

SILENT KILLERS: THE ALARMING IMPACT OF MICROPLASTICS POLYSTYRENE ON CATFISH LIVER HEALTH

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Abstract

Introduction: The presence of microplastics in the bodies of living things has become a matter of concern. One example is the widespread use of polystyrene microplastics, which have been widely used by industry even medical products. Styrene bonds and Benzyl Carbon in polystyrene have been identified as potential sources of free radical formation. Upon decomposition, these bonds release dissolved organic carbon, which has been identified as a significant contributor to chemical pollution. **Methods:** This study aims to determine the effect of microplastic polystyrene in experimental research (MP-PS measuring ± 0.5 mm) on catfish *Clarias gariepinus* with concentrations of 1, 1.5, 2, 2.5, 3 mg (K1-K5) for one month on fish liver histopathology. Histopathological observation of fish liver using paraffin method and statistical analysis using SPSS was tested using Anova. **Results and Discussion:** As determined by the analysis of variance (ANOVA) statistical test ($P > 0.05$), it showed that MP-PS had a significant effect on liver damage in catfish, resulting in the most severe liver damage and the least damage depending on the amount of MP-PS given to the fish. Additional data were obtained in the form of liver tissue abnormalities. **Conclusion:** The toxicological effects of polystyrene microplastics (MP-PS) on catfish liver health, demonstrating a dose-dependent relationship. Higher MP-PS concentrations led to severe histopathological damage, including several types of liver cell abnormalities. These findings emphasize the urgent need for stricter regulations on microplastic pollution, as its unregulated production threatens aquatic ecosystems and public health.

INTRODUCTION

It is predicted that 34 billion tons of plastic would be produced worldwide by 2050, with less than 10% of that amount currently being recycled (1). On the ocean's surface, there are roughly 250 million kilos (250 metric tons) of plastic debris. An estimated significantly greater volume enters the ocean annually, with around 800 to 2,400 kilotonnes from rivers and 4,800 to 23,000 kilotonnes from coastal areas (2). The presence of plastic waste has been observed to potentially be transported by wind or rain to sewer systems, subsequently spreading to rivers and exerting detrimental effects on the surrounding environment. Plastic waste undergoes a series of physical, chemical, and other processes that result in the formation of microplastic, defined as plastic

particles measuring less than 5 millimeters in size (3). The morphology of microplastic in the environment is highly diverse, exhibiting variations in shape, size, polymer type, and chemical composition, originating from various sources (4).

The objective of this study is concerned with the issue of exposure to polystyrene microplastics. Polystyrene (PS) is a synthetic material known for its strength, moisture absorption, and stable chemical properties. It is cost-effective and has a large surface area, making it widely used in various products (5). However, its environmental impact is a growing concern, particularly regarding its effects on the food chain, human, and marine health. Its popularity in industry is largely due to its affordability and durability. The presence

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of these substances is widely recognized in a variety of settings, including medical facilities, handwashing soaps, cleansers, toothpastes, and biomedical products (6). People coming into contact with microplastics is a major problem. Disposable water bottles, personal hygiene items, and food packaging can all cause this. A lot of firms utilize single-use plastics, which are bad for the environment and human health. For instance, there may be anything from 4,594 to 94,500 microplastic particles in face washes. It takes sophisticated techniques to filter the microplastics found in body exfoliants that reach the sewage system (7).

Styrene bonds in expanded polystyrene (EPS) break down easily when exposed to sunlight. This process, called photocatalytic oxidation, turns EPS into smaller particles (8). Smaller particles have more surface area, which speeds up the breakdown. Plastic surfaces can be a source of radical formation and inherent phototransformation (9). The benzyl carbon in styrene forms radicals under UV light, reacting with oxygen and water (10). As EPS breaks down, it releases dissolved organic carbon, making it a major source of chemical pollution (11). EPS can also produce flammable gases when heated or stressed chemically (12). Fragments of EPS can be found in rivers, seawater, soil, and in living creatures like fish, shrimp, rats, and even humans (13). While the degradation process of EPS and its presence in various environments have been thoroughly examined, there is limited research on how these degraded microplastic fragments affect freshwater organisms at the histopathological level, particularly their impact on liver function and health.

