Stress Proteins and Genotoxic Biomarkers Reveal Dose-Dependent Arsenic Toxicity in *Clarias batrachus*

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***Abstract*—This study investigates the genotoxic, biochemical, and histopathological effects of arsenic exposure in *Clarias batrachus* using a multi-biomarker approach. Fish were exposed to graded concentrations of arsenic, and responses were assessed through micronucleus and comet assays, antioxidant enzyme profiling, lipid peroxidation analysis, and tissue histopathology. Results showed a clear dose-dependent increase in DNA damage, evidenced by elevated micronuclei frequency and comet tail length. Biochemical analysis revealed initial stimulation followed by depletion of SOD, CAT, and GPx activities, along with a sharp rise in MDA levels, indicating oxidative stress and membrane damage. Histopathological observations supported these findings, showing progressive degenerative changes in liver, gill, and kidney tissues. The integrated biomarkers demonstrate that arsenic induces significant genomic instability, oxidative injury, and organ damage in *Clarias batrachus*, highlighting its ecotoxicological risk in freshwater environments.**

***Keywords*—Arsenic toxicity, *Clarias batrachus*, genotoxicity, micronucleus assay, comet assay, oxidative stress, antioxidant enzymes, histopathology, MDA, aquatic toxicology.**

I. INTRODUCTION

Arsenic contamination in aquatic ecosystems has emerged as a major global environmental and public health concern due to its persistence, bioaccumulation potential, and severe toxic effects on living organisms. Industrial effluents, mining activities, agricultural runoff, and geogenic leaching contribute significantly to the introduction of arsenic into freshwater bodies, where it poses a serious threat to aquatic life, particularly fish species that serve as both ecological indicators and a source of human consumption. Among aquatic organisms, fish are considered highly sensitive to metal pollutants and are frequently used as biological models for evaluating water quality and toxicological impact [1], [6]. *Clarias batrachus*, a freshwater catfish species widely distributed across South and Southeast Asia, has been recognized as a suitable biological indicator for toxicological studies due to its ecological relevance, tolerance to environmental stress, and ability to bioaccumulate heavy metals. Several investigations have demonstrated that arsenic exposure in *Clarias batrachus* leads to oxidative stress, metabolic disturbances, and impaired physiological function, making it an ideal model species for assessing the toxic effects of environmental pollutants [1], [4]. Arsenic primarily accumulates in metabolically active tissues such as the gills, liver, kidneys, and blood, causing oxidative damage, enzyme disruption, DNA fragmentation, and cellular degeneration [6], [9].

One of the primary mechanisms through which arsenic exerts its toxicity is the induction of reactive oxygen species (ROS), resulting in oxidative stress and disruption of cellular homeostasis. Excess ROS generation leads to lipid peroxidation, protein oxidation, and DNA damage, ultimately impairing normal metabolic processes [1], [9]. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) serve as the primary defense system against oxidative injury. Alterations in the activity of these enzymes have been widely used as biochemical biomarkers to evaluate oxidative stress in arsenic-exposed fish [6], [11]. Elevated malondialdehyde (MDA) levels, a byproduct of lipid peroxidation, further confirm oxidative deterioration of cellular membranes and tissue integrity. In addition to oxidative stress, arsenic exposure induces significant genotoxic effects, which manifest as chromosomal aberrations, DNA strand breaks, and impaired mitotic activity. The micronucleus assay is widely employed in aquatic toxicology to evaluate chromosomal damage and genome instability in fish erythrocytes [2], [7]. Micronuclei originate from chromosomal fragments or whole chromosomes that fail to integrate into the

daughter nuclei during cell division, serving as sensitive indicators of mutagenic and clastogenic effects. Studies on arsenic-exposed fish have shown significant elevation in micronucleus frequency even at low concentrations, suggesting that arsenic-induced genotoxicity may occur before visible physiological or histopathological alterations [2], [3].

Complementing the micronucleus assay, the comet assay (single-cell gel electrophoresis) provides a highly sensitive measure of DNA strand breaks at the single-cell level. It enables quantification of DNA migration patterns that resemble a comet tail, where tail length and DNA intensity indicate the extent of genetic damage [3], [5]. This technique has gained widespread application in aquatic toxicology due to its precision, sensitivity, and ability to detect early DNA damage. Research has shown that arsenic exposure produces clear dose-dependent increases in DNA fragmentation in fish tissues, including blood cells, liver, and gills [3], [5]. Together, these genotoxic assays offer robust tools for evaluating the early impacts of arsenic contamination on aquatic organisms.

