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Impact of Benzophenone UV Filter on Oxidative Stress Biomarkers and Antioxidant Defense in *Labeo rohita*

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ABSTRACT

Lately, disintegration of ozone layer has headed to an upsurge in ultraviolet radiation, and its adverse effects on humans and other organisms are well known. As the ultraviolet (UV) filters are the chemical complexes used to protect against UV irradiation, the proliferating use of these filters has led toward an increase in environmental concentrations. Benzophenone (BP) has started to gain the attention of researchers due to its widespread use in cosmetics and personal care products and also its prevalence in the aquatic environment due to its low degradation and high accumulative potential. This study evaluated the 96-h acute toxicity and chronic effects of BP on oxidative stress biomarkers and antioxidant defense in *Labeo rohita.* Fish having the same weight were subjected to increasing concentration of BP (100-1000µg/L) for acute toxicity testing and their 96-h LC_{50} and lethal concentration were estimated as 606.67 µg/L and 1033.25 µg/L respectively. Oxidative stress in terms of superoxide dismutase (SOD) and catalase (CAT) was determined in the gills, liver, kidney, heart and muscles of *L. rohita* after 75 days chronic exposure of sub-lethal ($1/3^{rd}$, $1/5^{th}$, and $1/7^{th}$ of LC_{50}) BP doses and sampling were done at 15, 30, 45, 60 and 75 days. A significant decline in CAT and SOD activity was observed during the exposure period showing excess reactive oxygen species (ROS) generation which is an indication of oxidative stress confirming the induced toxicity potential of BP.

Keywords Oxidative stress, Acute Toxicity, Chronic toxicity, Ultraviolet Filters, Antioxidant enzymes

Introduction

Aquatic environments are becoming suspectable to anthropogenic contamination, among which ultraviolet (UV) filters are gaining attention because of their extensive application in personal care products, cosmetics, and sunscreens. The concentration of UV filters permitted in cosmetics ranges from 0.1-10% (Kaiser et al., 2012). Only 14 kinds of UV filters are allowed to be used in cosmetics in the United States (Gao *et al*., 2015). Benzophenone (BP) based UV filters are one of the most commonly used UV filters for skin protection against UV-A and UV-B rays (Kim and Choi, 2014). However, their widespread use has resulted in increased concentration in water bodies through industrial discharge, wastewater effluents, and recreational activities like swimming and bathing (Giokas et al., 2007). As their concentration rises above the threshold in the waterbodies due to low biodegradability and inefficient removal, it becomes a health hazard for the aquatic organisms and disturbs the natural aquatic environment (Ekubo and Abowel, 2011). BP compounds easily accumulate in aquatic organisms due to their high bio-accumulative potential and lipophilic nature (Gago-Ferrero *et al.,* 2013) which results in physiological changes in the organisms. A wide range of ecotoxicological effects of BP filters have been reported in aquatic life from growth inhibition, mortality, oxidative stress, and organ damage, to interference in hormone signaling and reproduction ((Blüthgen et al., 2012; Du et al., 2017; Velanganni et al., 2021). BP filters have been categorized as a potential endocrine disruptor in fish and have been linked to coral bleaching (Weisbrod *et al.,* 2007). Aquatic invertebrates experienced disordered embryonic development and reproduction (Campos et al., 2019).

It has been reported that BP exposure accelerates the reactive-oxygen-species (ROS) formation in exposed organisms and induces oxidative stress: a state in which there is an imbalance between the amount of ROS produced and the cell's ability to activate antioxidant defense systems to reduce their effects (Velanganni and Miltonprabu 2021). ROS are essential for cell signaling but its high concentrations overwhelm the antioxidant system in the body and lead to cellular damage so it is regarded as an inevitable toxic byproduct of aerobic metabolism (Vaahtera *et al*., 2014). Important oxidative biomarkers are DNA oxidation, Lipid peroxidation (LPO), and protein carbonylation which are indicators of cellular injury (Choudhary *et al.,* 2007).

