



## Research Article

## The role of gallic acid in the protection of haematological and biochemical stress induced by malathion for 96 hrs. in freshwater fish *Clarias batrachus*

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## Abstract

Pesticide contamination poses a serious threat to aquatic ecosystems, particularly through organophosphate compounds that disrupt physiological functions in non-target species. This study evaluated the acute toxic effects of malathion (0.1 ppm) on the haematological and biochemical parameters of *Clarias batrachus* and assessed the protective potential of gallic acid as a natural antioxidant. Fish were divided into five groups: control (G1), malathion-treated (G2), and malathion combined with gallic acid at 50, 100, and 150 mg/L (G3– G5). After 96 hours of exposure, significant haematological alterations were observed in the malathion-treated group, including increased haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), alongside decreased red blood cell (RBC), total leukocyte count (TLC), and platelet levels. Biochemical analysis revealed reduced albumin, transaminases (SGOT, SGPT, AST), uric acid, and glucose levels, with elevated bilirubin, urea, creatinine, and total lipids, indicating hepatic dysfunction, renal stress, and metabolic imbalance. Gallic acid supplementation significantly ameliorated these alterations in a dose-dependent manner, with the highest recovery observed at 150 mg/L (G5). The findings demonstrate that malathion induces acute systemic toxicity in *Clarias batrachus*, while gallic acid confers substantial protective effects, likely through its antioxidant properties. This study highlights the potential application of natural antioxidants in mitigating pesticide-induced toxicity in aquaculture and emphasises the ecological risks associated with organophosphate contamination in aquatic environments

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**KEYWORDS:** Malathion toxicity, Gallic acid, Haematological parameters, Biochemical stress, *Clarias batrachus*.

## 1. INTRODUCTION

Pollution of aquatic ecosystems by extensive and intensive use of toxic chemicals by drift rounds of drainage and leaching has become one of the most important problems worldwide<sup>1,20</sup>. Commercial products for protecting plants and crops, including pesticides and their preparations, are currently available worldwide. Every year, 4 million tons of active ingredients in commercial pesticides are used<sup>1,2</sup>. Pesticides are the chemicals that are used for controlling the reproduction of unwanted organisms such as insects, fungi, and rodents in agriculture as well as public health<sup>2,3</sup>. Pesticide handling areas result in point-source pollution that can account for up to 90% of the total pesticide-loading invertebrates. The pesticide toxicity index (PTI) is a known tool to quantify the relative toxicity of a specific mixture to indicator species of aquatic organisms<sup>3,4</sup>. Pesticides are used extensively in agriculture and are one of the major water pollutants. Pesticides indirectly eradicate target and non-target economically important species by disrupting the biological chain or by protecting against toxic stress and chemical changes. As a result of the extensive application of pesticides, large-scale fish mortality has occurred widely<sup>5,7</sup>. Approximately 20,000 commercial products for the protection of plants and crops, including pesticides and their different preparations, are currently available worldwide<sup>1,6</sup>. Organophosphates are extensively used in agriculture, among the most hazardous chemicals for genetic disorders and physiological alteration. The major route of organophosphate to water ecosystems in urban areas is through rainfall runoff and atmospheric deposition<sup>6,13</sup>. Malathion is one of the most effective organophosphorus pesticides used for the control of pests in greenhouses, nurseries, homes, gardens, vegetables, field crops, fruit, and domestic animals<sup>7,21</sup>. Malathion is a light yellow or dark brown liquid with a strong offensive odor. It has low solubility in water and is slightly soluble in mineral oil, the most organic solvent. Malathion was one of the earliest organophosphate insecticides developed in 1950 and first registered for use in the United States in 1956 by the United States development of agriculture<sup>8,22</sup>. However, numerous malathion poisoning incidents, including acute and chronic cases, have been reported among workers and small children through accidental exposure<sup>9,23</sup>. Fish are susceptible to a wide variety of toxicants in water. Various species of fish show uptake and accumulation of contaminants or toxicants, such as pesticides. After accumulation in tissues, pesticides produce many physiological histological, and biochemical changes in the fish and other freshwater fauna by influencing the activity of different enzymes and metabolites<sup>5,7</sup>.

### Material and methods:

A batch of healthy and mature freshwater fish, scientifically identified as *Clarias batrachus* and commonly referred to as mangur fish, with an average size of 15±2 CM and weight of 80±5 Grams, was procured from the local fish market in Jhansi, Uttar Pradesh. Upon acquisition, the fish were carefully inspected for any signs of injury and subsequently immersed in a 1% KMnO<sub>4</sub> solution for a brief period to eliminate any

potential dermal infections. Following this, the fish underwent a 2-week acclimatization period in a spacious cement tank within a laboratory setting. During the acclimatization phase, the fish were provided with an artificial pellet diet twice daily. Additionally, the tank water was regularly replenished with clean water to remove waste and residual food, while a mechanical pump was employed to aerate the water and ensure an adequate oxygen supply. As per the experimental protocol, the fish were deliberately deprived of food for 24 hours and were not fed during the entire experimental duration. The required commercial grade of malathion (50% EC) was sourced from Jhansi and obtained through Indian Mart. The determination of the acute toxicity dosage, set at 0.1 PPM, was based on prior research findings and LC<sub>50</sub> assessments conducted by relevant authors. To prepare the malathion solution, the stock solution was diluted with acetone due to the compound's high solubility in acetone. Subsequently, the acclimatized fish were transferred to five glass aquariums, each measuring 60X30X38 CM, with each aquarium accommodating 10 fish. The five glass aquariums were organized into five distinct experimental groups for research. The initial group served as the control group and did not receive any chemical treatment, while the second group was subjected to contamination with 0.1 PPM (0.1 mg/L) of malathion. Third group was contaminated with .1PPM (0.1mg/L) of malathion and 50 mg/L of gallic acid treatment of fish, group four was contaminated with .1PPM (0.1mg/L) of malathion and 100mg/L of gallic acid treatment. Group five was contaminated with .1PPM (0.1mmg/L) of malathion and 150 mg/L of gallic acid treatment. Environment conditions were similar in all the five aquariums. In these five groups, three groups were treated with chemicals and treatment.

The experimental concentrations of the substances under study were refreshed every 24 hours for a total period of 96 hours. At each sampling time throughout the entire experimental period, two fish were carefully removed from each aquarium to conduct the necessary tests. Blood samples were meticulously obtained from the caudal vein of *Clarias batrachus* to facilitate comprehensive analysis. For hematological evaluations, heparinized vials were employed to prevent coagulation, allowing for accurate measurement of blood cell counts and other crucial hematological parameters. In contrast, plain vials were utilized for biochemical testing, enabling the assessment of metabolites, enzymes, and other biochemical markers that provide insights into the fish's overall health status and physiological condition. This systematic approach is essential for understanding the biological and environmental factors affecting this species. The serum was obtained by subjecting the fish blood samples to centrifugation at 3500 rpm for 15 minutes and then stored at a temperature of -20°C to preserve the integrity of the samples. The haematological indices examined included erythrocyte count (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and leukocyte differential counts were

determined according to the unified methods for haematological examination of fish (Abdul Rauf et al., 2015). Furthermore, the lipid profile was meticulously determined using a kit method, and liver and kidney function tests were carried out using a semi-automatic serum biochemistry analyzer to comprehensively assess the physiological responses of the fish to the experimental conditions.

