International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 04-04-2026, Accepted: 05-05-2026, Published: 20-05-2026
Volume 11, Issue 2, 2026, Page No. 254-261

Assessment of isoproturon-induced oxidative stress in freshwater fish *Cyprinus carpio*

Devika Rani H K[1], Parimala B[2*]
[1] Research Scholar, DOSR in Zoology, University College of Science, Tumkur University, Tumakuru, Karnataka, India
[2] Professor, Department of Zoology, University College of Science, Tumkur University, Tumakuru, Karnataka, India
Corresponding Author: Parimala B

Abstract
This study investigated the impact of lethal (4.5 mg/L) and sublethal (0.45 mg/L) concentrations of Isoproturon on oxidative stress responses in the freshwater fish *Cyprinus carpio*. Experimental fish were exposed to lethal concentrations for short durations (1–4 days) and to sublethal concentrations for extended periods (1, 5, 10, and 15 days) under controlled laboratory conditions. The study mainly focused on evaluating oxidative stress biomarkers and antioxidant defense mechanisms in the gill, liver, and kidney tissues. Exposure to Isoproturon caused significant biochemical and physiological alterations in the treated fish compared to the control group. Activities of antioxidant-related enzymes such as catalase and protease showed noticeable elevation, reflecting the activation of cellular defense mechanisms against herbicide-induced oxidative stress. Similarly, the levels of hydrogen peroxide, malondialdehyde, protein carbonyls, and free amino acids increased considerably in all examined tissues, indicating enhanced lipid peroxidation, oxidative protein damage, and metabolic imbalance. Conversely, total protein content decreased significantly, possibly due to increased protein degradation and higher energy demands under toxic stress conditions.
The observed responses were dependent on both concentration and exposure duration, with more severe effects recorded at higher concentrations and longer exposure periods. Among the tissues analyzed, the liver displayed the highest degree of oxidative damage, likely because of its central role in detoxification and metabolic activities. In addition to biochemical changes, exposed fish exhibited behavioral disturbances such as abnormal swimming patterns, reduced feeding behavior, excessive mucus secretion, and loss of equilibrium, which further confirmed the toxic influence of Isoproturon. The overall findings suggest that Isoproturon induces pronounced oxidative stress and impairs antioxidant defense systems in *Cyprinus carpio*. Therefore, oxidative stress biomarkers may be effectively used as reliable indicators for assessing herbicide toxicity and monitoring environmental contamination in freshwater ecosystems.

Keywords: Isoproturon, oxidative stress, antioxidant enzymes, lipid peroxidation, catalase activity, oxidative damage, environmental toxicology, aquatic pollution

Introduction
The rapid growth of agricultural activities and the increasing demand for enhanced crop productivity have led to the extensive application of pesticides and herbicides throughout the world. Although these agrochemicals play an important role in controlling weeds and improving agricultural yield, their indiscriminate and excessive usage has become a major environmental concern. Large amounts of herbicide residues enter aquatic ecosystems through agricultural runoff, irrigation discharge, and soil leaching, resulting in contamination of rivers, ponds, lakes, and reservoirs. Such contamination poses serious threats to aquatic organisms, particularly fish, which are highly sensitive to environmental pollutants and are widely used as bioindicators in ecotoxicological investigations. Exposure to toxic chemicals may alter physiological, biochemical, hematological, behavioral, and molecular functions in fish, thereby affecting their growth, metabolism, reproduction, and survival.
Among the commonly used herbicides, Isoproturon, a selective systemic phenyl urea herbicide, is extensively applied for controlling broadleaf weeds and grasses in cereal crops. Due to its widespread agricultural usage and moderate persistence in the environment, residues of Isoproturon are frequently detected in freshwater ecosystems. Once introduced into aquatic habitats, Isoproturon can accumulate in fish tissues and interfere with normal cellular and metabolic processes. Previous investigations have shown that herbicides such as Isoproturon induce oxidative stress, neurotoxicity, genotoxicity, endocrine disruption, and histopathological alterations in aquatic organisms. According to (Khan, 2006) [26], pesticides are capable of generating free radicals that cause severe toxic effects in tissues such as liver and kidney. Similarly, (Limon-Pacheco and Gonsebatt, 2009) [30] reported that pesticides act as prooxidants and disturb the oxidant antioxidant balance in multiple organs, thereby promoting oxidative damage.
Oxidative stress is considered one of the major mechanisms involved in herbicide-induced toxicity. Under normal physiological conditions, reactive oxygen species (ROS) generated during cellular metabolism are efficiently neutralized by antioxidant defense systems. However, exposure to toxicants such as Isoproturon enhances ROS production beyond the scavenging capacity of antioxidant enzymes, resulting in oxidative stress and cellular injury. Excessive ROS generation can damage essential biomolecules including proteins, lipids, carbohydrates, and nucleic acids, ultimately leading to membrane destabilization, enzyme inactivation, DNA strand breaks, and cell death. (Mates, 2000) [32] explained that ROS are highly reactive molecules capable of causing extensive cellular damage, (Kelly *et al.*, 1998) [24] emphasized that oxidative stress develops when ROS generation exceeds

