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Effects of malathion toxicity on the haematological, genotoxic and histological parameters of *Clarias batrachus* (Linnaeus, 1758)

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ABSTRACT

Pesticides are widely used in intensive agricultural practices to protect crops from various pest and diseases. Pesticide exposure may also be fatal to many non-target organisms like fish where it hampers its health through metabolism impairment. Malathion, is being extensively used as dust, emulsion, and vapour to control wide variety of insect pests under different conditions, which may induce many significant changes in fish. Present study is aimed to investigate the toxicological effects on hematological parameters, histopathological alterations, and chromosomal changes in fishes exposed to the organophosphate pesticide. Freshwater fish *Clarias batrachus* were exposed to two sub-lethal concentrations of Malathion (0.22mg/l and 0.44mg/l) and after 7 days of exposure, changes in haematological parameters, hepatic tissue histology, genotoxic effects were evaluated. The study showed that malathion induced different changes in the haematology, histology of liver and also induced nuclear abnormalities in erythrocytes of treated fishes. The haematological parameters showed some drastic changes like RBC count and haemoglobin content decreased whereas WBC count increased in the treated fish as compared to control group. The histopathological study of liver in the treated fishes reveals some significant changes like cell damage, distorted cell shape and vacuolations etc. Some significant genotoxic effects in the erythrocytes of treated fish were observed such as induction of micronuclei, lobed nuclei, irregularly shaped nuclei, notched nuclei and distorted nuclei. The results were statistically significant at $p<0.05$ level.

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1. Introduction

The aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman *et al*. 2002). Pesticides and insecticides can pollute water sources by two ways, through direct application of pesticides in aquatic systems and indirect uses such as erosion from agricultural lands and agricultural waste water infiltration and eventually washed into deep water environments and ecosystem (Dutta and Arends, 2003). The use of pesticides has become an inevitable feature of agriculture for pest control. It is assumed that 40% of the crop loss can be caused by pests and insects, which could be unbearable (Mrong *et al.* 2021). Pesticides are widely used in agriculture worldwide, and they may negatively affect non-target creatures like fish.

The organophosphorus family is the most popular class of pesticides among the main pesticide (insecticide) groups. Due to their low levels of breakdown and residues, organophosphorus pesticides (OPs) recently supplanted organochlorine pesticides (OCs) in agriculture. Such pesticides can have severe and long-lasting contaminating effects on aquatic animals that are not their intended targets, such as fish, and are particularly poisonous to aquatic life. Commonly used organophosphorus pesticides are Malathion, methyl parathion, dichlorvos, trichlorfon, chlorpyrifos, diazinon, fenitrothion, quinalphos, phosphamidon, etc. Malathion is a commonly used organophosphate insecticide useful to control economically important crops (Saeed *et al*. 2005), but are found very much hazardous to the aquatic flora and fauna and hence ultimately to human being as they depend on aquatic foods like fishes (Mellanby, 1967). Since the removal of the organochlorine insecticide owing to its ceaseless harmful effect, organophosphate (OP) insecticides have been chosen as the most widely favoured insecticide in today's world to make the crop free from pest for greater productivity.

The pesticide is used for the control of pest on greenhouses, nurseries, home and garden vegetables, field crops, fruits and domestic animals (Deka and Mahanta, 2016). According to reports, malathion harms fish even at low doses and also has low cumulative ability and short term persistent in nature but high pesticidal action (Svoboda *et al*. 2001). Therefore, monitoring the effects of these herbicides is crucial. Aquatic organisms, including fish, accumulate pollutants directly from contaminated water and indirectly via food chain (Sasaki *et al.* 1997). *Clarias batrachus* is one of the commercial important fish widely used for its food value of this fish in India, Burma, and Sri Lanka. Due to the ease in transportation of this fish and accessory respiratory organs it can survive for a long time out of water (Wasu *et al*. 2009). Fish live in very intimate contact with their environment and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Wilson and Taylor, 1993; Saravanan *et al.* 2010).

Circulating blood is the universal symbol of health and illness and any change in the biochemical or physical properties of the blood signals both haematological and non-hematological sickness. Haematological and blood biochemical parameters appear to be useful biomarkers for evaluating the physiological state of fish exposed to pesticides; however, they are not specific markers of intoxication (Lakshmaiah, 2017). Pesticide-induced

Pathophysiological changes in fish rely on various factors, including the active component and its concentration, the length of exposure, the type of fish, the habitat, etc. Currently, almost two million tonnes of pesticides are used annually. Blood parameters are considered as patho-physiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari and Sarkar, 2004).

