IMPACTS OF POLYETHYLENE TEREPHTHALATE MICROPLASTICS ON GROWTH PERFORMANCE AND BIOCHEMICAL BIOMARKERS IN ROHU, *Labeo rohita*

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ABSTRACT

Plastic wastes are usually dumped into water that ultimately break down into microplastics (MP), which are harmful to fish populations. Thus, studies on microplastic pollution in water bodies are essential to comprehend its effects and develop practical approaches to protect aquatic life and ecosystems. The current study investigated the effects of water-borne microplastics polyethylene terephthalate (PET-MPs) on growth, hematological indices, serum biomarkers (ALP, AST, ALT, and cortisol), and oxidative status of targeted organs (liver, gills, and intestine) of commercially important fish, *Labeo rohita (L. rohita)*. Healthy individuals of *L. rohita* (weight, 30 ± 2g; length, 10 ± 1 cm) were exposed to different concentrations (T_1-0, T_2-10, T_3-20, and T_4-40 mgL^{-1}) of PET-MPs with an average size of 546.91µm, respectively, for a period of 28 days. The results indicated the dose-dependent toxic effects of PET-MPs on growth and biochemical parameters of *L. rohita*. Growth performance was considerably ($p \leq 0.05$) decreased in PET-MPs exposure groups. Hematological results indicate that red blood cells, hemoglobin and hematocrit (HCT) values were significantly decreased as the concentration of PET-MPs increased. In the case of serum biomarkers, the levels of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and cortisol were significantly increased. Likewise, antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) activities and melano dialdehyde (MDA) level were also significantly increased in an organ- and dose-dependent manner. PET microplastics caused apparent dose-related physiological stress and organ toxicity in *L. rohita*, which showed their adverse effects on fish health and their potential threat to aquatic ecosystems and food safety. This study could help regulatory bodies to take appropriate steps to maintain healthy conditions of water bodies.

Keywords: Microplastics; Polyethylene terephthalate; *Labeo rohita*; Oxidative stress; Hematology; Cortisol; Liver enzymes

https://doi.org/10.36899/JAPS.2026.4.0093 Published first online May 06, 2026

INTRODUCTION

Plastics have become vital materials in society due to unique properties like inertness, durability, and corrosion resistance (Pilapitiya *et al.,* 2024). Globally, in 2018, about 360 million tons of plastic were manufactured and is predicted to rise by 1.1 billion tons in 2050 (Zhang *et al.,* 2022). As a result of an increase in manufacturing of plastic, a lot of plastic waste is being dumped into aquatic environments (Jiang *et al.,* 2022).

In aquatic ecosystems, several processes like chemical and thermal oxidation, photo-oxidation, biodegradation, and physical abrasion convert the plastic waste into smaller particles (Xiang *et al.,* 2022). Plastic particles that are less than 5 mm in size are referred to as microplastics. Based on the source of plastic waste, MPs are divided into primary and secondary MPs. Primary MPs are obtained from personal care and cosmetic products, while secondary MPs are generated when large plastics break down due to ecological elements (Jiang *et al.,* 2022).

Polyethylene terephthalate is one of the most produced plastics (Joseph *et al.,* 2024). It is used to make films, sheets, and fibers of food packaging and also used to make different parts of electronics like textiles, sports goods, and automotive parts (Singh *et al.,* 2018). Most commercially used glitters of size 50 to 6350 µm are also made of PET (Yurtsever, 2019). In aquatic environments, polyethylene terephthalate mainly disintegrates by hydrolysis and by photo-oxidation (Gewert *et al.,* 2015). Polyethylene terephthalate-derived debris has polluted various coastal areas; it mainly contains drinking bottles in trash (Munari *et al.*, 2016).

Among various aquatic environments, freshwater ecosystems are found to be an important source, sink, and also transporting medium for MPs (Pastorino *et al.,* 2023). These MPs have the potential to negatively impact aquatic life in a number of ways. Freshwater fish are a major food source for humans and other animals worldwide, so the harmful effects of MPs on these fish are very concerning. Above all, these polluted fish MPs may serve as an easy source through which these harmful MPs could reach humans via the food chain (Qaiser *et al.,* 2023). Numerous investigations have demonstrated that MPs may be harmful to fish and other aquatic life (Banaee *et al.,* 2023; Oza *et al.,* 2024).

Due to the small size of MPs, aquatic animals can unknowingly take these MPs as food through ingestion and respiration (Li *et al.,* 2021). MPs have the ability to build up in the gills by adhering to gill filaments, leading to structural harm and malfunction in processes like balancing of ions, transfer of gases, and osmoregulation (Hamed *et al.,* 2021; Zhang *et al.,* 2021). MPs also accumulate in the intestine and can decrease hunger, energy metabolism, and nutrient absorption by blocking the intestine, causing dysmotility and inflammation (Ding *et al.,* 2018). Likewise, MPs in these organs can also enter other parts of the body, like the liver and circulatory system, by passing through the lymphatics of intestine or endothelial cells (Jovanovi´ c *et al.,* 2018; Solomando *et al.,* 2021).