**RESULT**

The findings of the study demonstrate that exposure to malathion has significantly influenced the blood and biochemical parameters in *Clarias batrachus*. Specifically, it was observed that various blood components and enzyme levels

were altered due to this exposure. Furthermore, the research explored the therapeutic effects of gallic acid treatment on *Clarias batrachus*, assessing its potential to mitigate the adverse impacts caused by malathion. The fish exposed to malathion and different concentrations of gallic acid showed a decrease in Red Blood Cell (RBC), Hematocrit (HCT), Total Leucocyte Count (TLC), Neutrophil, and Mean Corpuscular Volume (MCV) values compared to the control fish (Table1). In this study, a significant increase in parameters such as haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), lymphocytes, and platelets was observed in *Clarias batrachus* after 96-hour malathion exposure and gallic acid treatment.

**Table 1:** Shows variations in different haematological parameters of *Clarias batrachus* in various experimental groups for 96 hours. Value is mean ± Standard Error (SE)

Haematological Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	0.1ppm Malathion	(0.1ppm Malathion + 50mg Gallic acid)	(0.1ppm Malathion + 100mg Gallic acid)	(0.1ppm Malathion + 150mg Gallic acid)
RBC (x10 <sup>6</sup> /mm <sup>3</sup> )	2.53 ± 0.13	1.39 ± 0.07	1.58 ± 0.08	1.84 ± 0.10	2.15 ± 0.11
Hb (g%)	10.1 ± 0.55	7.28 ± 0.40	8.25 ± 0.45	8.79 ± 0.48	9.12 ± 0.50
MCV (fl)	137.0 ± 7.5	95.5 ± 5.2	101.0 ± 5.5	108.0 ± 5.9	118.0 ± 6.5
MCH (Pg)	45.4 ± 2.5	30.9 ± 1.7	34.1 ± 1.8	37.4 ± 2.06	40.7 ± 2.2
MCHC (g/dl)	31.1 ± 1.7	23.2 ± 1.2	24.5 ± 1.3	26.2 ± 1.4	28.1 ± 1.5
PCV/HCT (%)	49.1 ± 2.7	41.4 ± 2.2	42.7 ± 9.3	44.9 ± 2.4	45.9 ± 2.5
TLC (/cmm)	8500.0 ± 498.8	1250.0 ± 621.9	10350.0 ± 572.1	9870.0 ± 545.6	9570.0 ± 529.0
PLT (thou/μl)	1.61 ± 0.08	1.21 ± 0.06	1.29 ± 0.07	1.34 ± 0.07	1.45 ± 0.08
Neutrophil (%)	720.0 ± 39.8	1080.0 ± 59.7	1062 ± 58.7	972.0 ± 53.7	881.0 ± 48.7
Lymphocyte (%)	6075.0 ± 335.8	8654.0 ± 478.4	8614.0 ± 476.1	877.0 ± 446.5	7333.0 ± 405.3
Eosinophil (%)	486.0 ± 26.8	932.0 ± 51.2	728.0 ± 40.2	687.0 ± 37.9	620.0 ± 34.2
Monocyte (%)	810.0 ± 44.7	1110.0 ± 61.3	1047.0 ± 57.8	1002.0 ± 55.3	994.0 ± 54.9
Basophil (%)	9.81 ± 0.54	19.4 ± 1.07	16.5 ± 0.9	14.5 ± 0.8	12.8 ± 0.7

The biochemical profile of blood can help predict physiological disturbances caused by pathological or chemical stress in organisms. Therefore, the toxic effects of malathion and the treatment effects of different doses of gallic acid were evaluated in terms of variation in fish serum biochemical parameters such

as total concentration of liver function tests (LFT), kidney function tests (KFT), and lipid profile in the serum of control and fish exposed to 0.1PPM of malathion and treated with different doses of gallic acid (50 mg/L, 100 mg/L, 150 mg/L) after a 96-hour experiment.

**Table 2:** Shows variations in different Biochemical parameters of *Clarias batrachus* in various experimental groups for 96 hours. Values are mean ± Standard Error (SE).

Biochemical Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	0.1ppm Malathion	(0.1ppm Malathion + 50mg Gallic acid)	(0.1ppm Malathion + 100mg Gallic acid)	(0.1ppm Malathion + 150mg Gallic acid)
SGOT	18.4 ± 1.01	58.9 ± 3.2	54.1 ± 2.9	42.1 ± 2.3	32.8 ± 1.8
ALP (IU/L)	89.1 ± 4.9	131.0 ± 7.2	125.0 ± 6.9	116.0 ± 6.4	106.0 ± 5.8
SGPT	30.7 ± 1.6	68.7 ± 3.7	62.4 ± 3.4	55.8 ± 3.08	46.8 ± 2.5
Bilirubin (md/dL)	1.68 ± 0.09	3.57 ± 0.19	3.21 ± 0.17	2.87 ± 0.15	2.54 ± 0.14
Albumin (mg/dL)	2.62 ± 0.14	1.73 ± 0.09	1.81 ± 0.10	1.98 ± 0.10	2.24 ± 0.12
Uric Acid (mg/dL)	3.52 ± 0.19	4.98 ± 0.2	4.65 ± 0.2	4.38 ± 0.2	4.01 ± 0.2
Creatinine (mg/dL)	0.79 ± 0.04	1.41 ± 0.07	1.21 ± 0.06	1.04 ± 0.05	0.92 ± 0.05
Blood Glucose(mg/dL)	87.2 ± 4.8	149.0 ± 8.2	126.0 ± 6.9	119.0 ± 6.5	105.0 ± 5.8
Cholesterol (mg/dL)	204.0 ± 11.2	305.0 ± 16.8	283.0 ± 15.6	269.0 ± 14.8	248.0 ± 13.7
Triglyceride (mg/dL)	237.0 ± 13.1	318.0 ± 17.5	298.0 ± 16.4	275.0 ± 15.2	263.0 ± 14.5
HDL (mg/dL)	52.4 ± 2.8	38.4 ± 2.1	41.1 ± 2.2	44.9 ± 2.4	48.7 ± 2.6
LDL (mg/dL)	98.0 ± 5.4	121.0 ± 6.6	117.0 ± 6.4	109.0 ± 6.02	105.0 ± 5.8
VLDL (mg/dL)	14.1 ± 0.7	22.7 ± 1.2	18.7 ± 1.03	17.2 ± 0.9	16.7 ± 0.9

- **LFT (Liver Function Test)**

The liver function test measured various parameters in the blood sample of *Clarias batrachus* fish after 96 hours of exposure to 0.1 PPM of malathion toxicant and different concentrations of gallic acid treatment (50 mg/L, 100 mg/L, 150 mg/L). The test revealed a reduction in the levels of albumin (measured in g/dl), AST (measured in IU/L), ALP (measured in IU/L), and ALT (measured in IU/L) compared to the control group of fish. Furthermore, after 96 hours of exposure, there was a significant increase in the levels of bilirubin (measured in mg/dl) compared to the control group of fish. These findings demonstrate the impact of the toxicant and gallic acid treatment on the liver function of *Clarias batrachus* fish.