Most animals, including fish, have developed mechanisms to mitigate the harmful effects of ROS. This mechanism consists of antioxidant enzymes such as peroxidase, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), as well as glutathione-S-transferases (GST), which

are found in many cell compartments. These enzymes are present in vertebrate tissues with a high level of activity in the liver, which is the organ of metabolism and detoxification. Disruption in antioxidant enzyme activity can be indicative of environmental stress. These enzymes can act as accurate molecular biomarkers of oxidative stress and provide insights into the extent of a population's response to prolonged xenobiotic exposure (Brown et al., 1998). Fish species have proven an efficient bioindicator of pollution because they are sensitive to contaminants in water bodies (Walia et al., 2015). *Labeo rohita* is one of the most used species in research as it plays an important role in maintaining ecological balance (FAO, 2018). It is a freshwater fish that is popular in Asian countries especially Pakistan, India, and Bangladesh because of its palatability as well as market demand (Sheikh et al., 2017) and can act as an ideal bioindicator for toxicological studies due to its high sensitivity to contaminants. However, there is no existing literature about the effects of BP on oxidative stress biomarkers in this species. In this sense, the objective of this work was to evaluate the oxidative stress biomarker and antioxidant enzyme defense in *L. rohita* during chronic exposure to sublethal BP concentrations.

MATERIALS AND METHODS

The experiment was performed at Fisheries Toxicology Lab, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan.

Maintenance of test organism

Before the experiment, healthy 90-day-old *L. rohita* fingerlings of equal weight and length were selected and acclimatized to laboratory conditions for ten days in a 70L glass aquarium with 50L of dechlorinated tap water. The fish were fed commercial pelleted feed twice daily, and aquarium water was changed with tap water daily during this period. Dead fish were removed from the aquarium regularly to avoid pollution in the test media.

Preparation of chemical

Benzophenone (Diphenylmethanon) CAS # 119-61-9, was sourced from Sigma-Aldrich, USA in 99% purity. It was dissolved in dimethyl sulfoxide to prepare a stock solution which was used to prepare the working solutions.

Acute toxicity

Control and treatment groups each having three replicates were established. Each replicate consisted of 10 fish and was subjected to increasing test concentrations of BP from 100 to 1000µg/L for 96-hr in the treatment group while the control group did not contain the test solution. The concentrations used was found in agreement with previous studies investigating BP toxicity in fish (Lima et al. 2022; Riaz et al., 2025). Fish were not fed during the acute toxicity trial while half of the test media changed to ensure the constant BP concentrations. Physicochemical parameters were regularly checked and maintained: temperature 27-29 °C, DO 6-8mgL^{-1}, pH 6.5-7, total hardness content 280ppm during the trial.

Oxidative stress biomarkers

To investigate the oxidative stress, fish was exposed to various sub-lethal doses: 1/3rd, 1/5th, and 1/7th of 96-h LC_{50} (Table 1) for 75 days. All the treatment groups were established in three replicates. Sampling was done after 15, 30, 45, 60, and 75 days of chronic exposure (Table 2), to assess the superoxide dismutase (SOD), and catalase (CAT) enzyme activities in the liver, kidney, gills, muscles, and heart of fish. All the organs were homogenized separately for approximately 15 minutes using mortar and pestle. After the homogenization, the mixture was stained, separated, and centrifuged (10,000 revolutions per minute, at 4°C for 15 min.). 10% glycerol was added and transparent supernatant was selected for further analysis. CAT and SOD activities were analyzed by Weydert and Cullen (2010) method with slight modification.