After ingestion, these MPs can decrease the development and metabolic processes (Wu *et al.,* 2023) obstruct the digestive tract (Zicarelli *et al.,* 2023), can cause death (Naidoo and Glassom, 2019), neurotoxicity (Ding *et al.,* 2018), change in growth of fish (Hasan *et al.,* 2024), also show alteration in biochemical markers (Hamed *et al.,* 2019), hepatotoxicity (Abbaszadeh *et al.,* 2024), and oxidative stress (Ding *et al.,* 2018). Reactive oxygen species (ROS), which have a high tendency to harm cellular components and cause oxidative stress, can be produced from pollutants (Proki´c *et al.,* 2019). In order to deal with this excess of reactive oxygen species, organisms activate the antioxidant system as a defense system and to protect cells from damaging effects (Box *et al.,* 2007). Certain defense enzymes, including CAT, SOD, and glutathione peroxidase, serve to remove H_2O_2 and other hydroperoxides produced by pollutants (Sureda *et al.,* 2018).

L. rohita is a significant omnivorous fish species among Indian major carps (IMCs). It is the most widely cultivable specie because of its rapid increase in size, high market value, enhanced resistance to diseases, and good flesh quality (Anand *et al.,* 2018). Indian major carps generate about 87% of all freshwater aquaculture, with *L. rohita* contributing to 35% of the overall output (Mir *et al.,* 2017). In 2018, Pakistan makes 46,102 tons of *L. rohita*, the country's second most significant fish (FAO, 2018). Thus, it is essential to protect the freshwater fish species *L. rohita* from the harmful effects of MPs. So, this research was designed to determine the negative effects of MPs on growth, hematological parameters, antioxidant responses, serum cortisol, and biochemical parameters of *L. rohita* exposed to waterborne PET-MPs.

MATERIALS AND METHODS

Fish collection and acclimatization: Healthy individuals of *L. rohita* (n = 120) (weight, 30.0 ± 2.0 g; length, 10.0 ± 1 cm) were collected from fish ponds, University of Veterinary and Animal Sciences Lahore, Pakistan,
(UVAS) during March 2024, and immediately transported to the Fish Seed Hatchery, Department of Fisheries and Aquaculture, (UVAS), in oxygenated polyethylene bags. Fish were kept in cemented rectangular tanks for two weeks for acclimatization according to Hunn *et al.*, 1968; OECD, 2025).

Microplastics source and stock solution preparation: Polyethylene terephthalate was used as a microplastic source as suggested by (Locher *et al.,* 2018). The glitter used in this study was purchased from a local stationery supply store. It was a mixture of normal particles of polyester that were around 0.2-0.6 mm in diameter. The stock suspension was freshly prepared by adding glitter particles to distilled water using sterile glassware. The suspension was vigorously shaken manually prior to dosing to ensure proper dispersion.Through Fourier transform infrared spectroscopy (FTIR), a polymeric type of microplastic was confirmed (Paradinas *et al.,* 2021). The particle size distribution of PET glitters was assessed using ImageJ software by randomly selecting 100 particles.

Experimental design: According to complete randomized design (CRD) 120 healthy individuals of *L. rohita* were randomly taken from rectangular tanks and divided into four glass aquaria (10 fish in each) having 300L water holding capacity and named as T_1, T_2, T_3 and T_4. The T_1 group was control and comprised of fish kept in microplastic-free clean water and under the same experimental conditions as all-other groups. While T_2, T_3 and T_4 fish group were exposed to 10 mgL^{-1}, 20 mgL^{-1} and 40 mgL^{-1} PET-MPs, respectively, for a period of 28 days. Although PET microplastic concentrations used in this study (10-40 mgL^{-1}) exceed typical freshwater levels (μgL^{-1} to <10 mgL^{-1}; Jin *et al.,* 2025; Zhao *et al*., 2026), these were selected to establish dose response relationships and assess sub-lethal mechanisms under controlled conditions, as supported by Yu *et al.,* (2023). Given the low toxicity of PET and lack of a defined LD_{50}, higher concentrations are necessary to induce measurable responses and reflect increasing microplastic pollution levels over time. A semi-static exposure system was used to conduct the experiment. In order to control the quality of water and

the constant concentration of microplastic, the entire amount (100%) of test water was replaced once a week. The respective concentrations of PET-MPs (10, 20, and 40 mg L^{-1}) were reintroduced after every renewal, and constant exposure was maintained during the study period. Continuous stirring was used to uniformly disperse PET microplastics in water and then added to experimental tanks. Whole experiment was done under controlled laboratory conditions viz pH, temperature, DO, ammonia and hardness at 7.5, 30◦C, 0.05 mgL^{-1} and 150 mgL^{-1} respectively in triplicates (Each treatment was conducted in three independent tanks, with multiple fish housed in each tank). Aerators were installed in each aquarium to maintain optimum oxygen levels. Fish were fed with commercial diet twice a day at 9.00 am and 15.00 pm, respectively up to satiation and feed was not withheld during the exposure period to maintain normal feeding behavior. At the end of the trial, two fish from each group were randomly selected and anesthetized with MS-222 (50 mgL^{-1}) to overcome handling stress. Blood was taken from the caudal vein of a fish using a 1 mL sterile hypodermic micro-syringe (Jiangsu Zhiyu Medical Instrumented Co. Ltd., Taixing City, China) and immediately analyzed for hematological parameters. After this, blood was centrifuged at 3000 g at 4°C for 10 min to extract serum, which was stored at -40°C for further analysis. After blood collection, fish were dissected and gills, liver, and intestine excised, stored in pre-labelled vials for determination of oxidative stress.

