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RESEARCH ARTICLE

Oxidative Stress, Antioxidant Enzymes, Genotoxicity and Histopathological Profile in *Oreochromis niloticus* Exposed to Lufenuron

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A B S T R A C T

Pesticides especially insecticides are widely used in the field of agricultural development all over the world to increase crop production. Moreover, exposure to such compounds does not only influence the intended targets but also induces adverse impacts on a number of unintended targets in animals. This research aimed to determine the toxic impacts of lufenuron (an insecticide) on the health status of fish in terms of measurement of oxidative stress, antioxidant enzymes, DNA damage and histopathological changes in Nile tilapia (*Oreochromis niloticus*) exposed to different concentrations. For this purpose, *Oreochromis niloticus* fish, weighing about 75-80g were arbitrarily separated into four different groups and then exposed to lufenuron @ 0.7, 1.2 and 1.7µg/ L for 39 days. The results showed that the fish exposed to the pesticide had significant changes in oxidative stress, antioxidant profile in gills and percentile rate of DNA damage in different visceral organs including liver, kidney, and gills. Results of light microscopic investigations indicated different histological changes in liver (necrosis of hepatocytes, degeneration of hepatocytes, vacuolar degeneration and congestion), gills (necrosis of lamellar epithelial cells, telangiectasia, and atrophy of secondary lamellae), heart (congestion, necrosis of neurons, microgliosis and intracellular edema), brain (congestion, myofibrosis, neutrophilic and myocarditis) and kidneys (necrosis of renal tubules, widening of urinary space, necrosis of renal tubular epithelial cells). A significantly escalate in oxidative stress while lower quantity of antioxidant biomarkers was documented in experimental fish. The findings of this study suggest that long-term exposure to lufenuron has negative health effects via induction of DNA damage, increased oxidative stress, lowering of enzymatic antioxidants profile and histological lesions in visceral organs of Nile tilapita (*Oreochromis niloticus*).

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INTRODUCTION

Seafood consists of many nutrients that are good for humans and are quickly absorbed by the digestive system. Because of this, seafood is one of the best sources of nutritional components (Da Rocha *et al.,* 2009; Aci *et al.,* 2020). Having an adequate number of benefits, fishes play a crucial role in the human diet as a source of protein and omega-3 polyunsaturated fatty acids since it lowers cholesterol and boosts the immune system (Hoseinifar *et al.,* 2019). Some economically essential fish species include rainbow trout (*Oncorhynchus mykiss*) and trout (*Salmo trutta*) from the family Salmonidae, catfish (*Clarias gariepinus*) from Clariidae family, common carp (*Cyprinus carpio*) from family Cyprinidae, Nile tilapia (*Oreochromis niloticus*) and perch (*Dicentrarchus labrax*) from the family Cichlidae and Moronidae respectively (Nur *et al.,* 2017; Aci *et al.,* 2020). The aquatic animals including fish

are regarded as the most important non-target organisms that can be harmed by pesticides and other aquatic contaminants that are found in aquatic habitats near to agricultural farms. Freshwater fish are frequently exposed to such pollutants, making them vulnerable to chemical toxicity as the gill tissues in these species are directly exposed to such toxicants (Li *et al.,* 2022; Mahmood *et al.,* 2022; Wang *et al.,* 2022). Studies have recorded that fish being non-target aquatic animals can be used as experimental biological model for estimation and screening of toxicological biomarkers (Tahir *et al.,* 2021; Jabeen *et al.,* 2021; Akram *et al.,* 2022; Kiran *et al.,* 2022). Routine blood biochemistry, pathophysiological ailments and genotoxicity are considered as reliable diagnostic tools for the evaluation of health status of fish in polluted ecosystems (Obiakor *et al.,* 2012; Ghaffar *et al.,* 2016; Taha *et al.,* 2020).

Due to their extreme toxic consequences such as carcinogenic, mutagenic, neurotoxic, and cytotoxic properties, the heavy metals, pesticides and other aquatic pollutants are the major threats in aquatic and other animals (Ahmad *et al.,* 2021; Merdana *et al.,* 2021; Mujahid *et al.,* 2021). Various toxins from industrial and agricultural water flow into canal as well as river, which eventually accumulate near water bodies (Naz *et al.,* 2021). Pollution of aquatic ecosystems due to various toxins has become a global threat causing health problems for both aquatic animals and human beings (Naz *et al.,* 2022). Pesticides bioaccumulation and detoxification in the liver, along with the pollutants, may have an impact on how cells behave physiologically (Xing *et al.,* 2012; Banaee *et al.,* 2014). Ecotoxicological research is essential for determining how aquatic species and people are being exposed (Kim and Kang, 2016). Exposure to pesticides may result in hepatic histological damage and subsequent physiological disturbance (Adel *et al.,* 2017; Ghelichpour *et al.,* 2017; Hoseini and Yousefi, 2019). It has been recorded that the processes of oxidation and reduction, synthesis, and hydrolysis activities in liver are all involved in the removal of xenobiotics (Franco, 1991; Maksymiv *et al.,* 2015).

Lufenuron is a benzoyl phenyl urea insecticide having the molecular formula [(RS)-1-[dichloro-4-(1, 1, 2, 3, 3, 3-hexafluropropoxy) phenyl] 3,6-diflurobenzoyl urea. Lufenuron is frequently employed in different fields including public health management, cereal crops to kill pests, insects, western flower thrips, and eriophyids in cotton, grapes, corn, citrus, sugar beet, potatoes, and ornamental plants (Vázquez *et al.,* 2014; Soares *et al.,* 2016). An unregulated use of lufenuron (LUF) insecticide from the benzoyl urea class damages the digestive system of insects. LUF prevents reproduction, polymerization, aggregation of chitin, moulting and development of insects (Vázquez *et al.,* 2014). LUF also induces tissue damage via changes in the antioxidant system, and saltwater adaptation in fish (Soares *et al.,* 2016; Ghelichpour and Mirghaed 2019). Pesticides can induce changes in fish hematological constituents. An alteration in blood parameters and histopathological changes are thought to indicate tissue damage, environmental stress, or an unwell state (Clemente *et al.,* 2013; Hoseinifar *et al.,* 2019).