Table 1: Testing of sub-lethal concentrations of BP during chronic toxicity exposure for *L. rohita*

Concentration µg/L^{-1}	Treatments	Exposure doses
BP	1/3rd of LC_{50}	202.22
	1/5th of LC_{50}	121.33
	1/7th of LC_{50}	86.66

Table 2: Fish distribution in groups and its sampling for antioxidant enzyme analysis

Group	15 days	30 days	45 days	60 days	75 days	Fish each replicate	Total fish (Three replicates each group)
1	3	3	3	3	3	15	45
2	3	3	3	3	3	15	45
3	3	3	3	3	3	15	45
4	3	3	3	3	3	15	45

1=Control; 2=1/3rd of LC_{50}; 3=1/5th of LC_{50}; 4=1/7th of LC_{50}

Statistical analysis

Probit analysis was employed to determine 96-hour LC_{50} and lethal concentrations of BP through the method of Hamilton et al. (1977). Analysis of variance (ANOVA) was used to determine statistical differences among variables and means were compared by Tukey's test. Data were analyzed by Statistix 8.1 and results were recorded in Mean±S.E. test media. BP toxicity increased with its increasing concentration evidenced by rising fish mortality (Figure 1). The 96-hr LC_{50} and lethal concentration of BP were estimated as 606.67 µg/L and 1033.25 µg/L respectively at 95% confidence interval.

RESULTS

Acute toxicity

Fish mortality was examined every 24-hr and dead fish removed immediately to avoid contamination in

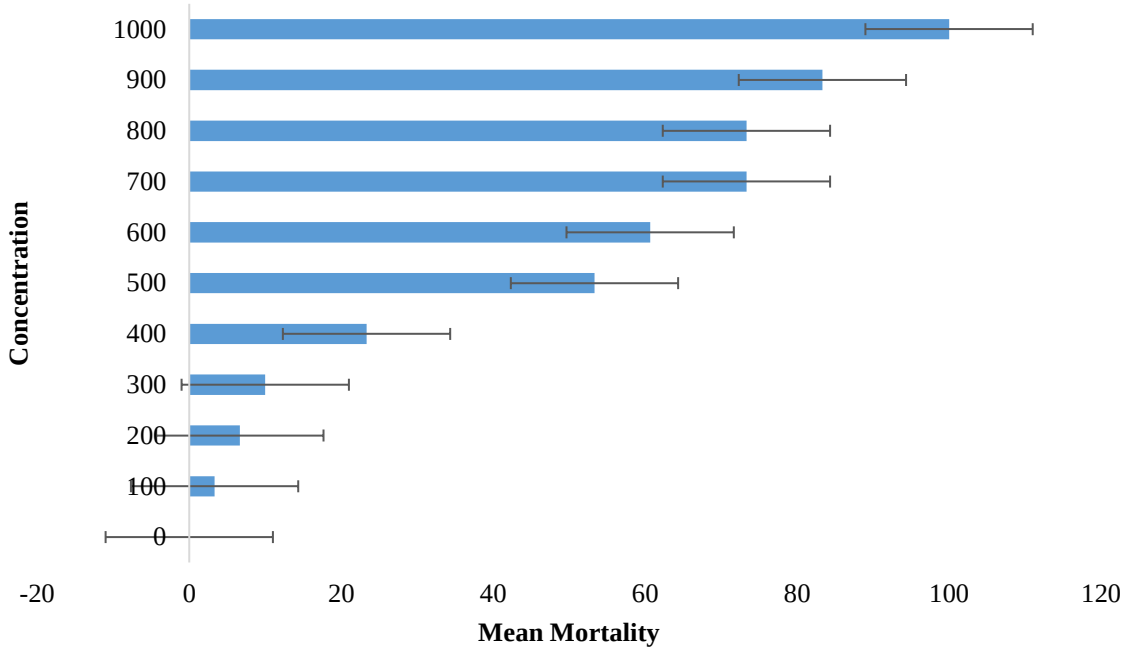
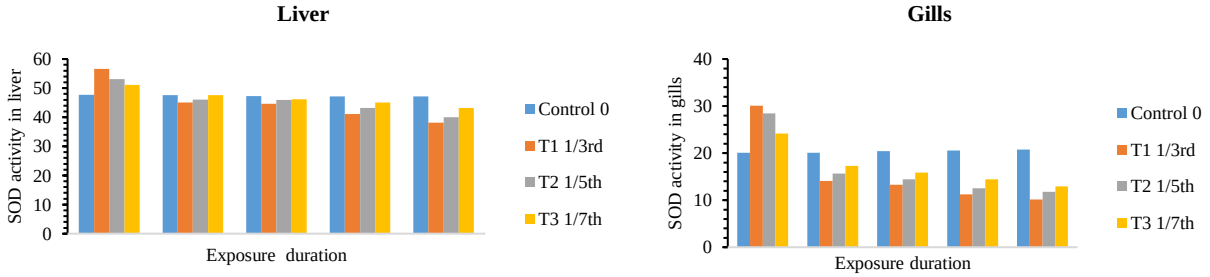


