JOURNAL OF VETERINARY AND APPLIED SCIENCES
VOLUME 16, ISSUE 1: 1340 – 1352 (2026)
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Exposure of juvenile *Clarias gariepinus* to sub-lethal levels of bisphenol A: Effects on oxidative stress marker levels and histology of the gills and muscles

Ebunoluwa R. Asenuga [1] and Osemudiamen A. Edene [2]

[1] Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin, Benin City, Edo State, Nigeria.

[2] Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

==========

Abstract

Aquatic pollution poses imminent risks to the wholeness of the ecology and food security, especially in environments where treatment of wastewater is inefficient or non-existent. The present study experimentally exposed African catfish (*Clarias gariepinus*) to sub-lethal levels of Bisphenol A (BPA) and evalauted its effects on the histology and levels of oxidative stress markers in the gills and muscles. One hundred and eighty fishes were used for the study. They were randomly assigned to six groups (Groups 1 – 6) of 30 each. Group 1 fishes were the Untreated Normal Control, while Group 2 fishes were exposed to the solvent (ethanol) used to dilute the BPA (Solvent treated control). Groups 3, 4, 5 and 6 were exposed to BPA at the levels of 2, 4, 6 and 8 mg/L, respectively. The BPA exposure lasted for 28 days. After day 28, the fishes were sacrificed and the gills and muscles were collected and used for assessment of levels of oxidative stress markers and also for histopathology, following standard procedures. Results showed that there was significant tissue (gills and muscles) toxicity which was dose-dependent. The concentrations of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), and the activity levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) in the gills and muscles of the treated groups were significantly ($p < 0.05$) higher than those of the control groups, while the levels of reduced glutathione (GSH) was significantly ($p < 0.05$) higher in the controls when compared to the treated groups. There was also a significantly ($p < 0.05$) lower concentration of proteins in the gills and muscles of the treated groups when compared to the controls. In the BPA treated groups, there was proliferation and thickening of epithelial cells covering the lamellae of the gills with pillar congestion, the severity of which was dose dependent. Atrophy of the myofibres and accentuation of the interstitial spaces of the muscles were recorded for the groups treated with higher levels of BPA. It was concluded that exposure of juvenile catfish (*Clarias gariepinus*) to BPA at the levels used in the study led to signifcant alterations in markers of oxidative stress and histopathology of the gills and muscles of the treated fishes.

Keywords: Bisphenol A (BPA); African catfish (*Clarias gariepinus*); Gills; Muscles; Oxidative stress markers; Histopathology; Toxicity.

*** Correspondence:** Ebunoluwa R. Asenuga; E-mail: ebunoluwa.olowu@uniben.edu; Phone: +2348052720219

Article History: Initial manuscript submission received – January 22, 2026; Final revised form received – March 26, 2026; Accepted for publication – March 30, 2026; Published – April 07, 2026.

Introduction

A vibrant aquatic ecosystem plays a vital role in the maintenance of human well-being by providing nourishment as well as means of income generation (Ferreira *et al*., 2023). Ichthyofauna (fish) is a major constituent of the aquatic ecosystem, because of their nutritive and economic relevance (Colvin *et al*., 2019). However, the aquatic biome is faced with serious threats from indiscriminate use of chemicals that pollute water, which eventually compromise biological diversity as well as the integrity of the aquatic ecosystem. Endocrine disrupting chemicals (EDCs) are one of the contaminants of concern; they have the ability to interfere with the biological pathway of the endocrine system in both wildlife and human populations, even at extremely low environmentally relevant concentrations, resulting in disruptive effects (Faheem and Bhandari, 2021).

Bisphenol A (BPA) is an EDC that has gained global recognition and attention, especially because of its extensive usage in polycarbonate plastics, industrial goods, epoxy resins, thermal papers, food packaging, flame retardants, etc. (Abraham and Chakraborty, 2020; Elizalde-Velázquez *et al*., 2023). The introduction of BPA into the aquatic environment can occur through inadequate or inefficient waste treatment, leaching and indiscriminate effluent discharge (Lin *et al.,* 2017; Hahladakis *et al.,* 2023). In developing nations like Nigeria, aquatic organisms are prone to the adverse effects of BPA, as a result of an insufficient wastewater disposal framework and inefficient regulatory structures (Mohammed *et al.,* 2024; Ologundudu *et al.,* 2025). As a result of the inherent persistence in the aquatic bodies, the bio-accumulative and concurrent toxicological effect of BPA, especially on non-target species, in particular fish, is of grave concern because of its importance in the food chain (Barboza *et al*., 2022).

In order, to carry out substantial eco-risk analysis, environmental conservation and formulate food hygiene regulations, it is essential to have a thorough understanding of the relationship between environmental pollution by BPA and its ability to induce oxidative stress in fish principally because of the societal relevance of fish (Diedrich *et al.,* 2019) especially with mounting dependence on aquaculture as a universal source of protein and income (Ceballos-Concha *et al.,* 2025).

The African catfish (*Clarias gariepinus*) has been identified as one of the most conspicuous freshwater ichthyofauna grown across the African continent, and which has made an impactful input to geographical aquafarming output and food security (Klimuk *et al*., 2024). The fact that African catfish has a rapid physical developmental rate, ability to resist harsh environmental conditions and widespread market receptiveness, makes it a popular species in both backyard and commercial aquafarming (Ojuwoni *et al,* 2020).

