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Histopathological Alterations in Vital Organs of African Catfish, Clarias gariepinus (Burchell 1822) Exposed to Sub-lethal Concentrations of Methylparaben

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ABSTRACT

Methylparaben (MeP), a widely used preservative in personal care products and pharmaceuticals, has raised environmental concerns due to its potential toxicity to aquatic organisms. This study investigated the effects of exposure to sub-lethal concentrations of MeP on the histological architecture of the gills, livers, kidneys, and intestines of Clarias gariepinus. Fifteen (15) adult C. gariepinus with an average weight of 1.15±0.21 kg were exposed to 0.74 or 1.48 mg/L MeP for 28 days after which the gills, livers, kidneys, and intestines were excised for histopathological study. The control fish was maintained in water not contaminated with MeP. There were no observable histopathological lesions in all the tissues in the control group. The fish exposed to 0.74 mg/L had mild fusion of the gill filament, focal haemorrhage of the liver parenchyma, moderate infiltration of inflammatory cells in the kidney interstitial spaces and lamina propria of the intestine. However, there was congestion and mild haemorrhage of the gill arch, severe glycogenic and lipid-filled hepatocytes, periglomerular infiltration of inflammatory cells of the kidneys, and severe infiltration of the lamina propria of the intestine with lymphocytes at the highest concentration (1.48 mg/L). The histopathological alterations observed in the tissues of C. gariepinus exposed to sub-lethal concentrations of MeP provided valuable insights into the toxicological effects of this preservative to aquatic animals. Hence, the use of methylparaben in these important products must be regulated.,,.

Highlights

- Methylparaben exposure caused organ specific damage in African catfish tissues.
- Gill, liver, kidney and intestinal structures were altered at sub-lethal MeP doses.
- Clarias gariepinus proved effective as a histolopathological biomarker in ecotoxicology.

1. Introduction

Methylparaben (MeP) is one of the most commonly used derivatives of parabens, as preservative in personal care products (PCPs), pharmaceuticals and food commodities (Merola et al., 2020). It is the most frequently used antimicrobial preservative in cosmetics due to the early toxicity assessment that reported its low toxicity in mice with a high lethal dose of more than 500 mg/kg (Mason et al., 1971; Wei et al., 2021). However, recent studies have shown that MeP can disrupt or modulate the endocrine system, even at low exposure levels,

MeP is considered an emerging concern (Kang et al., 2013; Lee et al., 2018; Karwacka et al., 2019; Merola et al., 2020; Wei et al., 2021).

Moreover, continuous production of cosmetics or PCPs with long shelf-life has inadvertently increased the widespread use of MeP which has led to the constant contamination of environmental matrices such as surface water (Zhao et al., 2019), wastewater (Haman et al., 2015), groundwater (Kapelewska et al., 2018), drinking water (Carmona et al., 2014), air, and dust (Chen et al., 2018) thus suggesting increased risk of

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both human and aquatic animals to MeP exposure. The use of histopathological study is, however, not yet fully exploited in the assessment of tissue-level damage in aquatic animals due to MeP exposures.

The African catfish (*Clarias gariepinus*) is a widely studied model species in ecotoxicology due to its resilience, economic importance, and high sensitivity to environmental pollutants (Omitoyin *et al.*, 2006; Adeyemi *et al.*, 2020; Aliu *et al.*, 2024; Imade *et al.*, 2024). As a benthopelagic species, *C. gariepinus* is frequently exposed to contaminants that accumulate in aquatic habitats, making it an ideal organism for assessing histopathological alterations induced by xenobiotics, including MeP (David and Pancharatna, 2009; Aliu *et al.*, 2024; Imade *et al.*, 2024). Some of the most commonly studied organs for histopathological studies in fish species include the gills, livers, and kidneys (Barse *et al.*, 2010; Ma *et al.*, 2023; Yin *et al.*, 2023; Akmal *et al.*, 2024).