Growth parameter: Following growth parameters were calculated according to Jobling *et al.* (1994).

$$WG\ \% = \frac{final\ body\ weight - initial\ body\ weight}{initial\ body\ weight} \times 100$$

$$SGR\ \% = \frac{\ln(final\ wet\ body\ weight) - \ln(initial\ wet\ body\ weight)}{number\ of\ days} \times 100$$

$$SR\ \% = \frac{no.of\ fish\ at\ the\ end\ of\ trial}{no.of\ fish\ at\ the\ beginning\ of\ trial} \times 100$$

Determination of oxidative status: 0.2 M phosphate buffered saline pH 7.4 at (1:4 w/v) ratio was used to homogenize gills, liver, and intestinal tissues of *L. rohita* in a homogenizer (Model: HG-15D). The supernatant was then extracted by centrifugation at 10,000 g for 30 minutes at 4°C. Supernatants were collected and stored at -80°C until analysis. Catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) assays were estimated as suggested by Chance and Maehly (1955), Winterbourn *et al*. (1975), and Ohkawa *et al*. (1979), respectively.

Determination of serum cortisol level: Serum cortisol level was determined through an ELISA reader at 450 nm by using the bioactive diagnostic kit as suggested by Sajjad *et al.* (2018).

Blood analysis: hematological and biochemical parameters*:* For hematological analysis, a Neubauer hemocytometer was used to manually count the red blood cells (RBCs × 10^{12} cells/L) and white blood cells (WBCs × 10^{9} cells/L) in blood that was diluted 1:200 in modified Natt-Herrick's solution. The cyanmethemoglobin method was used to measure hemoglobin (Hb). Blood films fixed with absolute methanol and stained with a modified Wright-Giemsa stain were used to measure the number of different blood cells according to Ellis (1976) and Ainsworth (1992). AST, ALT, and ALP were estimated by using bioactive diagnostic kits.

Statistical analysis: The normality and homogeneity of variance of the biochemical data were evaluated using the Kolmogorov–Smirnov test and Levene's test, respectively. Subsequently, the data were analyzed using one-way analysis of variance (ANOVA) with GraphPad Prism software (version 9.4.1). All results are presented as mean ± standard error (SE). Post hoc comparisons among treatment groups were performed using Tukey's honestly significant difference (HSD) test, and differences were considered statistically significant at $P \leq 0.05$ (Steel *et al.,*1997).

RESULTS

Characterization of microplastics: FTIR spectroscopy revealed that microplastics are composed of polyethylene terephthalate (Fig. 1). The average size of PET-MPs was 546.91µm measured by Image J software.

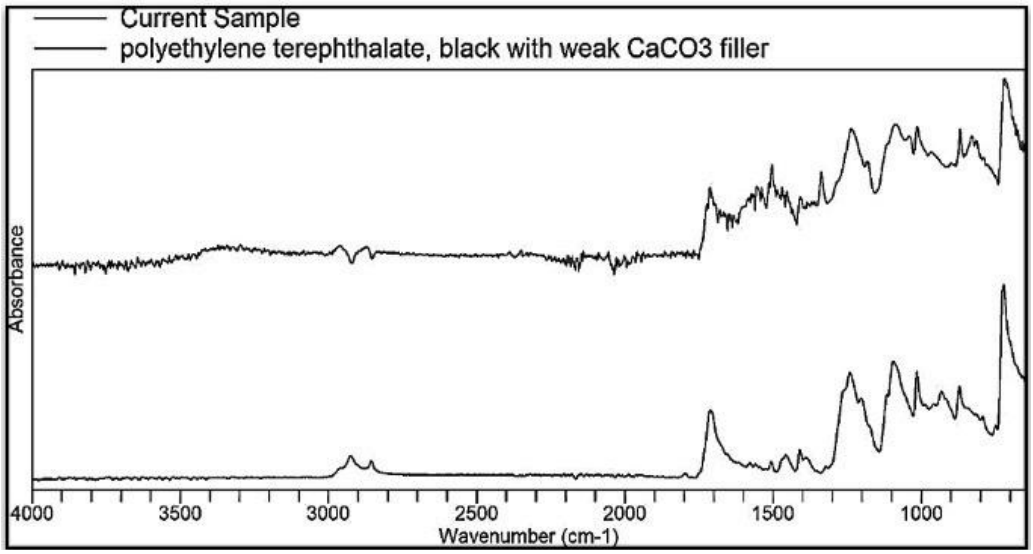

Figure. 1. Fourier Transform Infrared Spectroscopy (FTIR) of glitter

Growth parameters: Growth performance of *L. rohita* treated with PET-MPs is given in Table 1. Highest weight gain (WG) was observed in treatment T1 (15. 85 g) treated with 0 PET-MPs while there was a little weight gain in treatment T4 (2.95 g) treated with 40 mg/L of MPs. Furthermore, lowest SGR (0.33%) was found in treatment T4 followed by treatment T3 (0.67%) and then in T2 (1.05%) as compared to the control T0 (1.49%). No mortality was observed in any MP treated groups. The survival rate in all MP exposed fish groups and control group was 100%.