- **KFT (Kidney Function test)**

The real function test involved analysing the blood samples of various *Clarias batrachus* fish after they were exposed to Malathion for 96 hours. This test aimed to determine the physiological damage caused to the kidney. The results indicated a significant reduction in urea (mg/dl), uric acid (mg/dl), blood glucose (mg/dl), and creatinine (mg/dl) levels in the Malathion- treated group after 96 hours of exposure. Interestingly, in all treatment groups, the values for urea, uric acid, and blood glucose showed a continuous increase, while creatinine values exhibited a constant decrease compared to the control group.

- **Lipid Profile**

The lipid test was performed on the blood serum sample of *Clarias batrachus* after 96 hours of exposure to measure cholesterol and other fats in the blood. This test was used to assess the risk of heart disease, stroke, or cardiovascular disease. Lipid profile test exposure in fish. The elevation in LDL (mg/dl) and cholesterol (mg/dl) was continuous in all groups compared to control fish. Triglyceride and VLFL have numerous value increases in the group of malathion-treated fish. However, in three treatment groups, the value decreased compared to malathion-treated fish, but HDL (mg/dl) had no significant changes. The toxic effect of malathion & treatment effect of gallic acid is multidirectional and manifested by numerous changes in the body system's physiological and chemical processes. Significant variations were observed between various haematological and biochemical parameters with different concentrations of chemicals.

## DISCUSSION

The current research conducted an acute toxicity test on the freshwater fish *Clarias batrachus*. The test involved the administration of varying concentrations of gallic acid treatment and the assessment of malathion toxicity. Specifically, the acute toxicity of malathion was determined to be 0.1PPM at 96 hours. Additionally, different groups of fish were subjected to gallic acid treatment doses of 50mg, 100mg, and 150mg at 96 hours of exposure. The acute toxicity experiment was carefully divided into 5 groups for

comprehensive evaluation. Throughout the exposure period, changes in both haematological and biochemical parameters were meticulously assessed to provide a detailed understanding of the effects of the treatments on the fish. S.R. Rugazhvendan et al. (2009) reported that the results indicate that malathion is highly toxic to fish. Vishal Rajput et al. (2021) found that malathion is toxic and can disrupt the biochemical balance of test organisms. They also observed a decrease in malathion content in *Clarias batrachus* due to pesticide toxicity.

In a study published in 2019 by Jiayu Gao et al., it was reported that gallic acid, a trihydroxy benzoic acid, is a naturally occurring compound found in plant metabolites and is abundantly present across various plant species. This compound exhibits potent antioxidant properties and demonstrates free radical scavenging activities, making it a valuable agent for protecting biological cells, tissues, and organs from oxidative stress-induced damage. Kahlid Abdullah Al Ghanim (2012) reported that blood parameters are a useful tool for evaluating the effects of chemicals on fish. Past studies have found that changes in certain hematological parameters can indicate exposure to malathion. Blood cell indices such as MCV, MCH, and MCHC appear to cause changes in the fish's homeostatic system. Fluctuations in these indices correspond to similar responses noted in the RBC count, hemoglobin concentration, and PCV. Similar responses were observed in common carp and other freshwater fishes exposed to acute toxic levels of pesticides. In this study, a significant increase in Hb, MCV, MCHC, and PCV values was observed in group 2 (Malathion) compared to group 1 (control group), while RBC and PLT values decreased in group 2. Significant changes were also noted in Group 3 (Malathion + Gallic Acid 50 mg), group 4 (Malathion + Gallic Acid 100 mg), and Group 5 (Malathion + Gallic Acid 150 mg) treatment groups. G.V Venkataraman and P.N Sandhyarani (2013) reported a significant difference in blood parameters between the control group and each concentration. Fish blood reflects the pathophysiological status, and its parameters are important in diagnosing the structure and functional status of fish exposed to toxicants. Madhusudan Reddy Narra et al. (2015) reported that the reduction in RBC and Hb might be due to the inhibition of erythropoiesis, chemosynthesis, and osmoregulatory dysfunction due to the increased rate of erythrocyte destruction in the hematopoietic organ. Abdul Rouf (2015) reported that in the present study, the main hematological response of *C. mrigala* after acute exposure to malathion included significantly lower RBC count, hemoglobin concentration, hematocrit, and WBC compared to the control fish. Similar responses in these hematological indices provide evidence for supposed hematopoiesis, followed by anemia induction, which has previously been reported in fish exposed to other organophosphate pesticides. Indices after exposure to chlorpyrifos in *Clarias gariepinus* showed that malathion induced decreased RBC count, hemoglobin content, and hematocrit values in fish, which are indicators of anemia that can result from disruptive iron synthesis mechanisms, destruction of mature erythrocytes, and malfunctioning of the hematopoietic system.

In a study conducted by N. Silumbarasan and H. Hemalatha in 2015, it was observed that freshwater fishes undergo biochemical changes to adapt to common environmental stressors, particularly hypoxia. The researchers found that glucose levels in fish were impacted by a range of stress factors, including exposure to heavy metals. Interestingly, the study revealed that the glucose content in the two specific tissues under investigation exhibited a continuous decrease over the entire duration of the exposure period.

Fig. 1. Changes in Red Blood Cell (RBC) count of *Clarias batrachus* were exposed to an acute concentration (96 hrs.) of malathion.

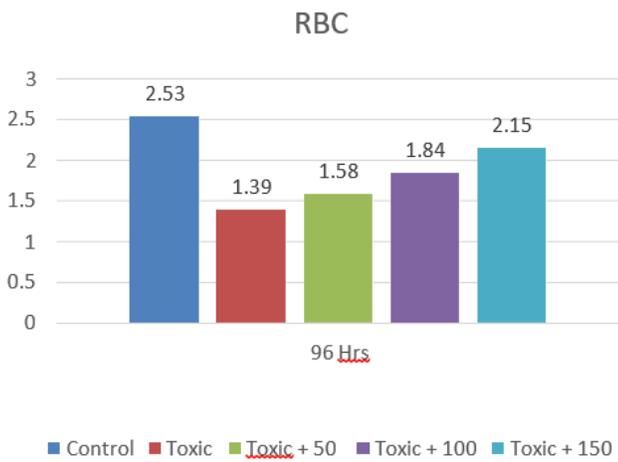


Fig. 2. Changes in Hb count of *Clarias batrachus* were exposed to an acute concentration of malathion (96 hrs).