Fig. 1 Recorded mortality of *L. rohita* with increasing BP concentration during acute testing

Oxidative stress biomarkers

Superoxide dismutase (SOD) activity:

For estimation of oxidative stress biomarkers, fish were exposed to sub-lethal doses of BP mentioned in Table 1 for 75 days and sampling was done after 15 days intervals. Fish showed a significant increase in SOD activity in the initial 15 days followed by a sudden decline at 30, 45, and 75 days relative to the control group shown in Figure 2. The decline in the SOD activity shows an increase in ROS generation in fish. Decline in SOD activity was observed in the following trend: liver>kidney>gills>heart>muscle



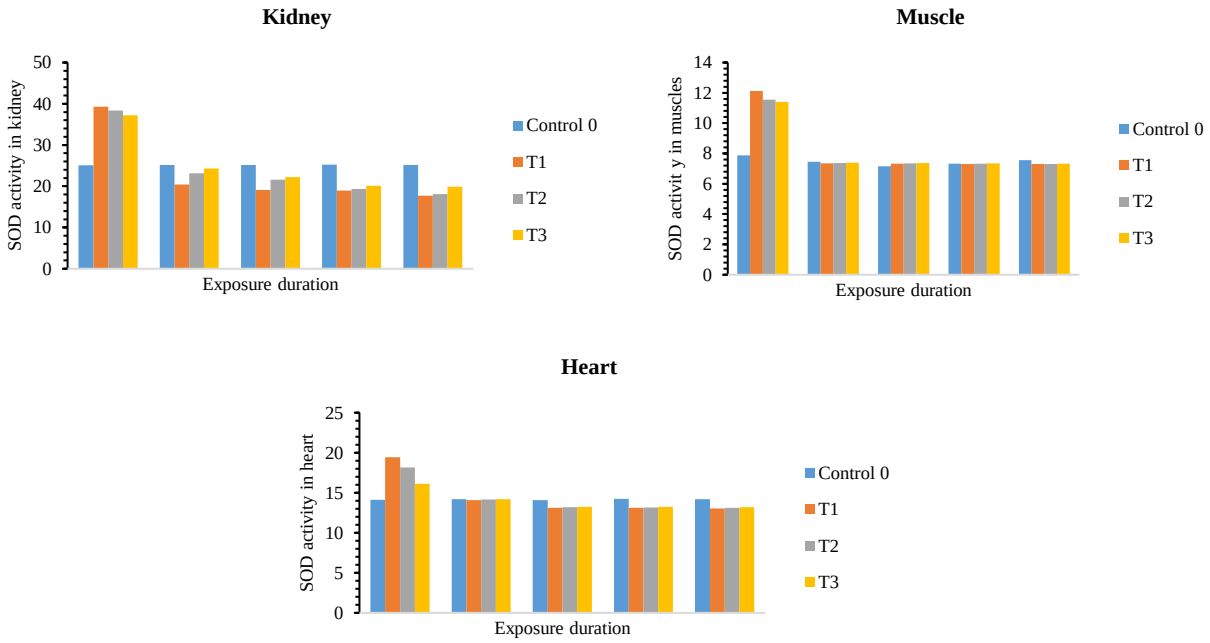
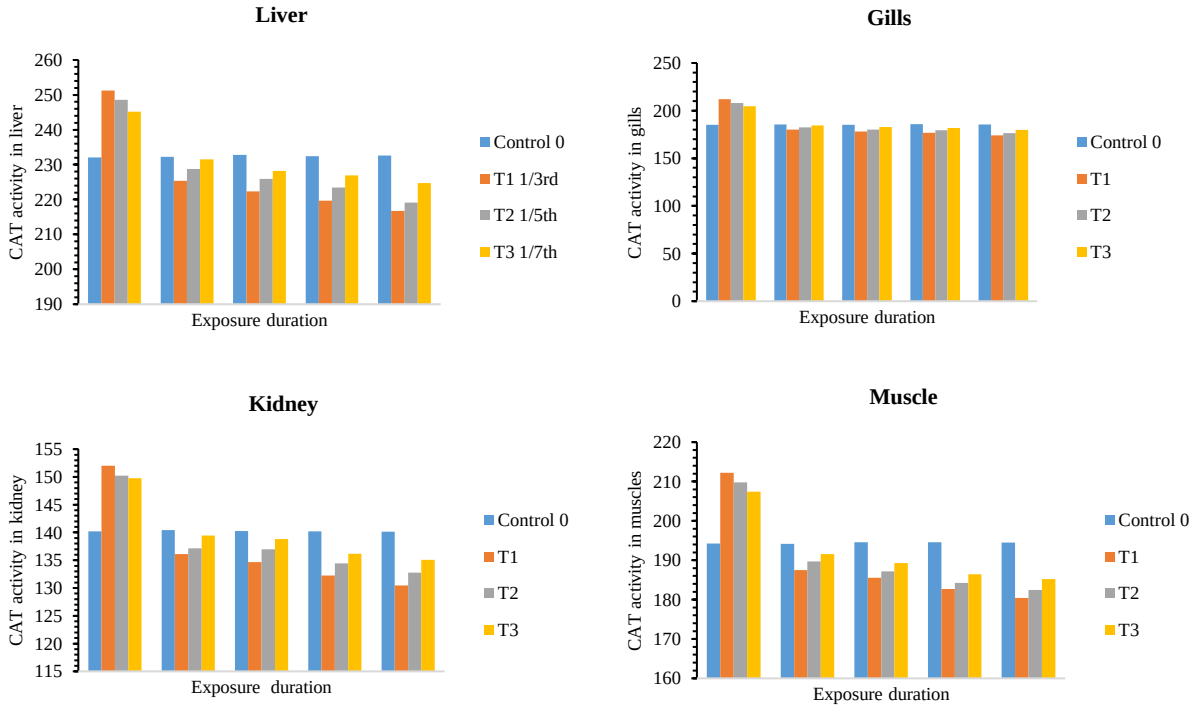


Fig 2. Effect of BP on SOD activity in organs of *L. rohita*. Values are means of three replications. (T1=1/3rd of LC_{50}; T2=1/5th of LC_{50}; T3=1/7th of LC_{50})

Catalase (CAT) activity:
Sub-lethal exposure to BP caused significant variability in CAT activity in fish organs. CAT activity followed the same trend as SOD, rising for the first 15 days and then decreasing afterward (Figure 3). The highest CAT activity fluctuations were observed in the liver while the lowest in the heart.