Biomarkers of estimation of injury due to production of free radicals in biological system and alterations in defense mechanisms of antioxidant biomarkers are the useful parameters of oxidative stress. Oxidative stress damages cellular structures in organisms (Akram *et al.,* 2022). Consequently, different pathological conditions occur that can lead to death (Nur *et al.,* 2022). Recent studies have employed variety of measures as indicators of oxidative stress including metabolic intermediates, catalase (CAT), superoxide dismutase (SOD), enzymes, and total antioxidants/oxidants capacity (TAS/TOS) (Nur *et al.,* 2022). Superoxide dismutase (SOD) and Catalase (CAT) are the most crucial enzymes for detoxification in all living thing (Slaninova *et al.,* 2009). Variability of antioxidant enzyme levels depends on the length and intensity of stressor exposure, concentration of xenobiotic, and also vulnerabilities of an exposed species of fish (Slaninova *et al.,* 2009). Oxidative stress is caused by numerous toxic agents, including pesticides used in agriculture or industry, the impact of pro-oxidant factors in the fish organism can be used to evaluate pollution (Kaya *et al.,* 2012). Comet assay is a useful method for measurement of genotoxicity in aquatic environments. This research aims to investigate the changes in antioxidant enzymes, the oxidative stress and histopathological profile of Nile tilapia exposed to insecticide lufenuron.

MATERIALS AND METHODS

The current experimental research was conducted at laboratory of Government Sadiq College women University Bahawalpur, Pakistan. All the research was done according to the standards for the use of laboratory animals for research established at Ethical Committee of Government Sadiq College Women university Bahawalpur.

Fish collection and management: Total 88 freshwater fish (*Oreochromis niloticus*), weighing between 75-80g each, were taken from the local Fish Seed Hatchery in Bahawalpur. All of the fish were brought to the testing lab in plastic bags with enough oxygen added and they were then put in glass tanks with 100 liters of water. For each concentration, three 100L tanks (each 36 inches long and 12 inches wide) containing four fish each were used. Prior to the trials start, all of fish had 15 days acclimatization period. All aquariums have enough aeration with continuous air pumps to provide enough oxygen to the exposed fish. Aquarium water was kept at a constant temperature. Prior to and following the experiment, measurement of the physio-chemical characteristics of water were made (Table 1).

Experimental design and treatment: After acclimatization, the experimental fish (60) were distributed into four equal treatment groups. Fish in each group (15) were placed at random (A-D) and then treated with insecticide lufenuron after adaptation to laboratory conditions. Fish of group A were known as control group while the fish of groups B-D were subjected to different concentration of Lufenuron. The environmental relevant concentrations (0.7, 1.2, and 1.7µg/L) of lufenuron were chosen according to earlier published data and used for the period of 39 days. Various tissues (gills, liver, brain, kidney, and heart) were obtained from each treated fish of all experimental groups at days 13, 26 and 39 of experiment and preserved until further analysis.

Assessment of histopathological alterations in visceral organs: At the days 13, 26, and 39 of the study, fish (4) from each group were randomly selected, euthanized, and dissected for histological abnormalities. Clove oil (4.5mg/L) was used to anaesthetize all the experimental specimens prior to sampling (Islam *et al*., 2019). Immediately after dissection, different organs (gills, liver, brain, kidney, and heart) of fish were taken, and stored in a 10% formaldehyde solution. All of the recovered tissues were processed and stained by using Hematoxylin and eosin staining procedure to record histological abnormalities (Hussain *et al*., 2019). About 15 dissimilar microscopic fields/tissues/fish were examined to measure the severity of histopathological changes with the help of light microscopic (Nikon Eclipse 80i, Nikon Co., and Tokyo, Japan).

Evaluation of oxidative stress parameters and enzymatic antioxidants in gills: Enzymatic antioxidants and oxidative stress markers were assessed in gills of exposed fish at days 13, 26 and 39 of the trial. Briefly, tissue homogenates were prepared from each organ separately using cold normal saline solution. Different biochemical profile (oxidative stress and antioxidant enzymes) was estimated using spectrophotometer. The quantity of ROS (Akram *et al.,* 2021; Hussain *et al.*, 2022; Raza *et al*., 2022) in gills of each fish was measured. The quantity of antioxidant enzymes such as catalase (CAT) (Ghaffar *et al.,* 2016), superoxide dismutase (SOD) (Kakkar *et al.,* 1995; Akram *et al.,* 2021, Raza *et al.,* 2022) and peroxidase (POD) (Ghaffar *et al.,* 2016), Glutathione reductase (GR) (Habig *et al.,* 1974) was measured in tissue homogenates of gills obtained from individual fish.