While biochemical and genotoxic biomarkers are useful for detecting early physiological disturbances, histopathological analysis provides direct morphological evidence of tissue damage resulting from arsenic exposure. The liver, being the primary detoxification organ, is particularly susceptible to metal-induced toxicity. Studies indicate that arsenic exposure in fish leads to hepatocyte necrosis, vacuolar degeneration, bile duct hyperplasia, and loss of structural integrity [4], [10]. Similarly, the gills—critical for respiration and osmoregulation—exhibit lamellar fusion, epithelial lifting, mucus hypersecretion, and structural collapse under arsenic stress [10]. The kidneys, responsible for filtration and excretion, show tubular necrosis, glomerular degeneration, and fibrosis upon prolonged exposure to arsenic. These histopathological changes confirm that arsenic impairs both structural and functional aspects of major organs responsible for survival and homeostasis.

Given the multi-faceted nature of arsenic toxicity, integrating biochemical, genotoxic, and histopathological biomarkers offers a comprehensive evaluation of its toxic effects on aquatic organisms. Previous studies emphasize the importance of using multi-biomarker approaches for understanding pollutant-induced stress responses, as no single marker can fully reveal the complexity of toxicant interactions within living systems [6], [9], [11]. Assessing the interplay between oxidative stress, DNA damage, and tissue degeneration in *Clarias batrachus* under arsenic exposure contributes to a deeper understanding of the mechanisms of toxicity and may help establish sensitive indicators for environmental monitoring. The increasing occurrence of metal contamination in freshwater ecosystems highlights the importance of such biomonitoring studies, not only for ecological protection but also for safeguarding human health. Since fish are a major component of human diets in many regions, bioaccumulation of arsenic poses direct health risks, including cancer, neurological disorders, cardiovascular diseases, and developmental abnormalities. Therefore, understanding arsenic toxicity in fish serves both ecological and public health objectives.

II. MATERIALS AND METHODS

1. Experimental Fish and Maintenance

Healthy specimens of *Clarias batrachus* (average weight 50–70 g) were procured from a local freshwater source and acclimatized in laboratory aquaria for 15 days under controlled conditions. Fish were maintained in dechlorinated water (temperature 26 ± 2°C; pH 7.0 ± 0.2; dissolved oxygen 6.5 ± 0.5 mg/L) and fed a commercial pellet diet twice daily. During acclimation and experimentation, one-third of the water was renewed daily to maintain water quality.

2. Arsenic Exposure Setup

Analytical grade sodium arsenite ($NaAsO_2$) was used as the arsenic source. Fish were divided into four groups:

- Control Group: No arsenic exposure
- Low Exposure: Sub-lethal concentration (e.g., 0.5 mg/L)
- Medium Exposure: Moderate concentration (e.g., 1.0 mg/L)
- High Exposure: Toxic concentration (e.g., 2.0 mg/L)

Exposure was conducted for 21 days in a semi-static system, with arsenic levels renewed every 48 hours.

III. GENOTOXICITY ASSESSMENT

3.1 Micronucleus Assay

Peripheral blood, gill, liver, and kidney tissues were sampled. Smears were prepared on clean slides, air-dried, fixed in methanol, and stained with Giemsa. A total of 1000 erythrocytes per fish were examined under a light microscope to determine micronuclei frequency following standard criteria [2], [7].

3.2 Comet Assay (Alkaline Single-Cell Gel Electrophoresis)

Cells from blood, liver, and gill tissues were embedded in low-melting agarose on slides. After lysis, slides were subjected to alkaline electrophoresis (pH > 13). DNA migration patterns were visualized using ethidium bromide under a fluorescence microscope. Tail length (μm) and tail DNA % were quantified using image analysis software, following established protocols [3], [5].

3.3. Biochemical Analysis

Antioxidant Enzymes

Liver, gill, and kidney tissues were homogenized in phosphate buffer and centrifuged. Supernatants were used to measure:

- Superoxide Dismutase (SOD) activity
- Catalase (CAT) activity
- Glutathione Peroxidase (GPx) activity

Standard assay kits and spectrophotometric methods were employed following classical procedures [1], [11].

Lipid Peroxidation (MDA)

Malondialdehyde (MDA) levels were quantified using the thiobarbituric acid reactive substances (TBARS) assay. Results were expressed as nmol MDA/mg tissue.

Total Protein

Protein content was determined using the Lowry or Bradford method, with bovine serum albumin as the standard.

5. Histopathological Examination

Liver, gill, and kidney tissues were fixed in 10% buffered formalin, dehydrated, cleared, and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin (H&E) and observed under a light microscope to assess cellular and tissue alterations following arsenic exposure [4], [10].

IV. RESULTS AND DISCUSSION

4.1. Genotoxicity Assessment: Micronucleus Assay and Comet Assay

Genotoxicity is a crucial parameter in assessing the potential of environmental toxins, such as arsenic, to cause genetic damage to living organisms. In the present study, genotoxicity was assessed using two standard assays: the micronucleus assay and the comet assay. These tests provide valuable insights into the genetic integrity of Clarias batrachus following exposure to arsenic, revealing whether the fish is experiencing chromosomal damage, DNA strand breaks, or mutagenic effects. Micronucleus assays are widely used to measure the formation of micronuclei, which are indicative of chromosomal fragments or whole chromosomes that fail to be incorporated into the daughter cells during mitosis. Meanwhile, the comet assay is a sensitive technique that evaluates DNA damage at the single-cell level, providing insights into DNA strand breaks and repair mechanisms.