The pollution of aquatic bodies with endocrine-disrupting chemicals (EDCs) constitutes a significant risk to the farming of cultivated catfish and wild populations. These environmental contaminants endanger fish well-being, reduce reproductive success, and eventually affect the quality of food (Adeogun *et al*., 2018). There is a convincing need therefore to determine with certainty the toxicological impact of sub-lethal concentrations of Bisphenol A (BPA) on the African catfish. The present study was an experiment designed to expose African catfish (*Clarias gariepinus*) to sub-lethal levels of Bisphenol A (BPA), and evaluate its effects on the histology and levels of oxidative stress markers in the gills and muscles of the fish.

Materials and Methods

A total of one hundred and eighty (180) juvenile African catfish (*Clarias gariepinus*) sourced from Fecundity farm, Ugbowo Benin-city, were used for the study. They were kept in tanks. The BPA used for the study was analytical grade BPA procured from Sigma Chemical Co., Saint Louis, MO, USA. The use of the fish for the study was approved by the Ethics Committee, Faculty of Pharmacy, University of Benin, Nigeria, with Approval Number EC/FP/025/003.

The 180 fishes were acclimatized for two weeks and randomly assigned to six groups (Groups 1 – 6) of 30 each (in triplicate of tens). The treatment groups were as follows: Group 1 fish were the untreated control that was not exposed to BPA. Group 2 fish were the solvent treated control that was exposed to the solvent (ethanol) used in diluting the BPA at 0.050ml/L. Fish in Groups 3, 4, 5 and 6 were exposed to 2, 4, 6 and 8 mg BPA/L of water, respectively. The doses of BPA used in this study was chosen based on the results obtained from a previous study (unpublished data) where higher doses were used and it was decided to use lower doses with extended exposure time to observe the effect. Exposure to the BPA lasted for 28 days.

During the exposure period, fish were fed pelletized feed twice daily, and half of the toxicant solution was replaced daily; a full water change was done every seven days in a semi-static system. Fish were observed daily for behavioural changes, sluggish movement and death. At the end of the exposure period, feed was withdrawn for 24 hours, following which the fish were sacrificed, dissected, and gills and skeletal muscle tissues extracted for analysis.

The extracted gills and muscle tissue were doused swiftly in cold normal saline, and filter paper was used to absorb excess saline. The extracted tissues were weighed. The gills and muscle were cut up and placed in 5 ml of phosphate buffer, pH 7.4 and homogenized. The homogenized sample was centrifuged at 10,000 revolutions per minute, at -4 °C for 10 minutes. The supernatant was aspirated and stored at -4 °C for biochemical evaluations.

The total protein levels of the supernatants were quantified following the Biuret method (Gornal *et al.*, 1949). Superoxide dismutase (SOD) activity was determined by estimating the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30 °C as described by Misra and Fridovich (1972). The concentration of reduced glutathione (GSH) was determined according to Ellman (1959). The activity of glutathione-S-transferase and glutathione peroxidase (GPx) was also assessed according to the methods of Habig *et al*. (1974) and Nyman (1959), respectively. The procedure of Buege and Aust (1978) was used to ascertain the concentration of malondialdehyde (MDA) in the tissues. While the hydrogen peroxide (H_2O_2) levels was determined using the procedure of Wolff (1994).

Some of the gills and muscle tissues collected were fixed in 10% buffered formalin and processed following standard histological procedures. The fixed tissues were first dehydrated in ascending grades of ethanol (70%, 90%, 96% and 100%) to remove water. They were then cleared in xylene to eliminate residual alcohol and enhance tissue transparency. Following this, the samples were impregnated with melted paraffin wax to allow infiltration. The impregnated tissues were submerged in new melted paraffin wax and allowed to set and form tissue blocks. A microtome was used to cut thin sections from the paraffin blocks, and the sections were carefully placed on clean glass slides and dried in a hot air oven (Drury and Wallington, 1980). The prepared tissue sections were stained using the haematoxylin and eosin (H & E) technique. Xylene was first used to dewax the slides, followed by rehydration using descending grades of ethanol (100%, 96%, 90%, and 70%). They were then stained with

haematoxylin and rinsed in water. 1% acid alcohol was used briefly for differentiation, after which warm water was used to counterstain the sections. Counterstaining was done using eosin, followed by rinsing in water. Thereafter, ascending grades of alcohol were used to dehydrate the sections, followed by clearing in xylene, after which DPX was used for mounting for microscopic examination (Drury and Wallington, 1980).

All the quantitative data collected were subjected to one way analysis of variance (ANOVA) using GraphPad Prism version 10.0 software. Variant means were separated using the least significant difference method. Significance was accepted at $p < 0.05$, and summary results were presented as mean ± standard deviation (SD).

Results

The total protein concentration in the muscle and gill tissues in all four fish groups exposed to BPA were significantly ($p < 0.05$) lower than the levels recorded for the two control groups (Table 1). The hydrogen peroxide concentration in the gills of all the fish groups exposed to BPA were significantly higher ($p < 0.05$) in a dose dependent manner than those of the two controls, but the muscle hydrogen peroxide levels of only the groups exposed to 6 and 8 mg/L of BPA (the higher doses) were significantly higher ($p < 0.05$) than those of the two controls (Table 2). Also, the levels of malondialdehyde in the gills and muscle tissues of all the fish groups exposed to BPA were significantly higher ($p < 0.05$) than the levels in the gills and muiscles of the fish in the two control groups (Table 3).