This study using *Clarias gariepinus* or African Catfish strain Mutiara, developed by the Research Institute for Fisheries Breeding (RIFB) in 2015 (14). The Mutiara strain Catfish is characterized by enhanced feed efficiency, a growth rate 10-40% higher than other strains, a lower feed conversion ratio of 0.8 to 1.0, and a 70% survival rate against *Aeromonas hydrophila* (15). Additionally, it thrives in diverse environmental conditions, tolerating temperatures from 15 to 35°C, pH levels between 5 and 10, low ammonia and nitrite concentrations. Due to its availability and adaptability (15), the African Catfish is an ideal model for studying MP toxicity and assessing various toxicological effects (16). The statement indicates selection of catfish as a model for observing the impact of polystyrene microplastics. This selection is due to the fact that catfish exhibit a high level of environmental tolerance, have a diet that is susceptible to microplastic exposure, and display quantifiable physiological responses. Furthermore, catfish are a fish species that is consumed by humans, thereby potentially

facilitating the spread of microplastics to humans.

Histological parameters serve as effective tools for assessing environmental contamination (17). The histological analysis presents a cost-effective and valuable method for ecological risk assessment, though it is inherently non-specific (18). To the best of our knowledge, the effects of Expanded Polystyrene (EPS) or Polystyrene (PS) fragments on the freshwater African Catfish Mutiara strain are still unknown. Determining the histological changes in *Clarias gariepinus* or catfish's hepatic organs and their growth performance as a result of MP with various types and chemical compositions of polystyrene pellets is the goal of the current investigation. The results of this study could advance our knowledge of how MP affects aquatic ecosystems, particularly freshwater ones.

METHODS

This research is an experimental method with a quantitative analysis approach to see the effect of polystyrene microplastics on liver histopathology and describe the type of damage. The research treatment was divided into 6 groups with different concentrations of PS microplastics and 4 repetitions were carried out based on the Federer formula to make the results more valid. Federer formula calculation is as follows: $(n-1)(t-1) > 15$ with n indicating a number of repetitions and t being a number of groupings (19). The groupings consisted of control without PS microplastics and five treatment groups, namely the fish group fed with polystyrene contamination at doses of 1, 1.5, 2, 2.5, and 3 mg.

Experiments on the Mutiara strain of African catfish were conducted from August to November 2024 while tissue preparations took place at the Animal Physiology Laboratory of Sunan Ampel State Islamic University Surabaya.

Experimental Fish and Design

The great stock of Mutiara Catfish, bred at Mojokerto Aquaculture Installation in Indonesia, produced healthy, active, and disease-free Mutiara strain catfish. The fish had an average weight of 27.5 grams and a length of 15.2 cm after one month of acclimatization monitoring. Then the fish were placed in tubular containers with a diameter of 25 cm and a height of 30 cm, each with 1 fish and a hollow lid to prevent the fish from escaping. Water quality maintenance in the container was checked weekly using parameters (pH, dissolved oxygen, and temperature) measured in a suitable range not less than the standard (19). pH testing using a pH meter (range 0.00-14.00, Accuracy ± 0.01 auto-calibration), the temperature was measured using a

Thermometer (TP3001-MC Tester Water) and dissolved oxygen was measured using (Model DO-5510, Lutron).

The catfish diet used in the study was commercial Hi-ProVite 781-2 containing 31-33% protein and fed at 3% of fish weight given 2 times a day, at 07.00 am and 07.00 pm following method (20). The fish were fed in stages so that the granules could be digested during the experiment to prevent regurgitation and stress (21). After exposure to the stipulated concentration, the fish were fed commercial fish food for one month. Then, a histopathological evaluation was conducted to ascertain the presence of liver damage. The experimental design involved two groups: control and group test. The control group (K0) consisted of animals fed a commercial diet, while the test groups (K1-K6) were fed a commercial diet supplemented with incremental concentrations of microplastics, ranging from 1 mg to 3 mg per animal. The concentration of microplastics was increased by a factor of 0.5 grams for each group. The microplastics utilized in the study were polystyrene microplastics mixed with modified feed.