The gills serve as the primary site for respiration, osmoregulation, and excretion in fish. It is also one of the fish organs that have direct contact with the immediate environment, making them highly vulnerable to toxic substances in water (Nowak *et al.*, 2018). Gills are major biomarkers used in assessing fish health (Nascimento *et al.*, 2012; Marinovic *et al.*, 2021). Liver is another vital organ that plays a central role in detoxification and metabolism of toxicants. It is a key target organ for toxicological studies and one of the most affected organs due to aquatic toxicity (Stoyanova *et al.*, 2020; Popović *et al.*, 2023; Akmal *et al.*, 2024).

The kidney also functions in osmoregulation, excretion, and waste filtration, making it highly susceptible to chemical toxicity. Studies have shown the relevance of the kidney in histopathological study in understanding various renal diseases including tumors, tubule interstitium and glomerular diseases (El-Zaatari et al., 2020; Kashem et al., 2025). The intestine is responsible for nutrient absorption and immune defense, making it another critical target for toxicant-induced histopathological changes (Boberg et al., 2010; Jing et al., 2023). The impact of xenobiotics including MeP on fish intestines is less documented. The present study therefore aimed at evaluating the histopathological effects of MeP on the gills, livers, kidneys, and intestines of African catfish, *C. gariepinus*.

2. Materials and Methods

Experimental animal

The experimental animals, adult African catfish, *C. gariepinus* (n = 15) with average weight and length of 1.15±0.21 kg and 44.46±2.82 cm were purchased from a reputable fish farm in Etioro, Ondo State, Nigeria, and transported to the Fish Hatchery Unit of the Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. They were acclimatized to laboratory conditions for two weeks in a Low Density Polyethylene (LDPE) tank containing 400 L of water at a stocking density of 5 fish per tank, prior to the exposure to MeP. The fish were fed twice daily (9 am and 4 pm) with a commercial diet, Nutreco® (30% crude protein) throughout acclimatization and experimentation periods. Unconsumed

feed and fecal pellets were removed daily while the water was changed twice a week.

Exposure of C. gariepinus to MeP

Methylparaben (MeP; CAS number: 99–76-3) was purchased from Xilong Chemical, China, and the stock solution (1000 mg/L) was prepared in absolute ethanol. The fish were divided into three groups and allocated to different experimental tanks (n = 5 per tank). The fish were exposed to 0 (control), 0.74, and 1.48 mg/L of MeP which represent $^{1}/_{20}$ and $^{1}/_{10}$ of the 96 h LC50, respectively, which was earlier determined as 14.79 mg/L. The fish exposure to MeP lasted for 28 days in a static-renewal assay in which the culture media were renewed every 48 h. On the 28th day of exposure, the fish were sacrificed and the organs such as the gills, livers, kidneys, and intestines were excised, rinsed in normal saline solution, and fixed in 10% formaldehyde for 24 h for further histopathological analyses.

Histopathological analyses of the gills, liver, kidney, and intestine

The histopathological analyses of the fixed tissues followed the procedures of Adeyemi *et al.* (2020). The fixed tissues were dehydrated in ethanol gradients and then cleared in xylene solution. The tissues were embedded in paraffin wax and the sections of the tissues were cut into clean microscope slides at 7 μ m thick using a rotary microtome. The cut sections were stained with Haematoxylin and Eosin dyes, and the stained slides were observed under a light microscope using ×40 objective. The photomicrographs of the desired views were then taken.

3. Results

Histopathology of the gills section

The sections through the gills of the fish in the control and 0.74 mg/L groups showed normal gill arch (Plates 1a and 1b, respectively). The cartilages were also normal. However, moderate fusion of gills filaments was observed in the gills of the fish exposed to 0.74 mg/L (Plate 1b). Moderate fusion of the gill filaments were also observed in the fish exposed to 1.48 mg/L MeP (Plate 1c) but the gills arch showed congestion and mild haemorrhage. Sloughing of the epithelial lining was also observed.

