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Toxicity Assessment of Synthetic Polycyclic Musk: Survival, Oxidative Stress and Histopathology of Freshwater Fish *Labeo rohita*

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ABSTRACT
Synthetic polycyclic musk compounds (PMCs) have been used in many industries as fragrance carriers to boost people's attractiveness found in all environmental compartments due to their uncontrolled use and high lipophilic nature, causing adverse effects on aquatic ecosystems and human health. The potential toxicity, bioaccumulation, and persistence of synthetic polycyclic musks have raised significant concerns regarding their impact on aquatic environments. The present research work investigated the toxicological responses of *Labeo rohita* induced by polycyclic musk compound glaxolide (HHCB). The acute toxicity evaluations regarding 96-h LC_{50} as well as lethal concentrations were determined as 547.86µg/L and 911.81µg/L respectively for experimental fish. After administration of sub-lethal doses of HHCB during 90 days chronic exposure, dose and time dependent oxidative stress regarding superoxide dismutase (SOD) and catalase (CAT) along with the histopathological changes in various organs of fish were evaluated. HHCB induced significant ($P<0.05$) dose and time dependent oxidative stress in organs of fish examined during chronic exposure. SOD enzyme activity of exposed fish was increased up to 60 days and then decreased while CAT enzyme activity was increased up to 15 days and then decreased afterward, till the end of the trial (90 days). Significant ($P<0.05$) alterations in the histopathology of examined organs were noted and all organs experienced detrimental changes. Meanwhile, organ tissues from unexposed groups exhibited normal and intact tissue structures. In conclusion, HHCB have substantial toxic impacts on *L. rohita* and increase with prolonged exposure duration.

Keywords: Glaxolide**,** *Labeo rohita***,** Acute toxicity, Chronic toxicity**,** Oxidative stress, Histopathological changes

1. Introduction

The concentration of emerging pollutants like persistent organic chemicals from personal care products (PCPs) in the aquatic environment has seen a significant and concerning rise worldwide in recent decades (Diao et al., 2024; Li et al., 2024). Synthetic musk compounds (SMCs), particularly polycyclic musk compounds (PMCs), are a significant type of aquatic pollutants often found in PCPs as additives. Since their novel production as fragrance alternative for naturally present musk, SMCs have been used in scents, detergents, fragrant sprays as well as candle waxes, etc., to increase people's attractiveness (Chen et al., 2024; Li et al., 2020; Peck and Hornbuckle, 2006). Currently, the most widely used synthetic musks are polycyclic ones which overlook the global marketplace, comparatively exceeding 90% (Guo et al., 2013). One of the most widely utilized PCMs is HHCB (1,3,4,6,7,8, hexahydro-4,6,6,7,8, hexamethylcyclopenta-(g)-2-benzopyran), market identity galaxolide. Since due to its combined yearly output the US Environmental Protection Agency (EPA) has included this substance on its list of overused chemicals (Vallecillos et al., 2015).

Consequently, SMCs has been discharged throughout the natural environment via the whole lifecycle of consumer products and solid waste; including manufacture, transportation, consumption, and treatment. Therefore, they are often found as contaminants in wastewater, surface water, and sediments (Balci et al., 2020; Chane et al., 2023; Couteau and Coiffard, 2010; Homem et al., 2015). Much research has been done regarding presence of synthetic musks in different components of environment, including effluent and fresh water, sewage detritus, biota samples, and even human samples (Chae et al., 2023; Diao et al., 2024; Moon et al., 2012). Solubility of PMCs for water varies widely, with high log K_{ow} in the range of 5.71 to 6.31, showing the lipophilic nature of these compounds make them bioconcentrate in organisms (Safford et al., 2015). Nevertheless, several investigations have shown the detrimental consequences caused by PMCs in aquatic species. HHCB found to be induce disruption of the antioxidant enzymatic system, disequilibrium of sex hormones, DNA damage, deterioration of the cytoplasm in hepatocytes and gills tissues which eventually leads to necrosis and infiltration of inflammatory cells (Ding et al., 2023; Ebrahimi and Taherianfard, 2010; Ehiguese et al., 2020; Li et al., 2013). Additionally, musk chemicals negatively impact fish and copepod growth and development (Blahova et al., 2018).

According to several case studies from Pakistan, a range of various PCPs are present in urban effluent, surface-water of canals, and all of their debris due to the inadequate wastewater treatment facilities (Ashfaq et al., 2019). Carps including *Labeo rohita* (Rohu), are a significant aquatic food source, with Pakistan's main commercial fish species. Unfortunately, the natural stock of this valuable fish species has diminished due to emerging pollutants (M et al., 2017). Very insufficient data about the LC_{50} and lethal toxicity of PMCs for *L. rohita* is acknowledged. Therefore, it is crucial to investigate the presence, destiny, and related ecological concerns regarding PMCs in the aquatic environment. Furthermore, determining the chronic impact of waterborne PMCs that could affect the antioxidant enzyme activity and histology of *L. rohita* was complemented the current research.

2. Materials and methods

2.1. Test chemical

The PMC, galaxolide (HHCB) (CAS No. 1222-05-5) was obtained from Sigma-Aldrich, UK. Fresh stock solution of HHCB was prepared in dimethylsulfoxide (DMSO) (CAS No. 67-68-5), and subsequent dilutions of the stock solution were prepared to create the working solutions

2.2. Test organism.

For current experiment Ninety-days-old *L. rohita* fingerlings of equal weight and length were kept at hatchery of Fisheries Research Farm, University of Agriculture, Faisalabad, Pakistan. Fish were acclimatized for fifteen days in cemented tanks having aerated tap water prior to the study. The fish were fed with commercial pelleted feed during the acclimatization stage.

2.3. Experimental layout

After acclimatization, ten fish were introduced into each glass aquarium having 50 litres of aerated tap water which were subsequently separated into different treatment and control groups to study the toxicological effects of HHCB on fish during acute as well as chronic studies. Aeration was ensured to the tanks during whole trial by the aid of capillary and automated pump system to avoid the deficiency of dissolved oxygen. Water temperature, pH and all other water quality parameters were recorded and maintained at optimal range throughout the experiment. Twelve hours of light and dark photoperiods were also maintained.

2.4. Acute toxicity assessment

Fish were exposed to 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 µg/L concentrations of HHCB to check the acute toxicity (LC_{50} and lethal concentrations). Three experimental replicates were exposed to all mentioned concentrations of HHCB for 96 hours and one group was set as the control group without exposure to toxicant. Fresh toxicant solution was introduced every 24 hours to maintain the fixed levels. Fish were remained deprived of feed throughout acute trail to avoid toxicant absorption in solid feed and fecal matter. Mortality of fish was recorded and noted on daily basis and dead fish were removed regularly to avoid contamination.