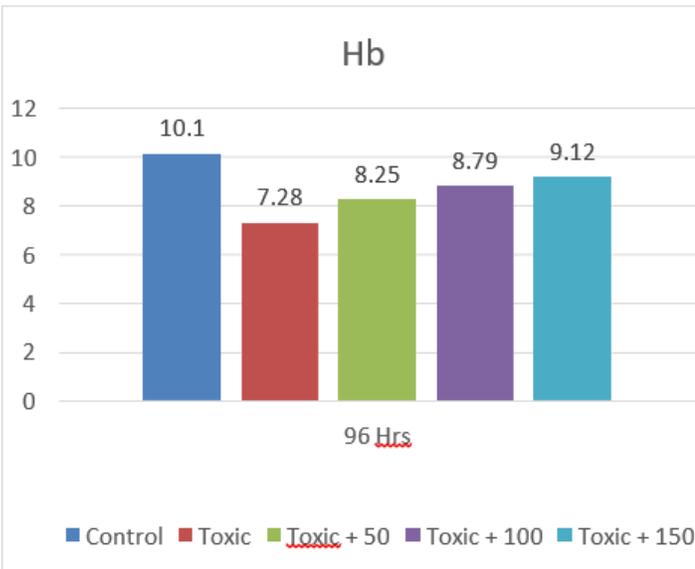


Fig. 3. Changes in PCV count of *Clarias batrachus* were exposed to an acute concentration (96 hrs) of malathion.

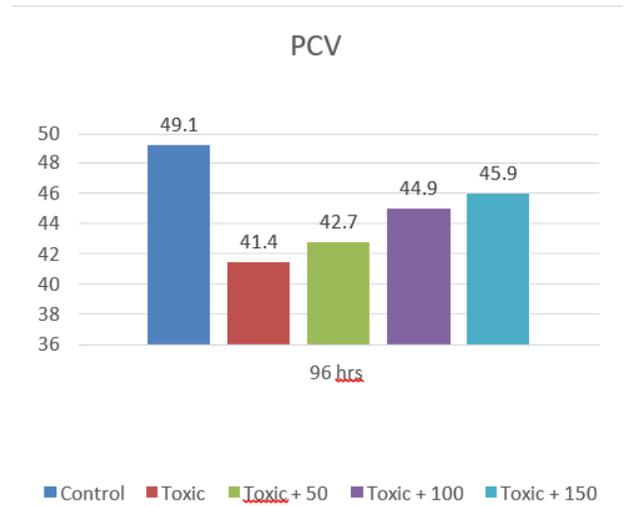


Fig. 4. Changes in MCV count of *Clarias batrachus* was exposed to an acute concentration (96 hrs) of malathion

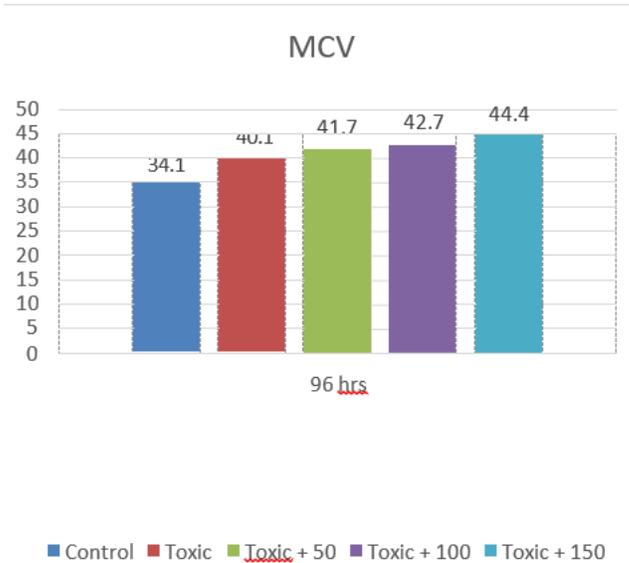


Fig. 5. Changes in MCH count of *Clarias batrachus* were exposed to an acute concentration (96 hrs.) of malathion.

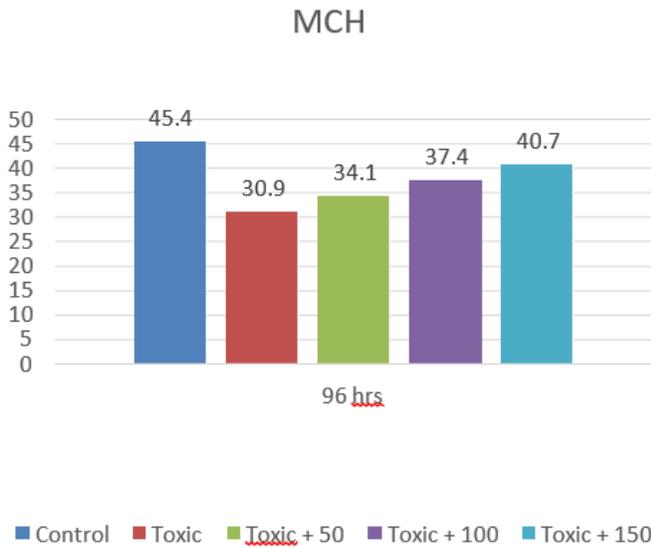


Fig. 6. Changes in MCHC count of *Clarias batrachus* was exposed to an acute concentration (96 hrs.) of malathion.

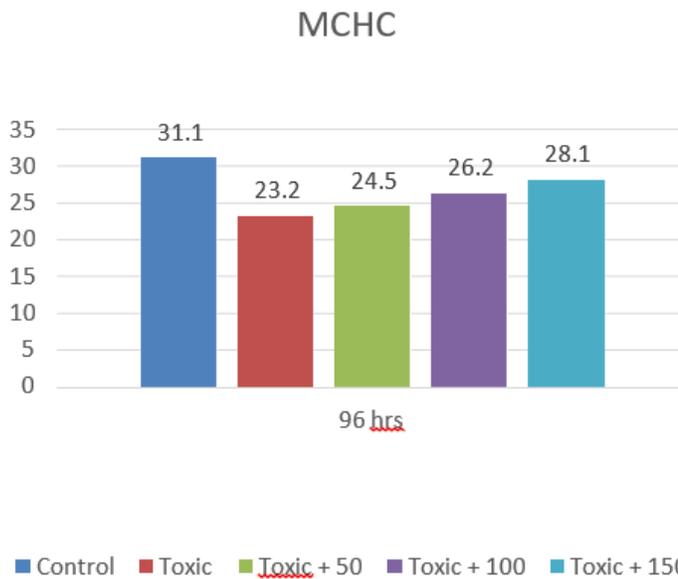


Fig. 7. Changes in TLC count of *Clarias batrachus* was exposed to an acute concentration (96 hrs.) of malathion.