The activity of SOD, GPx and GST in the gills and muscle were all significantly higher ($p < 0.05$) in the BPA treated groups in a concentration dependent manner, when compared to the two control groups (Figures 1, 2 and 3). In contrast, the levels of GSH in the gills and muscles were significantly lower ($p < 0.05$) in all the BPA treated groups in a concentration dependent manner, when compared to the two control groups (Figure 4).

Table 1. Total protein concentration of gill and muscles of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA).

Groups and their treatments.	Total Protein concentration in Gills (g/dl).	Total Protein concentration in Muscles (g/dl).
Normal Control group	0.88 ± 0.04	0.82 ± 0.05
Solvent Control group	0.90 ± 0.03	0.82 ± 0.017
Group exposed to 2 mg/L BPA	0.68 ± 0.04 ^{a,b}	0.57 ± 0.036 ^{a,b}
Group exposed to 4 mg/L BPA	0.65 ± 0.02 ^{a,b}	0.48 ± 0.023 ^{a,b}
Group exposed to 6 mg/L BPA	0.53 ± 0.03 ^{a,b}	0.49 ± 0.016 ^{a,b}
Group exposed to 8 mg/L BPA	0.53 ± 0.02 ^{a,b}	0.45 ± 0.22 ^{a,b}

Values are expressed as mean ± SD (n = 10). Values with different superscripts (^{a} and ^{b}) in a column are significantly different ($p < 0.05$) when compared to Normal Control and Solvent Control, respectively.

Table 2. Hydrogen peroxide (H_2O_2) concentration of gills and muscles of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA).

Groups and their treatments	H_2O_2 concentration in Gills (µg/ml)	H_2O_2 concentration in Muscles (µg/ml)
Normal Control group	39.4 ± 1.63	28.6 ± 0.81
Solvent Control group	39.9 ± 0.72	28.1 ± 0.54
Group exposed to 2 mg/L BPA	41.5 ± 1.42 [a,b]	28.4 ± 1.16
Group exposed to 4 mg/L BPA	46.2 ± 4.47 [a,b]	28.4 ± 0.22 [a]
Group exposed to 6 mg/L BPA	46.8 ± 1.01 [a,b]	32.7 ± 1.45 [a,b]
Group exposed to 8 mg/L BPA	56.3 ± 1.70 [a,b]	41.5 ± 1.50 [a,b]

Values are expressed as mean ± SD (n = 10). Values with different superscripts ([a] and [b]) in a column are significantly different ($p < 0.05$) when compared to Normal Control and Solvent Control, respectively.

Table 3. Malondialdehyde (MDA) concentration of gills and muscles of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA).

Groups and their treatments	MDA concentration in Gills (mol/g protein)	MDA concentration in Muscles (mol/ g protein)
Normal Control group	0.17 ± 0.009	0.17 ± 0.005
Solvent Control group	0.18 ± 0.003	0.18 ± 0.011
Group exposed to 2 mg/L BPA	0.36 ± 0.012 [a,b]	0.23 ± 0.008 [a,b]
Group exposed to 4 mg/L BPA	0.36 ± 0.02 [a,b]	0.23 ± 0.005 [a,b]
Group exposed to 6 mg/L BPA	0.38 ± 0.001 [a,b]	0.25 ± 0.005 [a,b]
Group exposed to 8 mg/L BPA	0.33 ± 0.008 [a,b]	0.24 ± 0.003 [a,b]

Values are expressed as mean ± SD (n = 10). Values with different superscripts ([a] and [b]) in a column are significantly different ($p<0.05$) when compared to Normal Control and Solvent Control, respectively.

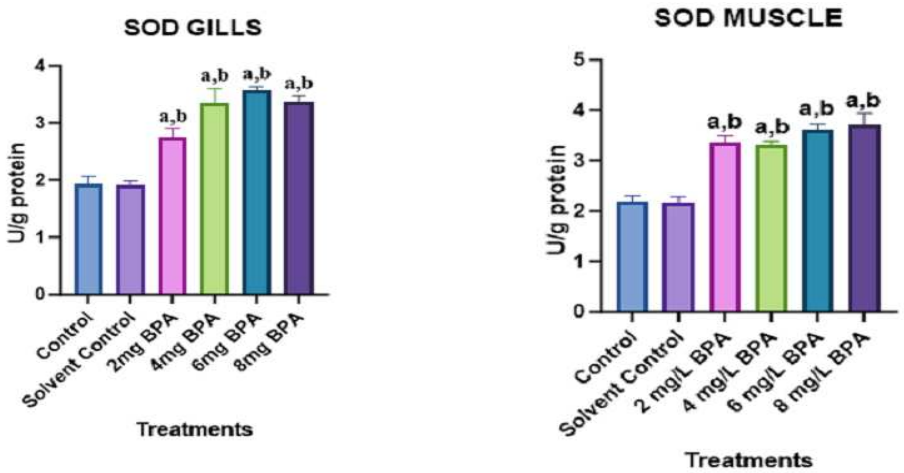

Figure 1. Superoxide dismutase (SOD) activity in gills and muscles of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA). [Values are expressed as mean ± SD. 'a' and 'b' indicate significant difference ($p < 0.05$) when compared to Normal control and Solvent control, respectively.]

