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EVALUATION OF LIPID PEROXIDATION AND ANTIOXIDANT ENZYME ALTERATIONS IN BRAIN AND GILL TISSUES OF ARSENIC-INDUCED CLARIAS BATRACHUS

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ABSTRACT

The present study of the freshwater fish Clarias batrachus (Magur) was collected from a local fish farm located near Patna, Bihar, and acclimatized in laboratory conditions prior to experimentation. The collected specimens were handled with minimal disturbance to prevent stress and transported in polythene bags filled halfway with aerated water. This method of transportation proved effective, as no mortality was observed during transit throughout the study period. On arrival, the fishes were transferred to glass aquaria containing dechlorinated tap water and acclimatized for 15 days under laboratory conditions with a 12-hour light/dark cycle. Water quality parameters such as temperature, pH, and dissolved oxygen were regularly monitored to ensure optimal conditions. The fishes were fed a commercial pellet diet once daily, and water was renewed on alternate days to maintain cleanliness. After acclimatization, healthy specimens of uniform size and weight were selected and divided into control and experimental groups. The experimental fishes were exposed to sub-lethal concentrations of sodium arsenite for a specific duration, and samples of brain and gill tissues were collected post-exposure for biochemical estimation of lipid peroxidation and antioxidant enzyme activities

Keywords: Arsenic toxicity, Clarias batrachus, Lipid peroxidation, Oxidative stress, Membrane damage.

I. INTRODUCTION

The poisoning of aquatic environments with heavy metals is a growing concern due to the effects of fast industrialization, urbanization, and agricultural runoff. Because of its great toxicity, persistence, and capacity to bioaccumulate in aquatic creatures, arsenic stands out among the other harmful elements as a potentially devastating environmental contaminant. Anthropogenic activities including mining, smelting, pesticide usage, and inappropriate waste management contribute to its discharge into water systems, while natural processes like volcanic emissions and weathering of arsenic-rich rocks also play a role. Fish, which are important indicators of water quality, have been linked to a wide range of physiological and biological problems due to long-term exposure to arsenic. One frequent freshwater teleost in Indian bodies of water is the walking catfish, or Clarias batrachus (Magur). In toxicological research, it serves as a model organism because of its ecological significance, economic worth, and resilience to many types of water. Understanding the effects of environmental toxins may be greatly aided by studying C. batrachus, a fish that plays a crucial role in freshwater ecosystem maintenance via its physiological responses to toxicants. Research on this species' susceptibility to arsenic poisoning sheds light on the metabolic and oxidative stress reactions experienced by aquatic creatures in the presence of contaminants.

Lipid peroxidation (LPO) is a powerful biochemical marker of oxidative stress because it shows how much damage reactive oxygen species (ROS) have caused to cell membranes. In a healthy body, enzymes including catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) efficiently neutralize reactive oxygen species (ROS), which are constantly produced as metabolic byproducts of cells. Nevertheless, oxidative stress occurs when the equilibrium between reactive oxygen species (ROS) generation and antioxidant defense is upset, as happens in organisms exposed to high concentrations of environmental stressors such as arsenic. The peroxidation of polyunsaturated fatty acids in cell membranes, which compromises membrane integrity and cellular function, is the end consequence of this imbalance. Malondialdehyde (MDA) is a persistent byproduct of lipid peroxidation and an accurate indicator of oxidative damage. To measure the extent of damage to membrane lipids, the LPO test measures MDA, usually utilizing the thiobarbituric acid reactive substances (TBARS) technique. Increased oxidative stress, as seen by an increased MDA level, might hinder important physiological functions as metabolism, osmoregulation, and respiration in fish. To evaluate the sub-lethal consequences of arsenic poisoning at the biochemical level, it is necessary to analyze LPO levels in arsenic-exposed Clarias batrachus.