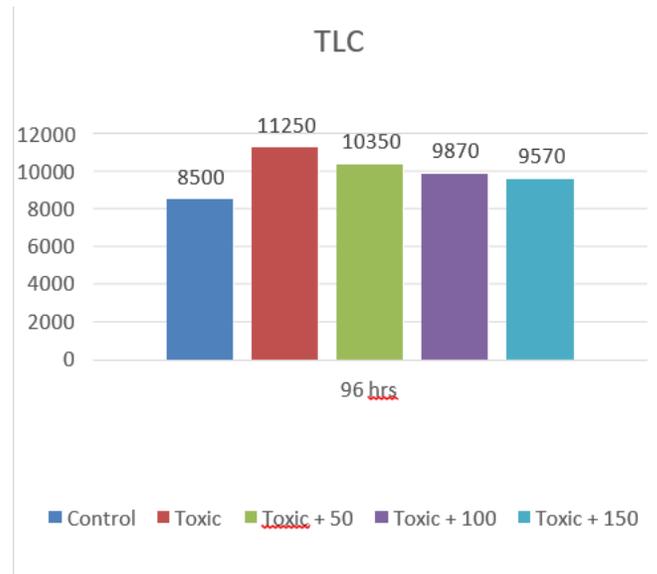
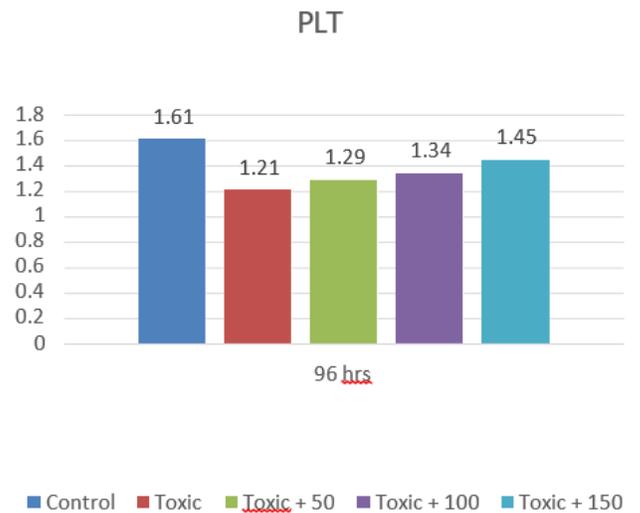
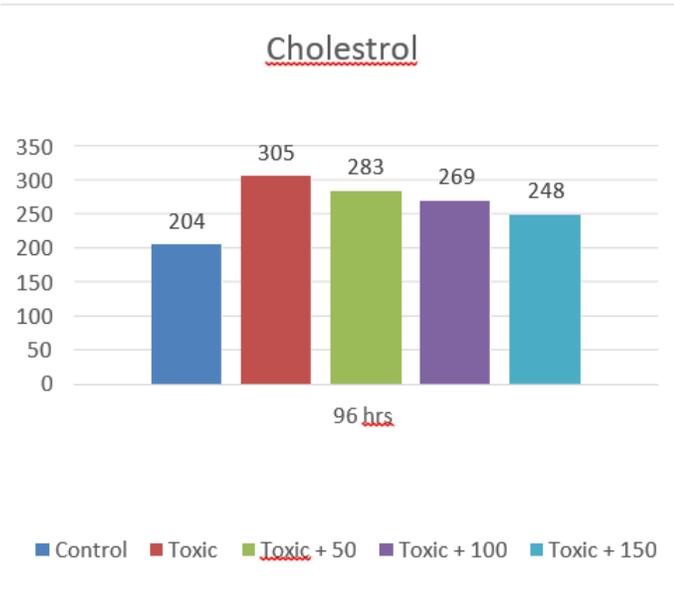


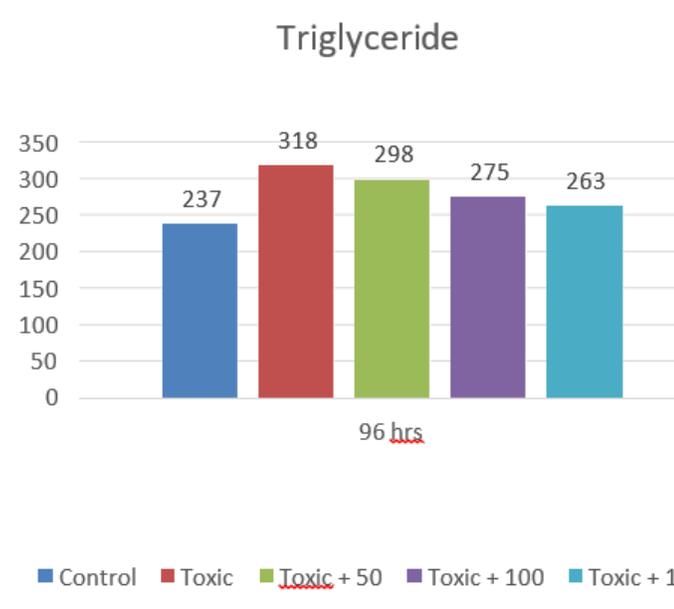
Fig. 8. Changes in PLT count of *Clarias batrachus* was exposed to an acute concentration (96 hrs.) of malathion.



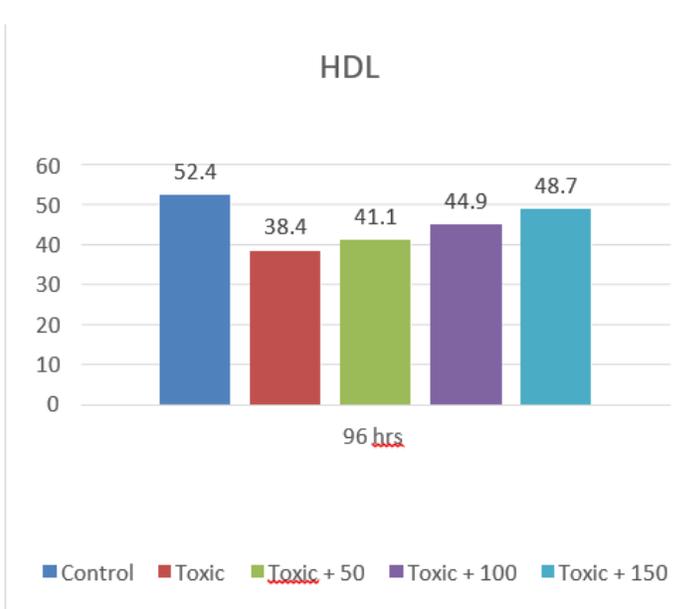
**Fig. 9.** Changes in the cholesterol count of *Clarias batrachus* were exposed to an acute concentration (96 hrs.) of malathion.



