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Hemato-Biochemical, Histological and Genotoxic Assessment of Methylparaben-Induced Toxicity in FreshWater Fish Rohu (*Labeo rohita*)

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Key words
Aquatic toxicology, Biochemistry, Fish, Genotoxicity, Histology, Oxidative stress, Paraben

ABSTRACT

Parabens, because of their antifungal and antibacterial properties, are used in many products such as cosmetics, baby care products, and pharmaceuticals. Because of their extensive use in many products, parabens are now considered serious aquatic pollutant. This study assesses the haemato-biochemical, histolegial and genotoxic effect of different concentrations of parabene on freshwate fish *Labeo rohita*. The fish were exposed to 16 mg methyl parabene/ L (group Ti), 22 mg/L (group T2) and 33 mg/L (group T3) for 21 days. We found that all haematological and biochemical components of fish blood were significantly increased. This was particularly seen in higher doses. The blood glucose level, liver function enzymes, blood proteins and lipid profile parameters were considerably increased. Histological changes were observed in the fish's liver, gills, and kidneys. Methylparaben causesd oxidative stress by inhibiting the activity of antioxidant enzymes. The diminished potential of antioxidant enzymes to counter oxidative stress is attributed to increased oxidative enzyme activity, suppressing antioxidant enzyme secretions. A comet assay detected DNA damage in fish blood cells. These findings suggest that the methylparaben is potentially genotoxic, causes tissue damage, alters metabolic parameters, and induces oxidative stress.

INTRODUCTION

Endocrine-disrupting compounds (ECDs) including parabens are an extensive class of chemicals that enter the aquatic environment from manufacturing numerous industrial and consumer items, agriculture, food and drug processing, wastewater treatment plants, and human waste. Some of the most potent endocrine disruptors share a chemical structure with parabens de Carvalho Penha *et al*. (2021). Parabens are widely used chemicals in pharmaceuticals and personal care products (Lincho *et al*., 2021). The use of parabens is due to their low production costs, high stability, and antimicrobial and antifungal activity. Methylparaben (MeP) is the most widely used paraben and, as such, one of the most frequently found in wastewater (de Carvalho *et al*., 2021). Al though MeP degrades quickly in aerobic environments (1 mg/L in water takes 19.3 min to break down), wastewater treatment plants procedures are insufficiently effective in eliminating all MeP. Moreover, only 59% of the world's population safely manages sewage, which means that poor sanitation contributes to the existence of MeP in the environment (Wu *et al*., 2017).

Human health may be negatively impacted by exposure to synthetic estrogens (such as parabens), especially in the reproductive cycle and reproductive function. It has previously been demonstrated that fish can imitate estrogen by being exposed to ethylene, propyl, and butyl parabens. The concentration of methylparaben in groundwater has been reported 5 µg/L in the UK (Stuart *et al*., 2012), 7.6 to 29.8 µg/L in the streams of Rio Grande and Morro Redondo, Brazil (Silveira *et al*., 2013) and 0.001 to 52.1 µg L^{-1} in various samples from aquatic environment (Czarczyńska-Goślińska *et al*., 2017).

Numerous studies have assessed how vulnerable aquatic species, such as fish, are to the harmful

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consequences of exposure to certain parabens. Histology is used to determine the impact of endocrine disruption in fish. There is evidence that histological alterations can indicate fitness both in populations and in specimens (Silva *et al*., 2018).

Little is known on the oxidative stress induced by parabens in aquatic species, even though parabens are found in the marine environment and are detrimental to organisms. It can cause lipid peroxidation, membrane instability, DNA damage, and cell protein degradation. Oxidative stress results from an imbalance in an organism's defenses against the creation of reactive oxygen species (ROS) (Silva *et al*., 2018). Examining oxidative stress markers is critical to assess the long-term consequences of paraben exposure. Exposure to MeP may cause changes in the antioxidant system and increase lipid peroxidation in fish. A few studies have been done on how MeP affect fish antioxidant markers (Ateş *et al*., 2018; Silva *et al*., 2018). Paraben causes biochemical alteration and fluctuations in enzymatic activity in fish (Akmal *et al*., 2024).

The current study aims to assess the harmful effects of MeP on freshwater fish, *Labeo rohita,* when the quantity of MeP in aquatic habitats rises, posing a threat to aquatic life. Fish is a source of protein and a component of the food chain, yet consumption after being captured from polluted water sources is detrimental to humans.