The liver is the largest gland of the body connected with several function. It is often designated as the body's detoxifying organ which is prone to a variety of infections and the structure can be damage by a variety of chemicals and drugs. It has no direct contact with the environmental pollutants dissolved in water but due to its contact with blood, its indirectly affected. Micronuclei (MN) are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that are not incorporated into the nucleus after cell division (Kalita and Khatun, 2018). Micronucleus assay is widely used to detect effect of many classes of chemicals, e.g., pharmaceutical, agricultural, food additives etc (Hayashi, 2016). Nowadays, hematological and histological parameters of fish is becoming important for toxicological research, environmental monitoring, and assessment of fish health conditions (Shah and Altindag, 2004). As not much work has been done on the effects of Malathion on fresh water fish *Clarias batrachus*. Therefore, the present study aimed to evaluate the effect of sublethal concentration of Malathion on haematological and histopathological changes of fresh water fish *Clarias batrachus.*

2. Materials and Methods

2.1 Collection of Experimental animal

Healthy fresh water catfish (*Clarias batrachus*) weighing about 120-150 grams were collected from the local market of Guwahati, Assam, India. All the collected fishes were transported to the laboratory and kept in three earthen pots, each containing non- chlorinated tap water. They were acclimatized for 3 days prior to the experimental study under laboratory conditions. The water was aerated properly during the experimental period and fish were fed daily with commercial fish feed.

2.2 Experimental chemical

The organophosphate, Malathion was collected from the local market of Guwahati. Malathion is an organophosphate which acts as an acetyl cholinesterase inhibitor. It is a man-made organophosphate insecticide commonly used to control mosquitoes and various insects that attack fruits, vegetables, landscaping plants, and shrubs. It can also be found in other pesticide products used indoors and on pets to control ticks and insects, such as fleas and ants.

2.3 Experimental Designs (Doses and Duration)

The LC_{50} value of malathion 96 hours was taken as 0.22 ppm (Diwakar *et al*. 2019). For the experiment, two sub lethal concentrations of 0.044 ppm and 0.022 ppm, which were one-fifth and one-tenth of the LC_{50} value, respectively was used to carry out the experiment. Fishes were divided into three groups of two fish each and treated as follows. Group 1 was treated as a control, Group 2 was treated with sub-lethal concentration of 0.022 mg/liter of Malathion, Group 3 was treated with a sub-lethal concentration of 0.044 mg/liter of Malathion.

2.4 Estimation of blood parameters

2.4.1 Estimation of red blood cells (RBC) and white blood cells (WBCs):

The total red blood cell (RBCs) and white blood cells (WBCs) were counted by microdilution method using Hemocytometer or Neubauer's chamber.

2.4.2 Estimation of haemoglobin:

Sahli's method, also called the acid hematin method, is the visual comparator method used to estimate hemoglobin.

2.4.3 Micronuclei and nuclear abnormality test using wrights stain

For nuclear abnormality test, peripheral blood samples were obtained by caudal vein puncture using a heparinized syringe. Blood was smeared immediately over the clean grease-free microscopic slides, air dried for 10-15 minutes. Each slide was stained with wright stain solution for 10 minutes. Three slides were prepared for each group. The slides were then washed under running slow tap water and then observed under the microscope.

2.5 Study of the effect of Malathion on the histology of liver tissue

Histological studies of liver of both control and treated groups of fish were conducted following the standard histological procedure (Gurr,1958). The sections were cut at 4 micron thickness. Double staining was done by using haemotoxylin and eosin. The histopathological changes were observed under a microscope.

2.6 Statistical Analysis

The results were expressed as means and standard deviation (SD). The means of parameter studied were subjected to student's t-test in MS Excel to determine the significant differences.

3. Results

3.1 Haematological Parameters

3.1.1 Total Count of RBC

The erythrocyte count of healthy control showed a mean value of 2.44 $\pm 0.47\times10^6$ mm-3. The fishes exposed to sub lethal concentrations of 0.22 mg/liter and 0.44 mg/liter of Malathion showed mean values of RBC as $1.64\pm0.21\times10^6$ and $1.21\pm0.06\times10^6$ mm-3 respectively (Table 1). The treatment with Malathion was found to cause a drastic decrease in the amount of RBC. The decrease caused was also found to be dose dependent as the higher the dose of Malathion, the sharp decrease in the amount of RBC is seen.