**Fig. 10.** Changes in Triglyceride count of *Clarias batrachus* were exposed to an acute concentration (96 hrs) of malathion.



**Fig. 11.** Changes in HDL count of *Clarias batrachus* was exposed to an acute concentration (96 hrs) of malathion.



**Fig. 12.** Changes in the LDL count of *Clarias batrachus* were exposed to an acute concentration (96 hrs) of malathion.

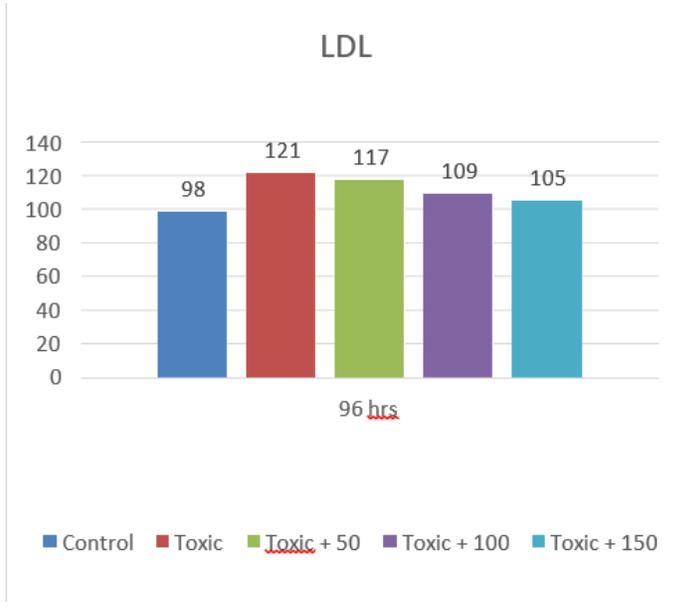


Fig. 13. Changes in VLDL count of *Clarias batrachus* were exposed to an acute concentration (96 hrs) of malathion.

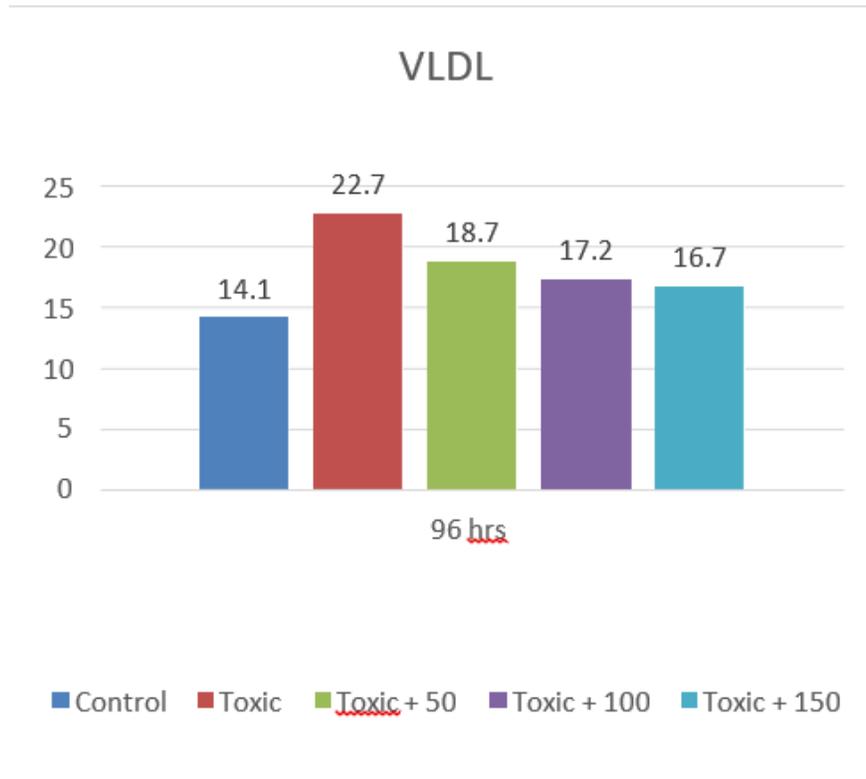


Fig. 14. Changes in Bilirubin count of *Clarias batrachus* was exposed to an acute concentration (96 hrs) of malathion.

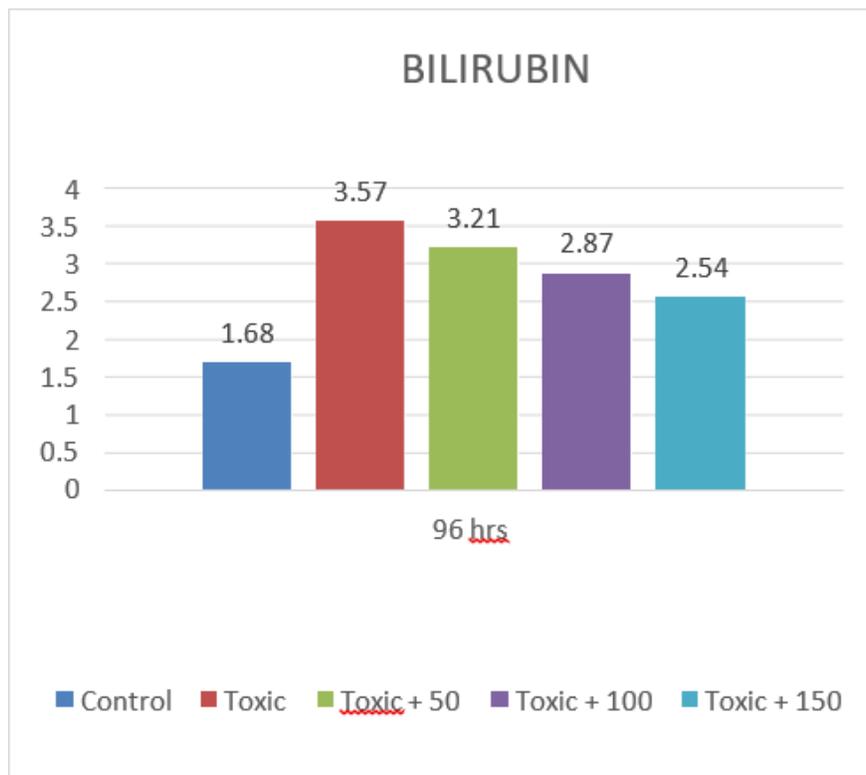


Fig. 15. Changes in Albumin count of *Clarias batrachus* were exposed to an acute concentration (96 hrs.) of malathion.