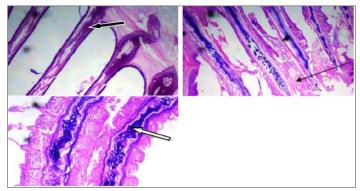


Plate 1a. Photomicrograph (x40 objective) of gills section of *C. gariepinus* (Control group), showing normal gill arch and the Lamellae or filament (black arrow) containing normal pilaster cells, chloride cells as well as normal epithelium (slender arrow). Normal cartilage of the arch is also seen (white arrow).

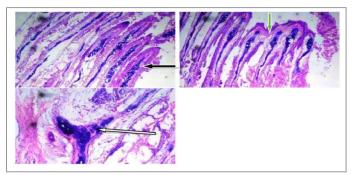


Plate 1b. Photomicrograph (x40 objective) of gill section of *C. gariepinus* exposed to 0.74 mg/L, showing normal gill arch (black arrow) and fused filament (green arrow). Normal cartilage of the arch is also seen (white arrow).

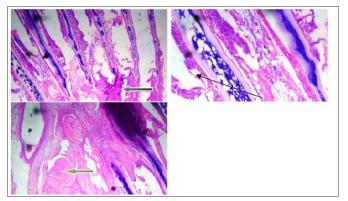


Plate 1c. Photomicrograph (x40 objective) of gill section of *C. gariepinus* exposed to 1.48 mg/L, showing moderate fusion of the gill filament at the apical part (black arrow), the epithelial lining of the filament is sloughed (slender arrow), the arch show congestion (green arrow) and mildly hemorrhagic.

Histopathology of the liver section

The sections through the liver of the control group showed normal central venules and there was no infiltration of inflammatory cells (Plate 2a). The hepatocytes of the fish exposed to 0.74 mg/L MeP, however, showed mild glycogenic cytoplasm. There was no infiltration of inflammatory cells in liver of the fish that were exposed to 0.74 mg/L and the central venule appear normal (Plate 2b). Also, the liver parenchyma showed focal haemorrhage. The central venules of the liver of fish exposed to 1.48 mg/L MeP were severely congested with glycogenic and lipidogenic hepatocytes (Plate 2c). However, there was no infiltration of inflammatory cells.

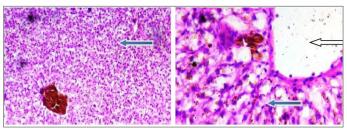


Plate 2a. Photomicrograph (x40 objective) of liver section of *C. gariepinus* (Control group), showing normal central venule (white arrow), the morphology of the hepatocytes show mild glycogenic cytoplasm (blue arrow), there is no infiltration of inflammatory cells.

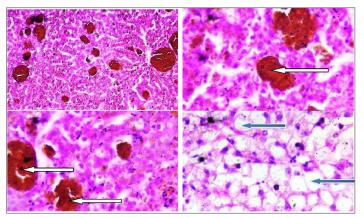


Plate 2c. Photomicrograph (x40 objective) liver section of *C. gariepinus* exposed to 1.48 mg/L, showing severely congested central venules (white arrow), the morphology of the hepatocytes show severe glycogenic and lipid filled cytoplasm (blue arrow X400), there is no infiltration of inflammatory cells.

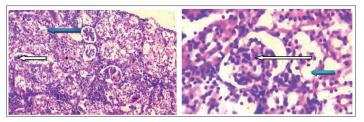


Plate 3a. Photomicrograph (x40 objective) of kidney section of *C. gariepinus* (Control group), showing normal glomeruli (white arrow), the renal tubules show moderate desquamation (blue arrow).

Histopathology of the kidney section

The control group showed normal glomeruli with moderate desquamation of the renal tubules (Plate 3a). However, the renal tubules of the fish exposed to 0.74 mg/L MeP showed hydropic and fatty degeneration. The interstitial spaces showed moderate infiltration of inflammatory cells (Plate 3b) while glomeruli of the fish exposed to 1.48 mg/L MeP showed peri-glomerular infiltration of the inflammed cells (Plate 3c). The renal tubules show moderate desquamation and some of the epithelia are fat-filled while interstitia were moderately infiltrated by inflammatory cells and hematopoietic cells.