2.5. Oxidative stress biomarkers

For the evaluation of dose and time dependent oxidative stress induced in different organs (gills, liver, kidney, heart and muscles), fish were exposed to three sub-lethal doses ($1/3^{rd}$, $1/5^{th}$ and $1/7^{th}$ of LC_{50}) of HHCB for ninety days. After the dissections, tissue sample from above mentioned organs were isolated fortnightly (at fifteen, thirty, forty-five, sixty, seventy-five and ninety days) to check catalase (CAT) and superoxide dismutase (SOD) enzyme activities. All organs of *L. rohita* were homogenized in saline phosphate buffer for approximately 15 min using a homogenizer. The resulting tissue mixtures from homogenization process were centrifuged at 10,000 revolutions/minute for fifteen minutes at 4°C. The supernatants were separated and CAT and SOD activities were accessed by following the methods of (Weydert and Cullen, 2010).

2.6. Histopathological biomarkers

Tissue samples from gills, liver and muscles of fish were obtained at the end (after 90 days) of chronic phase afterward exposing *L. rohita* to two sublethal doses viz. $1/5^{th}$ and $1/3^{rd}$ of LC_{50}, of HHCB to determine the histopathological changes. Sample tissues were fixed in 10% formalin, dehydrated in ethanol ascending grades, and then embedded in paraffin. 4-5 µm tissue slices were prepared using microtome. After hematoxylin and eosin staining tissues slices were examined under a light microscope and micrographs were captured to examine histopathological alterations.

2.7. Statistical analysis

Tolerance limits (96 hours LC_{50} and lethal concentration) of *L. rohita* for HHCB were analyzed following the probit analysis method of Hamilton et al. (1977). The statistical differences between parameters for each test concentration were investigated by analysis of variance (ANOVA) and further means were compared by post hoc test. Trend analysis was performed to evaluate treatment behavior and the relationship between variables was measured by correlation analysis using R software.

3. Results

3.1. Acute toxicity and mortality

For the determination of acute toxicity viz. median lethal (LC_{50}) and lethal doses of galaxolide (HHCB)

for *L. rohita*, gradually increasing concentrations of PMC were administered to fish. Ten fish were exposed to musk compound in each replicate and increasing mortality rate with increase in concentration was noted showing dose dependency (Fig. 1). Mean 96-hr LC_{50} and lethal concentration values of HHCB for *L. rohita* was recorded at P<0.05 with 95 percent confidence interval (Table 1). While physicochemical water quality parameters of test media were recorded regularly and the correlation between the parameters and concentration of toxicant during acute exposure represented the significant relationships (Fig. 2).

Table 1. Lethal concentrations of HHCB during acute toxicity exposure for *L. rohita*

HHCB Concentration (µg/L)		95% Confidence Interval (µg/L)	Deviance Chi-Square Value	Goodness of fit test, p
Median Lethal Concentration (LC_{50})	547.86±35.79	465.10-615.53	3.93	0.86
Lethal Concentration (LC_{99})	911.81±62.87	814.25-1087.11		

3.2. Oxidative stress biomarkers, catalase (CAT) activity

CAT activity in organs of *L. rohita* exposed to HHCB altered significantly in time and dose dependent manner from the unexposed group (control) during whole chronic exposure (Table 2-3). CAT activity in all fish organs significantly (P<0.05) increased up to 15 days and then decreased while lowest activity recorded after 90 days. In all selected body organs of fish significant fluctuations in trend of SOD enzyme activity was recorded (Fig. 3-7). Highest alterations were recorder in liver while lowest in muscle of *L. rohita*.

Table 2. Dose dependent catalase (CAT) activity (UmL^{-1}) in *Labeo rohita* exposed to galaxolide (HHCB)

PMC Treatment	Organs				
	Gills	**Liver**	**Kidney**	**Heart**	**Muscle**
Control	185.55±0.33 **a**	236.11±0.15 **a**	140.30±0.24 **a**	112.49±0.27 **a**	195.55±0.37 **b**
1/7th of LC_{50}	172.04±18.86 **d**	232.17±11.54 **c**	129.00±17.35 **b**	105.46±7.16 **b**	191.11±6.24 **d**
1/5th of LC_{50}	178.04±19.97 **c**	228.44±16.30 **d**	126.99±18.98 **c**	102.49±9.76 **c**	191.56±7.72 **c**
1/3rd of LC_{50}	184.02±20.36 **b**	234.60±21.50 **b**	122.44±25.32**d**	98.81±13.10 **d**	196.78±5.72 **a**

Table 3. Time dependent catalase (CAT) activity (UmL^{-1}) in *Labeo rohita* exposed to galaxolide (HHCB)

Exposure Duration	Organs				
	Gills	**Liver**	**Kidney**	**Heart**	**Muscle**
15 day	206.07±6.93 **a**	254.06±5.26 **a**	155.96±4.51 **a**	121.38±2.71 **a**	202.07±2.81 **a**
30 day	195.29±7.32 **b**	244.06±7.28 **b**	141.47±2.92 **b**	106.16±2.38 **b**	198.55±1.88 **b**
45 day	183.27±3.65 **c**	238.38±4.01 **c**	131.88±2.63 **c**	101.64±5.10 **c**	195.89±1.00 **c**
60 day	172.22±4.56 **d**	228.16±4.32 **d**	124.19±2.75 **d**	96.95±2.77 **d**	190.51±4.11 **d**
75 day	158.35±6.24 **e**	219.83±2.05 **e**	104.91±7.32 **e**	94.18±4.48 **e**	187.31±5.38 **e**
90 day	153.00±6.44 **f**	205.92±6.97 **f**	98.44±6.67 **f**	93.19±5.65 **f**	184.55±2.30 **f**

Means that do not share a letter are significantly different

3.3. Superoxide dismutase (SOD) activity

During exposure to sub-lethal doses of HHCB for 90 days, SOD activity in organs of *L. rohita* significantly (P<0.05) increased up to 60 days and then decreased up to end of trial. Dose as well as time dependent alterations were recorded (Table 4-5). After liver; gills, kidney, heart and muscles exhibited the high to low degree of alterations respectively. Significant fluctuating trends in all body organs were also recorded (Fig. 8-12).