Assessment of DNA damage in liver, gills, and kidneys: DNA damage estimation in various visceral tissues including, kidneys, gills, and liver was performed by using a reliable, suitable and sensitive technique (comet assay) under alkaline environments (Mitchelmore and Chipman, 1998; Hussain *et al*., 2019; Raza *et al.,* 2022). Briefly, liver, kidney, and gill tissues from each fish were obtained after necropsy of fish and were immediately placed in cold normal saline solution. After that about 0.2g tissue was triturated and centrifuged separately from all the visceral organs of each fish. The sediment having cells of each specimen were removed and processed for investigation of DNA damage (Hussain *et al.,* 2011; Raza *et al.,* 2022). Briefly, three layers were prepared from agarose (0.75% normal agarose and 0.5% low melting point agarose) dissolved in deionized water using frosted glass slides. About 5µl cells from each organ were suspended in low melting agarose (Raza *et al.,* 2022). All the prepared slides were placed in chilled lysing solution for unwinding of DNA and subsequently electrophoresis was done at 25V for 30 min (Singh *et al*., 1988; Kosz-Vnenchak and Rokosz, 1997). Finally, all the slides were neutralized and immediately stained using ethidium bromide stain. DNA damage (% of cell damage) was observed using fluorescent microscope by examining 550 cells/fish/slide.

Statistical analysis: The SPSS software, version 15 was used to refine statistical analysis through analysis of variance (ANOVA). To determine the degree of significance alterations among lufenuron treated and untreated fish, one–way analysis of variance (ANOVA) was utilized. All data were presented as mean ± SE. A significance threshold of $P<0.05$ was used.

RESULTS

No mortality was observed in fish of all experimental groups treated with lefenuron during the trial. Several behavioral ailments including irregular swimming, absence of organization, air guzzling and abnormal swimming were obviously detected in tilapia fish exposed to various concentrations of lufenuron (0.7, 1.2, and 1.7µg/L).

Quantity of oxidative stress and antioxidant enzymes: Results recorded that oxidative stress markers including thiobarbituric acid (TBARS) and reactive oxygen species (ROS) in gills of fish exposed to 1.7µg/L concentration of lufenuron increased significantly at days 26 and 39 of the study but ROS and TBARS values increased significantly when fish were exposed to 1.2µg/L concentration of lufenuron at day 39 of the study. The findings on several antioxidant enzymes in gills showed that the amount of antioxidant enzymes such as peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) significantly decreased in the gills collected from fish subjected to 1.2 µg/L and 1.7 µg/L concentration of lufenuron at days 26 and 39 of the recent study as compared to untreated fish (Fig. 1).

DNA damage: Results on genotoxic effects determined by comet assay (Fig. 3f) showed that the percentile rate of DNA damage in some visceral tissues (gills, liver, and kidneys) increased significantly in fish treated with lufenuron at days 26 and 39 of study. At days 26 and 39 of the experiment, the results on investigation on genotoxic potential of lufenuron in isolated cells of gills of tilapia fish exposed to 1.7µg/L concentration of lufenuron increased dramatically. The results exhibited that the rate of DNA damage in gills of treated fish was remarkably elevated in fish exposed to 1.2µg/L concentration of lufenuron at day 39 of the trial compared to control fish. DNA damage was remarkably increased all the visceral organs at days 26 and 39 in fish exposed to 1.7µg/L concentration of lufenuron than the fish of control group (Fig. 2).

Histopathological alterations: The gills, liver, heart, kidney, and brain of fish in the control group showed normal histological structures, but fish subjected to varied concentrations of distinct concentrations of the pesticide lufenuron showed distinct microscopic defects in the liver, gills, heart, kidney, and brain tissues (Fig. 3). When fish were exposed to higher concentration of lufenuron (1.7µg/L), the severity of alterations increased. The exposure of lufenuron (1.2µg/L and 1.7µg/L) causes moderate to severe histopathological alterations including, congestion, pyknosis of neurons and edema in the brain of exposed fish at day 39 of the study. The exposure of lufenuron (1.2µg/L and 1.7µg/L) resulted in moderate to severe histopathological changes in the gills including congestion in cartilaginous cores, disorder in arrangement of lamellae, atrophy of cartilaginous core, atrophy of lamellae, necrosis of lamellar cells, telangiectasia, disruption of primary lamellae, curling of secondary lamellae, uplifting of lamellae and lamellar disorganization at day 39 of the study.

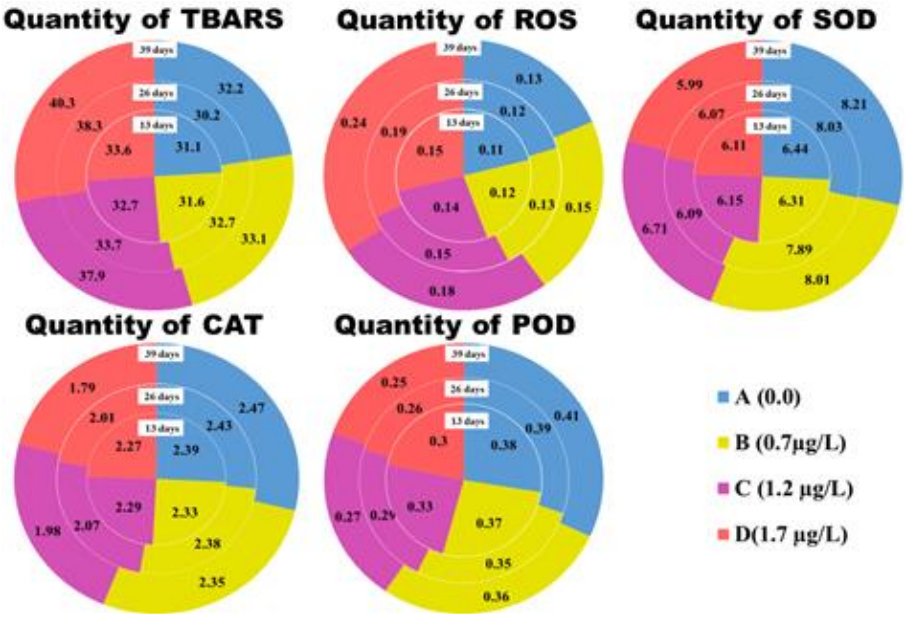

Fig. 1: Photograph showing comparison in concentrations of different oxidative and antioxidant profile of lufenuron treated and control fish