Microplastics Polystyrene (MP-PS) Mended Feed Preparation

The MP PS type utilizes styrofoam beads or granules measuring 0.5 mm in size, obtained from PT Panca Cipta Bersama, Indonesia. These beads contain approximately 85-87% polystyrene, which has undergone an expansion process but has not been molded. The granules are added into the commercial feed with a pellet pulverizer process using water then formed into small rounds and dried in the sun covered with clean net. so that it can float when in water.

Histopathologic Examination

The induction of anesthesia in fish is typically accomplished through the utilization of naturally derived substances, with clove oil being a prominent example. Extensive experimental evaluations have identified clove oil as a highly effective anesthetic agent for various applications, including long-distance transportation and dissection for research purposes. The optimal concentration of clove oil for these procedures ranges from 70 to 90 percent by weight (22). Subsequent incisions are made, extending from the anus in a ventral direction until reaching the lateral line. The final incision traverses the posterior head region, culminating at the base of the abdomen (23). The liver is then extracted for subsequent weighing and pathological preparations.

The catfish liver is located within the abdominal cavity, above the stomach and intestines. It is C-shaped and often reddish-brown in color due to its rich vascularization, indicating good nutritional status (24). In general, catfish liver comprises 3%-5% of the total fish body, and its weight can vary significantly based on the size and health of the fish (25).

Liver tissue samples were fixed in Neutral Buffer Formaldehyd (NBF 10%) fixative solution for 24 hours before analysis (26). The samples were then dehydrated using alcohol with increasing concentrations (70%, 80%, 90%, and 95% until absolute alcohol I, II, and III). This was followed by a clearing stage with xylene solution. Then, the tissue was infiltrated with paraffin and embedded in a paraffin mold. Finally, the tissue block was sectioned at a thickness of 4 μ m using a microtome. The stage of histological staining began. Hematoxylin-eosin was used to visualize liver tissue after it had been deparaffinized. Following alcohol dehydration, xylene was used to clean the tissue. In order to mount the tissue using Entellan (27). The specimen was evaluated with light microscopy.

Data Analysis

Hepatic slice preparations were evaluated using the Manja Roenigk histopathology scoring model, with a scale ranging from 1 to 4 in five fields of view. Each hepatic preparation was analyzed under a microscope in five different fields of view at 400x magnification in each field. Twenty cells were randomly selected and counted (28). The Manja Roenigk histopathology scoring model in Table 1 was used to assign a score to each cell. Each discussion was then multiplied by the score of each cell.

Note (Table 1): Scoring was conducted by multiplying the number of cells with the damage category. The possible minimum score is 100 and the possible maximum is 400 for necrotic cells. Criteria for liver histopathological assessment (28).

Furthermore, observations of damage to other cell types were made in order to ascertain the extent of damage using a 100x microscope magnification caused by MP-PS contamination. A statistical analysis was conducted using IBM SPSS Statistics 23.0, using a One-Way ANOVA with a P value lower than 0.05, indicating a significant difference between the dose groups. Subsequently, the dependent variable was associated with the dose that exerted the most significant influence.

