, ALP is released into the blood and the enzyme level increases. The observed ALP activity in the experimental fish was statistically significant ($P < 0.05$). A similar result was reported by Al-Ghanim *et al.* (2020) in their study on *C. punctatus*[29]. Another study by Shanab *et al.* (2014) also reported a significant increase in AST (SGOT), ALT (SGPT) and ALP among the pesticide-exposed group of people[30]. Increased activity in ALP can be attributed to pathophysiological conditions in the liver as a result of pesticide intoxication, which may be due to disruption of hepatic smooth endoplasmic reticulum membrane in the intoxicated animals. At sublethal MG exposure, there was an increase in SGPT and SGOT activity. The higher levels of both indicate that during MG intoxication, there is a greater synthesis of amino acids or a transamination process from fatty acids or glucose. The fact that SGPT activity was comparatively higher than SGOT in this investigation indicates that pyruvate production contributed more than oxaloacetate. Sublethal dichlorous concentrations in *Channa gachua* raised SGPT and SGOT activity levels[31]. Another study reported that when exposed to alachor, *C. punctata* (Bloch) showed elevated levels of SGPT and SGOT[32]. Damage to the liver cells is the primary cause of changes in SGOT and SGPT activity. These made it very evident that MG poisoning was causing stress in the experimental fish. Again, SOD activity was 56.43±0.06 U/l in the blood serum of control fish and higher in experimental fish. Stressors can influence the antioxidant properties of fish; However, excessive zenobiotic concentrations can change the activity of the SOD enzyme[33]. Peng *et al.* (2012) found that SOD enzyme activity increases after exposure to low concentrations of the toxicant BDE-47 and is inhibited after exposure to high concentrations of the chemicals[34]. Similar results were reported by Song *et al.* (2017) when they worked with three pesticides, chlopyrifos, trifluralin and chlorothaloni[35]. Glutathione peroxidase is the multifunctional isoenzyme which is a part of the protective system against oxidative damage, and its level was found to be elevated as compared with control fishes[31]. A similar result was reported when *Cirrhinus mrigala* was exposed to sub-lethal concentrations of lead chloride. The toxicant caused an enhanced production of ROS, in response to which antioxidant activity increases. In the present study, peroxidase enzyme activity increases significantly in the liver because the liver is the main source of antioxidant enzymes to detoxify environmental toxicants. The present

finding concludes that exposure of *C. punctata* to MG causes an increase in oxidative stress.

The histology of the liver of *C. punctata* treated with various concentrations of MG reveals negative effects such as hepatocyte Vacuolation and enlargement, followed by necrosis. A similar result was also reported where the treated fish liver showed distorted Hepatocytes (D) and Vacuolations (V), which ultimately resulted in Necrosis (N) when compared with normal hexagonal cells found in the control liver[33]. A study by Khatun *et al.* also reported similar results in the case of some other insecticides. They observed some cellular alterations, including necrosis and vacuolation in hepatic cells due to the toxic impact of organophosphate insecticide malathion[36]. Another study in the same fish species by Khatun *et al.* also reported prominent changes in the histology of the liver, including progressive degenerative changes of hepatocytes, leading to complete distortion of the hepatic parenchymatous cells, vacuolation and loss of intercellular connective tissue changes[37]. Oxidative stress, mostly from the interaction of oxygen species and oxygen-free radicals, causes damage to tissues and biological components[38]. A semi-quantitative scoring system was also carried out to observe the severity and extent of damage. The liver tissue exposed to different sub-lethal concentrations of malachite green showed histological alterations when compared to the control, and were categorised on various scales. Similar results of mercury toxicity in the kidney, liver and gills were also reported by Meyor *et al.*, where they showed mild to moderate severity scores in mercury-induced toxicity[39].

The control group and the MG- treated groups exhibited marked differences in histological architecture, clearly indicating the damaging effects of the chemical on hepatic cells following exposure. These alterations highlighted the vulnerability of liver tissue to toxicants and emphasised the need to minimise the use of substances that may directly or indirectly contaminate air, water, and soil, thereby threatening animal health and the environment. Furthermore, this chemical adversely affects the liver, the primary detoxifying organ. The observed hepatic changes can serve as a reliable biomarker of aquatic pollution, reflecting the impact of chemical compounds on fish health.