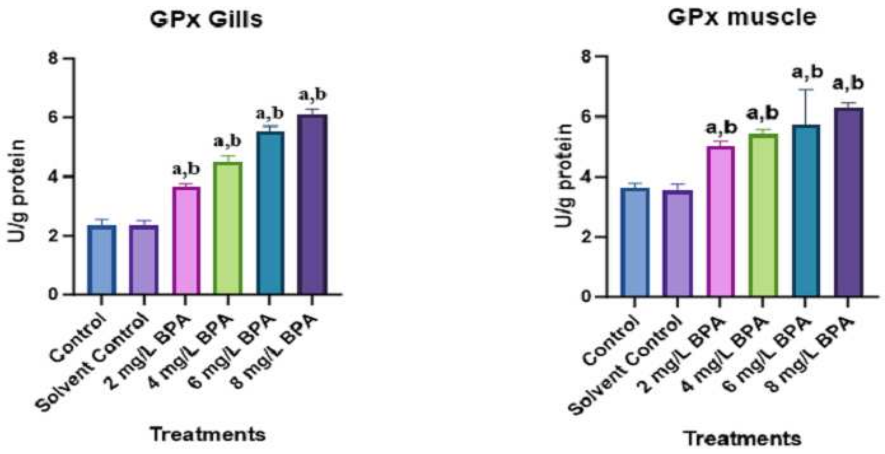

Figure 2. Glutathione peroxidase (GPx) activity in gills and muscles of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA). [Values are expressed as mean ± SD (n=10); 'a' and 'b' indicate significant difference ($p < 0.05$) when compared to Normal control and Solvent control, respectively.

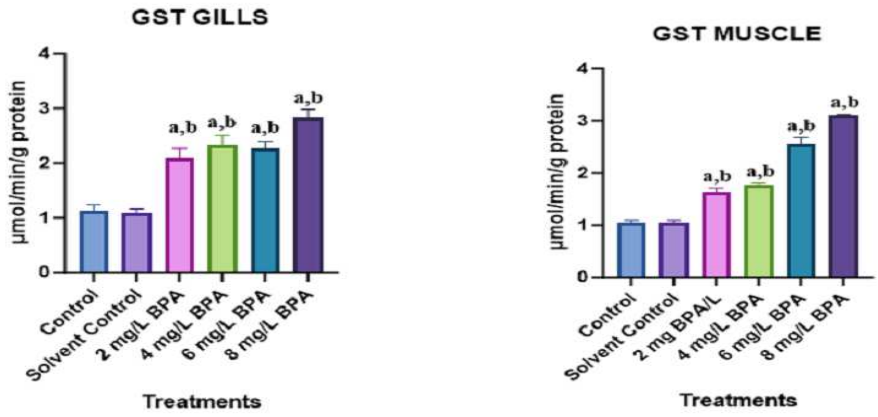

Figure 3. Glutathione-S-transferase (GST) activity in gills and muscles of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA). [Values are expressed as mean ± SD, (n=10); 'a' and 'b' indicate significant difference ($p < 0.05$) when compared to Control and Solvent control, respectively.

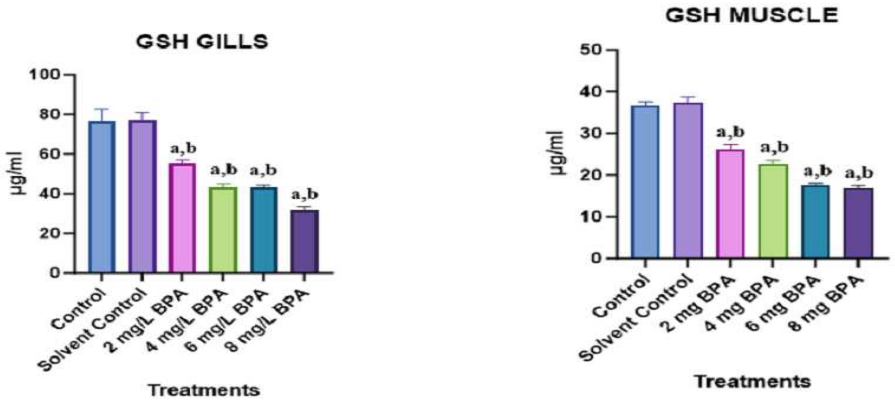

Figure 4. Reduced glutathione (GSH) concentration in gills and muscles of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA). [Values are expressed as mean ± SD (n=10); 'a' and 'b' indicate significant difference ($p < 0.05$) when compared to Control and Solvent control, respectively.

Histopathological examination of the gill tissue in the juvenile *C. gariepinus* (Figure 5) showed that there was no observable lesion seen in the fish exposed to water only (Normal control group). The fish in the Solvent control group showed moderate lamellae hyperplasia, proliferation and thickening of epithelial cells covering the lamellae led leading to the joining together of secondary lamellae. In the group exposed to 2 mg/L BPA and the group exposed to 4 mg/L BPA, there was proliferation and thickening of epithelial cells covering the lamellae; it was also observed that there was proliferation and thickening of epithelial cells covering the lamellae with pillar congestion in the group exposed to 6 mg/L BPA while in the group exposed to 8 mg/L BPA there was hyperplasia of lamellar cells, sub-epithelial oedema characterised by separation of the epithelial layer from the underlying pillar cells, forming sub-epithelial spaces (Figure 5).

Histopathological examination of skeletal muscle sections in the juvenile *C. gariepinus* revealed that there was no lesions observed in the Normal control, Solvent control and the group exposed to 2 mg/L BPA (Figure 6). However, in groups exposed to 4 mg/L BPA, expansion of the interstitial spaces was observed; and for groups exposed to 6 mg/L BPA, atrophy of myofibres was seen; while for groups exposed to 8 mg/L BPA, there was accentuation of the interstitial space (Figure 6).