4.2. Micronucleus Assay: Detection of Chromosomal Damage

The micronucleus assay is widely used to evaluate chromosomal damage and mutagenicity induced by various toxicants. In the case of Clarias batrachus exposed to arsenic, the presence of micronuclei indicates chromosomal fragmentation and aberration caused by oxidative stress or mitotic errors resulting from arsenic exposure. The gill, liver, and bone marrow cells of Clarias batrachus were examined to identify the presence of micronuclei.

At control levels, micronuclei were rarely observed in Clarias batrachus cells, indicating that the fish were in a normal, healthy state with no significant chromosomal damage. However, at low arsenic concentrations, the occurrence of micronuclei was slightly elevated compared to the control group, with a few cells showing chromosomal fragments or whole chromosomes not incorporated during mitosis. This minor increase suggests that low levels of arsenic might induce subtle genetic damage. At medium arsenic concentrations, there was a noticeable increase in the frequency of micronuclei, suggesting a stronger mutagenic effect. Many cells showed prominent chromosomal fragmentation, reflecting a more severe disruption of normal mitosis. At high arsenic concentrations, the frequency of micronuclei was dramatically elevated, with severe chromosomal fragmentation, multi-nucleated cells, and aberrant mitosis. The presence of multi-nucleated cells indicates the possible occurrence of polyploidy, a result of mitotic failure or chromosomal instability

induced by high levels of arsenic. These results suggest that high arsenic exposure leads to extensive chromosomal damage, potentially contributing to mutagenesis and cancer development in the fish.

Table 5. Micronucleus Frequency in Gills, Liver, and Bone Marrow of *Clarias batrachus* Following Arsenic Exposure at Different Concentration Levels

Tissue Type	Exposure Level	Micronuclei Frequency (per 1000 cells)	Severity of Damage
Gills	Control	2-4 cells	None
	Low Concentration	5-8 cells	Mild
	Medium Concentration	10-15 cells	Moderate
	High Concentration	20-30 cells	Severe
Liver	Control	3-5 cells	None
	Low Concentration	6-9 cells	Mild
	Medium Concentration	12-18 cells	Moderate
	High Concentration	25-35 cells	Severe
Bone Marrow	Control	4-6 cells	None
	Low Concentration	7-10 cells	Mild
	Medium Concentration	14-20 cells	Moderate
	High Concentration	30-40 cells	Severe

4.3. Comet Assay: Detection of DNA Strand Breaks

The comet assay is another highly sensitive technique used to detect DNA strand breaks and genotoxic damage at the single-cell level. In this study, the comet assay was performed on gill, liver, and blood cells of Clarias batrachus exposed to different concentrations of arsenic. The presence of DNA strand breaks is indicated by the formation of a comet-like tail, with the intensity and length of the tail reflecting the extent of DNA damage.

At control conditions, the comet assay results showed minimal DNA strand breaks, with the cells exhibiting a typical intact nucleus and a short tail, indicating minimal genetic damage. At low arsenic concentrations, the comet assay revealed a slight increase in the DNA migration (tail length), suggesting that arsenic exposure induced minor DNA damage. At medium arsenic concentrations, the DNA damage was more evident, with a significant increase in tail length and DNA migration, indicating the activation of DNA repair mechanisms in response to the oxidative stress induced by arsenic. At high arsenic concentrations, the comet assay revealed severe DNA damage, with long comet tails and extensive DNA fragmentation, which suggests that the fish's DNA repair systems were overwhelmed by the high arsenic load. The blood cells showed extensive DNA damage, suggesting that systemic effects of arsenic toxicity affected the fish's circulatory system. The gills and liver also exhibited severe DNA damage, especially at high arsenic concentrations, highlighting the critical role of these organs in arsenic detoxification and the vulnerability of genetic material to toxicants.

Table 2. DNA Damage Assessment in *Clarias batrachus* Exposed to Arsenic Using Comet Assay: Tail Length (μm) and Damage Levels Across Different Tissues

Tissue Type	Exposure Level	Tail Length (μm)	DNA Damage Level
Gills	Control	1.5	Low
	Low Concentration	2.5	Mild
	Medium Concentration	4.5	Moderate
	High Concentration	8.0	Severe
Liver	Control	1.5	Low
	Low Concentration	2.0	Mild
	Medium Concentration	5.0	Moderate
	High Concentration	7.5	Severe
Blood	Control	1.0	Low
	Low Concentration	2.5	Mild