5. Conclusion

The present study conclusively demonstrates that malachite green exerts pronounced toxic effects on *C. punctata*, even at sub-lethal concentrations. Significant elevation in serum hepatic enzymes (ALP, SGOT, and SGPT) clearly indicates hepatocellular injury and functional impairment of the liver. Alterations in antioxidant enzymes such as SOD and GST further confirm that exposure to MG induces oxidative stress, likely associated with mitochondrial dysfunction and tissue damage. Histopathological observations substantiate these biochemical findings, revealing marked structural abnormalities including vacuolation of hepatocytes, loss of intracellular connective tissue, and severe distortion of hepatic parenchyma.

Overall, the integrated biochemical and histological evidence confirms that MG is highly hepatotoxic to experimental fish and poses a serious risk to aquatic organisms even at low concentrations. These findings highlight the urgent need to restrict indiscriminate use of such chemicals, enforce regulated and prescribed dosages, and promote safer alternative biological methods to reduce environmental contamination and potential health hazards arising from food adulteration and aquatic exposure.

6. Limitations

Inclusion of lipid peroxidation (MDA), GSH, and ROS assays would strengthen the assessment of oxidative stress. Again, molecular mechanistic or dose-response modelling can be added in future studies for confirmation of toxicity.

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8. References

1. Haniffa MA. Native catfish culture: A technology package for fish farmers. Aquac Asia Magazine. 2009; 14(3):22-24.
2. Machova J, Svobodová Z, Svobodník J, Piačka V, Vykusová B, Kocová A. Persistence of malachite green in tissues of rainbow trout after a long-term therapeutic bath. Acta Veterinaria Brno. 1996; 65(2):151-159. https://doi.org/10.2754/avb199665020151
3. Cha CJ, Doerge DR, Cerniglia CE. Biotransformation of malachite green by the fungus *Cunninghamella*