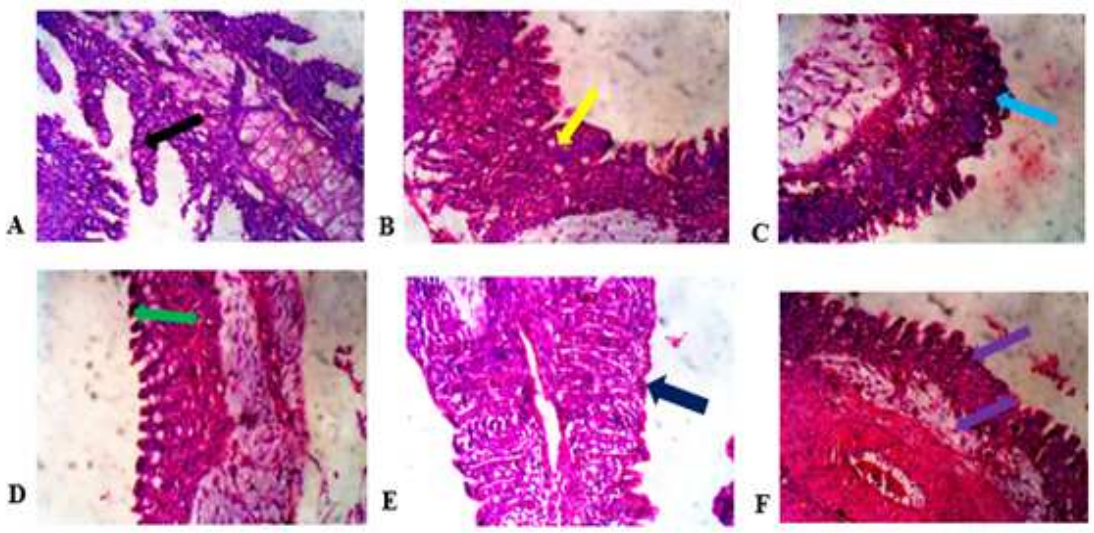

Figure 5: Photomicrographs of gill tissue of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA). Black arrow points to the gills villi where no lesions were observed; yellow arrow points to moderate lamellae hyperplasia; blue arrow points to the fusion of secondary lamellae due to proliferation and thickening of epithelial cells covering the lamellae; green arrow points to the proliferation and thickening of epithelial cells covering the lamellae; deep blue arrow points to the proliferation and thickening of epithelial cells covering the lamellae with pilar congestion; while purple arrow points to the hyperplasia of lamellar cells, subepithelial oedema characterised by separation of the epithelial layer from the underlying pillar cells forming subepithelial spaces. [H & E; ×400]

[A – Normal Control; B – Solvent control; C – Fish exposed to 2 mg BPA/L; D – Fish exposed to 4 mg BPA/L; E – Fish exposed to 6 mg BPA/L; F – Fish exposed to 8 mg BPA/L.

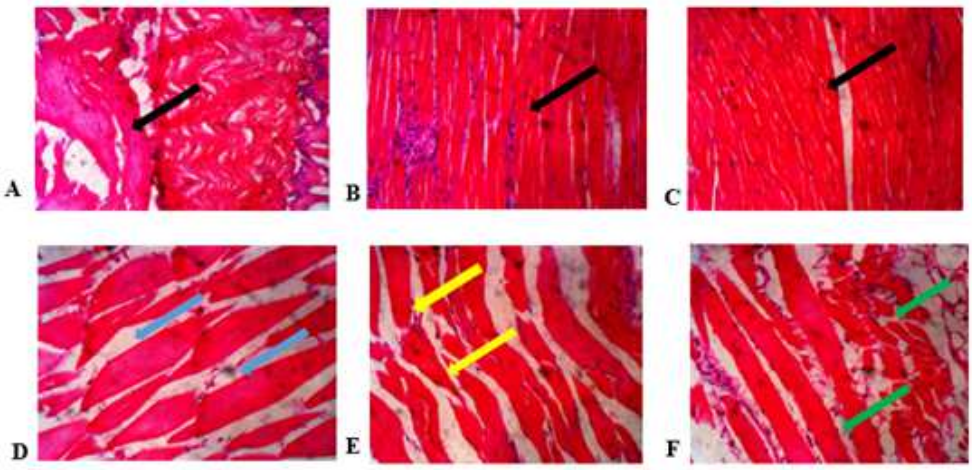

Figure 6: Photomicrographs of muscle tissue of African catfish (*Clarias gariepinus*) exposed to varied levels of bisphenol A (BPA). Black arrows point to the skeletal muscle where no lesions were observed; blue arrows point to the expansion of interstitial spaces; yellow arrows point to the atrophy of myofibres; while green arrows point to the accentuation of interstitial spaces. [H & E; ×400]

[A – Normal Control; B – Solvent control; C – Fish exposed to 2 mg BPA/L; D – Fish exposed to 4 mg BPA/L; E – Fish exposed to 6 mg BPA/L; F – Fish exposed to 8 mg BPA/L.