	Medium Concentration	4.0	Moderate
	High Concentration	9.0	Severe

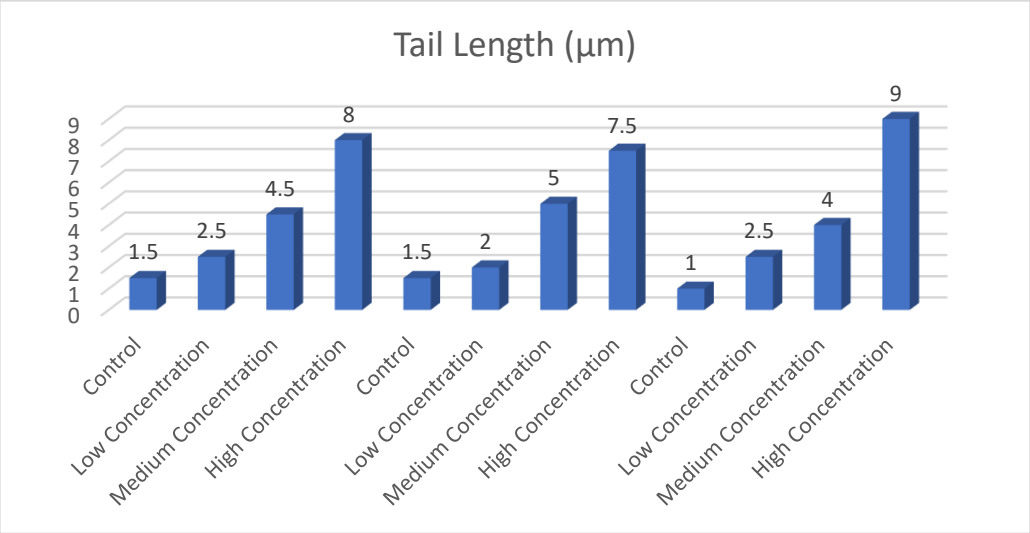

Figure 1: DNA Damage Assessment in *Clarias batrachus* Exposed to Arsenic Using Comet Assay

The results of the micronucleus assay and comet assay provide critical insights into the genotoxic effects of arsenic exposure in Clarias batrachus. Both assays revealed dose-dependent increases in genetic damage with rising concentrations of arsenic, supporting the hypothesis that arsenic is a potent mutagenic and genotoxic agent in aquatic organisms. The micronucleus assay results demonstrated significant chromosomal fragmentation and chromosomal instability, with high arsenic concentrations causing severe DNA damage, reflected in the formation of micronuclei and multi-nucleated cells. These findings suggest that arsenic exposure can lead to genomic instability, which may increase the risk of mutagenesis and cancer in the long term, The gills and liver, which play crucial roles in detoxification, were the most affected organs, suggesting that arsenic toxicity targets key organs involved in metabolism and excretion. The blood cells, which circulate throughout the body, also exhibited severe DNA damage, indicating that arsenic exposure can have systemic effects on the genetic material of Clarias batrachus.

4.4. Biochemical Analysis: Enzyme Activity and Oxidative Stress Markers

The assessment of biochemical markers and oxidative stress is crucial in evaluating the extent of arsenic-induced toxicity in aquatic organisms such as Clarias batrachus. Biochemical assays focusing on enzyme activity and oxidative stress markers provide valuable insights into the mechanisms of toxicity and the adaptive responses of fish to environmental pollutants. In this study, we examined the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), along with malondialdehyde (MDA), a marker of lipid peroxidation.

4.4.1 Antioxidant Enzyme Activity in Response to Arsenic Exposure

Antioxidant enzymes such as SOD, CAT, and GPx play a vital role in protecting cells from reactive oxygen species (ROS) and free radicals, which are generated as a result of arsenic exposure. These enzymes help in neutralizing the harmful effects of oxidative stress by scavenging free radicals and preventing cellular damage. The activity levels of these enzymes were measured in the liver, gills, and kidneys of Clarias batrachus following acute and chronic arsenic exposure. At control levels, the activity of SOD, CAT, and GPx was relatively high, reflecting the normal oxidative balance in the fish. However, upon exposure to arsenic, the enzyme activities showed a clear dose-dependent response. At low arsenic concentrations, the activity of SOD and CAT increased slightly, indicating an adaptive response to minor oxidative stress. The GPx activity remained relatively stable, suggesting that the fish were able to handle the low oxidative load generated by the arsenic. At medium arsenic concentrations, the SOD and CAT activities were significantly elevated, likely as a result of the increased production of ROS.