Table 4. Dose dependent superoxide dismutase (SOD) activity (UmL^{-1}) in *Labeo rohita* exposed to galaxolide (HHCB)

PMC Treatment	Organs Gills	Liver	Kidney	Heart	Muscle
Control	22.00±0.64 **c**	47.70±0.56 **c**	25.62±0.51 **d**	14.35±0.46 **d**	7.22±0.46 **d**
1/7th of LC_{50}	34.46±4.82 **b**	54.12±3.23 **ab**	31.92±3.40 **c**	20.46±3.63 **c**	12.08±2.75 **c**
1/5th of LC_{50}	37.55±5.84 **a**	53.91±3.66 **b**	35.38±4.28 **b**	23.55±4.00 **b**	13.65±3.09 **b**
1/3rd of LC_{50}	37.45±6.37 **a**	54.48±5.67 **a**	37.50±4.42 **a**	27.28±4.96 **a**	17.28±3.12 **a**

Table 5. Time dependent superoxide dismutase (SOD) activity (UmL^{-1}) in *Labeo rohita* exposed to galaxolide (HHCB)

Exposure Duration	Organs Gills	Liver	Kidney	Heart	Muscle
15 day	28.15±0.99 **e**	51.65±0.99 **d**	30.58±1.83 **d**	18.91±1.92 **e**	10.54±1.44 **e**
30 day	33.03±2.13 **d**	53.44±1.05 **c**	33.15±2.10 **c**	22.28±2.75 **d**	12.79±2.24 **c**
45 day	38.7±2.62 **b**	57.58±1.25 **b**	37.55±2.52 **b**	25.22±3.08 **c**	15.71±1.79 **b**
60 day	45.12±3.16 **a**	60.55±2.18 **a**	40.48±3.10 **a**	30.56±3.98 **a**	18.66±2.24 **a**
75 day	38.82±1.79 **b**	53.23±2.28 **c**	37.68±3.35 **b**	26.1±3.41 **b**	16.24±3.20 **b**
90 day	35.10±2.63 **c**	48.61±1.77 **e**	30.15±2.08 **d**	19.48±2.92 **e**	12.06±3.36 **d**

Means that do not share a letter are significantly different

3.4. Histopathological biomarkers, Histopathology of gills

HHCB high and low dose treated gill tissue exhibited significant damage ($P<0.05$) in form of club shaped primary lamella, epithelium necrosis, degenerated filaments, fused and spike lamella. Hypertrophy, inter-luminal debris, fusion of secondary lamella, ruptured lamellar epithelium and dilations of epithelial margins was also observed. Control group without any treatment of HHCB showed normal inter-filament lamellar spaces, healthy pillar and mucous cells and visible primary and secondary lamella (Fig. 13).

3.5. Histopathology of liver

Significant damage ($P<0.05$) induced in HHCB treated liver tissue from low and high dose showed clear necrosis, pyknotic nuclei, congestion, high vacuolar degenerations, vacuolization and melanomacrophagy was also observed. There was moderate (in low dose) and high (in high dose) degree of hyperemia, hydropic degenerations, granular degeneration, swelling and irregularity in shape of hepatocytes was observed. Hepatic vein damage was also occurred (Fig. 14).

3.6. Histopathology of muscles

HHCB induced significant ($P<0.05$) damage in treated muscle tissue from low and high dose showed shortening and high (in high dose) and low degree (in low dose) of degeneration of muscle bundle. Inter-luminal debris, Intramuscular oedema, cellular necrosis, dystrophic damage, vascular deformation and partial as well as complete vascular damage was also observed. Vascular necrosis and muscle fragmentation of intact structure was observed in low and high dose HHCB treated groups (Fig. 15).

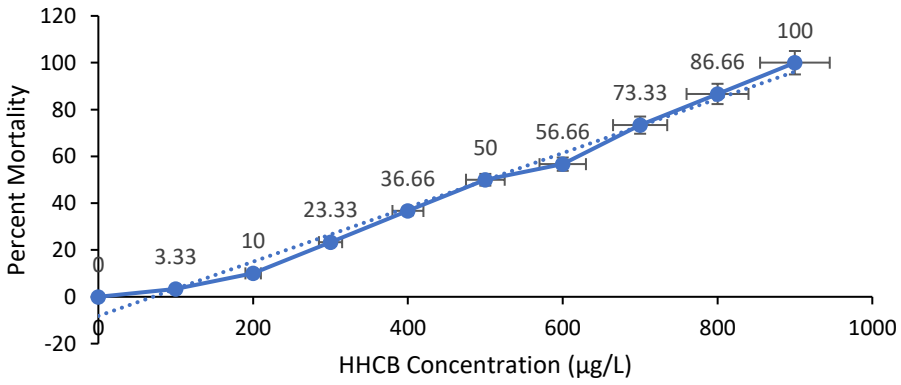

Fig. 1. Percent mortality of *L. rohita* during acute exposure of HHCB

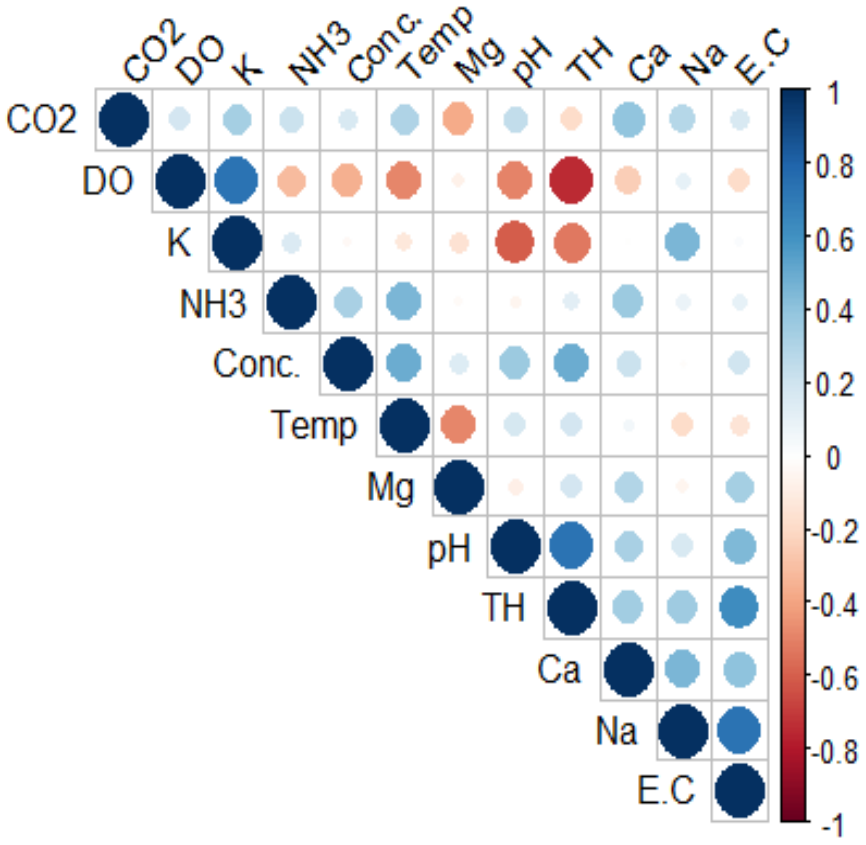

Fig. 2. Correlation between concentration of HHCB and physico-chemistry of experimental media Con.=Concentration (µg/L); Temp=Temperature (°C); T.H=Total Hardness (mgL^{-1}); NH_3=Ammonia (mgL^{-1}); DO=Dissolve Oxygen (mgL^{-1}); CO_2=Carbon Dioxide (mgL^{-1}); Na=Sodium (mgL^{-1}); K=Potassium (mgL^{-1}); Ca=Calcium (mgL^{-1}); Mg=Magnesium (mgL^{-1}); EC= Electrical Conductivity ($mScm^{-1}$).