Table 1: Effect of PET-MPs on the growth performance of *L. rohita*

Treatments	Growth parameters					
	IBW (g)	FBW (g)	WG (g)	WG %	SGR (%/day)	SR %
T1	30.51±1.28	46.36±1.05^{a}	15.85±0.61^{a}	52.03±3.68^{a}	1.49+0.08^{a}	100.00±0.00
T2	30.13±1.12	40.48±0.45^{b}	10.34±0.78^{b}	34.41±3.89^{b}	1.05+0.10^{b}	100.00±0.00
T3	30.55±0.52	36.88±0.89^{c}	6.33±0.39^{c}	20.71±0.98^{c}	0.67+0.02^{c}	100.00±0.00
T4	30.15±0.78	33.10±0.68^{d}	2.95±0.81^{d}	9.81±2.90^{d}	0.33+0.09^{d}	100.00±0.00

Different superscripts within columns indicate a significant variation ($p<0.05$). Data are mean±SD from three replicates. Abbreviations: IBW (Initial body weight); FBW (Final body weight); WG (Weight gain); WG (Weight gain percentage); SGR (Specific growth rate); SR (Survival rate) are acronyms

Serum biomarkers: PET-MPs exposure effect on serum biochemical parameters of *L. rohita* was presented in Fig 2: Liver enzymes. Upon MP exposure, there was a significant ($p\leq0.05$) increase in ALT, AST, and ALP activity. Highest AST, ALT and ALP values were observed in treatment T4 ($p\leq0.05$) exposed to a concentration of 40 mgL^{-1} of PET-MPs followed by treatment T3 and then in treatment T2 respectively, as compared to the control group with no MPs.

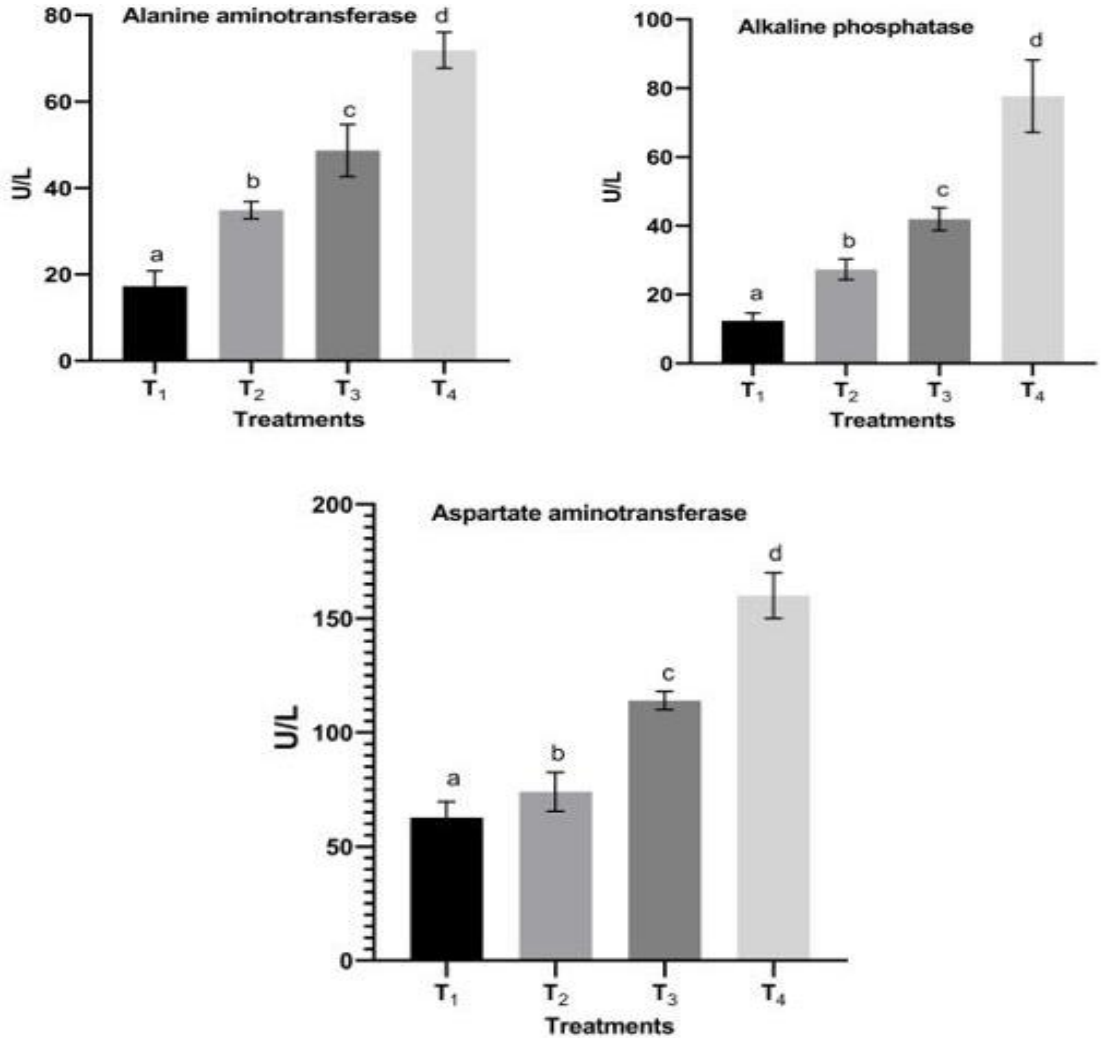

Figure 2: Liver enzymes activity (mean ± SD) of rohu, *L. rohita* exposed to PET-MPs

Cortisol level: PET-MPs exposure showed a remarkable increase ($p \leq 0.05$) on serum cortisol level of *L. rohita* ($p \leq 0.05$) in a dose-dependent manner as shown in Figure 3: Cortisol. Significantly highest cortisol level was found in treatment T4 exposed to 40 mgL^{-1} of PET-MPs then in treatment T3 and T2 respectively, while the lowest cortisol activity was observed in T1 (control).