Physiological, histological, and biochemical changes characterize arsenic poisoning in fish. Excessive production of reactive oxygen species (ROS) results from its interference with the mitochondrial electron transport chain. In addition, it interferes with antioxidant processes and deactivates vital enzymes by binding to sulfhydryl (-SH) groups on proteins. Caused by oxidative stress, which damages macromolecules including DNA, lipids, and proteins, cellular metabolism and tissue function are both compromised. Organs most often exposed to xenobiotics, such as the gills, liver, and kidneys, may undergo devastating pathological alterations due to arsenic-induced oxidative stress, such as necrosis, vacuolization, and altered enzymatic activity. Because it shows the beginning of stress-induced biochemical changes before morphological damage becomes obvious, the lipid peroxidation test is a useful diagnostic tool in environmental toxicology. Researchers want to learn more about the degree of oxidative damage and the organism's resilience to environmental stress by looking at MDA levels in arsenic-exposed C. batrachus tissues. Such discoveries may provide light on how fish adjust to metal stress and what level of toxicity poses a threat to aquatic ecosystems.

Additionally, there are wider ecological and public health implications to studying lipid peroxidation in arsenic-induced Clarias batrachus. Arsenic buildup in fish tissues may biomagnify across trophic levels, endangering the health of predators like humans—a crucial link in the food chain. Thus, biochemical evaluations like LPO tests not only provide light on the toxicity process but also act as early warning signs for environmental management and monitoring. To evaluate the sub-lethal consequences of arsenic exposure, this study integrates environmental toxicology with biochemical analysis, making it noteworthy. Bioindicator frameworks for pollution assessment in aquatic environments may be established by understanding membrane damage via lipid peroxidation. The research will also aid environmental authorities and legislators in their efforts to create long-term plans for water management by shedding light on potential mitigation techniques for arsenic pollution. In sum, studying the effects of arsenic on Clarias batrachus's LPO activity is an important first step in understanding the metabolic pathways that lead to oxidative stress caused by metals and the ecological fallout from pollution.

II. REVIEW OF RELATED STUDIES

Sinha, Gunjita. (2023). Anthropogenic activities including urbanization, industry, and transportation have contributed to a rise in arsenic trioxide pollution. Much like how mining and mountain erosion have polluted it. Catfish (Clarias batrachus) blood serum protein changed significantly as a result of

As2O3 exposure in this research. Arsenic trioxide has reduced the protein content of Clarias batrachus when monitored in As2O3 polluted water throughout the specified time frame. This suggests that As2O3 reduces protein content.

Jha, Deepak Kumar et al., (2022) A shift in the amount of oxidative stress parameters, including lipid peroxidation, is visible when arsenic exposure via drinking water is elevated. On the other hand, data on the effects of low-level arsenic exposure on oxidative damage is scarce. Lipid peroxidation is a well-established indicator of oxidative stress; this study set out to examine the oxidative stress producing potential of low-level arsenic exposure in the freshwater fish Channa puntatus. Sodium arsenite was used as a test material to expose fish specimens to levels of arsenic ranging from 10, 50 (low level exposure) to 500 µgL-1 (high level exposure). After 14 days of exposure, samples of several fish tissues were obtained, including gill, liver, kidney, and brain. To determine oxidative damage, the lipid peroxidation test was conducted using the TBARS assay to quantify malondialdehyde (MDA). Across the board, LPO levels were noticeably elevated. The gills were shown to be the most vulnerable organ in this investigation, exhibiting the highest level of peroxidative damage at an arsenic exposure level of 500 µgL-1.