MATERIALS AND METHODS

Fish handling

The experiments were performed in the laboratories of the Department of Zoology and the Department of Fisheries at the University of Okara, Pakistan. The chemicals and reagents were prepared according to the standard protocols and procedures. The fish were captured from the ponds located at Head Balloki, District Kasur, Pakistan, and transported to the laboratory using the Food and Agriculture Organization (FAO) standard protocol. Before acclimatization, fish were disinfected with 0.1% KMnO4 solution then placed in the water tanks for 7-day acclimatization. All the fish were fed with commercially prepared feed containing 20% protein from Optimum Tropical Fish Food Co., Thailand.

Experimental design

Four fish groups were formed for the experimental trial: a control group (T0) and three treatment groups (T1, T2, and T3), each with a triplicate. Each group included 15 fish (n=15). An ethanol solution of methylparaben (CH3(C6H4(OH)COO) CAS No. 99-76-3, 99.9% MACLIN, China) was produced using analytic-grade ethanol. Doses were prepared following after 48 h LC_{50} 67.11 (56.61–79.57) mg/L determined by Silva *et al*. (2018). Four fish groups, labeled T0, T1, T2, and T3 were exposed to different concentration of MeP: 0 µg/L, 16 mg/L, 22 mg/L, and 33 mg/L respectively, over a period of 21 days. Every other day, 90% of the water in each glass tank (35"×40"×25") was replaced with fresh water.

After 21 days of treated blood was collected for haematology, biochemical analysis and genotoxicity. Fish were dissected for histological study, tissues sections were prepared following the protocols given by Barse *et al*. (2010). The fish were anesthetized with clove oil at a 50 µl/L concentration and subsequently dissected. The fish (n = 3) tissues (gills, liver, kidney) were removed and then preserved in 90% alcohol until they were ready for further processing.

Histological study

Tissues were fixed in neutral buffered formalin and then transferred to 70% alcohol. The tissue was dehydrated using a gradual sequence of alcohol solutions before being embedded in paraffin. Thin sections (4-6µm) were stained with hematoxylin and eosin and seen using an Olympus BX40 microscope. The images were captured using a Nikon (SC 35 type 12) camera at a shutter speed of 30 sec.

Biochemical assay

The oxidative and antioxidant enzymatic and non-enzymatic activities of fish liver, kidney, and gills were assessed.

The reactivity of reactive oxygen species (ROS) was assessed by following the protocol of Tvrda (2019). The activity of thiobarbituric acid reactive substances (TBARS) was measured using the protocol of Yagi and Medicine (1982). The catalase (CAT) analysis was done using the standard protocol of Sinha (1972). The activity of superoxide dismutase (SOD) was calculated by following Beauchamp and Fridovich (1971) practice. The activity of glutathione (GSH) was assessed via the method developed by Carlberg and Mannervik (1975). Following Buege and Aust (1978), the activity of peroxidase (POD) in a homogenised mixture was assessed by the production of TBARS and quantified as malondialdehyde (MDA) equivalents.

Comet assay

The comet assay protocol described by Singh *et al*. (1988) was used. The slides were analysed using a fluorescence microscope at a magnification of 400X. The Comet IV software was employed to calculate the score of the comet (Chaubey *et al*., 2005).

Statistical analysis

Statistical analysis was done by applying

ANOVA to Graphic Prism Version 9.3.1 software at p< 0.05 level of significance. Graphic Prism Version 9.3.1 was used for graphical representation.

RESULTS

Effects of MeP on hematological parameters

The hematological parameters of *L. rohita* after exposure to MeP are shown in Table I. In the current study, increased red blood cells count, HGB concentration, and hematocrit were seen following exposure to various MeP concentrations to cope with increased oxygen demand. Hematological indicators such as WBC, MCV, MCH, MCHC, and platelet count increased significantly after 21 days of exposure to different concentrations of methylparaben.

Biochemical parameters

The results of blood glucose, aspartate aminotransferase (AST), alanine transaminase (ALT), and blood serum total protein, globulin, albumin are shown in Table II. Results of all these biochemical parameters showed significant increase after exposure to medium and high doses.

The lipid profile of *L. rohita* after treatement with MeP is shown in Table II. The results showed that after exposure to different concentrations of MeP, the lipid profile, including cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and VLDL cholesterol, increases significantly.

Table I. Effect of different concentrations of methylparaben on hematological parameters of *L. rohita*. All values are mean ± SD at p<0.05 level of significance.