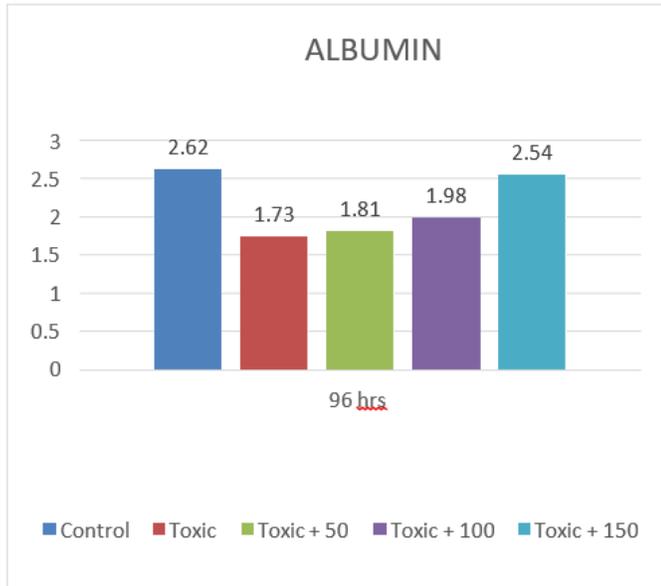


Fig. 16. Changes in Globulin count of *Clarias batrachus* were exposed to an acute concentration (96 hrs.) of malathion.

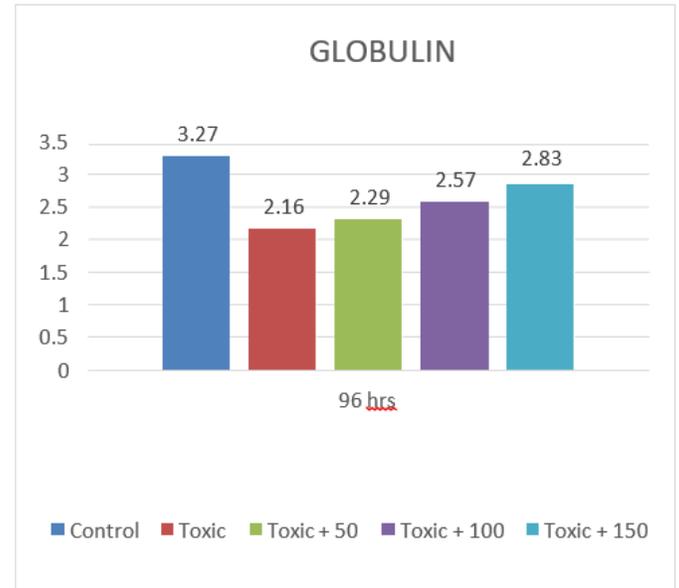


Fig. 17. Changes in Blood Glucose count of *Clarias batrachus* was exposed to an acute concentration (96 hrs.) of malathion.

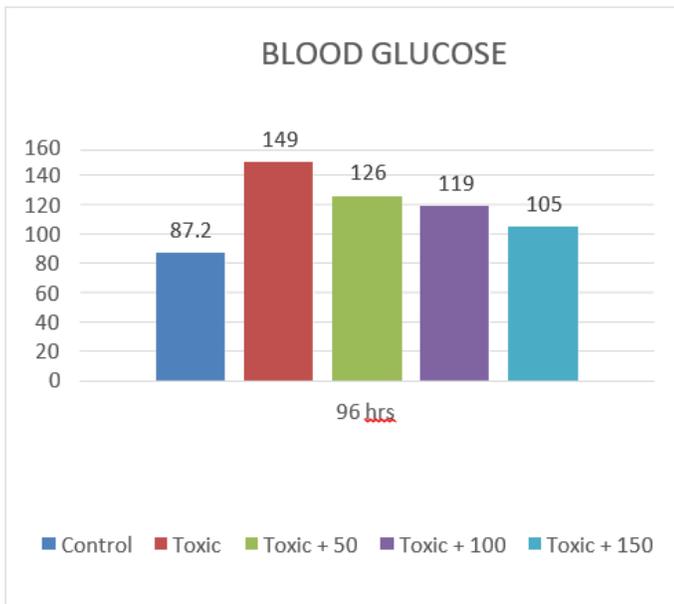


Fig. 18. Changes in SGOT count of *Clarias batrachus* was exposed to an acute concentration (96 hrs.) of malathion.

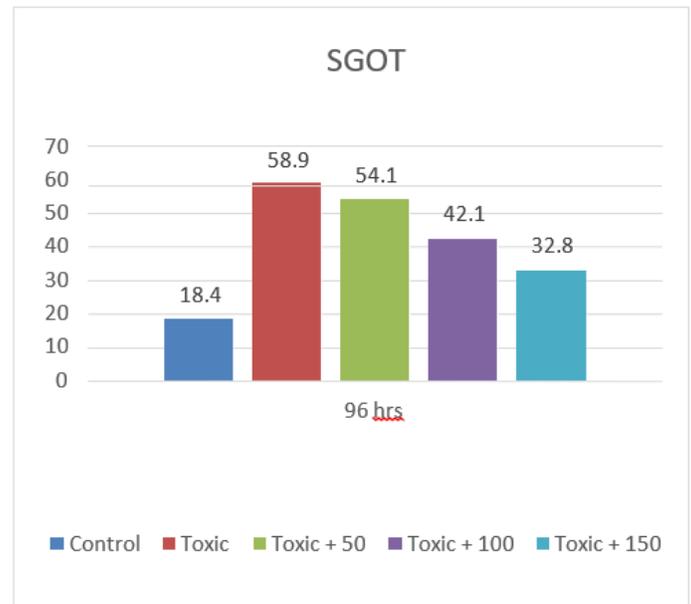


Fig. 19. Changes in SGPT count of *Clarias batrachus* were exposed to an acute concentration (96 hrs.) of malathion.

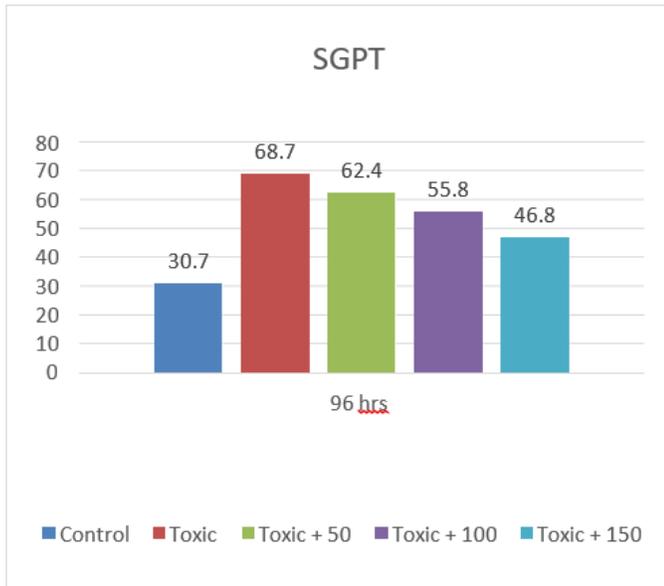


Fig. 20. Changes in ALP count of *Clarias batrachus* were to an acute concentration (96 hrs.) of malathion.