Koner, Debaprasad et al., (2021) Phylogenetically, the magur catfish (Clarias magur) is very close to other bony fishes with complete conservation of active site residues among piscine, amphibian, and mammalian species, according to the deduced amino acid sequences from the complete cDNA coding sequences of three antioxidant enzyme genes (sod1, sod2, and cat). The homology between the three-dimensional structures of three different antioxidant enzyme proteins and their mammalian homologues provides strong evidence that these enzymes have comparable functions. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) intracellular concentrations rose sharply in the beginning of exposure to ZnO NPs, indicating oxidative stress; however, these concentrations subsequently reduced. After a brief dip, the levels of glutathione (GSH) rose steadily across all of the tested tissues. According to biochemical and gene expression studies, the magur catfish can protect itself from oxidative stress caused by ZnO NPs by activating a network of antioxidant genes in different tissues, including the SOD/CAT enzyme system and GSH-related enzymes. In addition, it seems that NO stimulation, stemming from the expression of the nos2 gene, acts as a regulator to activate the SOD/CAT system in different tissues of magur catfish, which is an antioxidant approach, in response to NP-induced oxidative stress. The magur catfish likely has an effective antioxidant defense system that it uses to ward off oxidative stress and the associated cellular

damage that occurs when ZnO NPs enter its natural habitat.

Pichhode, Mohnish et al., (2020) the chemical form of arsenic is more hazardous than the organic form, and both forms are present all over the earth. After being exposed to a fatal dose (LC50) of arsenic trioxide for 24, 48, 72, and 96 hours, the effects of the poisonous substance on histological parameters of the catfish, Clarias batrachus, were studied. Kidney parenchymal inflammation, hemorrhage, rupture of Bowman's capsule, shrinkage of glomerulus, and hypertrophy of glomerulus are some of the severe histological changes caused by arsenic trioxide. In the liver, these changes include hepatocyte cell inflammation, macrophage infiltration, nucleus and cytoplasmic blabbing, disruption of normal architecture, and so on. Histological changes in the liver and kidneys of arsenic trioxide-exposed Clarias batrachus served as reliable markers of environmental pollution.

Marzouk, Mohamed et al., (2017) the purpose of this study was to determine the detrimental effects of heavy metals on the physiological and antioxidant statuses and histopathological alterations of these fish. Among the locations, there were significant differences in the levels of cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) in the water samples ($P < 0.05$). Sites that were contaminated with agricultural runoff, industrial runoff, and reference runoff made up the total pollution ranking. During the summer season, fish collected from the two polluted sites, particularly the Moshtahr site, had significantly higher serum levels of glucose, creatinine, urea, uric acid, aspartate transferase (AST), alanine transferase (ALT), and alkaline phosphatase (ALP) when compared to the reference site ($P<0.05$). However, compared to the reference site, fish obtained from the two contaminated locations had much lower blood levels of total protein, albumin, globulin, and A/G ratio across both seasons. Fish caught at the Moshtahr location showed the steepest decline in total proteins over the summer. The elevated levels of lipid peroxidase (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) were more noticeable in the summer at the Moshtahr site than in the spring at the other polluted location. When comparing the fish from EL- Qanater El- Khayreya to those from contaminated places, histopathological analysis of the liver tissues revealed vacuolar degeneration of the hepatocytes, hemorrhage, hemosidirin, and localized regions of necrosis. According to the research, African catfish, C. gariepinus, may serve as indicators for metal pollution in aquatic life monitoring due to changes in their biochemistry profile and antioxidant defense system.

Bhattacharyya, Anirban & Bhattacharya, Shelley. (2007) Excess H(2)O(2) production, a rise in the ratio of oxidized to reduced glutathione, and tissue lipid peroxidation were seen after 1-2 days of

exposure to two nonlethal doses of arsenic that were administered to Indian catfish, Clarias batrachus, over a duration of 10 days. In addition, within one day of exposure, antioxidant enzymes like catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) were more active in fish treated with arsenic, while glutathione reductase (GR) activity was decreased. This suggests that oxidative stress is being generated in fish at an early stage. It was shown that altered H(2)O(2) metabolism in the peroxisomes largely contributed to the oxidative stress, since these arsenic-induced reactions might be reversible after pretreatment with N-acetylcysteine. It follows that arsenic poisoning in C. batrachus may target peroxisomal H(2)O(2) metabolizing enzymes.