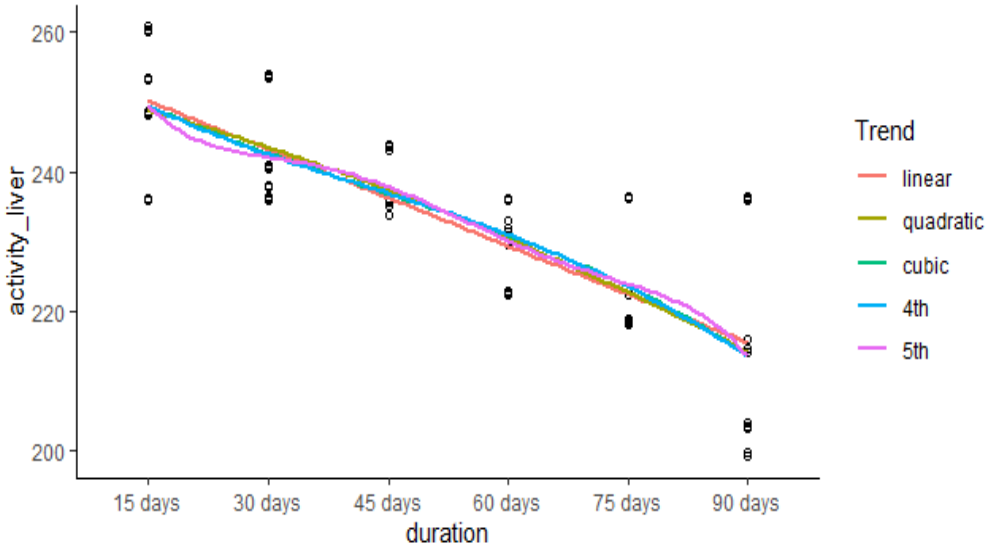

Fig. 3. Fluctuations in trends of HHCB treatment behaviour in catalase (CAT) activity of liver of *L. rohita*

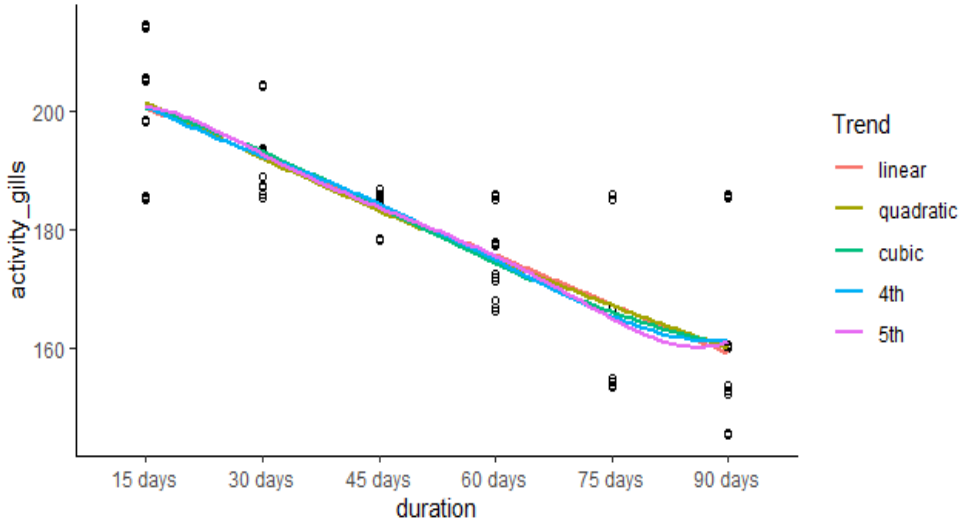

Fig. 4. Fluctuations in trends of HHCB treatment behaviour in catalase (CAT) activity of gills of *L. rohita*

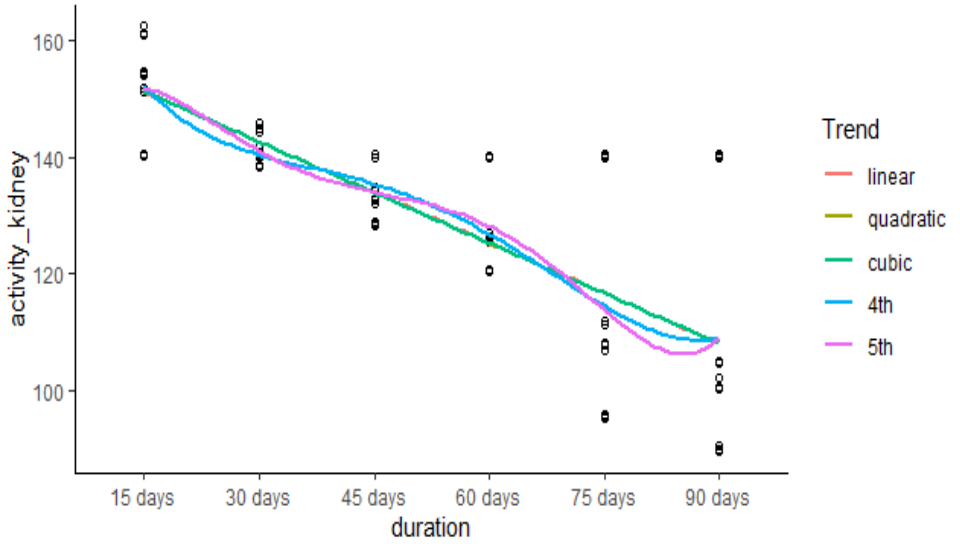

Fig. 5. Fluctuations in trends of HHCB treatment behaviour in catalase (CAT) activity of kidney of *L. rohita*