Parameters	Control (0mg/L)	Low (16mg/L)	Medium (22mg/L)	High (33mg/L)
HGB (g/dl)	2.127±0.53	2.183±0.45	3.06±0.17*	5.01±0.75**
WBC (x10^3/µL)	62.7±7.05	73.77±2.65*	87.70±6.26**	109.8±5.45**
RBC (x10^6/ µL)	0.97±0.13	1.11±0.12	1.18±0.079	2.31±0.79**
HCT (%)	9.08±0.27	9.92±0.96	12.23±1.23*	19.88±3.03**
MCV (FL)	65±6.08	68.33±5.68**	86.67±3.67**	127.7±6.11**
MCH (pg)	29.67±2.51	39.33±4.16	172.2±27.60**	258.7±31.21**
MCHC (g/dl)	20.67±4.62	27.15±2.96**	233.7±9.45**	306±4.58**
PLT (x103/ µL)	198±6.55	238±6.55	285.3±6.65**	834±67.82**

All values are mean ± SD at p<0.05 level of significance. *(* = p≤ 0.05, **= p≤ 0.01, ***= p≤ 0.001).*HGB, haemoglobin; WBC, white blood cell; RBC, red blood cell; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets.

Table II. Effect of different concentrations of methylparaben. on suger, liver function enzymes, blood protein, and lipid profile of *L. rohita*. Values are mean ± SD at p<0.05 level of significance.

Parameters	Control (0mg/L)	Low (16mg/L)	Medium (22mg/L)	High (33mg/L)
Sugar (mg/dl)	65.67±3.05	68.33±4.50*	123.0±16.70***	306.7±27.15***
ALT (U/L)	22.33±2.08	37.67±3.51*	65.33±6.65**	153.0±3.61***
AST (U/L)	105.0±5.0	131.7±7.03*	141.7±6.11*	153.0±2.65**
Total protein (g/dl)	2.267±0.25	3.183±0.73*	5.030±0.41**	9.237±0.21***
Albumin (g/dl)	0.7433±0.25	1.410±0.31*	2.407±0.35**	4.217±0.80***
Globulin (g/dl)	0.5933±0.12	1.480±0.57*	3.663±0.68***	4.683±0.83***
Cholesterol(mg/dl)	58.67±3.05	87.33±18.77**	240.7±61.53***	398.7±27.06***
Triglycerides(mg/dl)	139.3±48.63	221.7±34.99**	326.0±39.34***	732.0±9.0***
HDL Cholesterol (mg/dl)	16.67±3.51	31.33±4.93*	59.00±11.53***	84.67±9.71***
LDL Cholesterol (mg/dl)	41.67±10.41	76.00±4.73**	130.3±16.80***	228.0±28.51***
VLDL Cholesterol (mg/dl)	22.67±3.22	48.67±7.64**	87.67±5.51***	148.7±20.40***

All values are mean ± SD at p<0.05 level of significance. *(* =p≤ 0.05, **=p≤ 0.01, ***=p≤ 0.001).* ALT, alanine aminotransferase; AST, aspartate transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

Oxidative stress

The oxidative stress levels of ROS and TBARS were assessed in fish liver, kidney, and gills. The levels of the antioxidant enzymes GSH, SOD, CAT, and POD are depicted in Figures 1, 2 and 3. After subjecting *L. rohita* to methylparaben concentrations of 16mg/L, 22mg/L, and 33mg/L for 21 days, the levels of ROS and TBARS in the liver, gills, and kidney exhibited a notable and statistically significant rise when compared to the control group. The levels of all antioxidant indicators, namely GSH, SOD, CAT, and POD, exhibited a substantial decrease ($p \leq 0:05$) in the liver, gills, and kidneys.

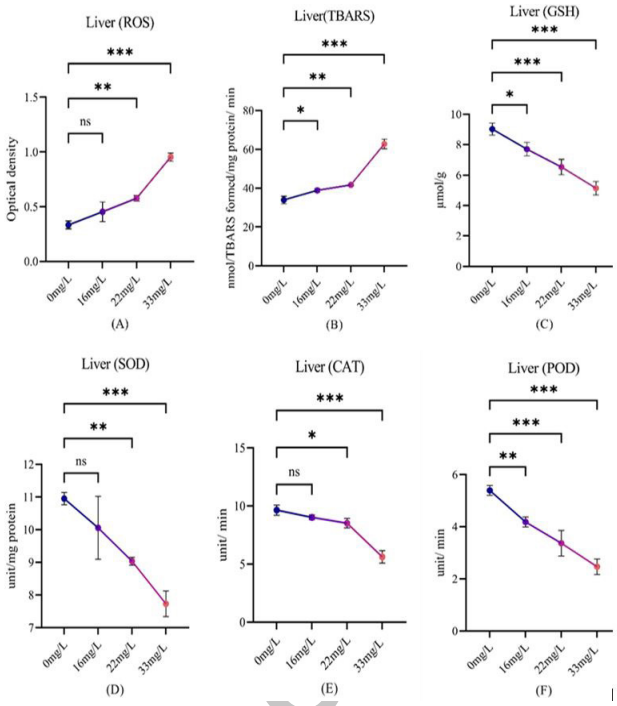

Fig. 1. Effect of different concentrations of methylparaben i.e. 0mg/L, 16mg/L, 22mg/L, and 33mg/L on ROS (A), TBARS (B), GSH (C), SOD (D), CAT (E), POD (F) activities in liver of fish. All values are mean ± SD at $p<0.05$ level of significance. (*= $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$). ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substance; GSH, glutathime; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase.