Bhattacharya, Shelley et al., (2007) to satisfy contemporary man's expectations, the environment is presently poisoned by hundreds of xenobiotics, or chemicals. This is the dark side of human civilization that we face daily, and yet, we have not reduced pollution rates much. Water is the last refuge for many pollutants, therefore while pollution affects the whole biosphere; it has a disproportionate impact on water resources. As a result, of all animal species, fish are the most susceptible. They have no choice but to deal with the poisoning of their environment, and they are unable to do anything about it. Still, fish can make it through very harsh environments when their normal habitat is severely damaged. Nevertheless, fish populations will steadily decline if pollution does not stop. This is because fish are very sensitive to even little changes in their habitat. On the other hand, there have been cases when the water has been cleaned up and various fish species are repopulating at a rapid pace, which has helped to restore ecological balance. Because of this, fish are seen as trustworthy indicators of water pollution. The study of fish ecotoxicology has gained a lot of traction recently, and the field of fish toxicology has successfully maintained its place in the field of xenobiotics research over the years. Arsenic (As) is now one of the most concerning metalloids in water pollution, and this article discusses some of the main signs of intoxication and detoxication shown by fish exposed to it.

III. MATERIALS AND METHODS

Magur, a kind of freshwater fish, was sourced from a fish farm close to Patna, Bihar. Transporting the specimens in polythene bags partly filled with aerated water was done with little disturbance to avoid stressing them. Every bag contained about a hundred fish, and a cylinder of pressured air was used to keep the fish aerobically. During the trial period, no deaths occurred while traveling using this means of transportation, proving its effectiveness.

To assess the acute toxicity of arsenic trioxide (*As_2O_3*), An analysis of static renewal toxicity was carried out in accordance with the protocols established by the American Public Health Association. As a whole, the median fatal dose (LC_{50}) in order to assess the toxicity of arsenic trioxide to Clarias batrachus. LC_{50} stands for the concentration of a poison that, when tested in a controlled environment, kills the test organisms 50% of the time. The reason this metric was chosen is because it shows toxicity in a consistent and repeatable way.

Based on initial findings, the test fishes died out when exposed to arsenic trioxide concentrations higher than 30 ppm. Hence, for the experimental setup, a range of arsenic trioxide concentrations from 1.0 to 30.0 ppm was created. Arsenic concentrations of1.0,2.5,5.0,7.5,10.0,12.5,15.0,17.5,20.0,25.0, and 30.0 ppm) were varied in each of the test tanks by adding ten fish to each. The effects of arsenic poisoning were seen by attentively monitoring the behavioral reactions of the fish at regular intervals. Quick action was taken to eliminate any fish that did not exhibit opercular movement or did not react to outside stimuli so that the remaining fish would not become victims of cannibalism. At 24, 48, 72, and 96 hours, mortality data were obtained, and the LC_{50} value was calculated using Finney's (1971) approach.

Research on the biochemical and physiological reactions of fish subjected to chronic arsenic stress was also carried out in sublethal toxicity tests. One tenth of the 96-hour concentration was chosen as the sublethal value LC_{50} value. In the present study, the 96-hour LC_{50} value for arsenic trioxide was found to be 1.89 ppm. Therefore, a sublethal concentration of 0.189 ppm (one-tenth of the LC_{50}) was used for a 7-day exposure period.

All fish, including those exposed to arsenic, were kept in the same circumstances throughout the trial. Dissecting the fishes and carefully removing parts like gills and liver for biochemical analysis was done at the conclusion of the exposure period. The importance of these organs in breathing, detoxifying the body, and controlling metabolism led to their selection. In order to determine the extent of membrane damage and the physiological effects of arsenic poisoning on *Clarias batrachus*, the biochemical investigations centered on measuring lipid peroxidation (LPO) and associated oxidative stress indicators.