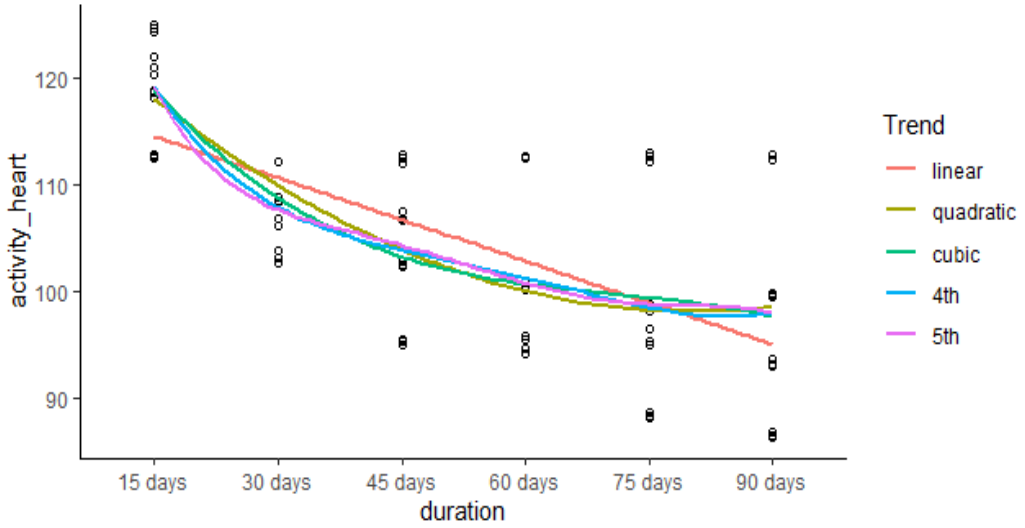

Fig. 6. Fluctuations in trends of HHCB treatment behaviour in catalase (CAT) activity of heart of *L. rohita*

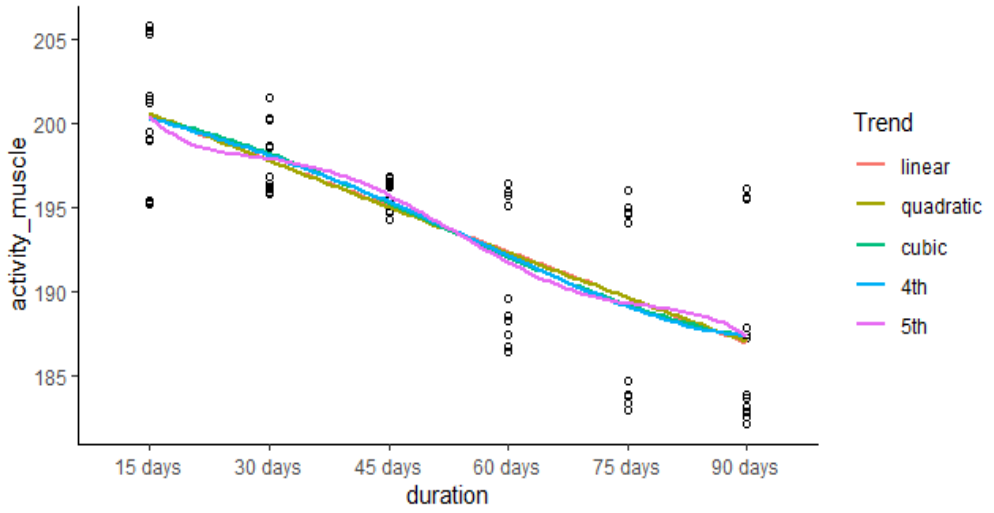

Fig. 7. Fluctuations in trends of HHCB treatment behaviour in catalase (CAT) activity of muscles of *L. rohita*

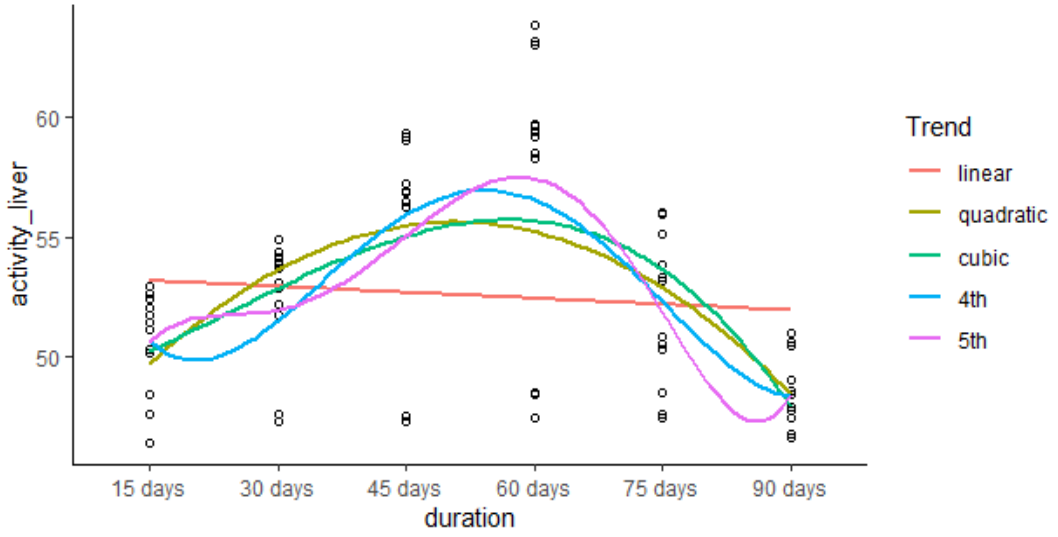

Fig. 8. Fluctuations in trends of galaxolide (HHCB) treatment behaviour in superoxide dismutase (SOD) activity of liver of *Labeo rohita*

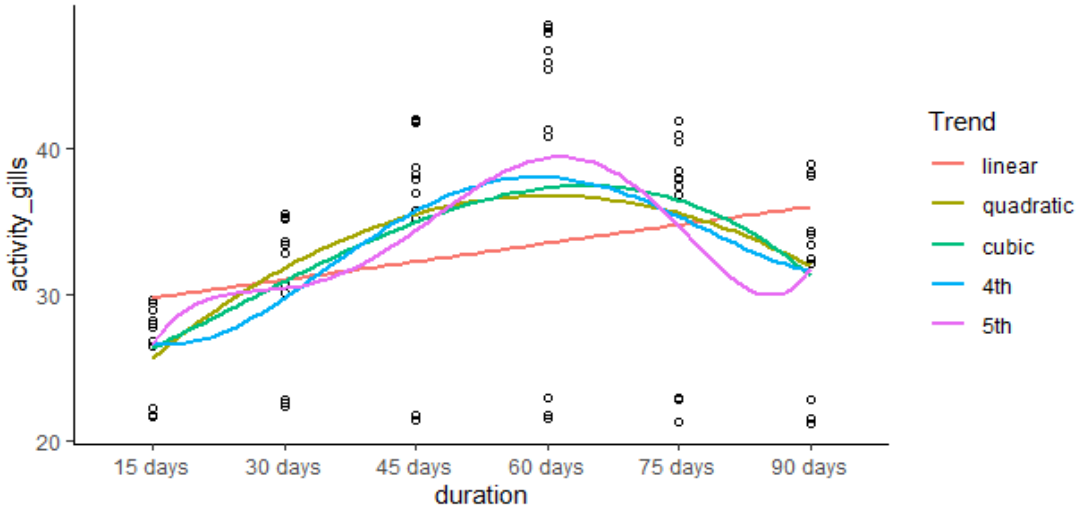

Fig. 9. Fluctuations in trends of galaxolide (HHCB) treatment behaviour in superoxide dismutase (SOD) activity of gills of *Labeo rohita*