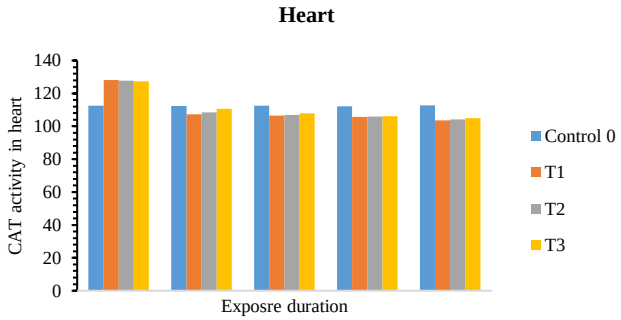


Fig 3. Effect of BP on CAT activity in organs of *L. rohita*. Values are means of three replications (T1=1/3rd of LC_{50}; T2=1/5th of LC_{50}; T3=1/7th of LC_{50})

Discussion

Aquatic contaminants disrupt the biological systems of exposed organisms and induce damage to both their structure and normal function (Johnson and Radhakrishnan, 2015). BP is one of those contaminants that pose threats to aquatic life due to its lipophilic nature and high accumulative potential (Kim and Choi, 2014). Determining tolerance and safety limits are important for evaluating contaminants' toxicity; acute toxicity testing has been proven crucial in this regard (Prentera et al., 2004). Our present study confirms the acute toxicity of BP for *L. rohita* as 606.67 µg/L. This result is consistent with the previous work done by Riaz et al. (2025), who determined 612µg/L as LC_{50} for the same test species and chemical. However, 96-hr LC_{50} values of BP-based UV filter were determined as 3.89mgL^{-1} and 633.00mgL^{-1} for *Brachydanio rerio* (Du et al., 2017). Differences in toxicity may be due to different exposure conditions and the test species. It is noted that the UV light elevates the toxicity of BP filters as indicated by Zhang et al. (2021) who studied zebrafish embryos in the absence and presence of UV light. Results showed the high toxicity of BP under UV light as their LC_{50} values decreased from 9.75 mgL^{-1}(without UV) to 4.52 mgL^{-1}.

Antioxidants play a crucial role in assessing water pollution, and enzymatic activity can serve as biochemical indications of potential injury to fish (Aziz et al., 2020). BP filter is reported to enhance the formation of ROS in the body to generate oxidative stress. Antioxidants keep the ROS at a threshold limit to protect the body from its negative effects. The present study found significant alterations in antioxidant enzymes in *L. rohita*. Their activity was observed to significantly increase in the first fortnight, and then gradually decrease in the next weeks of BP exposure and induced various toxic effects on fish organs. It was estimated that *L. rohita* hepatic cells have the highest enzymatic action of any fish organ, with higher levels of CAT and SOD. The liver is thought to be more severely impacted by BP than other organs. SOD and CAT levels were observed in the following trend: liver>kidney>gills>heart>muscle. Low SOD activity under high concentrations of contaminants can lead to more generation and accumulation of ROS in exposed organisms that cause cellular damage (Phull et al., 2018). Similarly, in another study, Abdel-Khalek (2015) found that disruption in CAT activity in different fish organs was attributed to its contact with toxicants and the free radical instability. These results are relevant to previously reported results by Liu et al. (2015) and Zhang et al. (2020) on *Carassius auratus* (goldfish) which demonstrated that BP-based UV filters, including BP-1, BP-2, BP-3, and BP-4, resulted in substantial alterations in the activities of antioxidant enzymes such as SOD, and CAT, as well as non-enzymatic antioxidants like glutathione (GSH). These studies indicated an initial increase in antioxidant activities at high tested concentrations, followed by a decline, suggesting an adaptive response to oxidative stress.

Conclusion

Our study investigated the acute toxicity of the BP UV filter and its sub-lethal impact on oxidative stress biomarkers and antioxidant defense in *L. rohita*. BP was found to disturb the functioning of antioxidant enzymes that act as a shield against excess ROS generation. After the exposure to sub-lethal BP doses, the CAT and SOD activity significantly altered and exhibited an organ-specific response. These results are in accordance with prior studies confirming BP toxicity in fish. Considering its prevalence in aquatic environments, it is important to limit the UV filters use and implement effective technologies to treat wastewater effluents.

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