Biochemical Studies

The fish from both the experimental and control groups were killed after the experiment. Isolated from the fish, the brain and gills were used for a variety of studies.

- **Estimation of lipid peroxidation in the tissue**

Nichans and Samuelson's (1968) approach was used to assess the lipid peroxidation. By reacting with thiobarbituric acid (TBA) in an acidic environment to produce a pink chromophore, which was then detected at 535 nm in a UV spectrophotometer, this technique assessed thiobarbituric acid reactive substances (TBARS). We made tissue homogenate in Tris-Hcl buffer (pH 7.5) after isolating all of the tissues. A clean test tube was used to combine 2.0 ml of TBA-TCA-HCl reagent with 1.0 ml of tissue homogenate, and the mixture was stirred well. For fifteen minutes, the concoction was heated in a water bath set to boiling point (60 C). Once the combination had cooled, the UV spectrophotometer was used to measure the pink chromophore at 535 nm in comparison to the reagent blank. The standard graph was constructed using 1,1,3,3-tetramethoxy propane. Wet weight of tissue represented as n moles/mg is used to represent the values.

Before being centrifuged at 2500 rpm for 5 minutes, the tissues were homogenized in phosphate buffer. A fresh test tube was used to collect 0.2 ml of the supernatant, which was then mixed with 1.8 ml of EDTA solution. Before centrifugation at 3000 rpm for 10 minutes, 3.0 ml of precipitating reagent was added and mixed properly. The mixture was then left for 5 minutes. The aliquot underwent filtering after centrifugation. The following was added to a clean test tube: 2.0 ml of the filtrate, 4.0 ml of a 0.3M disodium hydrogen phosphate solution, and 1.0 ml of DTNB reagent. The UV spectrophotometer detected a yellowish hue at 412 nm. To build the standard graph, reduced glutathione was used. The values are given as micromoles per milligram of tissues' moist weight.

- **Estimation of Glutathione peroxidase (GPX) in tissues**

Following the procedure described by author the glutathione peroxidase activity level was determined. Centrifuged at 2,500 rpm for 5 minutes, the tissues were homogenized in Phosphate buffer. After collecting 0.2 ml of the clear supernatant in a sterile test tube, the following enzyme combination was introduced. A phosphate buffer of 0.2 ml, EDTA of 0.4 nM, and sodium azide of 0.1 ml were all components of the enzyme combination. A thorough mixing of the reaction components and two minutes in an incubator set at 37 C were required for this reaction. Once the incubation time was up, the mixture was re-incubated at 37 C for precisely 10 minutes with the addition of 0.2 ml of reduced glutathione and 0.1 ml of H_2O_2. Adding half a milliliter of 10% TCA halted the process. The color was allowed to develop before being measured at 412 nm. To build the standard graph, reduced glutathione was used. Quantities are given in moles of GSH used each

minute per milligram of protein.

- **Estimation of catalase (CAT) in tissues**

Scientists used their methodology to find out how active catalase was. In the presence of hydrogen peroxide, the reaction of dichromate in acetic acid progressed to perchromic acid and finally to chromic acetate. The wavelength at which the chromic acetate was detected was 620 nm. Periods of time were varied during which the catalase preparation was left to split hydrogen peroxide. Using a calorimetric method, we were able to estimate the amount of leftover hydrogen peroxide as chromic acetic acid after stopping the reaction at various time intervals using a dichromate/acetic acid combination. Centrifuged at 2000 rpm for 10 minutes, the tissues were homogenized in phosphate buffer solution. A sterile, dry test tube was prepared by adding 0.9 ml of phosphate buffer, 0.1 ml of sample (tissue homogenate), and 0.4 ml of hydrogen peroxide. A combination of dichromate and acetic acid, measuring 2 milliliters, was added 30 to 60 seconds later. After ten minutes in a water bath set to 37 degrees Celsius, the tubes were removed and let to cool to room temperature. A UV spectrophotometer was used to measure the generated color at 620 nm. The standard graph was constructed using hydrogen peroxide. The values are expressed as µ moles H_2O_2 consumed/min/mg protein.