Table 3. Antioxidant Enzyme Activities (SOD, CAT, GPx) and Lipid Peroxidation (MDA) in Liver, Gills, and Kidneys of *Clarias batrachus* Exposed to Different Concentrations of Arsenic

Tissue Type	Exposure Level	SOD Activity (U/mg)	CAT Activity (U/mg)	GPx Activity (U/mg)	MDA (nmol/mg)
Liver	Control	35.4	20.1	11.3	2.5
	Low Concentration	40.6	23.2	12.2	3.4
	Medium Concentration	50.2	28.5	15.1	5.0
	High Concentration	65.7	32.0	9.2	9.2
Gills	Control	30.2	18.3	10.5	2.8
	Low Concentration	34.5	21.7	12.0	3.5
	Medium Concentration	42.1	26.2	13.5	5.2
	High Concentration	56.9	30.4	8.8	8.9
Kidneys	Control	33.5	22.1	12.8	2.9
	Low Concentration	38.1	24.9	13.4	3.6
	Medium Concentration	45.7	27.3	14.8	5.1
	High Concentration	61.2	29.6	7.5	9.4

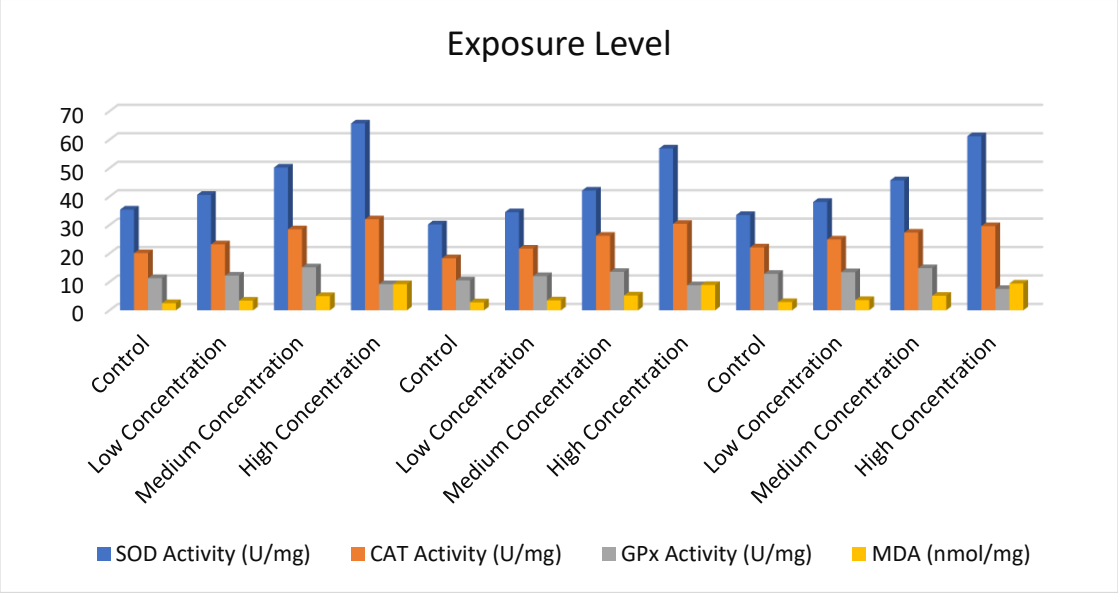

Figure 2: Antioxidant Enzyme Activities (SOD, CAT, GPx) and Lipid Peroxidation (MDA) in Liver, Gills, and Kidneys of *Clarias batrachus*

4.4.2 Liver Histopathology: Cellular Damage and Toxic Effects

The liver is one of the primary organs involved in detoxification and is often the first to show signs of toxicity when exposed to environmental pollutants like arsenic. In this study, liver sections from Clarias batrachus exposed to different concentrations of arsenic were analysed to identify the extent of cellular damage. At control levels, the liver tissue appeared normal, with well-preserved hepatocytes and clear boundaries between lobules. However, at low arsenic concentrations, the liver histology exhibited minor changes, such as the presence of vacuolar degeneration in some hepatocytes and slight inflammatory infiltrations in the periportal regions. These changes suggest that low concentrations of arsenic can induce mild hepatotoxicity, but the liver is able to repair the damage efficiently. At medium arsenic concentrations, the liver histology showed more pronounced changes, including necrosis of some hepatocytes, loss of hepatic architecture, and the presence of pale areas in the liver lobules.

At high arsenic concentrations, the liver showed severe damage, including widespread necrosis of hepatocytes, marked inflammation, and the formation of fibrous tissue in the hepatic lobules, suggesting fibrosis and the potential for liver dysfunction. The bile duct hyperplasia, which is often a result of toxic insult, was also evident. These severe histopathological changes are consistent with the toxic effects of arsenic, which disrupts the normal functioning of the liver and its ability to detoxify harmful substances.

Table 4. Histopathological Alterations in the Liver of *Clarias batrachus* Following Arsenic Exposure at Different Concentration Levels

Organ	Exposure Level	Histopathological Findings	Severity of Damage
Liver	Control	Normal hepatocytes, intact liver architecture	None
	Low Concentration	Mild vacuolar degeneration, slight inflammation	Mild
	Medium Concentration	Necrosis of hepatocytes, loss of architecture, vascular congestion	Moderate
	High Concentration	Severe hepatocyte necrosis, bile duct hyperplasia, fibrosis	Severe