Discussion

The significantly lower total protein content recorded for the groups exposed to BPA in the present study suggests that there was a metabolic dysfunction in the whole fish, probably reflecting a diversion of energy from growth to detoxification. The breakdown products of body cell mass proteins serve as substrates for glycogenesis and the formation of heat shock proteins (Almeida *et al.,* 2010). The concentration-dependent decline detected indicates that the body of the fish prioritises survival. The extensive loss of protein in the gills is noteworthy because this organ is dependent on proteins for exchange of gas, osmotic regulation and removal of some body waste. A previous study showed that BPA alters occluding junction proteins in the gills, distorting their integrity (Wang *et al.,* 2025).

The outcome of the evaluation of oxidative stress markers in the BPA treated fish showed that there was induction of significant oxidative stress in treated fish groups, with a concentration-response correlation in the examined tissues. An escalation of reactive oxygen species (H_2O_2) in both the gills and muscle is possibly due to the disruption of mitochondria (Ma *et al*., 2025). This further corroborates the ability of BPA to induce oxidative stress, while the absence of this increment in the muscle of fish exposed to 2 mg/L may suggest a local antioxidant threshold within the muscle tissue. In this study, the significantly higher H_2O_2 level in the gills at all doses of BPA exposure suggests that the gills are the main oxidative interface. On the contrary, in the gills and muscle, significant elevation of MDA was observed in all groups (2 – 8 mg/L), affirming extensive tissue lipid peroxidation. The presence of significantly higher concentration of MDA implies oxidative impairment to membranes resulting in compromised integrity and the function of the cell membrane (Juan *et al*., 2021). Previous studies have shown that amino acids can be oxidised by ROS and lipid peroxides, leading to the formation of protein carbonyl groups with resultant increased proteolytic degradation (Dalle-Donne *et al*., 2003). It is believed that the induction of

oxidative stress by BPA is capable of causing protein depletion and several health implications.

In this study, a direct antioxidant response to BPA exposure was observed with the concentration-dependent significantly higher activities of superoxide dismutase (SOD) in the gill and muscle tissues, which can be linked to the overproduction of superoxide anion. This increased SOD activity is central to the oxidative stress theory. SOD is the primary and first antioxidant enzyme that protects the body against reactive oxygen species, and which catalyses the breakdown of superoxide anion into hydrogen peroxide (H_2O_2) and molecular oxygen (Cojocaru *et al*., 2023). The tenacious upregulation of SOD across the entire exposure concentration range in both tissues implies that there was activation of the endogenous antioxidant system; however, it was insufficient to protect the tissues against free radical damage at higher concentrations. The observation above is in line with the study of Sies *et al*. (2022), where they reported that a prolonged period of high antioxidant activity indicates that failure to restore redox steady state results in continuous cellular disorderliness.

Glutathione peroxidase (GPx) activity was significantly higher in the gills and muscles of the BPA treated fish; this depicted an intense activity of the antioxidant defence system. The systemic elevation of the (GPx) activity corroborates the initially observed elevated levels of H_2O_2 and malondialdehyde in this study. The fact that SOD activity was also elevated showed a linked pathway: H_2O_2 is produced following SOD activities while GPx detoxifies H_2O_2 using reduced glutathione (GSH). Previous studies have shown that if the formation of H_2O_2 surpasses the body's clearing capacity, H_2O_2 accumulation energises the Fenton reaction, which leads to increased lipid peroxidation (Sies *et al*., 2022). This aligns with the elevated concentration of MDA observed in this study. This upregulation of GPx in this study may imply systemic oxidative injury as well as an effort to neutralise the intense oxidative burden.

The significantly higher activities of GST in the BPA treated groups is thought to be due to the activation of phase II biotransformation reaction in response to BPA exposure. GST is the antioxidant enzyme involved in catalysing the conjugation of electrophilic BPA metabolites to reduced glutathione (GSH) to make them more water-soluble for excretion via phase III transporters. This is a progressive detoxifying exercise to avert electrophiles and DNA/protein adduct production (Alnasser, 2024). The adaptive response of GST in both tissues (gills and muscle) implies the involvement of the whole body in summoning this defence system. The observed elevated GST activity is one of the chief molecular markers of phase II metabolism activation, though if prolonged can be metabolically devastating, resulting in cellular damage if detoxification capacity is lower than the toxicant load (Zapata-Vívenes *et al.,* 2020)

A significantly lower concentration of reduced glutathione (GSH) in gill and muscle tissues marks a major disturbance of the redox state. This depletion of GSH is the chief factor linking the increased expression of antioxidant enzymes (SOD, GPx, GST) and oxidative damage (elevated H_2O_2, MDA) previously observed. Redox buffering capacity is dependent on reduced GSH (Aoyama and Nakaki, 2015); a reduction in its concentration shows that the request for protection is greater than its cellular generation and salvaging within the body. The observed depletion of GSH is due to it serving as a substrate for GPx and GST; the dual use leads to a self-perpetuating disaster because of the rapid depletion by the two enzymes, leading to a compromise of the entire glutathione defence system, which explains why there is high antioxidant activity and yet oxidative damage is evident. The GSH depletion is the key that marks the shift from adaptive

response towards possible failure. These further reiterate that BPA-induced toxicity majorly involves the depletion of the antioxidant cellular defence system, making the tissues defenceless.