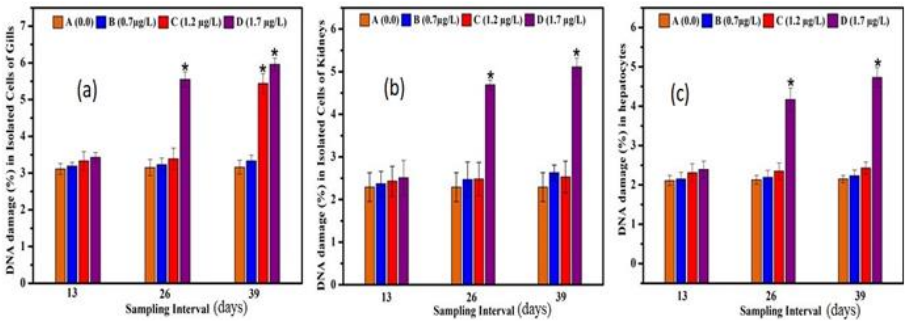

Fig. 2: Photograph showing DNA damage (%) in isolated cells of gills (a), kidneys (b) and liver (c) of lufenuron treated and control fish

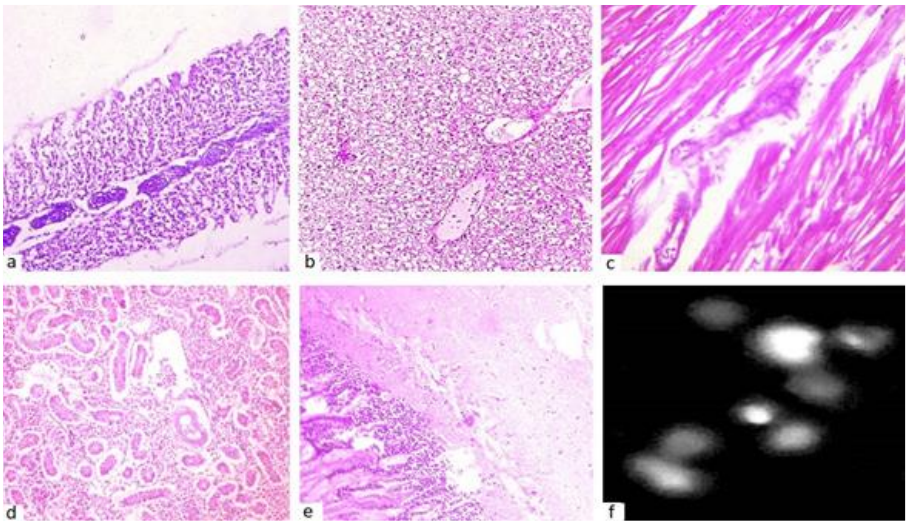

Fig. 3: Photomicrograph exhibiting histoarchitectural ailments a) Gills showing lamellar fusion, atrophy of lamellae, degeneration of cartilaginous core, telangiectasia and lamellar disorganization. b) Liver showing pyknosis, nuclear hypertrophy, vacuolar degeneration, hepatocytes with eccentric nuclei and edema. c) Heart showing edema, breakdown of myofibrilles and neutrophilic myocarditis; d) Kidneys showing necrosis of renal tubules, widening of urinary space, necrosis of renal tubular epithelial cells and degeneration of renal tubules. e) Brain showing necrosis of neurons, degeneration of neuron, atrophy of neuron and intracellular edema and f) DNA damages in isolated cells of gills.

Table 1: Physico-chemical analysis of water for experimental fish, *Oreochromis niloticus*

Parameters	Values
Water temperature (°C)	25.9±0.45
Electrical conductivity at 25°(µmhos/cm)	411.3±3.15
Dissolved oxygen (mg/L)	7.89±0.13
Alkalinity (mg/L)	171.5±1.43
Total dissolved solid (mg/L)	173.9±4.34
Ph	7.3±0.07
Total hardness (CaCO3, mg/L)	169.88±1.73
Potassium (mg/L)	1.61±0.13
Sodium (mg/L)	11.8±0.33

The liver tissues of tilapia fish exposed to lufenuron (1.2 and 1.7µg/L) showed moderate to severe (Table 2) histopathological alterations such as formation of ceroid, ccongestion, fatty change, pyknosis, hemorrhages, hypertrophy of nuclei, karyolysis, karyorrhexis, and hepatocytes containing eccentric nuclei at day 39 of the study (Fig. 3). Hearts of several fish exposed to lufenuron (1.2 and 1.7µg/L) were found to have moderate to severe histopathological abnormalities such as congestion, edema, myofibrosis and neutrophilic myocarditis (Fig. 3) at 39 of the study. The exposure of lufenuron (1.2 and 1.7µg/L) induces moderate to severe histopathological changes in kidneys (Fig. 3) including, necrosis of renal tubules, widening of urinary space, necrosis of renal tubular epithelial cells at day 39 of the study (Table 2).