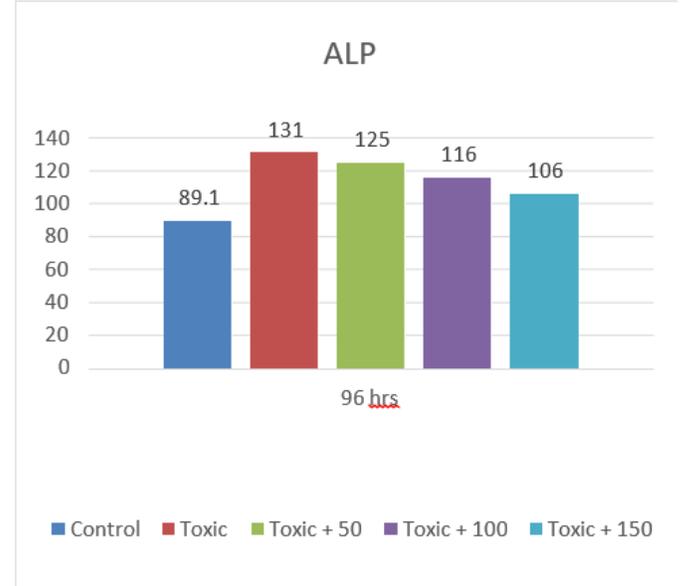


Fig. 21. Changes in LDH count of *Clarias batrachus* were exposed to an acute concentration (96 hrs.) of malathion.

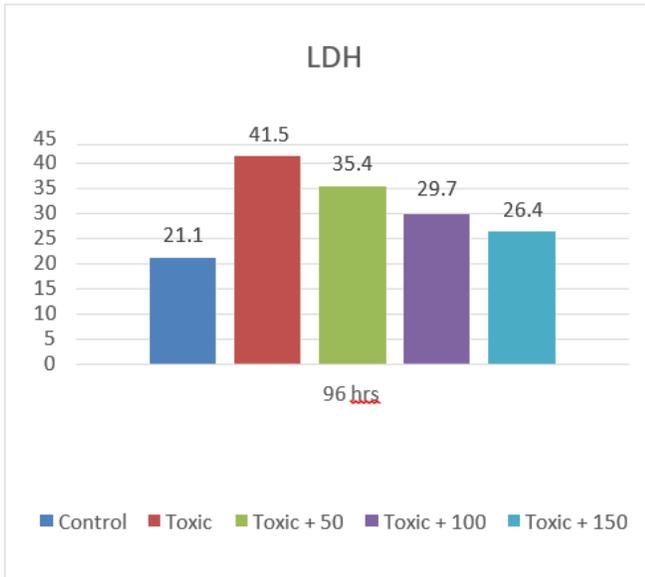


Fig. 22. Changes in Creatinine count of *Clarias batrachus* were to an acute concentration (96 hrs.) of malathion.

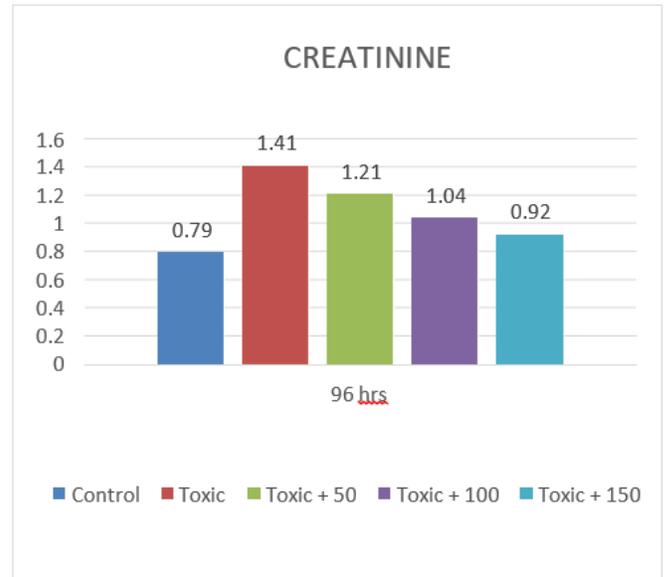
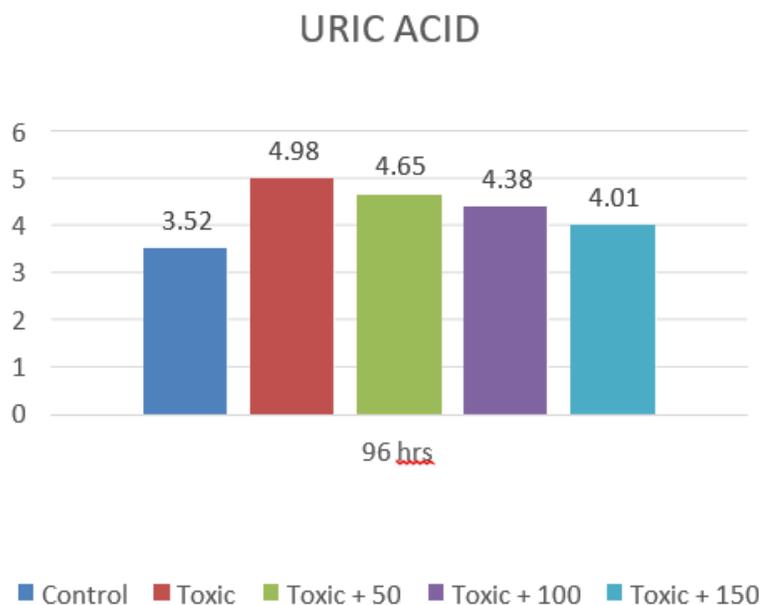


Fig. 22. Changes in Uric Acid count of *Clarias batrachus* was exposed to an acute concentration (96 hrs.) of malathion



## CONCLUSION

The present study improved our understanding of the biochemical and haematological changes in fish due to sublethal exposure to malathion and the treatment with gallic acid. Malathion appears to be moderately toxic to *Clarias batrachus*. In this investigation, we assessed the toxic effects of a low dose of 0.1 PPM of malathion and three different doses of gallic acid treatment on *Clarias batrachus* over a 96-hour

exposure period. The results indicate that fish are highly sensitive to malathion.

Acute toxicity studies are essential for determining the water quality requirements for fish. This information should be considered when malathion is used for pest control in agricultural fields surrounding natural freshwater reservoirs.

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