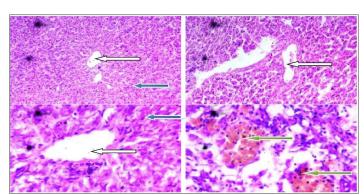


Plate 2b. Photomicrograph (x40 objective) of liver section of *C. gariepinus* exposed to 0.74mg/L, showing normal central venule (white arrow), the morphology of the hepatocytes appear normal (blue arrow),

there is no infiltration of inflammatory cells. The liver parenchyma show focal haemorrhage (green arrow).

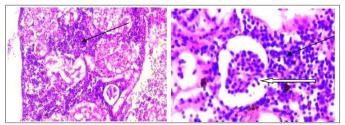


Plate 3b. Photomicrograph (x40 objective) of kidney section of *C. gariepinus* exposed to 0.74 mg/L, showing glomeruli (white arrow) and the renal tubules showing hydropic and fatty degeneration, the interstitial spaces show moderate infiltration of inflammatory cells (slender arrow).

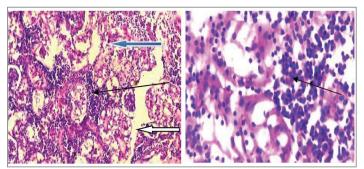


Plate 3c. Photomicrograph (x40 objective) of kidney section of *C. gariepinus* exposed to 1.48 mg/L, showing glomeruli with peri glomerular infiltration of inflammatory cells(white arrow), the renal tubules show moderate desquamation and some of their epithelia are fat filled(blue arrow), the interstitium is moderately infiltrated by inflammatory cells (slender arrow) amidst hematopoietic cells.

Histopathology of the intestinal section

The mucosal layer of the intestinal sections of the control group has well preserved villi. The glands and the sub-mucosal layers appeared normal (Plate 4a). However, the mucosal layer showed moderately preserved villi in the intestine of fish exposed to 0.74 mg/L MeP (Plate 4b). The glands appeared normal but the lamina propria was mildly infiltrated by inflammatory cells. Other layers such as the sub-mucosal and circular muscularis layer appeared normal. The lamina propria of the fish exposed to 1.48 mg/L MeP is severely infiltrated by lymphocytes (Plate 4c) while the mucosal, sub-mucosal and circular muscularis layer appear normal.

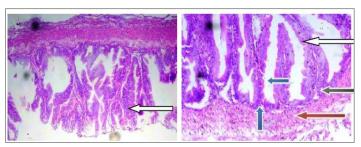


Plate 4a. Photomicrograph (x40 objective) of intestine section of *C. gariepinus* (Control group), showing normal mucosal layer with well-preserved villi (white arrow), the glands appear normal (blue arrow), the

submucosal layer appear normal (black arrow), the circular muscularis layer (red arrow) appears normal.

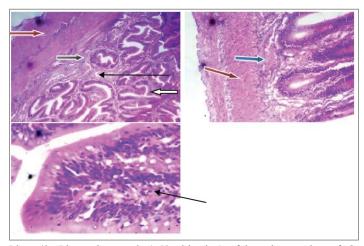


Plate 4b. Photomicrograph (x40 objective) of intestine section of *C. gariepinus* exposed to 0.74 mg/L, showing normal mucosal layer with moderately preserved villi (white arrow), the glands appear normal (blue arrow) but the lamina propria is mildly infiltrated by inflammatory cells (slender arrow), the submucosal layer normal (black arrow), the circular muscularis layer (red arrow) appears normal.

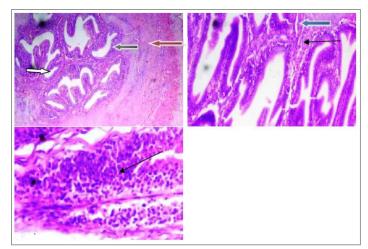


Plate 4c. Photomicrograph (x40 objective) of intestine section of *C. gariepinus* exposed to 1.48 mg/L, showing normal mucosal layer with well-preserved villi (white arrow), the glands appear normal (blue arrow), the lamina propria is severely infiltrated by lymphocytes (slender arrow), the submucosal layer appear normal (black arrow), the circular muscularis layer (red arrow) appears normal.