Statistical analysis

After doing an analysis of variance (ANOVA), the Duncan Multible Range Test (DMRT) was used to determine statistical significance.

IV. RESULTS

The amount of lipid peroxidation was 2.01 ±1.21 nmole/g wet weight of tissue in the brain tissue of healthy fish. The amount of lipid peroxidation was found to be 3.19±1.27 nmole/g wet wt. of tissue during the sublethal dosage of arsenic, in comparison to the control group. Lipid peroxidation was measured at 1.91± 0.92 nmole/g wet weight of tissue in the control group's gill tissue. Fig. 1 shows that the gill tissue exhibited an elevated tendency of lipid peroxidation at sublethal arsenic concentrations, with a value of 2.26±1.82 nmole/g wet weight of tissue.

Table 1: Level of lipid peroxidation (LPO) in the selected tissue of fresh water fish with sub-lethal concentration of arsenic

Condition	Brain	Gill
Control	2.0	1.9
Treated	3.2	2.6

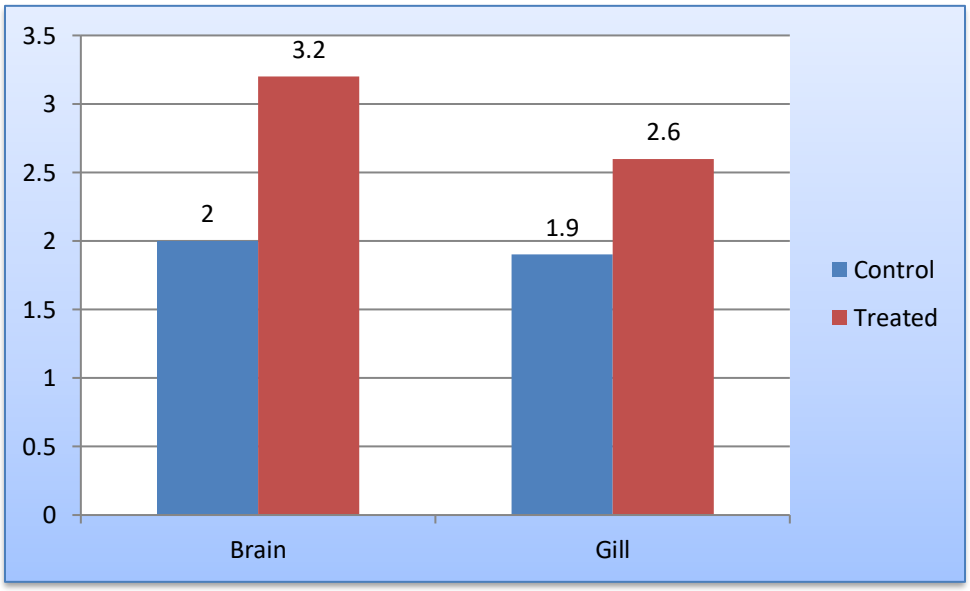

Figure 1: Level of lipid peroxidation (LPO) in the selected tissue of fresh water fishwith sub-lethal concentration of arsenic

Glutathione levels were 18.81±1.63 µ mg/g wet weight of tissues in the brains of healthy fish. In comparison to the control group, the amount of glutathione in tissues was reduced to 10.62 ±1.79 µg/g wet weight during the sublethal dosage of arsenic. In the control group, the gill tissue had a glutathione level of 17.40 ±1.79 µmoles/g wet weight of tissue. The gill tissue exhibited a declining trend in glutathione levels (11.20 ±1.63 µmoles/g wet weight of tissue) at sublethal concentrations of arsenic.