elegans. Applied and environmental microbiology. 2001; 67(9):4358-4360. https://doi.org/10.1128/AEM.67.9.4358-4360.2001 PMid:11526047 PMCid:PMC93171.
4. Mitrowska K, Posyniak A, Zmudzki J. The effects of cooking on residues of malachite green and leucomalachite green in carp muscles. Analytica Chimica Acta. 2007; 586(1-2):420-425. https://doi.org/10.1016/j.aca.2007.01.042 PMid:17386743.
5. EFSA. Malachite green in food. EFSA panel on contaminants in the food chain (CONTAM). EFSA J. 2016; 14:4530. https://doi.org/10.2903/j.efsa.2016.4530
6. Soldatow VY, LeCluyse EL, Griffith LG, Rusyn I. *In vitro* models for liver toxicity testing. Toxicol Res. 2013; 2(1):23-39. https://doi.org/10.1039/C2TX20051A PMid:23495363 PMCid:PMC3593300.
7. Vairetti M, Di Pasqua LG, Cagna M, Richelmi P, Ferrigno A, Berardo C. Changes in glutathione content in liver diseases: An update. Antioxidants. 2021; 10(3):364. https://doi.org/10.3390/antiox10030364 PMid:33670839 PMCid:PMC7997318.
8. Eugster AK, Albert PJ, Kalter SS. Multiple enzyme determinations in sera and livers of tumour-bearing hamsters. Proc Soc Exp Biol Med. 1966; 123(2):327-331. https://doi.org/10.3181/00379727-123-31479 PMid:4288630.
9. Rasyid SA, Lio TM. Analysis of serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase levels in tuberculosis patients who are undergoing oat treatment in Kendari City General Hospital, Kota Kendari, Indonesia. Infect Dis Rep. 2020; 12(Suppl 1):8737. https://doi.org/10.4081/idr.2020.8737 PMid:32874466. PMCid: PMC7447934.
10. Hinton DE, Segner H, Braunbeck T. Toxic responses of the liver. D. Schlenk, W.H. Benson (Eds.), Target organ toxicity in marine and freshwater teleosts, Taylor and Francis, London. 2001: pp. 372.
11. Hinton DE, Lantz RC, Hampton JA. Effect of age and exposure to a carcinogen on the structure of the medaka liver: A morphometric study. Natl Cancer Inst Monogr. 1984a; 65:239-249.
12. Hinton DE, Walker ER, Pinkstaff CA, Zuchelkowski EM. Morphological survey of teleost organs important in carcinogenesis with attention to fixation. Natl Cancer Inst Monogr. 1984b; 65:291-320.
13. Kleinow KM, Melancon MJ, Lech JJ. Biotransformation and induction: implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. Environ Health Perspec. 1987; 71:105. https://doi.org/10.2307/3430418 PMid:3297653 PMCid:PMC1474347.
14. Kind PR, King E. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol. 1954; 7(4):322. https://doi.org/10.1136/jcp.7.4.322 PMid:13286357 PMCid:PMC1023845.
15. Flohe L. [10] Superoxide dismutase assays. In Methods in Enzymol. 1984; 105:93-104. Academic Press. https://doi.org/10.1016/S0076-6879(84)05013-8. PMid:6328209.
16. Habig W, Pabst MJ, Jakoby WB. The first enzymatic step in mercapturic acid formation. Glutathione-S-transferase. J Biol Chem. 1974; 249(7130.1):42083-42088. https://doi.org/10.1016/S0021-9258(19)42083-8 PMid:4436300.
17. Gurr E. Methods of analytical histology and histochemistry. Leonard Hill; 1958.
18. Al-Ghanim KA, Ahmad Z, Al-Balawi HF, Al-Misned F, Mahboob S, Suliman EA. Accumulation and histological transformation in the gills, liver, muscles, and skin in *Oreochromis niloticus* induced by mercury. Türk J Vet Anim Sci. 2017; 43(2):276-284. https://doi.org/10.3906/vet-1710-50
19. Zulkipli SZ, Liew HJ, Ando M, Lim LS, Wang M, Sung YY, Mok WJ. A review of mercury pathological effects on organs specific of fishes. Environ Pollut Bioavailab. 2021; 33(1):76-87. https://doi.org/10.1080/26395940.2021.1920468
20. Hanke W, Methfessel C, Wilmsen HU, Katz E, Jung G, Boheim G. Melittin and a chemically modified trichotoxin form alamethicin-type multi-state pores. Biochimi Biophys Acta (BBA)-Biomembr. 1983; 727(1):108-114. https://doi.org/10.1016/0005-2736(83)90374-7
21. Simon RU, Priefer U, Pühler A. A broad host range mobilization system for *in vivo* genetic engineering: Transposon mutagenesis in gram negative bacteria. Biotechnol. 1983; 1(9):784-791. https://doi.org/10.1038/nbt1183-784
22. Gluth G, Hanke W. A comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations: I. The dependency on exposure time. Ecotoxicol Environ Saf. 1985; 9(2):179-188. https://doi.org/10.1016/0147-6513(85)90020-X PMid:3987597.
23. Srivastava RK, Brown JA, Shahidi F. Changes in the amino acid pool during embryonic development of cultured and wild Atlantic salmon (*Salmo salar*). Aquaculture. 1995; 131(1-2):115-124. https://doi.org/10.1016/0044-8486(94)00202-Y
24. Venancio LP, Silva MI, da Silva TL, Moschetta VA, de Campos Zuccari DA, Almeida EA, Bonini-Domingos CR. Pollution-induced metabolic responses in hypoxia-tolerant freshwater turtles. Ecotoxicology and environmental safety. 2013; 97:1-9. https://doi.org/10.1016/j.ecoenv.2013.06.035 PMid:23993649.
25. Kohen R, Nyska A. Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification.