antioxidant defense capacity. (Ozyurt *et al.,* 2004) [34] demonstrated that oxidative stress induced by toxic compounds can damage proteins, lipids, and DNA, thereby contributing to tissue degeneration and inflammatory responses. (Barzilai and Yamamoto, 2004) [5] further suggested that abnormal ROS generation serves as an important indicator of oxidative cellular injury.

Fish possess efficient antioxidant defense mechanisms that protect cells against ROS-mediated damage. These antioxidant systems include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and peroxiredoxins, along with non-enzymatic antioxidants such as reduced glutathione (GSH). (Pi *et al.,*2010) reported that antioxidant enzymes play a crucial role in neutralizing toxic free radicals generated during xenobiotic exposure and maintaining cellular redox balance. Therefore, alterations in antioxidant enzyme activities are widely recognized as reliable biomarkers of oxidative stress in aquatic organisms exposed to environmental contaminants.

Lipid peroxidation is one of the most important consequences of oxidative stress and is extensively used as an indicator of free radical-mediated cellular damage. Aquatic organisms are particularly susceptible to lipid peroxidation because their tissues contain high levels of polyunsaturated fatty acids that are highly prone to oxidation. Reactive oxygen species attack membrane phospholipids and initiate chain reactions that result in the formation of lipid peroxides and secondary products such as malondialdehyde (MDA) and 4-hydroxynonenal. Among these, MDA is considered one of the most sensitive biomarkers for evaluating oxidative damage in biological systems. (Volodymyr, 2011) [39] explained that measurement of thiobarbituric acid reactive substances (TBARS) is widely used to estimate lipid peroxidation levels in aquatic toxicology studies. Increased MDA levels in fish tissues indicate membrane damage and oxidative deterioration caused by toxic pollutants.

Protein oxidation is another major consequence of ROS-induced cellular injury. Oxidative modification of proteins results in the formation of carbonyl groups, which are considered reliable markers of oxidative stress. Spectrophotometric determination of protein carbonyl content using dinitrophenylhydrazine has become a widely accepted method for assessing oxidative protein damage in aquatic organisms. Elevated protein carbonyl levels reflect oxidative alteration of structural and functional proteins, which may impair metabolic and enzymatic activities within cells (Volodymyr, 2011) [39]. In addition to protein oxidation, alterations in free amino acids and total protein levels are also important indicators of metabolic disturbances caused by toxicants.

Aquatic ecosystems receive numerous agricultural and industrial pollutants that can disturb free radical generation and antioxidant defense systems in aquatic organisms. Fish absorb these pollutants through water, food, sediments, and suspended particulate matter, making them highly vulnerable to toxic effects. Recent advances in aquatic toxicology have demonstrated that oxidative stress biomarkers are highly effective tools for evaluating pollutant-induced toxicity and ecological risks in freshwater environments. (Aluru *et al.,* 2024) [2] observed significant proteomic and oxidative stress alterations in zebrafish exposed to herbicides, while (Hu *et al.,* 2026) reported that environmental contaminants induce oxidative and metabolic disturbances in fish tissues. Studies conducted on freshwater fishes such as *Danio rerio*, *Oreochromis niloticus*, *Labeo rohita*, and *Cyprinus carpio* have shown significant increases in lipid peroxidation, hydrogen peroxide accumulation, and protein carbonyl levels along with alterations in catalase and superoxide dismutase activities following herbicide exposure. These findings suggest that oxidative stress biomarkers are highly sensitive indicators of aquatic pollution.