Histological analysis

Figures 4, 5, and 6 show the histology of the gills, liver, and kidneys of *L. rohita*. The gill histology of the control group (4a) showed normal morphological structures found in healthy fish, i.e., secondary lamellae coated with simple squamous epithelial cells. Fish exposed to different methylparaben doses (4b-4d) showed lamellar disorganization, lamellar aneurysm, edema, curved gill lamellae, and bone cell deformities.

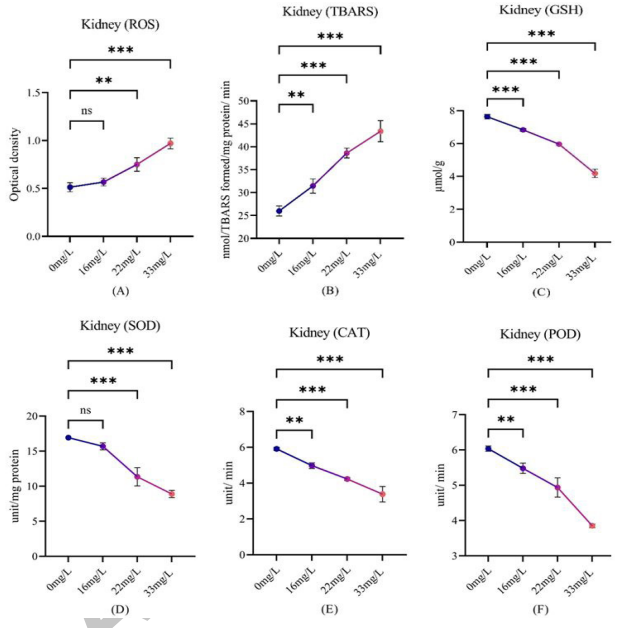

Fig. 2. Effect of different concentrations of methylparaben i.e. 0mg/L, 16mg/L, 22mg/L, and 33mg/L on ROS (A), TBARS (B), GSH (C), SOD (D), CAT (E), POD (F) activities in kidney of fish. All values are mean ± SD at $p<0.05$ level of significance. (* =$p \leq 0.05$, **=$p \leq 0.01$, ***=$p \leq 0.001$). For abbreviations see Figure 1.

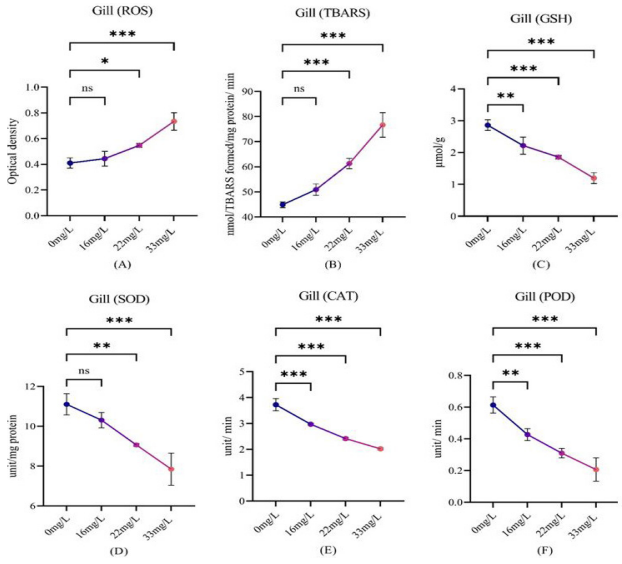

Fig. 3. Effect of different concentrations of methylparaben i.e. 0mg/L, 16mg/L, 22mg/L, and 33mg/L on ROS (A), TBARS (B), GSH (C), SOD (D), CAT (E), POD (F) activities in gill of fish. All values are mean ± SD at $p<0.05$ level of significance. (* =$p \leq 0.05$, **=$p \leq 0.01$, ***=$p \leq 0.001$). For abbreviations see Figure 1.