4. Discussion

The histopathological analysis of fish organs is considered as a suitable tool for evaluating the effects of pollutants on the organisms at the tissue level (Adeyemi, 2013; Okomoda *et al.*, 2013). Gills, which serve as the primary site for gas exchange, are highly sensitive to toxicants in the aquatic environments due to its direct contact with the environment (Nowak *et al.*, 2018). The histological changes in the gills of *C. gariepinus* in this study were consistent with the observations in *Labeo rohita* that were exposed to a similar concentration of ethylparaben (Akmal *et al.*, 2024). The observation of visible histopathological lesions in the gills of MeP-exposed fish could be an

early sign of structural alterations, which can affect the proper functioning of the gills especially those related to gaseous exchange (Turko *et al.*, 2020).

Liver is a crucial metabolic organ in animals playing important roles in xenobiotics biotransformation as well as detoxification (Darbre and Harvey, 2008). The results from the study revealed the hepatotoxic effects of MeP in *C. gariepinus* which was in form of various histological changes in the liver structures. The results of this study were supported by reports from similar studies that have shown histological changes in the liver tissues following exposure to toxicant substances (Li *et al.*, 2022; Calisir *et al.*, 2025).

The kidney plays a key role in osmoregulation and excretion, making it a target for chemical toxicity (David and Pancharatna, 2009). Normal glomeruli and renal tubules were observed in the control group, though with moderate desquamation. This is, however, a natural process of shedding of dead outer cells. The signs of hydropic and fatty degeneration in the renal tubules, along with moderate inflammatory infiltration in the interstitial spaces of the fish exposed to 0.74 mg/L MeP indicated renal stress and impaired filtration capacity (Oishi, 2002). Increased nephrotoxicity was evident in the kidney of the fish exposed to 1.48 mg/L MeP with periglomerular infiltration of inflammatory cells, fat accumulation in tubules, and significant interstitial inflammation. The damage to the kidney tissues by MeP in this study corroborates reports of similar studies with *Labeo rohita* and *Orchorynchus mykiss* (Akmal *et al.*, 2024; Calisir *et al.*, 2025).

Intestine is essential for nutrient absorption and immune responses, making it a relevant tissue for toxicological evaluation (Boberg *et al.*, 2010). The impact of MeP on fish intestinal architecture is less documented. Observation of normal mucosal structure, well-preserved villi, intact glands, and normal submucosal, and muscular layers in the control group indicated a healthy gut environment. Although, the villi and glands appeared structurally intact in the intestine of the fish exposed to 0.74 mg/L MeP, however, mild infiltration of inflammatory cells in the lamina propria suggested an early immune response to paraben exposure, which has been observed in other studies on chemical-induced gut inflammation (Silva *et al.*, 2018). Moreover, the severe lymphocytic infiltration within the lamina propria of 1.48 mg/L group indicated an ongoing inflammatory response, which can lead to intestinal dysfunction over prolonged exposure (Wald, 2010).

5. Conclusion

The findings from the study indicated that methylparaben has the potentials to cause histological changes in the vital organs of *C. gariepinus* with the severity of the tissue damages at higher exposure concentration. These organs play important roles in the biochemical and physiological activities of fish, as such changes to their structure would adversely affect their functions since the functions of a particular organ are dependent on its structure. These observations therefore call for a stricter regulation on the usage and disposal of methylparaben in the environment, especially in aquatic environment.

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Competing Interest

Authors declared that they have no conflicting interest.

Data Availability Statement

All data reported in this study are presented in the results section in the manuscript.

Ethics Approval

The experimental procedures were approved by the Research Ethical Committee of the Adekunle Ajasin University, Akungba-Akoko, Nigeria (Approval Number: 210323ERC1).

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