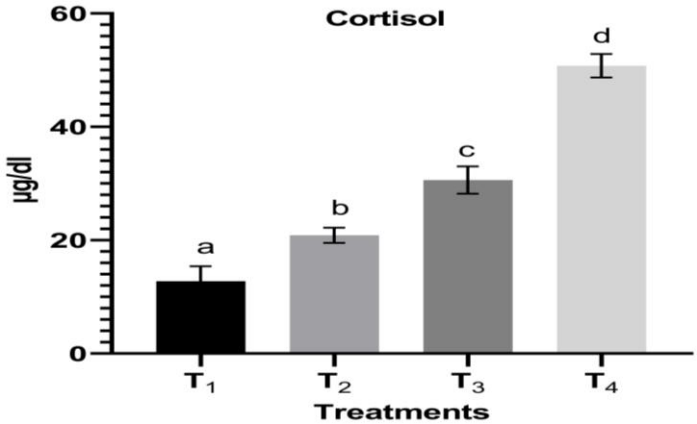

Figure 3: Cortisol level of rohu, *L. rohita* exposed to PET-MPs. Values were expressed as mean ± S.D.

Oxidative status: The effect of microplastics exposure on antioxidant enzymes (SOD and CAT) and LPO level in tested organs (gills, liver, and intestine) of *L. rohita* is presented in Figs. 4: SOD, 5: CAT and 6: Melano dialdehyde. With the increase in dose of PET-MPs, there was a remarkable elevation ($p \leq 0.05$) in antioxidant enzymes and LPO level in a dose-dependent manner. Highest SOD activity was observed in treatment T_4 and then in treatment T_3 and T_2, respectively, of intestinal tissue ($p \leq 0.05$) (Fig. 4). Catalase activity was significantly increased ($p \leq 0.05$) in the liver tissue when exposed to 40 mgL^{-1} MP treated group (Fig. 5). The lipid peroxidation (LPO) activity in the liver was significantly increased ($p \leq 0.05$) in treatment $_{T4}$ than in treatment T_3 and T_2 respectively (Fig. 6).

Oxidative status: The effects of PET-MPs exposure on antioxidant enzymes (SOD and CAT) and lipid peroxidation in the gills, liver and intestine of *L. rohita* are shown in Figures 4 (SOD), 5 (CAT) and 6 (MDA). In all examined organs, PET-MP exposure resulted in a significant increase ($p \leq 0.05$) in oxidative stress biomarkers compared to the control group, with a clear dose-dependent response.

SOD activity increased significantly ($p \leq 0.05$) in the liver, followed by the intestine and gills of *L. rohita* across all PET-MP exposure groups (T_2–T_4). The highest SOD activity was recorded in the T_4 group (40 mg L^{-1}), followed by T_3 (20 mg L^{-1}) and T_2 (10 mg L^{-1}), demonstrating a clear dose-dependent increase compared with the control (Fig. 4).

CAT activity increased significantly ($p \leq 0.05$) in the liver, followed by the gills and intestine of *L. rohita* across all PET-MP exposure groups (T_1–T_3). The highest CAT activity was observed in the T_4 group (40 mg L^{-1}), followed by T_3 (20 mg L^{-1}) and T_2 (10 mg L^{-1}), indicating a clear dose-dependent elevation compared with the control. The increase was most pronounced in the liver, while moderate but significant enhancements were recorded in the gills and intestine (Fig. 5).

LPO levels, increased significantly ($p \leq 0.05$) in the liver, followed by the intestine and gills of *L. rohita* across all PET-MP exposure groups (T_1–T_3). The highest MDA levels were recorded in the T_4 group (40 mg L^{-1}), followed by T_3 (20 mg L^{-1}) and T_2 (10 mg L^{-1}), indicating a clear dose-dependent escalation in membrane lipid damage compared with the control (Fig. 6).