3.1.2 Total Count of WBC

The total count of WBC revealed that the mean amount of white blood cells (WBCs) in a healthy control *Clarias batrachus* is $70.18\pm0.12\times10^3$ mm-3. The fishes exposed to a sub-lethal concentration of 0.22 mg/liter and 0.44 mg/liter showed the mean value of white blood cell as

Table 1. changes in total number of RBC, WBC and hemoglobin in Malathion-treated fish (*Clarias batrachus*)

Concentration of malathion	Total Number of RBCs (10^6 mm^{-3})	Total number of WBCs (10^3 mm^{-3})	Haemoglobin (gm dl^{-1})
Control (group 1)	2.44± 0.47	70.18 ± 0.12	8.015± 0.37
Treated (group 2)	1.64± 0.21	81.423±0.22	7.203 ± 0.26
Treated (group 3)	1.21±0.06	99..725± 0.45	6.265±0.12

81.423±0.22×10^3 and 99.725±0.45×10^3 mm-3 respectively (Table 1).

3.1.3 Total Haemoglobin

The control fish showed mean value of 8.015±0.37 gm dl^{-1} for hemoglobin. The fishes exposed to sub lethal concentrations of 0.22 mg/liter and 0.44 mg/liter showed the mean value of the concentration of 7.203±0.26 and 6.265± 0.12 gm dl^{-1} respectively (Table 1). The value showed a significant decrease when compared to control.

3.2 Analysis of Micronuclei and nuclear abnormality using Wright's stain

3.2.1 Control group:

Normal RBC (Erythrocytes) having well-defined boundaries, centrally placed rounded nuclei and clear cytoplasm were observed in the control group (Fig. 1) .

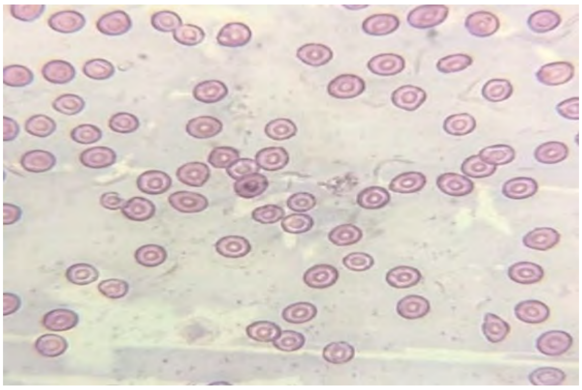

Fig. 1. Photomicrograph of normal peripheral erythrocytes of *Clarias batrachus* in control group. Wright's Stain, magnification × 40; RN: Rounded nuclei

3.2.2 Experimental group (Low Dose)

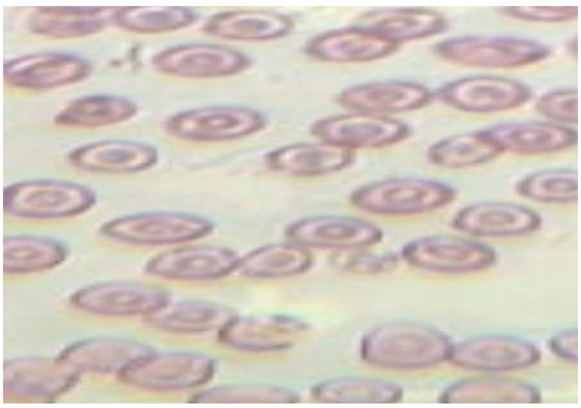

Fig. 2. Photomicrograph of nuclear abnormalities in erythrocytes of *Clarias batrachus* treated with 0.022 mg/L concentration of Malathion showing notched nuclei (NN) in peripheral blood erythrocytes. Wright's Stain, magnification × 40

3.2.3 Experimental group (High Dose)

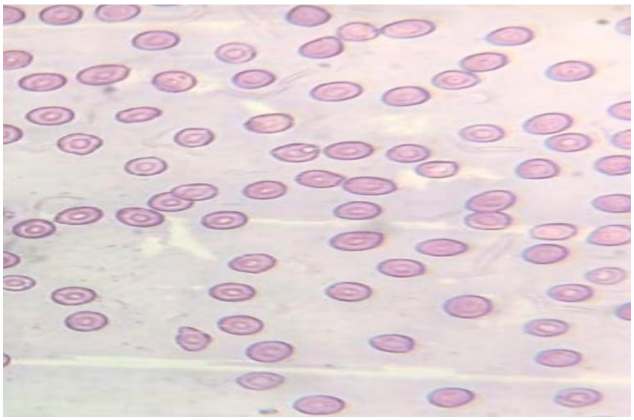

Fig. 3. Photomicrograph of nuclear abnormalities in erythrocytes of *Clarias batrachus* treated with 0.044 mg/L concentration of Malathion showing micronucleus and distorted nuclei (DN) in peripheral blood erythrocytes. Wright's Stain, magnification × 40

3.3 Histological study of liver

Histological findings:

The hepatic cells of the liver, of the control group of fishes show a normal typical and compact architecture with no histological abnormalities (Fig. 4). However, the two different treated group of fishes show some cellular alterations (Fig. 5 & 6), the most prominent of which are:

1. Necrosis in hepatocytes (NH) and nucleus degeneration (DH) in group 2 (Fig. 5)
2. Hepatocytes damage, vacuolation in hepatic cells (VH) and cell necrosis in liver tissues in treated group 3 (Fig: 6)

3.3. 1 Group 1 (Control)

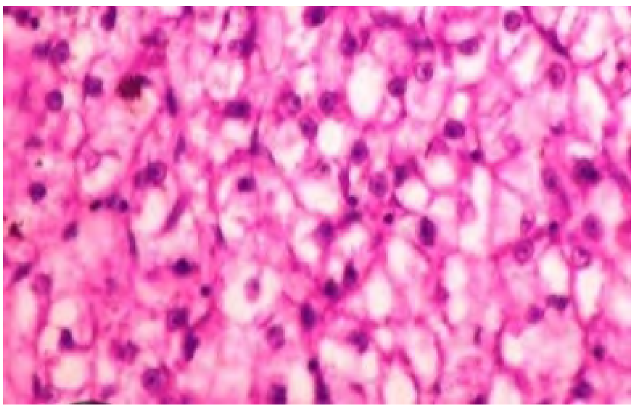

Fig. 4. Showing well defined hepatocytes (H) without any tissue degeneration and cell necrosis

3.3.2 Experimental group (low dose)

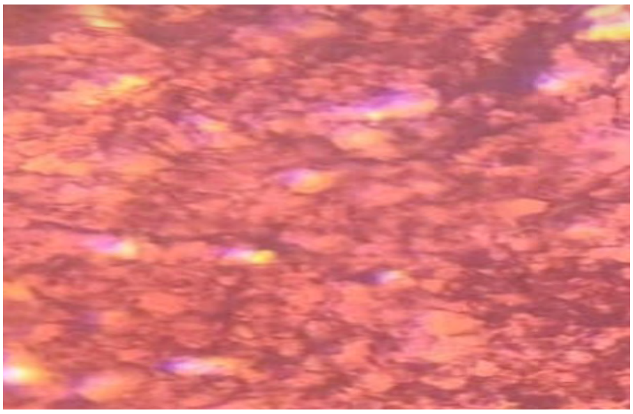

Fig. 5. *Clarias batrachus* treated with 0.022 mg/L concentration of Malathion showing degeneration of hepatocytes (DH) and necrosis of hepatic cells (NH) in liver tissues. Magnification × 40

3.3.3 Experimental group (high dose)

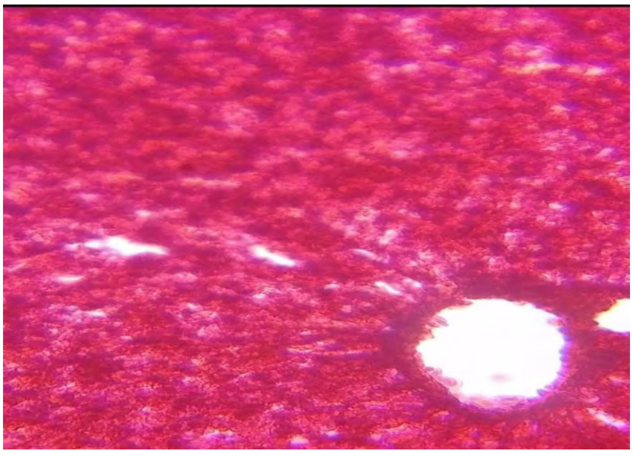

Fig. 6. *Clarias batrachus* treated with 0.044 mg/L concentration of Malathion showing hepatocytes damage, vacuolation in hepatic cells (VH) and cell necrosis in liver tissues. Magnification × 40

4. Discussion

Haematological parameters of fish constitute an important system of survival. Insecticidal contamination to aquatic ecosystems can affect the haematological parameters of fish (Summarwar, 2012). The study showed a decrease in the count of RBCs and haemoglobin content in the treated fish while WBCs count increased. This was perhaps, a typical defensive response of the fish against a toxic invasion and second most common probability may be leukemia (Sudha, 2012). The significant decrease in the amount of RBC in the present study can be attributed to hemolysis and shrinkage of blood cells by the toxic effect of the insecticide. Also, a decrease in the amount of hemoglobin may be the release of immature cells from the hematopoietic tissues into the blood as well as the disruption of iron metabolism that can lead to defective hemoglobin synthesis. These alterations in haematological parameters were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in hemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to lead (Shah, 2006).