Table 2: Level of glutathione (GSH) in the selected tissue of fresh water fish Clarias batrachus exposed with sub-lethal concentration of arsenic

Condition	Brain	Gill
Control	18.5	17.0
Treated	10.5	11.0

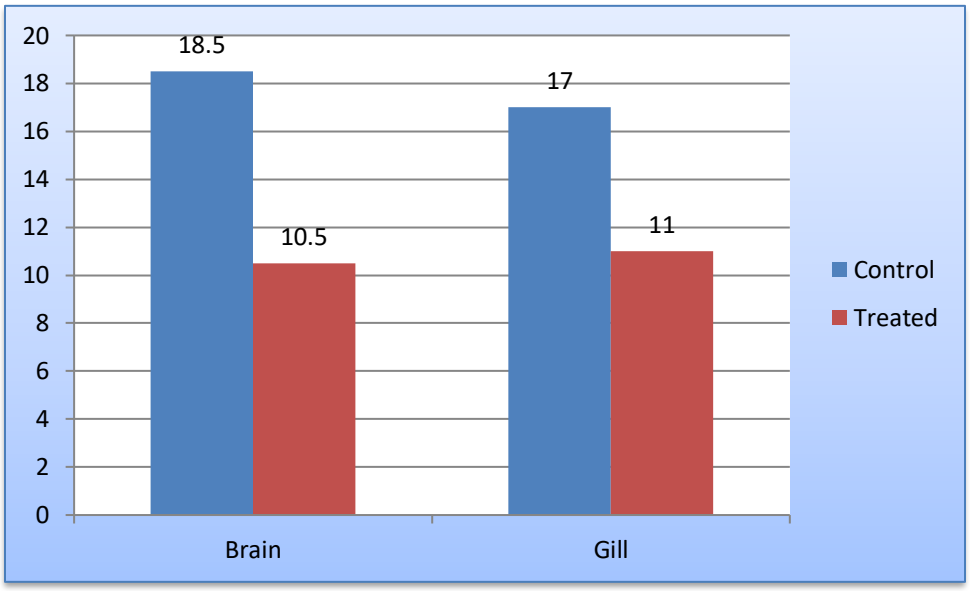

Figure 2: Level of glutathione (GSH) in the selected tissue of fresh water fish Clarias batrachus exposed with sub-lethal concentration of arsenic

The level of glutathione peroxidase was 0.120±1.72 µmoles/mg of protein/min in the brain tissue of healthy fish. The amount of glutathione peroxidase was reduced to 0.112±1.62 µmoles/mg of protein/min at the sublethal dosage of arsenic, in comparison to the control. Within the control group, the gill tissue had a glutathione peroxidase level of 0.131±1.68 µmoles/mg of protein/min. The gill tissue exhibited a declining trend of glutathione peroxidase (0.091±1.79 µ moles/mg of protein/min) at sublethal arsenic concentrations.

Table 3: Level of glutathione peroxidase (GPx) in the selected tissue of fresh water fish Clarias batrachus exposed with sublethal concentration of arsenic