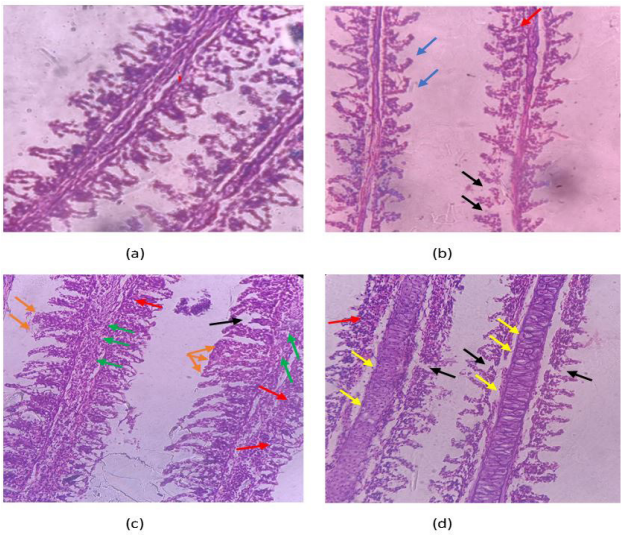

Fig. 4. Effect of different concentrations of methylparaben for 21 days on histological structure of *L. rohita* (H and E stain, 40X). The gills of the control group (A) showed normal histology, while the other treated groups showed lamellar disorganization (black arrow), lamellar aneurysm (red arrow), edema (yellow arrow), curved gill lamella (blue arrow) and bone cell deformities (green arrow). B, 16mg/L; C, 22mg/L; D, 33mg/L.

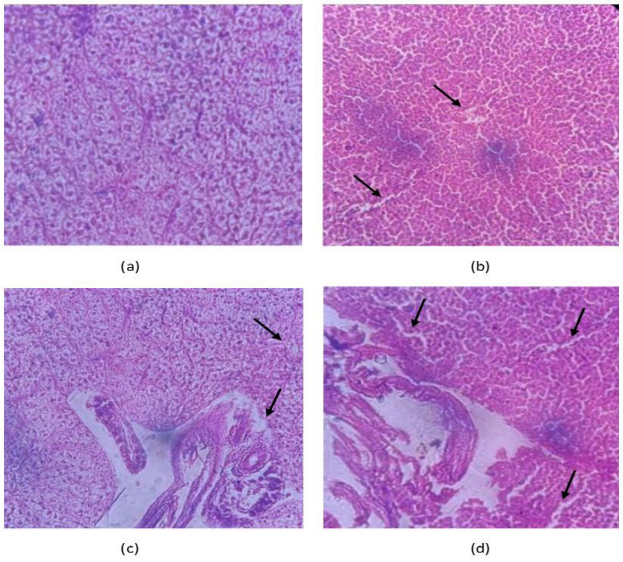

Fig. 5. Effect of different concentrations of methylparaben for 21 days on histological structure of fish liver (H and E stain, 40X). The liver of the control group (A) showed normal histology, while the other treated group's histology showed sinusoidal spaces (black arrow), pyknotic nuclei (red arrow), necrosis (light blue arrow), eosinophilic granules (yellow arrow) and melano macrophages (green arrow). B, 16mg/L; 22mg/L; 33mg/L.

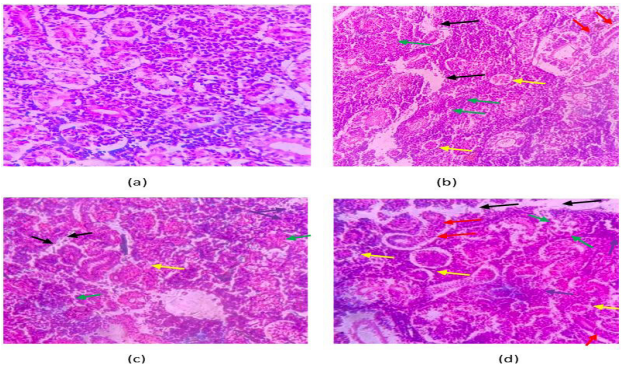

Fig. 6. Effect of different concentrations of methylparaben for 21 days on histological structure of fish kidney. The kidney of the control group showed normal histology, while the other treated groups 16mg/L, 22mg/L, and 33mg/L histology showed sinusoidal spaces (black arrow), elongated tunules (red arrow), damaged paranchyma cells (green arrow), cluster nuclei formation (purple arrow), and melano macrophages (yellow arrow).

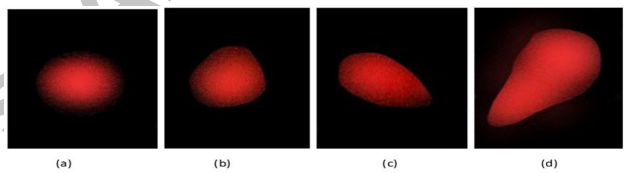

Fig. 7. Genotoxicity of methylparaben in RBCs. Comet assay: (a) reference control olive and (b-d) reference treated comet.