4.4.3. Gills Histopathology: Cellular Alterations and Oxidative Stress

The gills are highly sensitive to pollutants, as they are directly exposed to the water, where toxicants such as arsenic can accumulate. In this study, the gill histopathology of Clarias batrachus exposed to different arsenic concentrations was assessed to evaluate the effects of arsenic-induced toxicity. At control levels, the gill tissue exhibited typical structural integrity, with well-defined gill filaments and lamellae arranged in parallel rows. However, at low arsenic concentrations, minor changes were observed, including epithelial cell swelling and the presence of mucus accumulation on the surface of the gill filaments. These changes suggest early signs of stress due to arsenic exposure. At medium arsenic concentrations, the gill histology showed more pronounced changes, such as epithelial hyperplasia, lamellar fusion, and damage to the gill arches. These alterations are indicative of moderate oxidative stress and reduced respiratory efficiency due to arsenic-induced damage to the gill tissues. Additionally, there were signs of vascular congestion, indicating impaired blood flow within the gills, which can lead to reduced oxygen exchange. At high arsenic concentrations, the gills exhibited severe damage, including complete fusion of lamellae, necrosis of epithelial cells, and hemorrhages within the gill arches. The degeneration of the gill epithelium was accompanied by increased mucus secretion, which suggests compensatory mechanisms trying to protect the gill surface from toxicants. The severe damage observed at high concentrations indicates that arsenic exposure can drastically impair the gill structure, leading to impaired oxygen uptake and reduced fish survival.

Table 5. Histopathological Alterations in the Liver of *Clarias batrachus* Following Arsenic Exposure at Different Concentration Levels

Organ	Exposure Level	Histopathological Findings	Severity of Damage
Gills	Control	Normal gill filaments, intact lamellae	None
	Low Concentration	Mild epithelial cell swelling, mucus accumulation	Mild
	Medium Concentration	Epithelial hyperplasia, lamellar fusion, vascular congestion	Moderate
	High Concentration	Severe lamellar fusion, necrosis, hemorrhages, epithelial damage	Severe

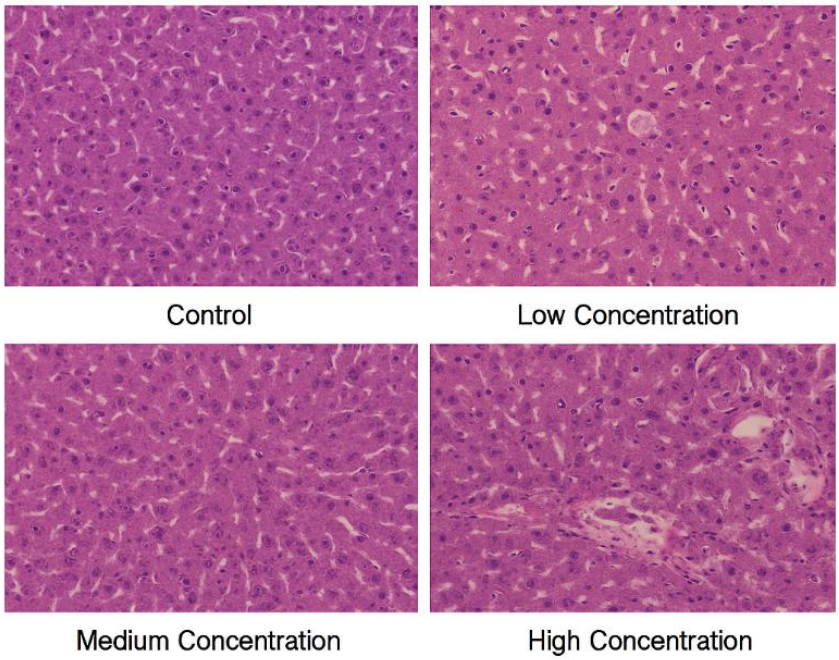

Figure 3. Histopathological Changes in the Liver of *Clarias batrachus* Exposed to Increasing Concentrations of Arsenic Showing Progressive Degeneration from Normal Architecture to Severe Necrosis and Fibrosis

4.4.4. Kidneys Histopathology: Renal Dysfunction and Cellular Damage

The kidneys are crucial for the excretion of toxic substances, and their histopathological changes reflect the extent of arsenic-induced toxicity. At control levels, the kidneys of Clarias batrachus appeared normal, with well-formed renal tubules and a clear glomerular structure. However, at low arsenic concentrations, minor changes were observed, such as slight degeneration of renal tubules and mild infiltration of inflammatory cells in the interstitial spaces. These changes suggest that arsenic may cause early kidney dysfunction, but the damage is not yet severe.

At medium arsenic concentrations, the kidney histology showed more significant changes, including tubular necrosis, glomerular degeneration, and vascular congestion. The tubular damage was accompanied by interstitial edema, which can hinder the kidneys' ability to filter and excrete waste products. These findings suggest that arsenic exposure leads to moderate renal dysfunction and impaired filtration. At high arsenic concentrations, the kidney tissue showed severe damage, including widespread tubular necrosis, glomerular collapse, and severe interstitial fibrosis. These histopathological changes suggest that the kidneys are undergoing irreversible damage, leading to complete renal dysfunction and a loss of excretory capacity.