Condition	Brain	Gill
Control	0.12	0.13
Treated	0.11	0.09

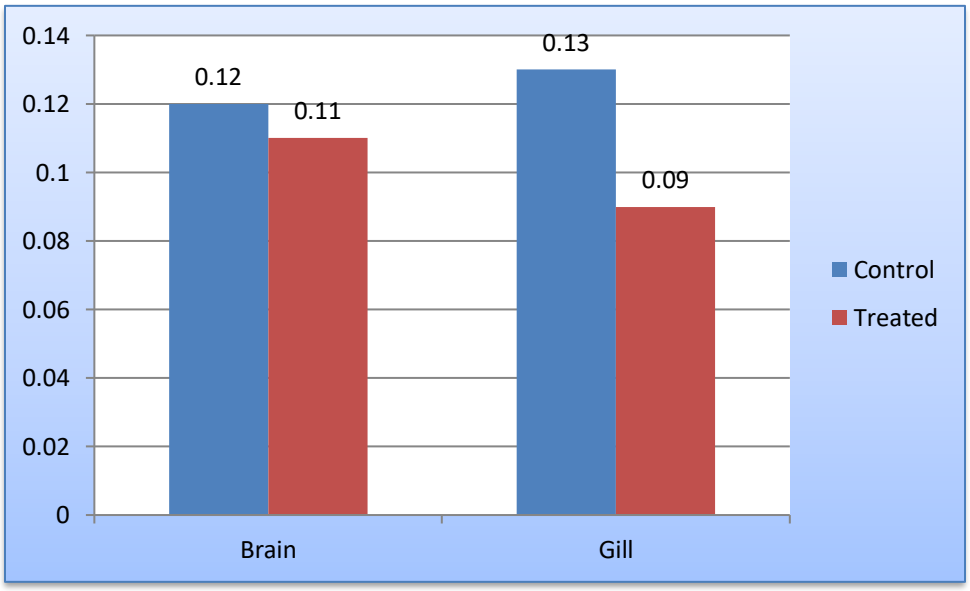

Figure 3: Level of glutathione peroxidase (GPx) in the selected tissue of fresh water fish Clarias batrachus exposed with sublethal concentration of arsenic

Approximately 6.91±1.65 moles of catalase per milligram of protein per minute were found in the brain tissue of healthy fish. The level of catalase was reduced to 3.86±0.98 µmoles/mg of protein/min at the sublethal dosage of arsenic, in comparison to the control. In the control gill tissue, the catalase level was 6.40±1.02 µmoles/mg of protein/min. The gill tissue demonstrated a declining trend of catalse at sublethal concentrations of arsenic (4.21±0.92 µmoles/mg of protein/min).

When heavy metals build up in fish tisses, they may trigger processes that release reactive oxygen species (ROS), which can cause oxidative stress in the environment. Many mammalian species, including fish and other aquatic creatures, have defensive systems to offset the effect of reactive oxygen species (ROS). Enzymes from the glutathinone S-transferase family have detoxifying effects

on lipid hydroperoxides produced by heavy metals, and there are superoxide dismutases that catalyze the dismutation of superoxide radicals to hydrogen peroxide.

The current research found that after 7 days of treatment with a sub-lethal dosage of arsenic, the glutathione, GPx, and CAT levels in the brain and gill tissues of CLARIAS BATRACHUS dramatically declined, while the LPO content level rose. This finding may be associated with changes in lipid peroxidation and antioxidant enzyme activity, both of which may lead to biochemical dysfunction in tissues. According to the results of this study, an increase in the concentration of LPOs in the mitochondria may account for the excess ROS production caused by mercury toxicity, which can alter the mitochondrial electron transport chain and block the permeability transition pore. The uncoupling of oxidative phospharilation and the consequent rise in ROS generation are caused by these processes.

V. CONCLUSION

The current investigation delves deeply into the biochemical impacts of arsenic exposure in Clarias batrachus, with a particular emphasis on the lipid peroxidation (LPO) assay-measured membrane damage. Researchers were able to determine how much arsenic-induced oxidative stress and cellular homeostasis disrupted by measuring levels of malondialdehyde (MDA). To better understand how aquatic creatures react biologically to environmental pollution, these discoveries are crucial. Fish physiological resistance and susceptibility to pollution may be better understood by analyzing the oxidative stress responses that are seen as indicators of arsenic poisoning. Additionally, the research enhances the use of Clarias batrachus as a metal toxicity assessment model, which is a boon to aquatic toxicology. The results provide environmental monitoring programs with baseline data and improve our knowledge of the oxidative processes caused by arsenic. In order to protect aquatic life and guarantee ecological sustainability, the study concludes that reducing arsenic contamination is crucial. This work establishes a connection between biochemical changes and environmental stress, which may be used to better identify and manage risks in order to save freshwater ecosystems and human health.

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