Concentration-dependent histopathological evidence of induced toxicity in the gills and muscle of the exposed fish by BPA was observed clearly from this study. The persistent oxidative and metabolic alterations noticed in this study can be related to the modest hyperplasia observed in lower concentrations, to extreme epithelial detachment and oedema at higher concentrations, illustrating a clear anatomical consequence. Earlier studies by Pramanik and Biswas (2024) reported that there was lamellar fusion and epithelial hyperplasia, which is a protective adaptive response to uptake of toxicants by the gills. Findings in this present study concur with this report. The observed alterations in the gills' architecture will lead to impairment of gas exchange due to reduced respiratory surface area, haemodynamic instability and localised hypoxia as the concentration of BPA increases. This aligns with the oxidative stress noticed in this study, in which there is an accumulation of H_2O_2 and MDA that can probably damage the vascular endothelium and ultimately affect circulatory activities. These findings directly reveal a progression from metabolic stress to structural collapse. The absence of abnormal changes in gills structure of both control groups is a reflection of BPA being the causative agent of the above alterations.

In addition, progressive damage to the structural integrity of the skeletal muscle was observed in groups exposed to BPA in a concentration-dependent manner. The presence of anatomical alteration in the skeletal tissues provides evidence of systemic toxicity that goes beyond the point of toxicant entry in the gills. There are no structural alterations in both controls and groups exposed to 2 mg/L BPA. However, in groups exposed to 4 mg/L BPA, the interstitial spaces were expanded, which may be as a result of oedema, which is an early morphological indication of stress, which can be due to inflammation and oxidative stress affecting the exchange of nutrients. Atrophy of myofibres was observed in groups exposed to 6 mg/L BPA, which is a critical reflection of structural breakdown and can be related to the protein loss measured biochemically. It was noticed that there was accentuation of interstitial spaces in groups exposed to 8 mg/L BPA, which is a key to the onset of severe oedema and possibly tissue fibrosis. These findings concur with those of Lushchak and Lushchak (2021). This final stage pathology observed in the muscle with simultaneous structural alteration in the gill is a pointer to a multi-organ failure syndrome.

Conclusion: This study established that exposure of juvenile *Clarias geriepinus* to Bisphenol A (BPA) at the levels used, resulted in generalised toxicity involving oxidative distress and metabolic dysregulation. It appears that there was a bioenergetic interchange resulting in the redirection of energy meant for growth to power the detoxification activity, which ultimately led to a significant reduction in protein reserves. The compensatory expression of the antioxidant defence system (SOD, GPx, GST, GSH) was eventually overwhelmed, resulting in extensive lipid peroxidation. Subsequently, exposure to BPA eventually alters the cellular integrity and health.

Acknowledgements

This Research was funded by TETFund Institutional-Based Research Fund (2024), and we sincerely appreciate the financial support to successfully carry out this research.

Conflict of Interest

All authors declare that there is no competing financial interest or personal relationship with other people or organisations that could inappropriately influence the reported work in this manuscript.

References

Abraham A and Chakraborty P (2020). A review of sources and health impacts of bisphenol A. *Reviews on Environmental Health*, 35(2): 201 – 210.

Adeogun AO, Ibor OR, Imiuwa ME, Omogbemi ED, Chukwuka AV, Omiwole RA and Arukwe A (2018). Endocrine disruptor responses in African sharptooth catfish (*Clarias gariepinus*) exposed to di-(2-ethylhexyl)-phthalate. *Comparative Biochemistry and Physiology, Toxicology and Pharmacology*, 213: 7 – 18.

Almeida JR, Oliveira C, Gravato C and Guilhermino L (2010). Linking behavioural alterations with biomarkers responses in the european seabass Dicentrarchus labrax L. Exposed to the organophosphate pesticide fenitrothion. *Ecotoxicology,* 19: 1369 – 1381.

Alnasser SM (2024). The role of glutathione S-transferases in human disease pathogenesis and their current inhibitors. *Genes & Diseases,* 12(4): 101482.

Aoyama K and Nakaki T (2015). Glutathione in cellular redox homeostasis: Association with the excitatory amino acid carrier 1 (EAAC1). *Molecules,* 20(5): 8742 – 8758.

Barboza LG, Cunha SC, Monteiro C, Fernandes JO e Guilhermino L (2022). Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. *Journal of Hazardous Materials*, 393: 122419.

Buege JA and Aust SD (1978). Microsomal lipid peroxidation. *Method in Enzymology*, 30: 302 – 310.

Ceballos-Concha A, Asche F y Cárdenas-Retamal R (2025). Salmon aquaculture in Chile: Production growth and socioeconomic impacts. *Reviews in Aquaculture*, 17(1): e12993. https://doi.org/10.1111/raq.12993

Cojocaru KA, Luchian I, Goriuc A, Antoci LM, Ciobanu CG, Popescu R, Vlad CE, Blaj M and Foia LG (2023). Mitochondrial dysfunction, oxidative stress, and therapeutic strategies in diabetes, obesity and cardiovascular disease. *Antioxidants* (Basel). 12(3): 658.

Colvin SA, Sullivan SM, Shirey PD, Colvin RW, Winemiller KO, Hughes RM and Eby L (2019). Headwater streams and wetlands are critical for sustaining fish, fisheries, and ecosystem services. *Fisheries*, 44(2): 73 – 91.

Dalle-Donne I, Giustarini D, Colombo R, Rossi R, and Milzani A (2003). Protein carbonylation in human diseases. *Trends in Molecular Medicine,* 9(4): 169 – 176.