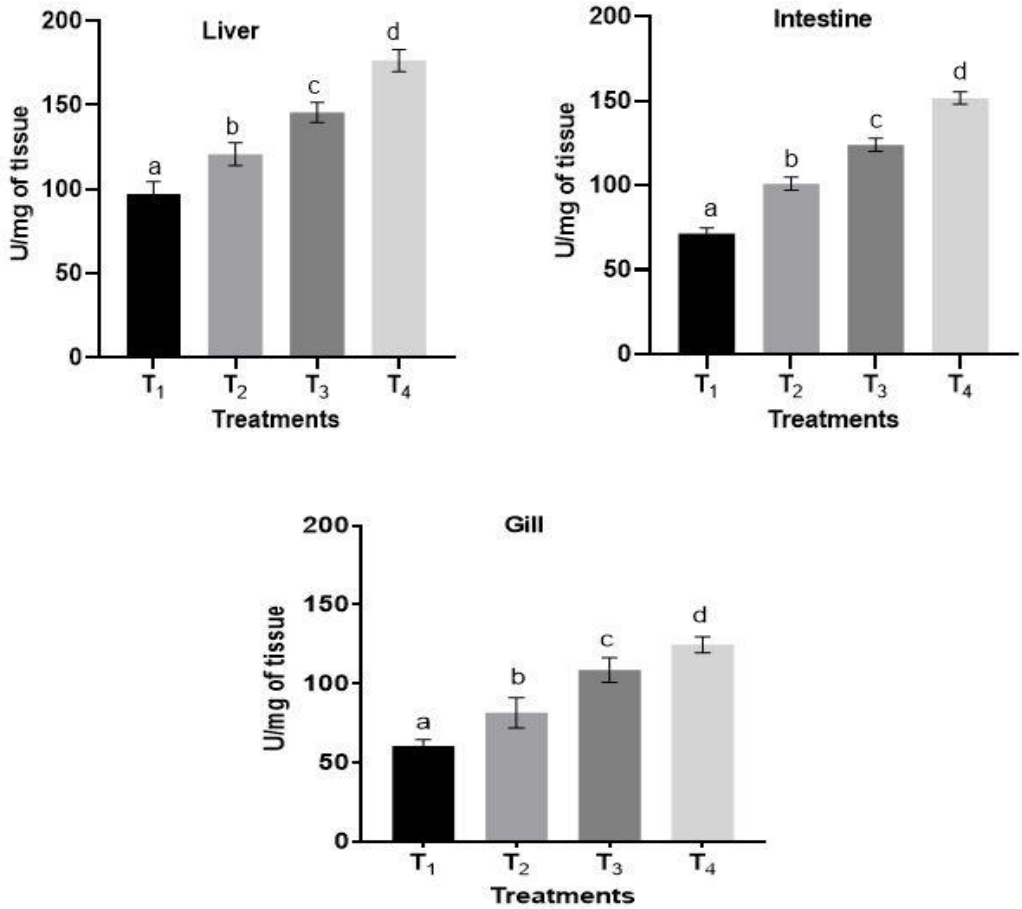

Figure 4: SOD activity in *L. rohita* exposed to PET-MPs for 28 days. Error bars expressed as standard deviation. Different letters represent significant difference between treatment groups.

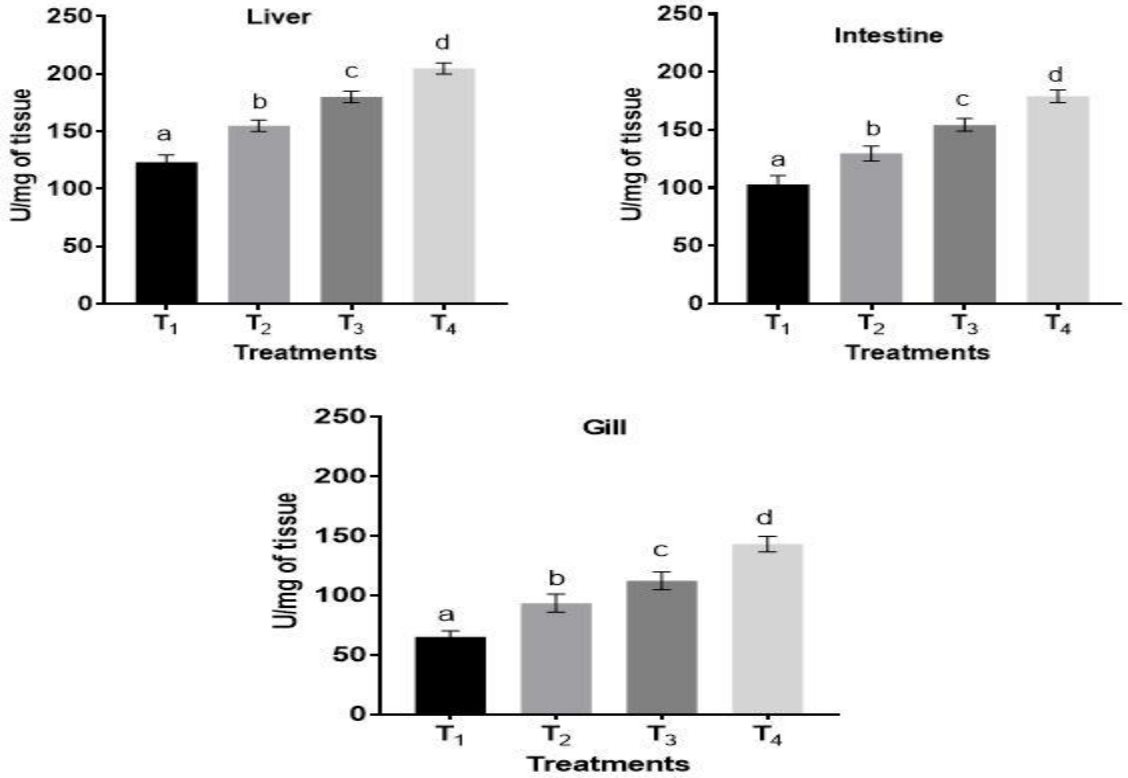

Figure 5: CAT activity in *L. rohita* exposed to PET-MPs for 28 days. Error bars expressed as standard deviation. Different letters represent significant difference between treatment groups.