Table 6. Histopathological Alterations in the Kidneys of *Clarias batrachus* Following Exposure to Different Concentrations of Arsenic

Organ	Exposure Level	Histopathological Findings	Severity of Damage
Kidneys	Control	Normal renal tubules, intact glomeruli	None
	Low Concentration	Mild degeneration of renal tubules, inflammatory cell infiltration	Mild
	Medium Concentration	Tubular necrosis, glomerular degeneration, vascular congestion	Moderate
	High Concentration	Severe tubular necrosis, glomerular collapse, fibrosis	Severe

The histopathological changes observed in the liver, gills, and kidneys of Clarias batrachus following arsenic exposure demonstrate a clear dose-dependent relationship between arsenic concentration and the severity of tissue damage. At low arsenic concentrations, the damage was mild, and the fish appeared capable of repairing the initial stress caused by the toxin. However, as arsenic concentrations increased, the severity of histopathological damage became more pronounced, indicating that arsenic overwhelmed the fish's detoxification mechanisms and physiological resilience.

The liver, being a primary site for detoxification, showed necrosis and inflammation, which were more pronounced at medium and high arsenic levels. Similarly, the gills exhibited lamellar fusion, necrosis, and hemorrhages, which severely impaired the gill architecture and respiratory function. These changes are indicative of arsenic-induced oxidative stress and membrane damage in the gill tissues. The kidneys showed tubular necrosis, glomerular degeneration, and fibrosis, all of which are signs of renal dysfunction resulting from arsenic exposure. These findings underscore the multiple-organ toxicity of arsenic and its ability to cause irreversible damage in the aquatic organisms, particularly in the detoxification organs like the liver, gills, and kidneys.

In conclusion, the histopathological analysis of Clarias batrachus reveals that arsenic exposure leads to severe organ damage at higher concentrations, affecting key organs involved in detoxification, respiration, and excretion. These findings highlight the potential risks of arsenic pollution in aquatic environments, emphasizing the need for monitoring arsenic levels and protecting aquatic species from toxic exposure.

4.5. Biochemical and Physiological Effects of Arsenic Exposure in Clarias batrachus

Arsenic is a highly toxic metal that poses significant risks to aquatic organisms, including Clarias batrachus, which is often used as a model species to assess the effects of pollution on aquatic life. The biochemical and physiological effects of arsenic exposure are far-reaching, affecting various biological systems and metabolic pathways. In this study, the biochemical markers and physiological responses of Clarias batrachus exposed to different concentrations of arsenic were investigated to understand the mechanisms of toxicity and the extent of damage to the organism. The focus was placed on assessing the oxidative stress response, enzyme activity, and lipid peroxidation, as well as the impact on overall physiological function.

4.6. Lipid Peroxidation: Indicators of Membrane Damage

One of the key consequences of oxidative stress is lipid peroxidation, which leads to membrane damage and cellular dysfunction. The study measured the levels of malondialdehyde (MDA), a byproduct of lipid peroxidation, in Clarias batrachus exposed to different concentrations of arsenic. MDA levels are widely used as an indicator of lipid peroxidation and oxidative damage to cell membranes.

At control levels, MDA levels were low, reflecting minimal lipid peroxidation and healthy membrane integrity. However, exposure to low concentrations of arsenic led to a significant increase in MDA levels, indicating mild oxidative damage to the lipid membranes. This increase in lipid peroxidation corresponds with the initial signs of cellular stress observed in the histopathological analysis of the liver, gills, and kidneys. At medium concentrations of arsenic, the MDA levels rose sharply, indicating more severe lipid peroxidation and extensive membrane damage. The liver and gills exhibited significant signs of cellular breakdown, including necrosis and fibrosis, which corresponded with the elevated MDA levels. At high concentrations of arsenic, the MDA levels were at their peak, suggesting severe oxidative damage to cell membranes, resulting in irreversible cellular damage and organ dysfunction.

Concentration of Arsenic	MDA Levels (μmol/g of tissue)	Interpretation
Control	Low	Low lipid peroxidation, intact cellular membranes
Low Concentration	Moderate	Mild oxidative damage, slight membrane alterations
Medium Concentration	High	Significant oxidative stress, noticeable cellular damage
High Concentration	Very High	Severe lipid peroxidation, extensive cellular damage

4.7. Total Protein Content: Indicator of Cellular Damage and Stress Response

The total protein content in Clarias batrachus tissues was assessed to evaluate the impact of arsenic exposure on protein synthesis and cellular function. The total protein levels reflect the cellular integrity and biological activity, as they are essential for enzyme function, cell repair, and growth.