Table 2: Different histopathological changes in various tissues of *Oreochromis niloticus* exposed to various concentrations of Lufenuron

Histopathological lesions	Groups		
	B (0.7µg/L)	C(1.2 µg/L)	D (1.7 g/L)
Brain			
Congestion	+	++	+++
Necrosis of neurons Intracellular edema	+	++	+++
Intracellular edema	+	+++	++++
Gills			
Congested cartilaginous core	+	++	+++
Lamellar fusion	+	++	++++
Congestion	+	+++	++++
Degeneration of cartilaginous core	+	+++	++++
Lamellar atrophy	+	+++	++++
Necrosis of lamellar pillar cells	+	++	+++
Telangiectasia	+	++	+++
Disruption of primary lamellae	+	++	+++
Curling of secondary lamellae	+	++	++
Uplifting of lamellae	+	++	++
Lamellar disorganization	+	+++	++++
Liver			
Congestion	+	++	+++
Ceroid formation	+	++	+++
Vacuolar degeneration	+	++++	++++
Hemorrhages	+	++	+++
Pyknosis	+	++	++++
Nuclear hypertrophy	+	++	++++
Karyorrhexis	+	+++	++++
Karyolysis	+	++++	++++
Hepatocytes with eccentric nuclei	+	++++	++++
Heart			
Congestion	+	++	++
Edema, Myofibrosis Neutrophilic myocarditis	+	+++	++++
Myofibrosis	+	++	+++
Neutrophilic myocarditis	+	++	+++
Kidneys			
Necrosis of renal tubules	+	++	+++
Widening of urinary space	+	+++	++++
Necrosis of renal tubular epithelial cells	+	+++	++++

DISCUSSION

Investigation on different species of fish encounter physiological, biochemical, histopathological ailments and DNA damage during the acclimatization period due to exposure to several toxic pesticides. Hence, this study investigated effects of lufenuron on status of oxidative stress markers, enzymatic antioxidants, genotoxicity and histopathological ailments of tilapia fish.

Pesticides usually lead to aquatic organisms under oxidative stress, which ultimately induce overproduction of reactive oxygen species and disruption of mechanisms of antioxidant enzymes. Oxidative stress also alters the molecular based functions of different visceral organs (Akcha *et al.,* 2003; Slaninova *et al.,* 2009; Lukaszewicz-Hussain 2010). Antioxidant enzymes assist fish in environmental stresses in adopting to stress conditions and changing their activity; the extent of alteration relies on the intensity, species, and router of exposure to the stressful events (Clempus and Griendling, 2006; Gravato *et al.,* 2006). Hydrogen peroxide and anions of superoxide must be detoxified using the volatile antioxidant enzymes CAT and SOD. The antioxidant enzymes greatly protect organisms living in aquatic and terrestrial ecosystems from natural and artificial oxidative stress (Rao 2006; Nwani *et al.,* 2010). The enzyme SOD is of vital important which counteract the free radicals. Thiobarbituric Acid Reactive Substance (TBRAS), a significant secondary oxidative stress product is valuable for monitoring oxidative stress along with oxidative enzymes. It is made when polyunsaturated fatty acids are oxidized (PUFA). The results showed that values of TBARS and ROS increased significantly during the study. The aquatic animals particularly different species of fish have the capacity to combat the oxidative stress (Fazelan *et al.,* 2020). It has been recorded that SOD play important and crucial roles being the first line of defense mechanism during the stress induced by any environmental pollutants (Jindal and Kaur, 2014). The gills are the primary organs that have direct contact with water and toxicant dissolved in water. In the current study, activity of SOD, POD and CAT in gills of fish exposed to lufenuron declined dramatically at 39 day of treatment. Similar findings were made in earlier experiment using *Carassius auratus* gibelio (Zikić *et al.,* 2001), *Prochilodus lineatus* (Simonato *et al.,* 2011). Different several published studies have investigated that pesticides are the major pollutants which induce oxidative stress via overproduction of free radicals in fish (Mahmood *et al*., 2022; Veedu *et al*. 2022; Wang *et al*., 2022). It has also been recorded that antioxidant enzymes like SOD, GST CAT and GPx play crucial role in prevention of injuries of exposed tissues due to pesticides in aquatic organisms (Veedu *et al*., 2022; Li *et al*., 2022).

Results from the comet assay for determination of genotoxic effects (Fig. 1) demonstrated that treated fish had considerably higher frequency of DNA damage in different tissues (gills, kidneys and liver) at days 26 and 39 of the research. Studies have recorded that comet assay is a suitable, appropriate, frequently used, sensitive, and reliable technique measurement of DNA damage in different tissues of both terrestrial and aquatic life (Lee and Steinert, 2003; Ghisi *et al.,* 2011; Raza *et al*., 2022). Formerly, no information could be found on DNA damage due to exposure to lufenuron in different tissues of tilapia. In our trial, the results on genotoxic investigation exhibited significantly increased DNA damage potential of lufenuron in different tissues of tilapia fish. The higher values of DNA damage in visceral tissues of tilapia fish could be due to rapid generation of free radicals leading to induction of oxidative stress (Kawaguchi et al., 2008; Gaivão *et al.,* 2009; Hussain *et al*., 2011; Raza *et al*., 2022).

Various moderate to severe microscopic ailments in gills including congestion of cartilaginous core, fusion of lamellae, congestion, degeneration of cartilaginous core, lamellar atrophy, necrosis of lamellar pillar cells, telangiectasia, disruption of primary lamellae, curling of secondary lamellae, uplifting of lamellae and lamellar disorganization have been observed at day 39 of the recent study. Previously, several histopathological irregularities have been observed in gills of *Colossoma macropomum* (Bernet et al., 1999; Soares *et al*., 2016) induced by lufenuron. Because of their close proximity to water, gills are crucial for osmoregulation gas exchange, excretion of nitrogenous waste, acid-base balance, but they are also the first to experience the negative effects of various pollutants and pesticides (Bortner and Cidlowski, 1996; Ghelichpour *et al.,* 2017). These alterations can be explained as an adaptive strategy to sustain physiological processes since the gills were exposed to toxic substances. However, this could have unintended consequences including respiratory and ionic disruptions that could harm the structural