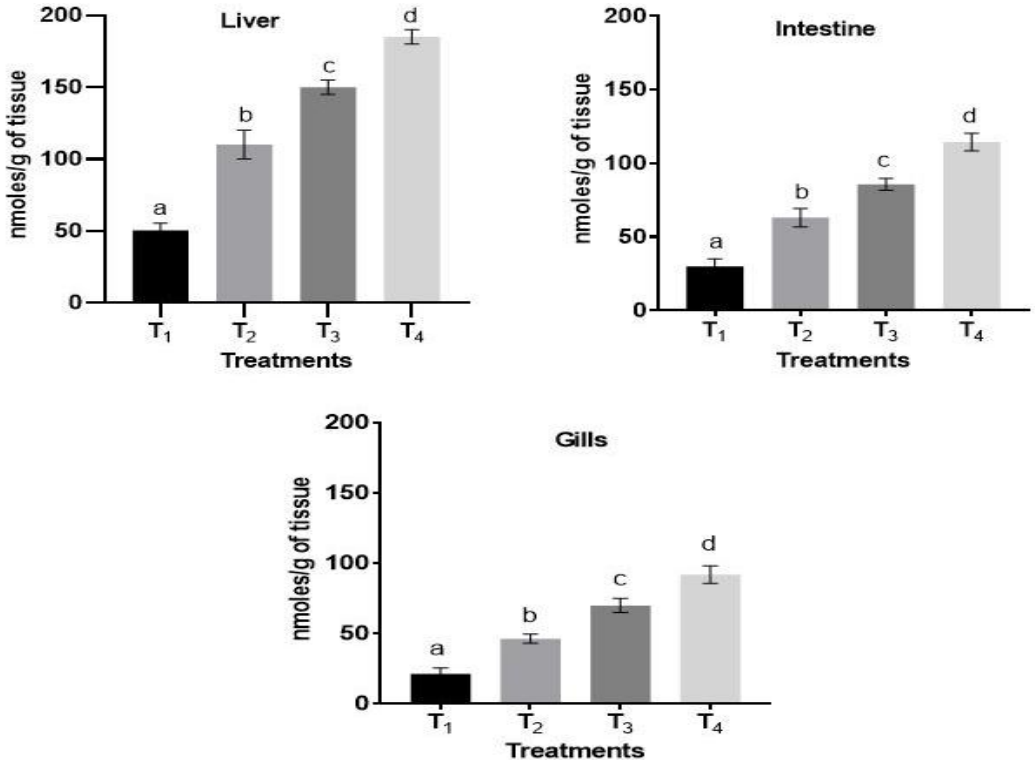

Figure 6:MDA levels in *L. rohita* exposed to PET-MPs for 28 days. Error bars expressed as standard deviation. Different letters represent significant difference between treatment groups.

Hematological indices: The effect of microplastics exposure was found to cause significant ($p \leq 0.05$) changes in hematological profile of *L. rohita as* presented in table 2. A remarkable decrease ($p \leq 0.05$) in red blood cells, hemoglobin, and HCT values was observed in *L. rohita* with the increase in the concentration of PET-MPs. Lowest RBCs value was observed in treatment T4 than in treatment T3 and T1respectively. While there was a remarkable increase ($p \leq 0.05$) in the level of WBCs with an increase in the dose of PET-MP exposure. Highest WBCs level was observed in T4 followed by T3 and T2 respectively.

Table 2: Effect of microplastics exposure on the hematological indices of *L. rohita*

Treatments	Hematological parameters			
	WBCs×10^3/µL	RBCs (10^6/µL	Hemoglobin g/Dl	HCT%
T1	7.17±2.76^d	2.81±0.168^a	7.73±0.3^a	22.73±0.89^a
T_2	14.69±2.38^c	2.35±0.126^b	5.67±0.41^b	17.74±2.85^b
T_3	25.31±2.9^b	2.07±0.2^b	5.16±0.6^b	14.69±1.06^b
T_4	34.37±4.34^a	1.40±0.23^c	3.08±0.3^c	9.01±0.89^c

Different superscripts within columns indicate a significant variation ($p<0.05$). Variables are expressed as mean± standard deviation from three replicates.

DISCUSSION

MPs are emerging, relatively unknown environmental contaminants of worldwide concern (Umamaheswari *et al.,* 2021). Since plastic polymers can withstand hundreds to thousands of years in nature due to their low biological decomposition. As time passes, they eventually break into microplastics, which are particles with a diameter of ≤5 mm (Andrady, 2011) and pose adverse effects to humans and many other aquatic organisms. Therefore, the current study can offer valuable findings on the toxicity of PET-MPs, as it shows that the exposure to PET-MPs at the concentrations of 10-40 mgL^{-1} can cause considerable changes in various physiological parameters of *L. rohita*. The concentrations of PET microplastic in this study (10-40 mgL^{-1}) are more than normal environmental concentrations but are typically used in laboratory toxicity experiments to determine the dose response relationships and explore sub-lethal mechanisms. Similalry, Yu *et al*. (2023) administered similar concentrations to determine the effects of microplastic on physiological and oxidative stress. Even though it is not reflective of the typical environmental conditions, such levels can offer some useful mechanistic information on microplastic toxicity.

The current research study has shown that the growth rate of *L. rohita* was significantly lower due to exposure to PET-MPs as opposed to the control group. Micro- and nano plastics have also been reported to cause similar growth retardation in aquatic organisms, such as lower development and survival in juvenile sea cucumbers exposed to nano plastics (Naidoo and Glassom, 2019), lower growth and survival in *Clarias gariepinus* (Permatasari *et al.,* 2023), and shorter body length in *Epinephelus coioides* and *Larimichthys crocea* that had been exposed to nano plastics (Wang *et al.*, 2022: Gu *et al*., 2020) also proved that the specific growth rate of *L. crocea* was significantly decreased in the case of short-term exposure to polystyrene nanoplastics (100 nm).