Table 1. Criteria for Liver Histopathological Manja Roenigk Assessment

Score	Cell Destruction Type	Criteria	Reference
1	Normal (N)	Normally hepatocyte	(28)
2	Parenchymatous Degeneration (PD)	Cloudy, swollen hepatocyte with yellow cholesterol	
3	Hydropic Degeneration (HD)	This condition is marked by hepatocyte cells that have absorbed excess water. The hepatocytes appear larger than in other conditions but retain the same color	
4	Necrotic (NC)	Dead cells, with or without a nucleus, and a clear cell membrane	

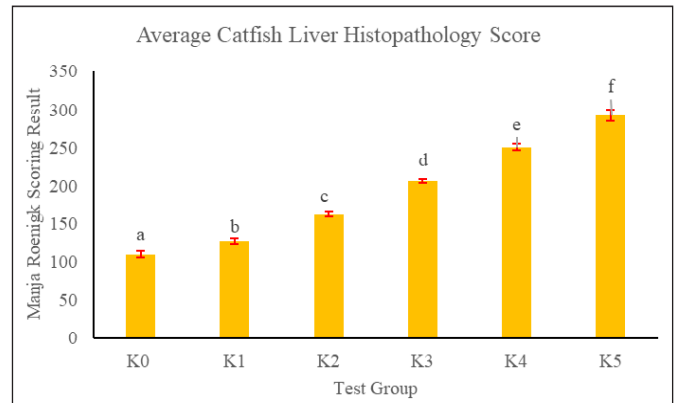
RESULTS

The objective of the analysis was to determine which concentration of MP PS contaminants exerted the most significant impact on catfish liver damage. The discovery of the type of histopathological damage was multiplied according to the Manja Roenigk scoring and the average of each repetition was calculated (Table 2). The analysis revealed that the K5 concentration exhibited the most severe damage, with an average value of 292.75, significantly exceeding the values observed for other concentrations. In contrast, the K1 group exhibited the least damage, with an average value of 127.75. The remaining test groups (K2-K4) exhibited damage scores that remained within the 100-200 range, indicating less severe damage.

Table 2. Scoring Results of Catfish Liver Histopathology Damage Based on Manja Roenigk's Scoring Method

Treatments	Damage Based on Scoring Manja Roenigk				Mean \pm SD
	N (x1)	PD (x2)	HD (x3)	NC (x4)	
K0	93.75	7.5	4.5	4	109.75 \pm 4.78 ^a
K1	81.75	26.25	15	8	127.75 \pm 3.59 ^b
K2	61.25	40	33.5	28	162.75 \pm 3.06 ^c
K3	41.25	49.5	60	56	206.75 \pm 2.90 ^d
K4	21.25	60	74.25	96	251.50 \pm 3.97 ^e
K5	11.25	43.5	90	110.5	292.75 \pm 7.08 ^f

The results presented in the table have been obtained through the execution of a one-way ANOVA analysis with normally distributed data ($P > 0.05$). A value of 0.683 ($P > 0.05$) from the Levene's test for homogeneity supported the homogeneity assumption. The results of the ANOVA test showed that H1 was accepted and H0 was rejected, with a value of 0.00 ($P < 0.05$). This suggests an effect of microplastic contamination concentration on fish liver histology. The Duncan Test value indicates significant differences among groups of MP PS contamination (K0-K5), with superscripts a-f denoting each test group (Table 2 & Figure 1).