Histology of the liver of the control group (5a) showed no change in *L. rohita*. The hepatic parenchyma showed sinusoid-limited hepatocyte plaques. The hepatocytes have polygonal shapes, granular cytoplasm, and rounded nuclei. They were arranged in cord form. Fish from low to high doses (5b-5d) showed sinusoidal spaces, pyknotic nuclei, narcosis, eosinophilic granules, and melano-macrophages.

The typical arrangement of glomeruli in the kidney of *L. rohita* was observed during the histology of the control group (6a). The histology of fish after being exposed to different concentrations (6b-d) showed sinusoidal spaces, elongated tubules, damaged parenchyma cells, cluster nuclei formation, and melano-macrophages.

Genotoxic effect of methylparaben

Alkaline comet assay were performed to evaluate the potential of methylparaben to cause genotoxicity (Fig. 7). Blood cells of *L. rohita* were used for the comet assay. The DNA damage in blood cells after exposure to MeP was observed in an alkaline comet assay. This damage gradually increased with the increase in the MeP exposed

concentration. The percentage of tail DNA observed at 13.49±0.74 after exposure to the high dose, compared with the control group at 1.21±0.25, reveals a significant difference. By increasing concentrations of ethyl paraben in A (0µg/L), B (16mg/L), C (22mg/L), and D (33mg/L), the difference increases significantly. Olive tail moment (OTM) shows substantial variations as well; at the high dose (33mg/L), it was 7.60±2.50, whereas at the control (0mg/L), it was 0.66±0.16. Tail DNA and olive tail moment (OTM) increase with the increase in the concentration of MeP (Fig. 8).

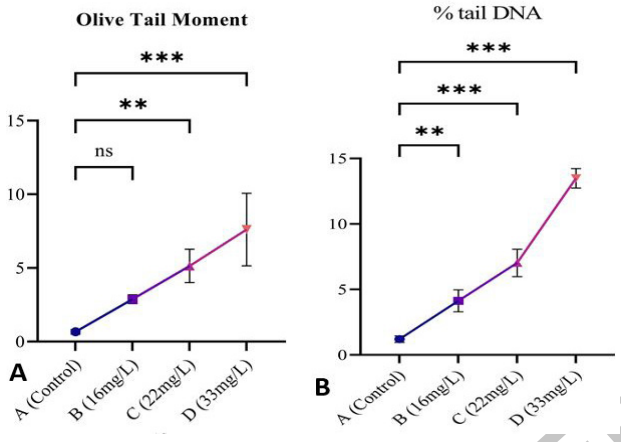

Fig. 8. (e) % of tail DNA and (f) Olive tail moment. All values are mean ± SD at p<0.05 level of significance. (* = p ≤ 0.05, **=p ≤ 0.01, ***=p ≤ 0.001).

DISCUSSION

Aquatic toxicity became major problem in recent years due to large amount of emerging contaminate secreted unchecked in aquatic environment. Most of these toxicant have anthropogenic effects on human and are carcinogenic. These toxicant causing environmental disaster and have created localized and regional problem in worldwide such as parabens (Adeogun and Aina Olubukola, 2012; Adeogun *et al*., 2011; Chapin and Vitousek, 2011).

The parabens including MeP have been used extensively in pharmaceuticals and personal care products due to their antifungal and antibacterial action. Due to their extensive uses has prompted concerns about their influence on humans and the environment. They are present in soil, sludge, variety of organisms, aquatic organisms and surface water. They are raising concern about their potential harms and adverse effects on variety of organisms including fishes and human. Parabens are very toxic to aquatic organisms (Nagar *et al*., 2020).

The hematological changes in *L. rohita* can be understood as adaptive responses that enhance the ability to carry oxygen and ensure efficient gas exchange. The mentioned modifications have been documented by Sweilum (2006) and Gaber *et al*. (2013). The spleen and liver stimulate the production of red blood cells in low oxygen conditions to make up for the higher need for oxygen in the outer tissues (Gaber *et al*., 2013). The current study found elevated levels of red blood cell count, HGB concentration, MCH, MCV, MCHC, and Hct following exposure to varying doses of methylparaben. This response was observed as a means to meet the increased demand for oxygen. Inkaya *et al*. (2022) observed that parabens had a positive impact on hematology in male rats, resulting in increased levels of RBCs, HGB, and Hct. Park *et al*. (2023) also noted comparable elevations in RBCs, HGB, Hct, MCH, MCV, and MCHC. The present investigation revealed a substantial reduction in platelet count as the concentration increased from low to high levels. Exposure to MeP leads to an increase in platelet count, resulting in inflection when compared to the control group. The rise in platelet count results from thrombocytosis, a disorder characterized by an elevated concentration of platelets during an infectious state (Hafsari and Ridha, 2022). An epidermal rash was seen while collecting blood from the fish.