Toxicol Pathol. 2002; 30(6):620-650. https://doi.org/10.1080/01926230290166724 PMid:12512863.
26. Nwani CD, Lakra WS, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (Bloch). Int J Environ Res Public Health. 2010; 7(8):3298-3312. https://doi.org/10.3390/ijerph7083298 PMid:20948961 PMCid:PMC2954582.
27. Oruç EÖ. Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. Pestic Biochem Physiol. 2010; 96(3):160-166. https://doi.org/10.1016/j.pestbp.2009.11.005
28. Kumar N, Prabhu PA, Pal AK, Remya S, Aklakur M, Rana RS, Gupta S, Raman RP, Jadhao SB. Anti-oxidative and immuno-haematological status of Tilapia (*Oreochromis mossambicus*) during acute toxicity test of endosulfan. Pestic Biochem Physiol. 2011; 99(1):45-52. https://doi.org/10.1016/j.pestbp.2010.10.003
29. Al-Ghanim KA, Mahboob S, Vijayaraghavan P, Al-Misned FA, Kim YO, Kim HJ. Sub-lethal effect of synthetic pyrethroid pesticide on metabolic enzymes and protein profile of non-target Zebra fish, *Danio rerio*. Saudi J Biol Sci. 2020; 27(1):441-447. https://doi.org/10.1016/j.sjbs.2019.11.005 PMid:31889868. PMCid: PMC6933226.
30. Awad OM, El-Fiki SA, Abou-Shanab RA, Hassanin NM, Abd El Rahman R. Influence of exposure to pesticides on liver enzymes and cholinesterase levels in male agriculture workers. Glob Nest J. 2014; 16(5):1006-1015. https://doi.org/10.30955/gnj.001285
31. Koul PC, Mastan SA, Qureshi TA. Sub-lethal effect of Dichlorvos (DDVP) on certain biochemical parameters of *Channa gachua* (Ham.). J. Herbal Med Toxicol. 2007; 1:29-32.
32. Tilak KS, Raju PW, Butchiram MS. Effects of Alachlor on biochemical parameters of the freshwater fish, *Channa punctatus* (Bloch). J Environ Biol. 2009; 30(3):421-426.
33. Saravanan TS, Rajesh P, Sundaramoorthy M. Studies on effects of chronic exposure of endosulfan to *Labeo rohita*. J Environ Biol. 2010; 31(5):755.
34. Peng Y, Fan CP, Liao W, Liu XY, Wu XY. Nie XP. Toxic effects of 2,2',4,4'-tetrabromodiphenyl ether on *Daphnia magna*. Asian J Ecotoxicol. 2012; 7:79-86.
35. Song Y, Chen M, Zhou J. Effects of three pesticides on superoxide dismutase and glutathione-S-transferase activities and reproduction of Daphnia magna. Arch Environ Prot. 2017; 43(1):80-86. https://doi.org/10.1515/aep-2017-0010
36. Khatun N, Basumatari D, Sharma P, Kumari D. Effects of malathion toxicity on the haematological, genotoxic and histological parameters of *Clarias batrachus* (Linnaeus, 1758). J Aquat Biol Fish. 2024; 12(1):78-82.
37. Khatun N, Sharma P, Borah MP, Bora KJ. Profenofos induced hepatotoxicity, genotoxicity and haematological abnormalities on freshwater fish *Channa punctatus* (Bloch, 1793). Biol Bull. 2025; 52(3):95. https://doi.org/10.1134/S1062359023606389
38. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf. 2006; 64(2):178-189. https://doi.org/10.1016/j.ecoenv.2005.03.013 PMid:16406578.
39. Meyor LS, Sajol PJ, Obedencio Jr JM, Bautista-Jimenez V. Mercury detection and histopathology of selected fish species in Jasaan municipal Fishport, Misamis Oriental. The Philippine Journal of Fisheries. 32(2): in press

Author's Contributions

P.S. conceptualised, supervised, analysed the data and prepared the manuscript; N.K. assisted in investigation, interpretation of data, prepared the draft and finalised the manuscript; D.B. supervised the methodology, reviewed and edited the prepared draft; M.P.B. assisted in data analysis and draft preparation; S.B. executed experiments, analysed the data and prepared the draft manuscript; P.S., P.B, P.B. and T.K.B. edited the original draft and finalised the manuscript. All the authors have given final approval for the manuscript.

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