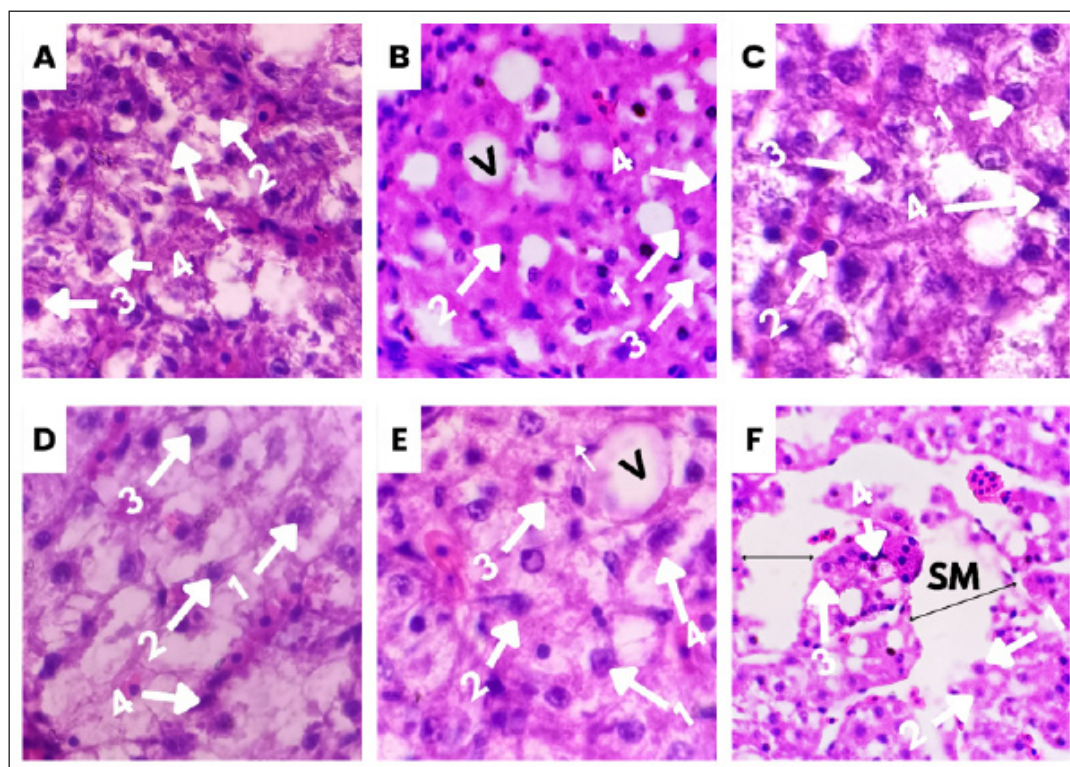
**Figure 1. Difference in the effect of PS MP Contamination on Each Test Group**

Hepatocyte Observation of Catfish Liver Histopathology Results

Hepatocytes, which comprise the majority of hepatic tissue, undergo necrosis or morphological changes, thereby contributing to the dark pigmentation of the fish liver. Hepatocytes, a specialized type of cell that constitutes the majority of the liver's composition, are situated between the sinusoids (29). As shown in Figure 2, the liver cells of catfish in the experimental groups have shown signs of hepatocyte damage. These levels of damage include normal, parenchymal degeneration (cell swelling and a cloudy cytoplasm), hydropic degeneration (enlarged cells due to water accumulation in the cytoplasm), and necrosis (nuclear damage, pycnosis, and clumped cytoplasm).

In the absence of significant necrosis, liver cell degeneration and subsequent necrosis are normal and don't count as pathological processes (30). Substantial necrosis can trigger pathological processes, however. Liver damage in the control group may result from the normal regeneration of cells. Cell turnover is essential for protecting body tissues. The normal process of cell death, called apoptosis, occurs during development until the death of all body tissues. The distinction between apoptosis and necrosis lies in the type of damage and number of dying cells (30).

Necrotizing death cells are more uncontrolled due to acute cell damage and are irreversible (31). The type of necrosis damage can be caused by several factors, such as cell size shrinking due to the compacting and thickening of the cell nucleus chromosomes following damage, the rupture of the cell nucleus, the pale coloration of the cell nucleus, and the blurring or indistinctness of its shape (32). Furthermore, the phenomenon of hydropic degeneration, characterized by its duration and intensity, can impede cellular compensation, ultimately resulting in irreversible alterations, manifesting as cell necrosis



(A) K0, (B) K1, (C) K2, (D) K3, (E) K4, (F) K5
1) Normal, 2) Parenchymatous Degeneration (PD), 3) Hydropic Degeneration (HD), 4) Necrotic (NC),
V) Vacuolation, SM) enlarged sinusoids

Figure 2. Histopathologic Features of Catfish Liver Hepatocytes

Parenchymal degeneration is marked by mild degeneration, causing swelling and turbidity of the cytoplasm, along with granules due to protein deposition. Since this type of degeneration only happens in the mitochondria and endoplasmic reticulum during oxidative processes, it is reversible. Parenchymal degeneration is marked by cells swollen, cloudy appearance due to their inability to effectively excrete water (33). It is distinguished by protein deposits. However, these deposits are reversible, suggesting that the cells retain their original structural integrity (34).