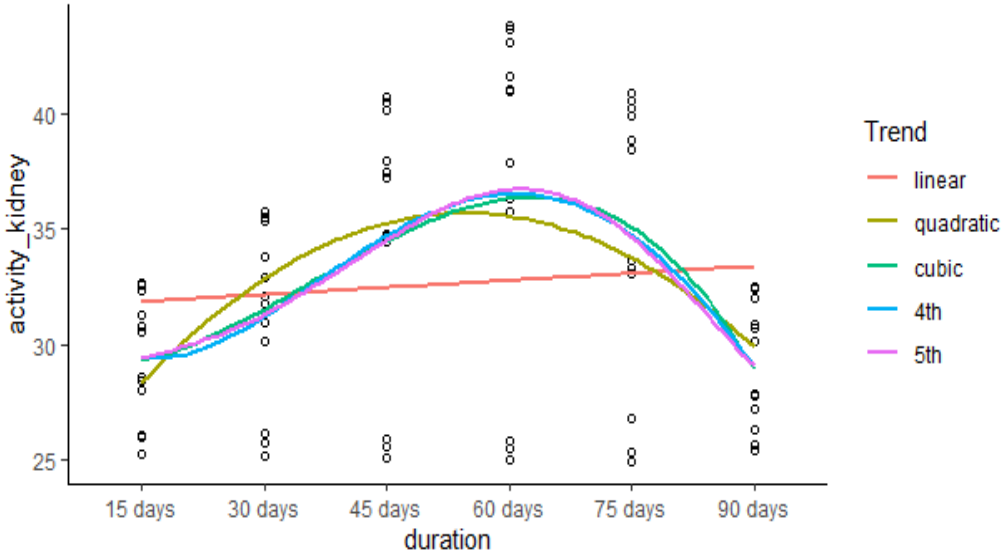

Fig. 10. Fluctuations in trends of galaxolide (HHCB) treatment behaviour in superoxide dismutase (SOD) activity of kidney of *Labeo rohita*

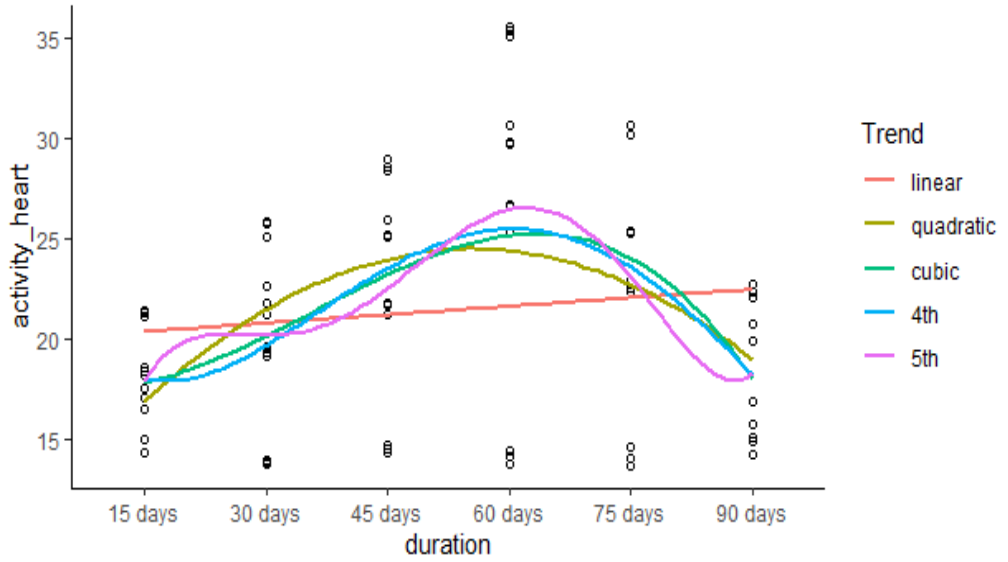

Fig. 11. Fluctuations in trends of galaxolide (HHCB) treatment behaviour in superoxide dismutase (SOD) activity of heart of *Labeo rohita*

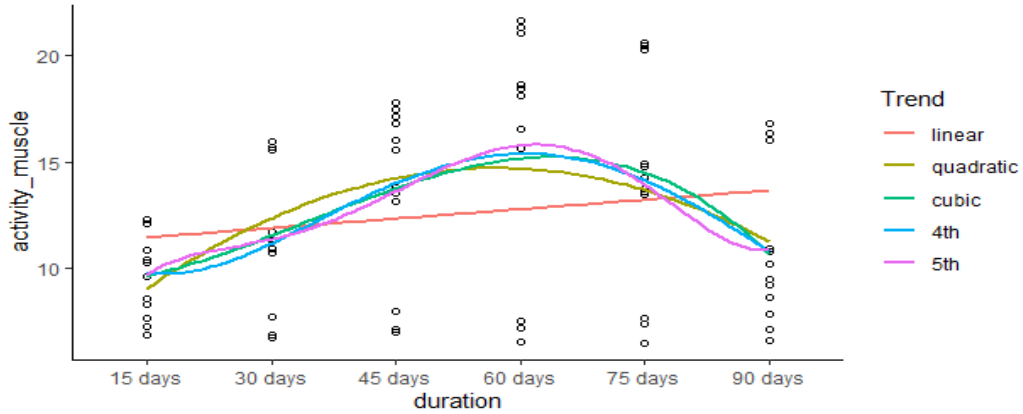

Fig. 12. Fluctuations in trends of galaxolide (HHCB) treatment behaviour in superoxide dismutase (SOD) activity of muscles of *Labeo rohita*