integrity of gills (Kim and Kang, 2016; Aldoghachi *et al.*, 2016). The liver of tilapia fish showed histopathological alterations induced by higher concentrations of lufenuron (1.2 and 1.7µg/L) such as ccongestion, ceroid formation, vacuolar degeneration, hemorrhages, pyknosis, nuclear hypertrophy, karyorrhexis, karyolysis and hepatocytes with eccentric nuclei. In prior studies, the similar histopathological alterations were observed in *Cyprinus carpio* (Ghelichpour *et al*., 2020). Hearts of several fish were found to have histopathological abnormalities such as congestion, edema, myofibrillosis and neutrophilic myocarditis. The exposure of lufenuron causes congestion, necrosis of neurons and intracellular edema, which were mild to moderate histological alterations in the brain of fish. The exposure of lufenuron induces histopathological changes in kidneys including necrosis of renal tubules, widening of urinary space and necrosis of renal tubular epithelial cells. Previously, there was no published data was found regarding the induction of toxic histopathological alterations in heart, kidney, and brain of various fish species due to lufenuron.

Conclusions: From the findings it may be suggested that lufenuron causes adverse effects in different tissue of fish, even at very low levels. Histopathological analysis of gills, kidneys, livers, brain, and hearts of *Oreochromis niloticus* exposed to lufenuron at increased level indicated severe alterations. It was also observed that lufenuron induced DNA damage and oxidative stress in exposed fish. This study investigated the mechanisms of toxicity of lufenuron and concerns regarding the use and disposal of lufenuron for environmental pollution and mitigation measures to prevent contamination in aquatic populations.

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Authors contribution: SI, RH and SN plan and executed the trial. FAAS, MHS, AMMC, AG, and RA involved in data analysis and results interpretation. RH and SN prepared the paper and edited the manuscript.

REFERENCES

Aci R, Muderrisoglu S, Duran U, *et al*., 2020. Content of minerals in muscle tissue of diploid rainbow trout (*Oncorhynchus mykiss*) in freshwater (Derbent Dam) and seawater (The Black Sea). J Elementol 25:1375-84.

Adel M, Dadar M, Khajavi SH, *et al*., 2017. Hematological, biochemical and histopathological changes in Caspian brown trout (*Salmo trutta caspius* Kessler, 1877) following exposure to sublethal concentrations of chlorpyrifos. Toxin Reviews 36:73-79.

Ahmad L, Gul ST, Saleemi MK, *et al*., 2021. The effect of different repeated doses of cypermethrin on the behavioral and histological alterations in the brain of rabbits (*Oryctolagus cuniculi*). Int J Vet Sci 10:347-54. doi: 10.47278/ journal.ijvs/2021.092.

Akcha F, Vincent Hubert F and Pfhol-Leszkowicz A, 2003. Potential value of the comet assay and DNA adduct measurement in dab (*Limanda limanda*) for assessment of in situ exposure to genotoxic compounds. Mutation Research/Genetic Toxicol Environ Mutagen 534:21-32.

Akram R, Ghaffar A, Hussain R, *et al*., 2021. Hematological, serum biochemistry, histopathological and mutagenic impacts of triclosan on fish (bighead carp). Agrobiol Rec 7:18-28. doi: 10.47278/journal.abr/2021.009

Akram R, Iqbal R, Hussain R, *et al*., 2021. Evaluation of Oxidative stress, antioxidant enzymes and genotoxic potential of bisphenol A in fresh water bighead carp (*Aristichthys nobils*) fish at low concentrations. Environ Pollution 268:115896.

Akram R, Iqbal R, Hussain R, *et al*., 2022. Effects of bisphenol a on hematological, serum biochemical, and histopathological biomarkers in bighead carp (Aristichthys nobilis) under long-term exposure. Environ Sci Poll Res 29:21380–21395

Aldoghachi MA, Azirun MS, Yusoff I, *et al*., 2016. Ultrastructural effects on gill tissues induced in red tilapia *Oreochromis* sp. by a waterborne lead exposure. Saudi J Biol Sci 23:634-41.

Banaee M, Nematdoust HB and Ibrahim A, 2014. Sub-lethal toxicity of chlorpyrifos on Common carp, *Cyprinus carpio* (Linnaeus, 1758): Biochemical response. Int J Aquat Biol 1:281-88.

Bernet D, Schmidt H, Meier W, *et al*., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J Fish Dis 22:25-34.

Bortner CD and Cidlowski JA, 1996. Absence of volume regulatory mechanisms contributes to the rapid activation of apoptosis in thymocytes. American J Physiol-Cell Physiol 271:950-C61.

Clemente Z, Castro VL, Feitosa LO, *et al*., 2013. Fish exposure to nano-TiO2 under different experimental conditions: Methodological aspects for nanoecotoxicology investigations. Sci Total Environ 463-464:647-56.

Clempus RE and Griendling KK, 2006. Reactive oxygen species signaling in vascular smooth muscle cells. Card Res 71:216-25.

Da Rocha CaM, Dos Santos RA, Bahia MDO, *et al*., 2009. The Micronucleus Assay in Fish Species as an Important Tool for Xenobiotic Exposure Risk Assessment—A Brief Review and an Example Using Neotropical Fish Exposed To Methylmercury. Rev Fish Sci 17:478-84.

Fazelan Z, Vatnikov YA, Kulikov EV, *et al*., 2020. Effects of dietary ginger (*Zingiber officinale*) administration on growth performance and stress, immunological, and antioxidant responses of common carp (*Cyprinus carpio*) reared under high stocking density. Aquaculture 518:734833.

Franco G, 1991. New perspectives in biomonitoring liver function by means of serum bile acids: experimental and hypothetical biochemical basis. British J Industrial Med 48:557-61.