Hydropic degeneration is a more severe form of degeneration than parenchymal degeneration because there is no fat or glycogen in the cytoplasmic vacuoles. As a result of fluid buildup and metabolic diseases like poisoning, the cytoplasm general swelling and a pale complexion (35). Hydropic degeneration is marked by the presence of distinct vacuoles in the cytoplasm, causing swelling, pallor, and clarity. This condition is typified by an abundance of water and a lack of fat or glycogen. Enlarged sinusoids may be due to the release of microplastic chemicals that collect in the liver and physically clog liver capillaries (18).

Histopathologic Description of Catfish Liver Tissue Damage

Damage to catfish liver tissue other than hepatocytes is found in each test group (K0-K5). Some

damage and cell abnormalities are used as other support related to the effect of MP PS on catfish liver (Figure 3).

The flow and accumulation of microplastics in the liver will release styrene monomers from microplastic degradation. Through the production of Reactive Oxygen Species (ROS) in immune cells, exposure to acids and proteolytic enzymes can have significant effects that merit careful consideration, as well as microbiological degradation, which can result in oxidative stress on liver tissue. In an effort to counteract ROS, which may be responsible for the presence of protein degradation, and cell crumble or death, hepatocytes trigger an inflammatory response (36).

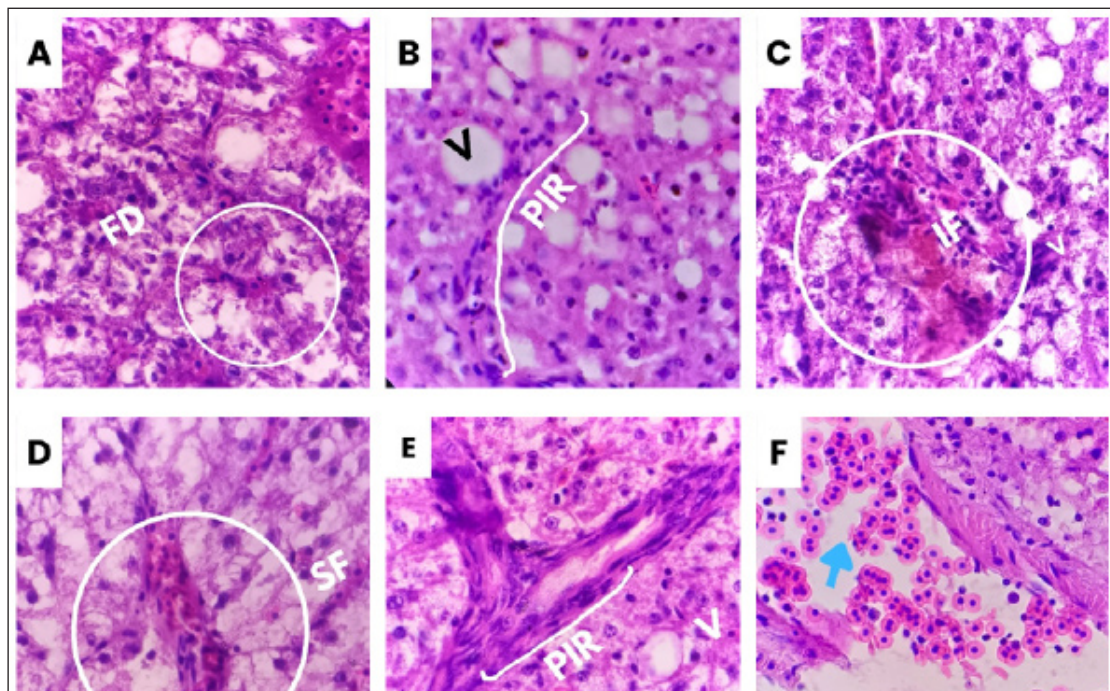
Hepatocytes, the liver's main cells, sit between sinusoids at the intersection of the portal vein and the hepatic artery. These sinusoids are visible when blood is present. The distinctive red color of the sinusoids likely results from cell infiltration or bleeding (37). Fatty degeneration disrupts fat cells, leading to liver cell swelling or enlarged liver cells. This condition is formally termed "fatty degeneration" within the domains of pathogen science and pathology, signifying the presence of excessive fat deposits within liver cells (37).