MeP, as an endocrine disruptor, disturbs the normal functioning of various processes and causes alterations in the immune system, lipid homeostasis, glucose levels, and thyroid functioning (Błędzka *et al*., 2014). Hormones such as testosterone and estrogen are derived from lipids. The lipid profile results indicate a notable elevation in cholesterol, HDL cholesterol, LDL cholesterol, and VLDL cholesterol levels following exposure to MeP compared to the control group. Kim and Chevrier (2020) observed a consistent elevation in both male and female cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and VLDL cholesterol levels. Lipids cannot decompose within the bloodstream and require transportation to and from cells using LDL and HDL. High-density lipoprotein cholesterol (HDL-C) facilitates cholesterol transportation from the arteries to the liver. Hepatic impairment can elevate serum cholesterol levels (Gupta *et al*., 2008). Triglycerides function as a reservoir of readily available and stored energy. Fish exhibit elevated triglyceride levels due to increased energy requirements during periods of stress. MeP exposure leads to elevated blood glucose levels. Bellavia *et al*. (2019) observed comparable blood glucose level elevations across pregnant women exposed to factors that increased their susceptibility to gestational diabetes.

This study assesses the nephrotoxicity and hepatotoxicity of MeP. ALT, AST, total protein, albumin, globulin, and bilirubin are valuable biomarkers for liver

function, while urea and serum creatinine are good indicators of kidney function. These biomarkers can effectively detect renal and hepatic toxicity. The study observed increased liver enzymes ALT and AST levels with increasing doses of MeP exposure, indicating changes in serum biochemistry. Compared to the control group, there were significant elevations in total protein, albumin, and globulin levels. The current study evaluates the toxic effect of MeP on hepatic tissues. For the liver, ALT and AST, blood sugar, total protein, albumin, and globulin are promising biomarkers for hepatic toxicity. These results were supported by the findings of Beriry *et al*. (2022) and Darbre and Harvey (2008). The elevation of liver enzymes directly results from hepatocyte liver damage induced by MeP. The liver mainly filters harmful substances and generates antioxidant enzymes like catalase, which decomposes hydrogen peroxide (Friedman, 2010). Beriry *et al*. (2022) found that administering parabens increases urea and creatinine levels while also causing a decrease in glomerular filtration. This supports the notion that elevated urea and creatinine levels are associated with paraben administration. Creatinine is a metabolic byproduct of muscle creatine that is eliminated from the body through the kidneys via urine. When renal function is impaired following an injury, these functions become dysfunctional, and their level is elevated. Following exposure to MeP, hepatic cells have impaired metabolism of hem, resulting in elevated levels of bilirubin (BUN). These results are consistent with the data documented by Beriry *et al*. (2022).

The histological changes observed in this investigation validate the biochemical modifications. Gills in fish are the main organs that come into contact with water and play a role in osmoregulation, breathing, and excretion. Therefore, they continue to be the main focus for harmful substances. The histology analysis of the gills demonstrated that methylparaben exposure resulted in lamellar disorganization, fusion of primary lamellae, lamellar aneurysm, hyperplasia of epithelial cells, and abnormalities in the bone cells of secondary lamellae. Camargo and Martinez (2007) also documented changes in gills, such as the raising of epithelial cells, an increase in cell growth, and the merging of epithelial cells. Our present work is supported by the findings of Beriry *et al*. (2022), who observed a comparable change in the histology of the gills following exposure to paraben.

The liver is the main organ responsible for detoxifying and metabolizing harmful substances in the body. Consequently, the liver is highly susceptible to water toxicity, as Zhang *et al*. (2023) stated. A healthy liver typically consists of hepatocytes with uniform shapes, cytoplasmic vacuolation, and a nucleus positioned laterally. Hepatocytes cytoplasm contains lipids and glycogen stored in vacuoles, which are utilized for regular metabolic processes. Under stressful circumstances, the glycogen stored within hepatocytes is exhausted due to its role as a reserve for glucose. Hepatocytes are created due to sinusoidal spaces (Behrens *et al*., 2023). The rise in vacuolization signifies metabolic harm resulting from the harmful effects of MeP. The liver histology exhibited pyknotic nuclei, eosinophilic granules, sinusoidal gaps, necrosis, and melano-macrophages.