Diedrich A, Blythe J, Petersen E, Euriga E, Fatchiya A, Shimada T and Jones C (2019). Socioeconomic drivers of adoption of small-scale aquaculture in Indonesia. *Sustainability*, *11*(6): 1543. https://doi.org/10.3390/su11061543

Drury RA and Wallington EA (1980). Carleton's Histological Techniques. 5th Edition. Oxford University Press, New York; pp. 195.

Elizalde-Velázquez GA, Gómez-Oliván LM, Herrera-Vázquez SE, Rosales-Pérez KE, SanJuan-Reyes N, García-Medina S, Galar-Martínez M (2023). Acute

exposure to realistic concentrations of Bisphenol-A trigger health damage in fish: Blood parameters, gene expression, oxidative stress. *Aquatic Toxicology*, 261: 106610.

Ellman GL (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics,* 82(1): 70 – 77.

Faheem M and Bhandari RK (2021). Detrimental effects of bisphenol compounds on physiology and reproduction in fish: A literature review. *Environmental Toxicology and Pharmacology*, 81: 103 – 497.

Ferreira V, Bini LM, González Sagrario MD, Kovalenko KE, Naselli-Flores L, Padial AA and Padisák J (2023). Aquatic ecosystem services: an overview of the Special Issue. *Hydrobiologia*, 850(12): 2473 – 2483. https://doi.org/10.1007/s10750-023-05235-1

Gornal AG, Bardawill JC and David MM (1949). Determination of serum proteins by means of biuret reaction. *Journal of Biological Chemistry*, 177: 751 – 766.

Habig, WH (1974). Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry*, 249: 7130 – 7139.

Hahladakis JN, Iacovidou E and Gerassimidou S (2023). An overview of the occurrence, fate, and human risks of the bisphenol-A present in plastic materials, components, and products. *Integrated environmental assessment and management*, *19*(1): 45 – 62.

Juan CA, Pérez de la Lastra JM, Plou FJ and Pérez-Lebeña E (2021). The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, Lipids and Proteins) and induced pathologies. *International Journal of Molecular Science*, 22(9): 4642.

Klimuk AA, Beketov SV and Kalita TL (2024). Physiological and ecological features of cultivation African catfish *Clarias gariepinus*. *Advances in Current Biology*, 144(6): 705 – 716.

Lin Z, Wang L, Jia Y, Zhang Y, Dong Q and Huang C (2017). A study on environmental bisphenol A pollution in plastics industry areas. *Water, Air and Soil Pollution*, 228(3): 98.

Lushchak VI and Lushchak O (2021). Interplay between reactive oxygen and nitrogen species in living organisms. *Chemico-Biological Interaction,* 349: 109680.

Ma W, Zeng W, Zhang D, Zhou Y, Huang Y and Hong Y (2025). Oxidative Stress in Aquaculture: Pathogenic Mechanisms and Preventive Strategies in Farmed Aquatic Animals. *Current Issues in Molecular Biology,* 47(11): 873.

Misra HP and Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry,* 247(10): 3170 – 3175.

Mohammed JN, Okaiyeto K, Haruna S, Wan WR, Oguntibeju OO and Ekundayo TC (2024). Systematic assessment on the remediation of Bisphenol A in the global environments: a mixed method analysis of research outputs. *Discover Environment*, *2*(1): article id.15.

Nyman M (1959). Serum haptoglobin; methodological and clinical studies. *Scandinavian Journal of Clinical and Laboratory Investigation,* 11(39): 1 – 169.

Ojuwoni AA, Akinwole AO and Faturoti EO. (2020). Evaluation of The Growth Of Tilapia And African Catfish In Ponds Fertilized With Poultry Manure And Cow Dung. *Nigerian Journal of Fisheries*, *17*(1): 1859 – 1867.

Ologundudu OT, Msagati TA, Popoola OE and Edokpayi JN (2025). Bisphenol A in Selected South African Water Sources: A Critical Review. *ACS omega*, 10(7): 6279 – 6293.

Pramanik S and Biswas JK. Histopathological fingerprints and biochemical changes as multi-stress biomarkers in fish confronting concurrent pollution and parasitization. *iScience,* 27(12): 111432.

Sies H, Belousov VV, Chandel NS, Davies MJ, Jones DP, Mann GE, Murphy MP, Yamamoto M, Winterbourn C (2022). Defining roles of specific reactive oxygen species (ROS) in cell signaling and biology. *Nature Reviews Molecular Cell Biology,* 23(7): 499 – 515. https://doi.org/10.1038/s41580-022-00456-z

Wang X, Zhou S, Dong J, Wei Z, Liu Y, Huang Y, Sui J and Zhu L (2025). The impact of bisphenol A on gill health: A focus on mitochondrial dysfunction induced disorders of energy metabolism and apoptosis in Meretrix petechialis. *Aquatic Toxicology*, 279: 107259.

Wang Y, Wu J, Wang D, Wan M, Li X, Zhang L and Lu H (2024). BPA induces hepatotoxicity in zebrafish through oxidative stress and apoptosis pathways. *Fish Physiology and Biochemistry*, *50*(2): 403 – 412.

Wolff SP (1994). Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods in Enzymology*, 233: 182 – 189. http://dx.doi.org/10.1016/S0076-6879(94)33021-2

Zapata-Vívenes E, Nusetti O, Marcano L, Sánchez G, Guderley H (2020). Antioxidant defenses of flame scallop *Ctenoides scaber* (Born, 1778) exposed to the water-soluble fraction of used vehicle crankcase oils. *Toxicology Reports,* 7: 1597 – 1606.