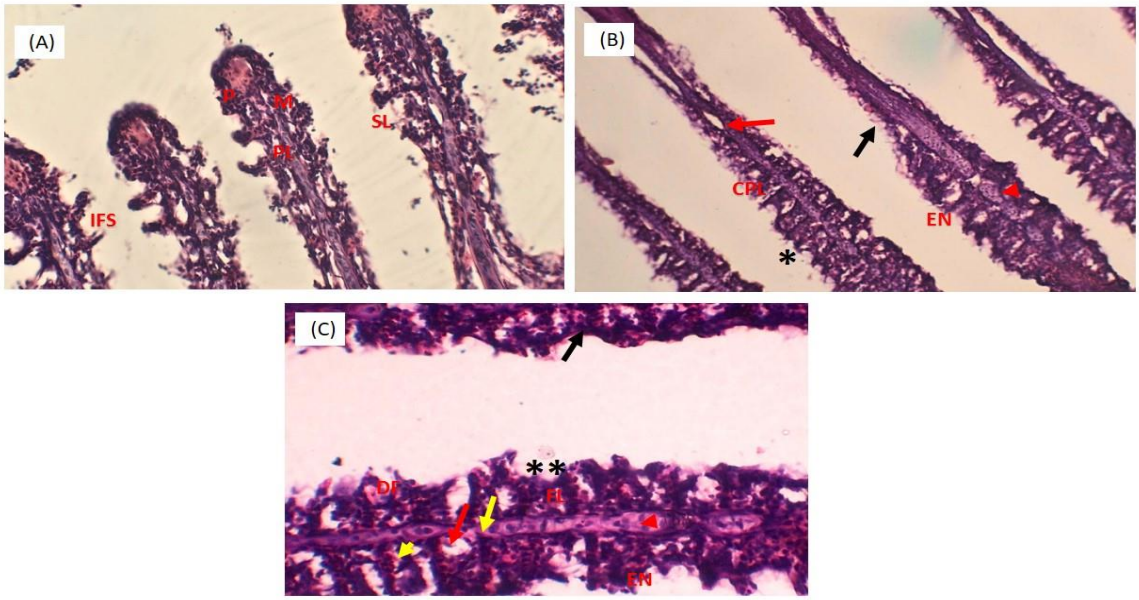

Fig. 13. Histology of HHCB treated *Labeo rohita* gills (A) Control (unexposed); (B) Low dose treated (1/5th of LC_{50}); (C) High dose treated (1/3rd of LC_{50}); IFS=Inter-filament lamellar spaces; P=Pillar cells; M=Mucous cells; PL=Primary lamella; SL=Secondary lamella; *=Significant damage; **=Highly significant damage; CPL=Club shaped primary lamella; EN=Epithelium necrosis; DF=Degenerated filaments; FS=Fused lamella; Black arrow=Necrosis/ Complete necrosis of filaments; Red arrow=Spiked lamella; Yellow arrow=Hypertrophy; Yellow Arrow Head=Inter-luminal debris; Red arrow head=Ruptured lamellar epithelium (Hematoxylin & Eosin staining, 40X)

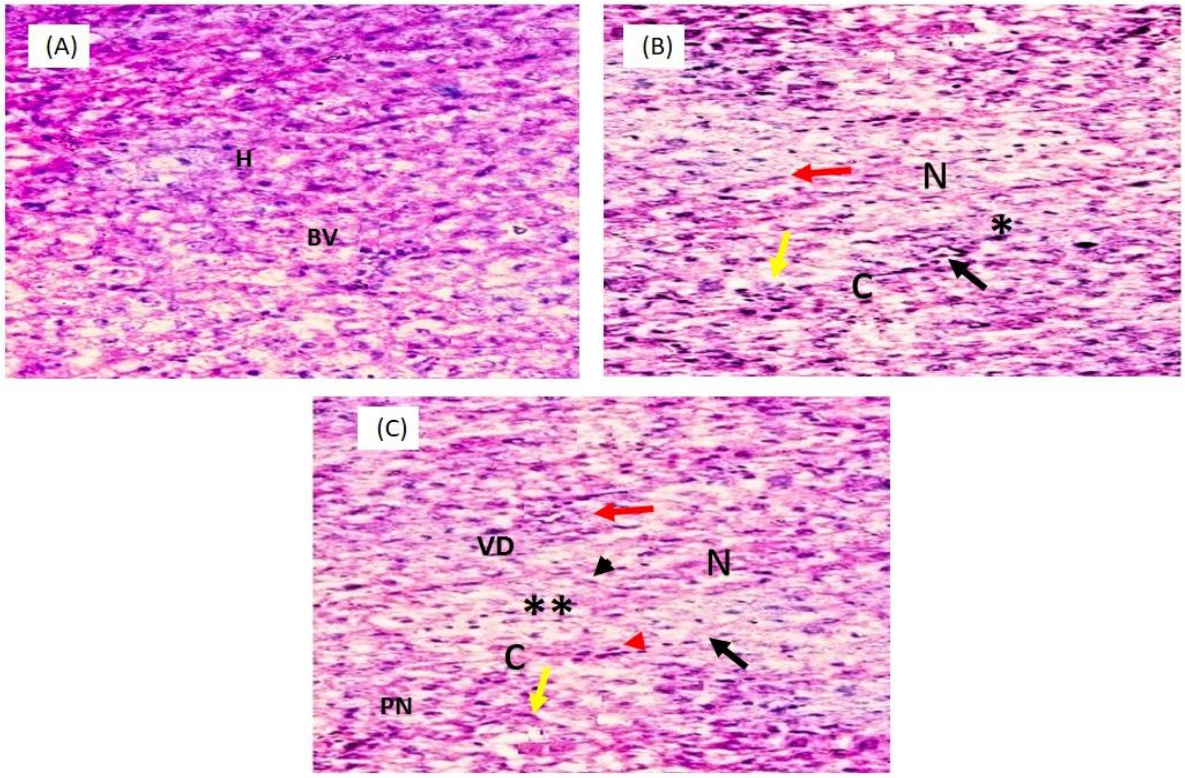

Fig. 14. Histology of HHCB treated *Labeo rohita* liver (A) Control (unexposed); (B) Low dose treated (1/5th of LC_{50}); (C) High dose treated (1/3rd of LC_{50}); H=Hepatocytes; BV=Blood vessels; N=Necrosis; PN=Pyknotic nuclei; C=Congestion; VD=Vacuolar degeneration; *=Significant damage; **=Highly significant damage; Red arrow=Vacuolization; Black arrow=Melanomacrophagy; Yellow arrow=Hyperemia; Red arrow head=Hydropic degeneration; Black arrow head=Granular degeneration (H&E 40X)