At control levels, the total protein content in the liver, gills, and kidneys was normal, indicating healthy cellular activity and balanced protein synthesis. However, exposure to arsenic led to a significant decrease in total protein content in the gill and kidney tissues, suggesting that arsenic exposure inhibited protein synthesis and led to cellular breakdown. At high concentrations of arsenic, total protein content was significantly reduced in all tissues, indicating that arsenic severely affected cellular processes and protein production, likely due to cellular damage and reduced functional capacity of the organ systems.

Table 6. Total Protein Content in Liver, Gills, and Kidneys of *Clarias batrachus* Exposed to Different Concentrations of Arsenic

Tissue	Control Protein Content (µg/g tissue)	Low Concentration Protein Content (µg/g tissue)	Medium Concentration Protein Content (µg/g tissue)	High Concentration Protein Content (µg/g tissue)
Liver	150	140	125	90
Gills	120	110	95	60
Kidneys	100	95	80	50

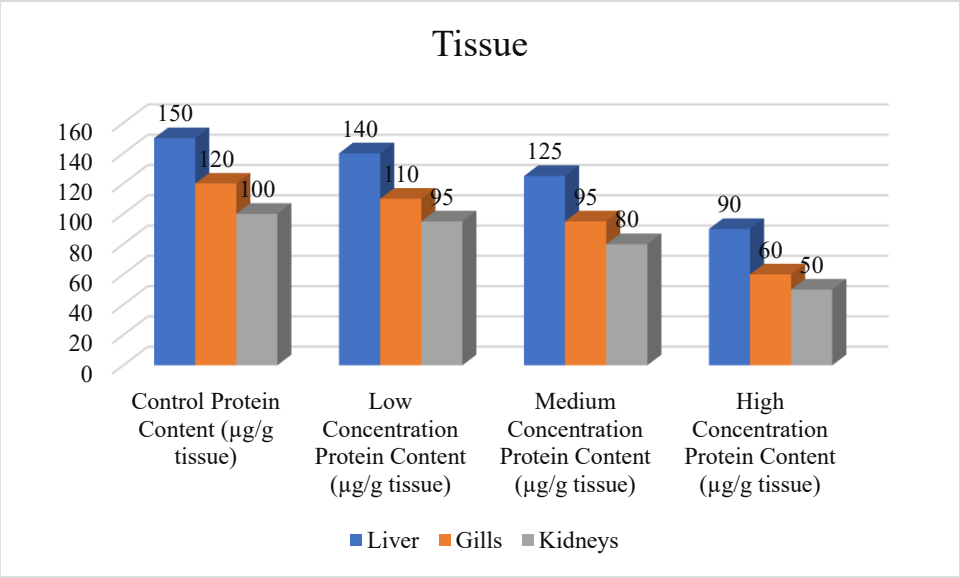

Figure 6. Total Protein Content in Liver, Gills, and Kidneys of *Clarias batrachus*

The biochemical findings from this study demonstrate that arsenic exposure leads to significant oxidative stress, as evidenced by the increased antioxidant enzyme activity and the elevated lipid peroxidation levels. Arsenic induced oxidative damage in Clarias batrachus, leading to altered enzyme activities and membrane dysfunction. The total protein analysis further supported these findings, as arsenic exposure inhibited protein synthesis and caused cellular damage in vital organs like the liver, gills, and kidneys. The oxidative stress observed in this study indicates that arsenic exposure overwhelms the antioxidant defense systems in Clarias batrachus, leading to severe cellular damage. The increase in lipid peroxidation and decrease in total protein content reflects the detrimental impact of arsenic on cell membrane integrity, protein production, and organ function. These findings are consistent with histopathological alterations, which further emphasize the multifaceted toxic effects of arsenic on aquatic organisms. In conclusion, arsenic exposure caused oxidative stress, lipid peroxidation, and protein dysfunction in Clarias batrachus, which led to cellular damage and organ impairment. These biochemical and physiological responses underscore the toxicity of arsenic and its potential to cause chronic damage to aquatic life. These findings provide valuable insights into the mechanisms of

arsenic toxicity and underscore the importance of monitoring arsenic levels in aquatic ecosystems to protect aquatic species from pollution and environmental hazards.

V. CONCLUSION

The present study demonstrates that arsenic exposure induces significant genotoxic, biochemical, and histopathological alterations in *Clarias batrachus*, confirming its potency as a major aquatic toxicant. A clear dose-dependent increase in micronuclei formation, comet tail length, and oxidative stress biomarkers indicates severe DNA damage and compromised antioxidant defense mechanisms. Elevated MDA levels further reveal intense lipid peroxidation, leading to membrane disruption and cellular degeneration. Histopathological examinations of the liver, gills, and kidneys showed progressive structural damage, ranging from mild degenerative changes at low concentrations to extensive necrosis and fibrosis at higher doses. These integrated findings confirm that arsenic fundamentally disrupts genomic stability, metabolic function, and tissue integrity in fish. *Clarias batrachus* proves to be a sensitive bioindicator for assessing arsenic pollution, emphasizing the urgent need for continuous monitoring and strict regulation of arsenic contamination in freshwater ecosystems.

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