Interlobular connective tissue is the structural arrangement of the liver around the portal triad (bile duct, portal vein, hepatic artery) and within the portal area. Damage to the interlobular connective tissue is

associated with cirrhosis, hepatitis, and fatty liver disease (38). The distance between sinusoids widens due to fatty acid degeneration from the formation of fatty vacuoles, which create empty spaces in the sinusoids due to an increase in fatty acids (38).

Vacuolation is defined as damage to the cell nucleus and cytoplasm that is no longer visible in hepatocytes. Vacuolation damage in histology is characterized by the presence of empty circles, which occur due to the accumulation of excess fat

in the hepatopancreatic tubules (39). The causes of vacuolation include toxic substances, insufficient oxygen, or excessive feed that can't be efficiently converted into energy. Vacuolation can also result from biochemical imbalances leading to the formation of small flocculent glycogen and the accumulation of lipids within the hepatocyte cytoplasm (40). Certain infections can trigger an inflammatory response that leads to vacuolation. Toxic conditions can also cause vacuolar changes in immune cells as part of the immune response.



A) K0, B) K1, C) K2, D) K3, E) K4, F) K5, FD) Fatty Degeneration, IF) Inflammatory Cell, SF) Sinusoid Reddening, C1) Breakage Interlobular Connective Tissue, V) Vacuolation, PIR) Pericellular inflammatory response. Blue arrow) Abnormalities blood cells.

Figure 3. Histopathology of Catfish Liver Tissue Damage

DISCUSSION

The observed damage to fish liver tissue is probably due to the interaction between the digestive system and the liver, especially the liver-gut axis, which is triggered by exposure to MP PS, either through ingestion or via modification feed. The liver, being the primary organ to encounter blood from the gastrointestinal (GI) tract via the gut-liver axis, is significantly impacted by gut microbiota and its byproducts, which are the predominant catalysts of hepatic pathological responses (41). The intestinal barrier, comprising the intestinal mucosa and vascular endothelium, functions as a conduit between the intestine and the liver. This barrier plays a crucial role in regulating the systemic dissemination of microbes and toxins (42).

The presence of microplastic (MP) in the digestive system can lead to the accumulation of MP PS, which may break down and potentially enter the liver-gut

axis barrier, releasing chemical compounds. It can lead to an increase in the bioaccumulation of reactive oxygen species (ROS), liver inflammation, and cell damage, which can contribute to the development of chronic liver disease (CLD). Additionally, alterations in bile acids and microbe-derived molecules can exacerbate liver damage (41).

Chemicals can trigger oxidative stress, which can lead to health complications like liver cancer, hepatitis, and liver damage (43). ROS formation produces superoxide ($O_2^{\cdot-}$), which is converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). This process produces reactive hydroxyl radicals ($\cdot OH$). The body has defense mechanisms to neutralize ROS, including SOD, glutathione peroxidase, and catalase, which break down these molecules into water and oxygen. Excessive ROS can damage lipids, proteins, and DNA. Lipid peroxidation, a hallmark of oxidative stress, causes cell membrane deterioration and cellular dysfunction.

Protein modification can cause proteins to misfold and break down. This can lead to DNA damage, mutations, and genetic instability, which can cause diseases (43).