The kidney, as a crucial organ involved in the control of osmotic balance and the elimination of waste, is highly susceptible to hazardous substances (Thophon *et al*., 2003). Water toxicants commonly result in tubule degeneration, capillary dilation, and parenchyma cell degeneration in the kidney (Irnidayanti *et al*., 2023). Analysis of kidney histology in *L. rohita* revealed several notable changes, including glomerular expansion and the absence of Bowman's capsule, hypertrophied nuclei in tubular cells, sinusoidal spaces, cluster nuclei formation, presence of melano-macrophages, and damaged parenchyma cells. These changes were observed in comparison to the control group, which exhibited a typical arrangement of the glomerulus in the kidney of *L. rohita*.

The enzymatic fluctuations have been observed which indicates the biochemical alterations in fish after exposure to methyl paraben. The damaged cause by ROS and TBARS such as O_2^{-}, H_2O_2 and OH^{-}, is evaluated through oxidative stress. The bodies' enzymatic and non-enzymatic antioxidant system consists of SOD, catalase, POD, and GSH, which prevent the body from damage caused by free radicles. Certain xenobiotics including methylparaben prompting oxidative stress due to toxic effect, due to excessive oxidative activity free radicles accumulates. The accumulation of free radicles is due to inadequate antioxidant activity which damages large number of cellular components like DNA, carbohydrates (Shah *et al*., 2022).

TBARS assay is a primary biomarker for lipid peroxidase in tissue damage. TBARS assay measures malondialdehyde (MDA) (Akbari *et al*., 2023). An increased MDA is a biomarker for lipid peroxidase and, hence, oxidative stress caused by certain xenobiotics (Garcia *et al*., 2020). Parabens, including MeP, are lipophilic and can easily cross the plasma membranes. Inside cell increases MDA level, which in turn increases ROS. ROS accumulation due to overproduction or failure of the antioxidant system causes oxidative stress (de Almeida *et al*., 2022).

Accumulations of ROS and failure or decreased antioxidant system change the redox potential of the plasma membrane and cause oxidative stress in soft

tissues. MeP exposure elevates lipid peroxidase, which was observed in different tissues of fish. Similar elevations of lipid peroxidase were previously recorded by Shah and Verma (2011) and Silva *et al*. (2018).

GSH is a main antioxidant enzyme that works as a free radical scavenger and prevents damage caused by reactive oxygen species, lipid peroxidase, and free radicals (Carmo *et al*., 2022). Significantly decreased levels of GSH have been observed after methylparaben exposure. Our results are supported by Tastan *et al*. (2020) and Shah and Verma (2011). The decrease in GSH levels indicates a high concentration of free radicles. The amount of free radicals exceeds the scavenging capabilities of GSH. The enzymatic antioxidant system, including catalase, SOD, and GSH, works as the first line of defense ROS induced damage (Czlapka-Matyasik and Gut, 2023). A significant decrease has been observed in all of these antioxidants, SOD, GSH, and catalase, which is due to the protein oxidation caused by MeP. Increased amounts of peroxidase interact with enzymes, reducing enzyme activity and causing histidine residue alterations and protein crosslinking (Griffiths *et al*., 2002). The results of oxidative stress and antioxidant enzymes show that the antioxidant system's free radical scavenging activity has decreased. Thus, methylparaben induces oxidative stress in *L. rohita*.

The comet assay in *L. rohita* demonstrated DNA strand breaking, as evidenced by measures such as Olive tail moment (OTM) and percentage tail DNA to blood cells. The comet assay results confirmed that MeP is genotoxic. Previous research by Güzel-Bayülken *et al*. (2019) assessed the genotoxicity of parabens in human lymphocytes, which is consistent with our findings. Similarly, Martín *et al*. (2010) assessed the genotoxicity of parabens in monkey liver cells. Another study by Akmal *et al*. (2024) also showed similar DNA damage in fish blood cells when exposed to paraben.

CONCLUSION

Our study indicates that exposure to methylparaben has detrimental effects on the DNA and overall health of fresh water rohu (*Labeo rohita*). After a comprehensive evaluation, the hematological and biochemical indicators showed notable physiological disruption. Histological tests revealed that Methylparaben damaged kidney, gill, and liver tissues.

Elevated AST and ALT levels indicate damage to liver cells, while decreased antioxidant enzyme activity suggests the presence of oxidative stress and a weakened defense system against ROS. MeP has been found to cause damage to the DNA of blood cells, which raises concerns regarding its potential long-term genetic consequence.

These findings indicate that MeP should be tightly regulated to minimize its impact on aquatic environments. Further investigation is required to comprehend and mitigate its toxicity, and these studies demonstrate this necessity. By increasing public knowledge about the potential risks associated with methylparaben exposure, we may safeguard aquatic environments and the organisms inside them.

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Ethical statement

The University of Okara's Ethical Research Committee (ERC) granted ethical approval for fish handling.

Statement of conflict of interest

The authors have declared no conflict of interest.

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