Mechanistically, both physical and systemic physiological stress might be involved in the mechanism of development of growth inhibition in PET-MP-exposed *L. rohita*. Direct contact of the microplastics with the gills and intestinal tissues may result in epithelial damage and subsequent uptake of the particles. The deposition of MPs in the intestine can cause changes in the gut morphology, the expansion of goblet cells, and the composition of gut microbes, and thus disrupt nutrient absorption and metabolic activities (Huang *et al.,* 2020). Such physical disturbances lower the efficiency of nutrient utilization and lead to low performance in growth.

After uptake, PET-MPs cause systemic inflammation, demonstrated by increased WBCs counts, which is an indication of an immune response to exposure to microplastics. At the same time, PET-MPs also enhance oxidative stress by causing excessive generation of ROS, resulting in the redox imbalance (Kim *et al.,* 2021). The current study has shown a high level of upregulation of the antioxidant enzymes (superoxide dismutase and catalase) in the liver, gills and intestine after 28 days of exposure. The liver demonstrated the highest level of antioxidant reaction because this organ is at the center of detoxification and metabolism, whereas gills and intestine demonstrated the mid-level responses because the organisms are directly exposed to water-borne MPs (Jandu and Vashishat, 2021). Increases in SOD and CAT activities, including those after MP exposure, have been observed to be similar in *Symphysodon aequifasciatus* (Wen *et al.,* 2018) and zebrafish (*Danio rerio*) (Lu *et al*., 2016).

Overproduction of ROS also led to lipid peroxidation as depicted by high levels of MDA in the liver and the intestine. Higher MDA is an indication of an oxidative attack on lipids and cellular structures in the membrane (Linhart

et al., 2014). Similar rises in MDA were found in red tilapia and guppy after exposure to microplastics (Ding *et al*., 2020; Huang *et al.,* 2020), and in *Sparus aureus* after exposure to polyethylene microplastics (Capo *et al.,* 2021). These results show that the antioxidant defenses, despite being triggered, were too weak to counter the oxidative damage during prolonged exposure to PET-MP.

The final effect of oxidative stress and inflammation is cellular and tissue damage especially in the liver which is seen in changed biochemical and hematological parameters. An increase in WBC, decreased levels of RBCs, hemoglobin and HCT are signs of immune activity, oxidative damage and anemia-like states in *L. rohita* (Ahmed *et al.,* 2020; Abdel-Zaher *et al.,* 2023). *Carrasius carassius, Pseudobagrus fulvidraco*, and *Clarias gariepinus* have also been reported to exhibit similar hematological disturbances when exposed to microplastic (Sayed *et al.,* 2021; Lee *et al.,* 2023; Yu *et al.,* 2023), although species-specific tolerance is also reported (Wen *et al.,* 2018).

All these results suggest a mechanistic process whereby physical damage to gills and intestine by the uptake of particle increase systemic inflammation (increased WBC and oxidative stress) and caused cell death by PET-MP exposure. The overall metabolic price of immune response, antioxidant defense and tissue repair redirects energy towards somatic growth in *L. rohita* resulting in the observed dose-dependent growth inhibition. This combined reaction agrees with the known models of pollutant-stress effect in fish (Rashid *et al.,* 2025).

Some of the most common biomarkers of stress in fish include corticosteroid hormones especially cortisol. In the current experiment, cortisol concentration in *L. rohita* was gradually rising with the increase in PET-MP concentration, which suggests that the hypothalamic-pituitary-interrenal axis was activated by the microplastic-induced stress. Other species of fish have also been shown to experience similar increases in cortisol after exposure to phthalate plasticizers and microplastics, which is a reflection of the interference with endocrine stress regulation (Revathy and Chitra, 2019; Yang *et al.,* 2020). Despite the fact that the observed rise in the number of white blood cells indicates an acute inflammatory reaction, the chronic exposure to elevated cortisol levels during chronic exposure to PET-MP can have the opposite effect: a series of immunosuppressive effects on individual immune functions can lead to physiological susceptibility. Further, long-term cortisol stimulation has been known to enhance metabolic reprogramming, which diverts energy resources towards offsetting stress, detoxification, and homeostasis, and not somatic growth. This endocrine-immune-metabolic interaction would probably enhance oxidative stress, hepatotoxicity, and hematological abnormalities seen in PET-MP-exposed fish, which eventually leads to growth retardation. All these results indicate that PET-MP exposure causes a complex, cortisol-stressed systemic stress that depletes immune fitness, modulates energy distribution, and has adverse impacts on the general health and fitness of *L. rohita* (El-Houseiny *et al*., 2023).

Conclusions: This study confirmed that exposure to PET-MPs cause an adverse effect on the growth and on various biochemical parameters of *L. rohita.* The significant elevation of biomarkers like SOD, CAT, MDA, and serum cortisol level served as reliable indicators of induce oxidative stress. Furthermore, the increase in liver enzymes and WBC counts clearly demonstrates the systemic toxicity of microplastics in *L. rohita.* Further research should investigate on how microplastics interact with other pollutants across all life stages and genders, focusing on cellular and genetic impacts to gain a more complete picture of their toxicity.

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