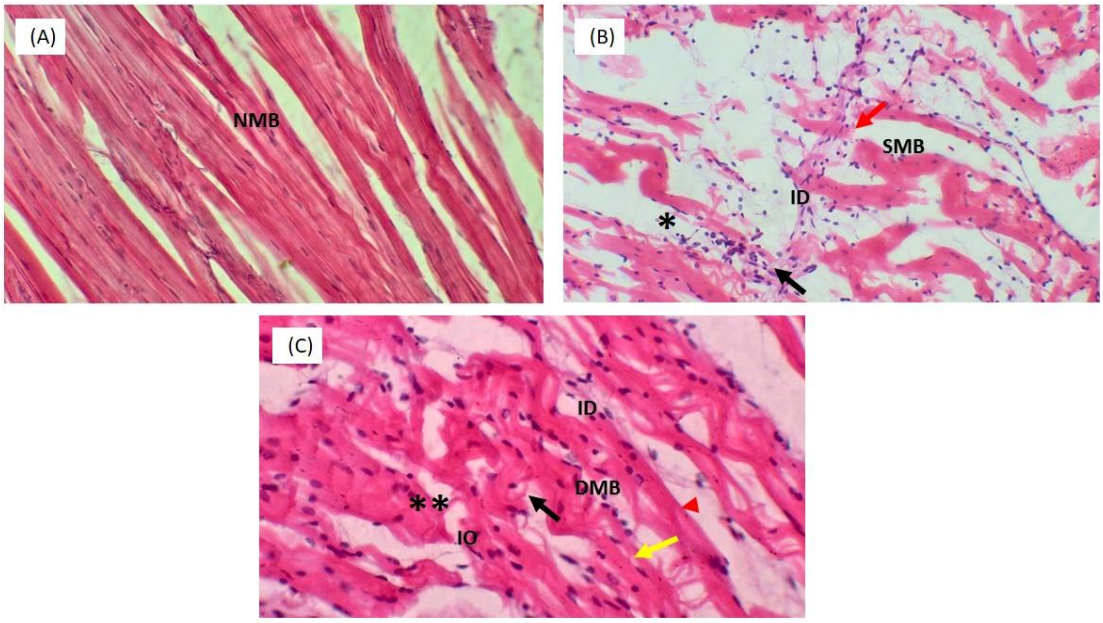

Fig. 15. Histology of HHCB treated *Labeo rohita* muscle (A) Control (unexposed); (B) Low dose treated (1/5th of LC_{50}); (C) High dose treated (1/3rd of LC_{50}); NMS=Normal muscle bundle; SMB=Short muscle bundle; ID=Inter-luminal debris; DMB=Degenerated muscle bundle; IO=Intramuscular oedema; *=Significant damage; **=Highly significant damage; Yellow arrow=Cellular necrosis; Black arrow=Dystrophic damage; Red arrow=Vascular deformation; Red arrow head=complete vascular damage (H&E 40X)

4. Discussion

Water pollution resulting from a variety of pollutants raises concerns about their effect on aquatic life (Carlsson et al., 2013; Chen et al., 2024; Sehonova et al., 2019). Synthetic musk compounds (SMCs), particularly polycyclic musk compounds (PMCs), are an important class of aquatic pollutants (Diao et al., 2024; Li et al., 2018). Relative permanence and low biological and chemical breakdown rates of PMCs in the environment increase bioaccumulation (Li et al., 2024; Safford et al., 2015). PMCs have been found multiple times in aquatic organisms (Chae et al., 2023; Cunha et al., 2015). One suitable way to demonstrate the toxicity of a particular environmental contaminant is the 96-hour LC_{50} test. This test allows us to determine the percentage of fish that survive exposure to various concentrations of contaminants (Hedayati et al., 2010). During the present study, 96-h LC_{50} as well as lethal concentrations of *L. rohita* against galaxolide (HHCB) was determined. Fish species showed a variable response on $p < 0.05$ for PMC, regarding 96-h LC_{50} as well as lethal concentration. HHCB short term toxicity testing was examined on all aquatic species. Significant concentration-dependent reactions were seen in the growth of *Paracentrotus lividus* as well as *Mytilus galloprovincialis* larvae, also the death of *Sparus aurata* (p 0.01) has been recorded (Ehiguese et al., 2021).

Polycyclic musk affects aquatic organisms by the induction of oxidative stress (Chen et al., 2011, 2015). The induction of oxidative stress by polycyclic musk has been shown in fish, earthworms (Chen et al., 2012; Liu et al., 2011) and wheat (Chen and Cai, 2015). Repored elevation of reactive oxygen species (ROS) which include $O^{\bullet-}_2$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ are forms of oxygen which exist as atmospheric oxygen in its excited or partly reduced state (Davies, 2018; Land, 1990). In present research work, significant alterations in antioxidant enzyme activities were observed during the 90 day chronic exposure of HHCB. Superoxide dismutase (SOD) and catalase (CAT) activity was highly fluctuating and induced oxidative stress and consequently various toxic effects on fish organs. The toxicity of simulated urban runoff containing polycyclic musks and cadmium were assessed in *Carassius auratus* using oxidative stress biomarkers (Chen et al., 2012). The activity of antioxidant enzymes including SOD, CAT and the content of malondialdehyde (MDA) in the liver of *C. auratus* were analyzed. The results showed that the activity of antioxidant enzymes and the content of MDA increased significantly exposed to the simulated urban runoff containing HHCB alone or mixture of HHCB and Cd. The activity of the investigated enzymes and the content of MDA then returned to the blank level over a longer period of exposure. The damage was reported in antioxidant and genomic systems of Zebra Mussel (*Dreissena polymorpha)* when subjected to environmental relevant quantities of HHCB (Chen et al., 2015). They observed *D. polymorpha* suffered from oxidative and genetic harm after twenty one days exposure to different concentrations of galaxolide that are often

reported in aquatic habitats. HHCB cause oxidative stress and genotoxicity in marine creatures (Ehiguese et al., 2020).

The current study examined histological changes brought on by repeated exposure to HHCB. Alterations in the histopathology of the gills, liver and muscles were noted. According to study all fish organs experience the detrimental changes. Fish tissues exposed to HHCB demonstrated factors that contribute to the condition include epithelium necrosis, degenerated filaments, fused and spiked lamella in gill tissues. Swelling and irregularity in shape of hepatocytes was observed. Hepatic vein damage was also occurred in fish liver exposed to PMC. Thickening of muscle bundle was observed and inter-luminal debris was significant in both PMC treated tissues of fish muscle. Similar histological abnormalities were seen in the liver, gills and muscles of fish who received PMCs. The physiological effects of the musk chemical were studied in rainbow trout. Peroxidation in the caudal renal tissue came from histological pictures (HODKOVICOVA et al., 2020). Different concentrations of PMC resulted in histopathological changes in liver, kidney, skin, or gill. Histological examination revealed some pathological changes, such as cytoplasmic vacuolation of hepatocytes and focal epithelial hyperplasia and hyperaemia of gills (Blahova et al., 2018). Single intra-peritoneal injection of galaxolide in the European Sea Bass changes the histology of gonads (Fernandes et al., 2013).

5. Conclusion

HHCB intoxication caused mortality in fish species at low concentration, induced oxidative stress and significant changes in tissue formation in fish organs. Moreover, antioxidant enzyme activity in selected fish organs showed fluctuating trends by increasing and then decreasing at lowest levels. Gills, liver and muscle histology deviated from normal formation and altered significantly. The findings of study suggested that PMCs are detrimental to aquatic populations and fish health.

CRediT authorship contribution statement

Mina Jamil: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Software, Writing – review & editing, Writing – original draft. Sajid Abdullah: Conceptualization, Formal analysis, Project administration, Resources, Supervision, Validation, Visualization. Shakeela Parveen: Conceptualization, Project administration, Resources, Supervision, Validation, Visualization. Muhammad Kashif: Formal analysis, Software, Validation, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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