Gaivão I, Piasek A, Brevik A, *et al*., 2009. Comet assay-based methods for measuring DNA repair in vitro; estimates of inter- and intra-individual variation. Cell Biol Toxicol 25:45-52.

Ghaffar A, Hussain R, Aslam M, *et al*., 2016. Arsenic and urea in combination alters the hematology, biochemistry and protoplasm in exposed rahu fish (*Labeo rohita*)(Hamilton, 1822). Turk J Fish Aquat Sci 16:289-96.

Ghelichpour M and Mirghaed TA, 2019. Effects of sublethal exposure to new pesticides lufenuron and flonicamid on common carp, *Cyprinus carpio*, hydromineral balance to further saltwater exposure. Int J Aquat Biol 7:195-201.

Ghelichpour M, Mirghaed AT, Hoseini SM, *et al*., 2020. Plasma antioxidant and hepatic enzymes activity, thyroid hormones alterations and health status of liver tissue in common carp (*Cyprinus carpio*) exposed to lufenuron. Aquaculture 516: 734634.

Ghelichpour M, Taheri Mirghaed A, Mirzargar SS, *et al*., 2017. Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of *Cyprinus carpio* (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb. Aquacul Res 48:5666-76.

Ghisi NDC, Ramsdorf WA, Ferraro MVM, *et al*., 2011. Evaluation of genotoxicity in *Rhamdia quelen* (Pisces, Siluriformes) after sub-chronic contamination with Fipronil. Environ Monit Assessment 180:589-99.

Gravato C, Teles M, Oliveira M, *et al*., 2006. Oxidative stress, liver biotransformation and genotoxic effects induced by copper in *Anguilla anguilla* L. – the influence of pre-exposure to β-naphthoflavone. Chemosphere 65:1821-30.

Habig WH, Pabst MJ, Fleischner G, *et al*., 1974. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proc National Acad Sci United States America 71:3879-82.

Hoseini SM and Yousefi M, 2019. Beneficial effects of thyme (*Thymus vulgaris*) extract on oxytetracycline-induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (*Oncorhynchus mykiss*). Aquacul Nutrition 25:298-309.

Hoseinifar SH, Zou HK, Van Doan H, *et al*., 2019. Can dietary jujube (*Ziziphus jujuba* Mill.) fruit extract alter cutaneous mucosal immunity, immune related genes expression in skin and growth

performance of common carp (*Cyprinus carpio*)?. Fish Shellfish Immunol 94:705-10.

Hussain R, Ali F, Javed MT, *et al*., 2019. Clinico-hematological, serum biochemical, genotoxic and histopathological effects of trichlorfon in adult cockerels, Toxin Reviews 1556-9551.

Hussain R, Ghaffar A, Abbas G, *et al*., 2022. Thiamethoxam at sublethal concentrations induces histopathological, serum biochemical alterations and DNA damage in fish (*Labeo rohita*). Toxin Reviews 41:154-64.

Hussain R, Mahmood F, Khan MZ, *et al*., 2011. Pathological and genotoxic effects of atrazine in male Japanese quail (Coturnix japonica). Ecotoxicology 20: 1-8.

Islam SM, Rahman MA, Nahar S, *et al*., 2019. Acute toxicity of an organophosphate insecticide sumithion to striped catfish *Pangasianodon hypophthalmus*. Toxicol Reports 6: 957-962.

Jabeen G, Manzoor F, Arshad M, *et al.,* 2021. Effect of cadmium exposure on hematological, nuclear and morphological alterations in erythrocyte of fresh water fish (Labeo rohita). Continental Vet J 1:20-24

Jindal R and Kaur M, 2014. Phenotypic alterations in erythrocytes of *Ctenopharyngodon idellus* (Cuvier & Valenciennes) induced by chlorpyrifos: SEM study. Internat J Fish Aquacul Sci 4:23-30.

Kakkar R, Kalra J, Mantha SV, *et al*., 1995. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Mol Cell Biochem 151:113-19.

Kawaguchi S, Nakamura T, Honda G, *et al*., 2008. Detection of DNA Single Strand Breaks Induced by Chemical Mutagens Using the Acellular Comet Assay. Gen Environ 30:77-85.

Kaya İ, Karapehlivan M, Yilmaz M, *et al*., 2012. Investigation of effects on plasma nitric oxide, malondialdehyde and total sialic acid levels of glyphosate in Kars creek transcaucasian barb (*Capoeta capoeta* [Guldenstaedt, 1773]) in Turkey. Fres Environ Bull 21:123-26.

Kim J-H and Kang J-C, 2016. The toxic effects on the stress and immune responses in juvenile rockfish, *Sebastes schlegelii* exposed to hexavalent chromium. Environ Toxicol Pharmacol 43:128-33.

Kiran H, Kousar S, Ambreen F, Ilyas R, *et al.,* 2022. Effect of plant-based feed on the antioxidant enzymes, biochemical and hematological parameters of Oreochromis niloticus. Cont Vet J 2:67-75.

Kosz-Vnenchak M and Rokosz K, 1997. The "comet" assay for detection of potential genotoxicity of polluted water. Folia Biol 45:153-6.

Lee RF and Steinert S, 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat Res/Reviews Mut Res 544:43-64.

Li X, Naseem S, Hussain R, *et al.,* 2022. Evaluation of DNA damage, biomarkers of oxidative stress and status of antioxidant enzymes in freshwater fish (*Labeo rohita*) exposed to pyriproxyfen. Oxidat Med Cell Long 5859266: https://doi.org/10.1155/2022/5859266

Lukaszewicz-Hussain A, 2010. Role of oxidative stress in organophosphate insecticide toxicity – Short review. Pest Biochem Physiol 98:145-50.