Many workers reported similar findings, the carbofuran caused the reduction in the value of hemoglobin in teleosts fishes (Singh and Srivastava, 2010). However, a significant rise in the concentration of leucocytes was reported in *C. punctatus* due to the toxic effect of Malathion (Magar and Duve, 2012). White blood cells play an important role in the body's defence mechanism. The increase in WBC results from sensitization of the immune system to tissue damage caused by Malathion. This was perhaps a typical defensive attack of the fish in response to toxicity induced by Malathion. Ahmad, 2012 in his study the effects of sub-lethal exposure of malathion on haematological parameters of *Clarias gariepinus* observed decreased value of RBC and WBC counts, hemoglobin (Hb) concentration and haematocrit (Ht) values as compared to the control fish after exposed to sublethal doses of malathion. Venkataraman and Sandhya Rani, 2013 studied acute toxicity and blood profile of freshwater fish, *Clarias batrachus* exposed to sub-lethal concentration of malathion and have calculated the 96 h LC_{50} of malathion as 1 ppm. They had noticed that with increase in the concentration and exposure period of malathion, there were significant reductions in Hb (haemoglobin), haematocrit (PCV), RBC (Red Blood Cell Count) and MCV (Mean Corpuscular Volume) levels and increase in WBC (White Blood Cell Count), MCHC (Mean Corpuscular Haemoglobin Concentration) and MCH (Mean Corpuscular Hemoglobin) levels in the malathion treated fish in contrary to the normal values of haematological parameters in control group. Kalita and Khatun (2018) stated that fishes that were exposed to 6.5mg/L and 13mg/L of rogor for 7 consecutive days a decreased number of RBC and decreased Haemoglobin concentration was observed in both the treated groups in comparison with the control. Increased number of WBC in comparison with the control was also observed in the treated groups.

In the study, the histological structure of the liver in the control group of fish showed a normal typical and compact architecture with no histological abnormalities, while treated groups showed changes like necrosis in hepatocytes. Similar findings were reported by Shukla *et al*. (2005) where they noticed that when the catfish *Clarias batrachus* is exposed to the increased concentration of the organophosphate pesticide Nuvan, the hepatocytes exhibited a reduction in their size and peripheral accumulation of cytoplasm and the nuclei of the hepatocytes lost their rounded appearance. Mandal and Kulshrestha studied the effects of sublethal concentration of sumithion on liver of *Clarias batrachus* and observed liver necrosis, vacuolization and breakdown of the cell boundaries. Again, Elezaby *et al*. (2001) studied the effect of Malathion on the fish *Oreochromis niloticus* and has observed that Malathion induced many histopathological changes in the liver and gills of the fishes. These changes were hemorrhage, necrosis and destruction of lamellae of the lungs, and necrosis and lipidosis in the liver. Couch (1975) reported perivascular lesions in the liver of fishes exposed to organic contaminants and pesticides. According to Gingerich (1982) the vacuolation of hepatocytes might indicate an imbalance between the rate of synthesis and rate of substance release in hepatocytes.

A study on the effect of malathion on liver of fresh water fish *Channa punctatus* exposed to sublethal concentration of malathion exhibited histopathological alterations and showed degeneration of cytoplasm and vacuolization of hepatocytes (Magar and Shaikh, 2013). Pugazhvendan *et al.* 2009, observed normal histological architecture of liver in control fish but the histopathology of experimental fish liver showed proliferation of ducted cells and appearance of small spaces between hepatic cells. The effects on liver that were observed in the study stated that liver reduce the general state of health of the fish at sub-lethal concentrations. It may, therefore, be said that a sub-lethal concentration may be safe; however, it cannot be used indiscriminately.

5. Conclusion

The results of the present study stated that Malathion, a commonly used pesticide in field is moderately toxic to fish. Some hematological changes like declination of RBC and Hb in treated fish were observed in the study which were not too drastic and suggested a moderate Malathion toxicity to the fishes' haematological parameters. Again, the amount of white blood cells in the blood has increased in order to combat the various infections induced by Malathion on the fishes. The control group and groups treated with low dose and high dose of Malathion showed a different histology and suggest the damaging effect of Malathion on the hepatic cells of the liver when exposed to low and high doses of Malathion with the higher dose being more toxic. Therefore, we should try to minimize the use of substances that could directly or indirectly contaminate the air, water, and land and harm animal health and the environment. It can also be stated that organophosphorous insecticide like malathion affect the detoxifying organ, liver which could be used as a good response of aquatic pollution with effect of organophosphorous compound on fish.

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