Liver damage can be triggered by toxic substances, chemicals, administered dose, and duration of exposure. High concentrations of compounds exert a substantial influence on the toxic response. This response may manifest as necrosis or dysfunction. Damage to liver cells is rarely direct; it's usually due to the byproducts of toxic substances. Many compounds are metabolized in the liver, increasing the risk of liver damage (44). Imbalanced metabolic processes can lead to various diseases, including liver problems. Liver cell deposition causes their transformation (44)

Free radicals released during microplastic (MP) degradation reduce oxygen levels, decreasing adenosine triphosphate (ATP) production. A decrease in ATP disrupts the active transport process, causing sodium ions to accumulate in the intracellular fluid and form edema. Continued disruption causes swelling of organelles, including mitochondria and the endoplasmic reticulum. This disrupts cell membrane regeneration (34). Initial toxicity damages the cell membrane, then the cell nucleus. Initial damage triggers further degeneration, and prolonged exposure leads to cell death. Polyunsaturated fatty acids in the cell wall undergo lipid peroxidation, forming long chains that damage the cell membrane (34). Figure 1 shows a statistically significant disparity among the test groups (K1-K5). This is due to varying concentrations of MP. Adding PS beads (0.5 mg) to the mixture had a clear effect, probably because of the light nature of EPS or PS beads, which are affected by wind during the blowing process. More beads were needed to reach the target concentration. The study about toxicity of polystyrene microplastics on juvenile *Onchorhynchus Mykiss* (Rainbow Trout) reported that the release of MP PS from the feces of exposed fish can lead to the formation of sharp MP fragments. These fragments have the potential to cause damage to the abrasion skin and disrupt normal physiological activity (45).

The results of the histopathological damage scoring (Table 2) indicate that the highest damage scoring value (K3-K5) is 200-300, which indicates mild to moderate damage. The utilization of *Clarias gariepinus* catfish as a bio-indicator is plausible due to its robust immune system, extensive geographic distribution, ease of acquisition, and vulnerability to xenobiotics. Furthermore, catfish exhibit omnivorous tendencies, frequently inhabiting sedimentary environments, which renders them susceptible to microplastic exposure, a factor that has the potential to exacerbate metal

accumulation. This characteristic renders them susceptible to bioaccumulation of metals (46).

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AUTHORS' CONTRIBUTION

All authors contributed to the research and writing of the manuscript and have approved its content. The specific roles of each author are as follows: HRPS: Conceptualization, Methodology, Data Collection, Writing-Preliminary Draft, Statistical Analysis, Data Interpretation, Writing-Reviewing, and Editing. MIH: Data Curation, Statistical Analysis, Data Interpretation. Funding Research, Supervision, A-Z: Visualization, Validation, Data Interpretation, Supervision.

CONCLUSION

This study demonstrates the significant toxicological impact of polystyrene microplastics (MP-PS) on the hepatic health of *Clarias gariepinus*. The findings indicate a dose-dependent relationship, wherein increasing concentrations of MP-PS resulted in progressive histopathological damage, including hepatocyte necrosis, hydropic degeneration, and structural abnormalities. The highest concentration (K5) elicited the most severe hepatic damage, highlighting the potential risks associated with sustained exposure to microplastics in aquatic organisms.

These results emphasise the urgent need for a comprehensive regulatory framework to mitigate microplastic pollution, particularly in industries producing primary and secondary plastics. The absence of strong policies governing the production, use, and disposal of microplastics currently poses a significant threat to aquatic ecosystems and, by extension, public health. Future research should further investigate the chronic implications of microplastic exposure across diverse biological systems and explore potential mitigation strategies to reduce its ecological and physiological impact.

The problem of microplastics should be of concern to the public, industrial companies, and the government level. This is because the threshold related

to the production of primary plastics such as microbeads used in beauty, health, and construction products has not been established which can damage environmental stability. In addition, it is necessary to pay attention to the content of secondary plastics or processed plastics that are more environmentally friendly. Further research is needed regarding the extent of the impact of microplastics on living things because microplastics are starting to enter the human body.

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