Mahmood Y, Hussain R, Ghaffar A, *et al*., 2022. Acetochlor affects bighead carp (*Aristichthys nobilis*) by producing oxidative stress, lowering tissue proteins, and inducing genotoxicity. BioMed Res Internat 9140060:. https://doi.org/10.1155/2022/9140060

Maksymiv IV, Husak VV, Mosiichuk NM, *et al*., 2015. Hepatotoxicity of herbicide Sencor in goldfish may result from induction of mild oxidative stress. Pest Biochem Physiol 122:67-75.

Merdana IM, Watiniasih NL, Sudira IW, *et al.,* 2021. The effect of ethanolic extract of *Myrmecodia pendans* on gentamicin induced nephrotoxicity in Wistar Rats. Int J Vet Sci 10:96-101. doi: 10.47278/ journal.ijvs/2021.025

Mitchelmore CL and Chipman JK, 1998. DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. Mutation Res/Fund Mol Mech Mutagen 399:135-47.

Mujahid Q, Khan A, Saleemi MK, *et al*., 2021. Allethrin induced Toxicopathological alterations in adult male albino rats. Agrobiol Rec 5:8-14. doi: 10.47278/journal.abr/2020.019.

Naz S, Hussain R, Ullah Q, *et al*., 2021. Toxic effect of some heavy metals on hematology and histopathology of major carp (*Catla catla*). Environmental Sci Poll Res 28:6533-39.

Naz S, Mansouri B, Chatha AMM, *et al*., 2022. Water quality and health risk assessment of trace elements in surface water at Punjnad Headworks, Punjab, Pakistan. Environmental Sci Poll Res 29:61457-69.

Nur G, Caylak E, Kilicle PA, *et al*., 2022. Immunohistochemical distribution of Bcl-2 and p53 apoptotic markers in acetamiprid-induced nephrotoxicity. Open Med (Wars) 17:1788-1796.

Nur G, Yilmaz M, Karapehlivan M, *et al*., 2017. The effect of tebuconazole on serum paraoxonase and aminotransferase activities in *Cyprinus carpio* (L. 1758). Fresenius Environment Bulletin 26:6212-16.

Nwani CD, Lakra WS, Nagpure NS, *et al.,* 2010. Toxicity of the Herbicide Atrazine: Effects on Lipid Peroxidation and Activities of Antioxidant Enzymes in the Freshwater Fish *Channa Punctatus* (Bloch). Int J Environmental Res Public Health 7:3298-312

Obiakor M, Okonkwo J, Nnabude P, *et al*., 2012. Eco-genotoxicology: micronucleus assay in fish erythrocytes as in situ aquatic pollution biomarker: a review. J Animal Sci Advan 2:123-33.

Rao JV, 2006. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. Pest Biochemist Physiol 86:78-84.

Raza GA, Ghaffar A, Hussain R, *et al.,* 2022. Nuclear and Morphological Alterations in Erythrocytes, Antioxidant Enzymes, and Genetic Disparities Induced by Brackish Water in Mrigal Carp (*Cirrhinus mrigala*). Oxid Med Cellul Longevit 4972622.

Simonato JD, Fernandes MN and Martinez CB, 2011. Gasoline effects on biotransformation and antioxidant defenses of the freshwater fish *Prochilodus lineatus*. Ecotoxicology (London, England) 20:1400-10.

Singh NP, McCoy MT, Tice RR, *et al*., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experiment Cell Res 175: 184-191.

Slaninova A, Smutna M, Modra H, *et al*., 2009. A review: oxidative stress in fish induced by pesticides. Neuro Endocrinol Lett 30 Suppl 1:2-12.

Soares RLP, Lucas Corrêa De Andrade A, Pinheiro Santos T, *et al*., 2016. Acute and chronic toxicity of the benzoylurea pesticide, lufenuron, in the fish, *Colossoma macropomum*. Chemosphere 161:412-21.

Taha MG, El-Hamamsy SMA, Ahmed NS, Ali MM, *et al.,* 2020. Amelioration effect of *Carica papaya* fruit extracts on doxorubicin – induced cardiotoxicity in rats. Int J Vet Sci 9:349-54.

Tahir R, Ghaffar A, Abbas G, *et al*., 2021. Pesticide induced hematological, biochemical and genotoxic changes in fish: A Review. Agrobiol Rec 3:41-57. doi: 10.47278/journal.abr/2021.005

Vázquez MMP, Vázquez PP, Galera MM, *et al*., 2014. Comparison of two ionic liquid dispersive liquid–liquid microextraction approaches for the determination of benzoylurea insecticides in wastewater using liquid chromatography–quadrupole-linear ion trap–mass spectrometry: Evaluation of green parameters. J Chromatograph A 1356:1-9.

Veedu SK., Ayyasamy G, Tamilselvan H, *et al.,* 2022. Single and joint toxicity assessment of acetamiprid and thiamethoxam neonicotinoids pesticides on biochemical indices and antioxidant enzyme activities of a freshwater fish *Catla catla.* Comp Biochem Physiol, Part C: Toxicol Pharmacol 257: https://doi.org/10.1016/j.cbpc.2022.109336.

Wang JQ, Hussain R, Ghaffar A, *et al.,* 2022. Clinico-hematological, mutagenic, and oxidative stress induced by pendimethalin in freshwater fish bighead carp (*Hypophthalmichthys nobilis*). Oxidat Med Cell Long 2093822: https://doi.org/10.1155/2022/2093822

Xing H, Li S, Wang Z, *et al*., 2012. Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. Chemosphere 88:377-83.

Zikić RV, Stajn AS, Pavlović SZ, *et al*., 2001. Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish (*Carassius auratus* gibelio Bloch.) exposed to cadmium. Physiol Res 50:105-11.