Among freshwater fishes, *Cyprinus carpio* is widely recognized as an important experimental model in aquatic toxicology because of its economic importance, wide distribution, adaptability to laboratory conditions, and sensitivity to environmental contaminants. Previous studies have reported that exposure of *Cyprinus carpio* to herbicides causes behavioral abnormalities such as erratic swimming, excessive mucus secretion, reduced feeding activity, surfacing behavior, and loss of equilibrium. In addition, biochemical and physiological disturbances have been observed in vital organs such as liver, kidney, and gills, which are directly involved in detoxification, respiration, osmoregulation, and excretion. Therefore, evaluation of oxidative stress biomarkers in these tissues can provide valuable information regarding herbicide-induced toxicity in freshwater environments.

Recent developments in environmental toxicology emphasize that no single biomarker alone is sufficient to comprehensively evaluate oxidative stress. Hence, multiple oxidative stress indices are generally analyzed together to obtain a better understanding of toxicant-induced damage (Volodymyr, 2011) [39]. Biomarkers such as catalase activity, hydrogen peroxide levels, malondialdehyde content, protein carbonyls, free amino acids, protease activity, and total protein levels are widely used for assessing oxidative stress responses in fish exposed to pesticides and herbicides. These biochemical parameters provide important information regarding the integrity of antioxidant defense systems and the extent of cellular damage caused by toxicants.

Therefore, the present study was undertaken to investigate the effects of lethal and sublethal concentrations of Isoproturon on oxidative stress responses in different tissues of *Cyprinus carpio*. The study focuses on evaluating alterations in antioxidant defense mechanisms and oxidative damage biomarkers including catalase activity, protease activity, hydrogen peroxide, malondialdehyde, protein carbonyls, free amino acids, and total protein levels. The extent of lipid peroxidation was also assessed because ROS can attack membrane phospholipids and initiate oxidative degradation of fatty acid chains. Evaluation of these biomarkers may provide valuable insights into the toxic effects of Isoproturon and its ecological impact on freshwater aquatic ecosystems.

Materials and methods

Sample collection, maintenance and acute toxicity

Healthy and disease-free fingerlings of *Cyprinus carpio* were obtained from the Fisheries Department, Turvekere, Tumakuru, Karnataka, India. The fish were transported to the laboratory in well-aerated plastic containers to reduce transportation stress and mortality. After reaching the laboratory, the fish were carefully transferred to large plastic tubs for acclimatization under laboratory conditions. The

experimental fish, *Cyprinus carpio*, with an average body weight of 5±1 g and length of 7.5 ± 0.25 cm, were acclimatized to laboratory conditions for 20–30 days prior to the commencement of the experiment. Healthy and active fish were selected for the study, while injured or unhealthy individuals were discarded. During the acclimatization period, the fish were maintained in 100-L glass aquaria containing dechlorinated tap water with continuous aeration and were fed daily with commercially available pellet feed. A constant photoperiod of 12 h light and 12 h dark was maintained throughout both acclimatization and experimental periods.

The physicochemical characteristics of the experimental water were monitored regularly according to standard procedures prescribed by (APHA, 2005) [3]. Water quality parameters were maintained as follows: temperature 24 ± 2 °C, pH 8.0 ± 0.2, dissolved oxygen 9.6 ± 0.8 mg/L, free carbon dioxide 6.3 ± 0.4 mg/L, total hardness 23.4 ± 3.4 mg as $CaCO_3$/L, phosphate 0.39 ± 0.002 µg/L, specific gravity 1.001, and conductivity below 10 µS/cm throughout the study period.

To maintain optimum water quality and avoid accumulation of metabolic wastes, the aquarium water was renewed completely every day, and dead fish, if any, were removed immediately. The fish were regularly observed for normal swimming behavior and healthy physiological condition before being utilized for toxicity experiments. Feeding was discontinued 48 hours prior to the initiation of exposure studies to minimize contamination of the test medium and reduce variations in metabolic activity during the experimental period.

The herbicide Isoproturon (75 % WP) used in the present study was purchased from a local agrochemical store in Tumakuru, Karnataka, India. Before the experiment, the quality and expiry date of the chemical were carefully checked. Fresh stock solutions of Isoproturon were prepared using dechlorinated tap water whenever required for the experiment.

For the acute toxicity assay, the fish were divided into groups of ten individuals and exposed to different concentrations of Isoproturon in 20 L of water. A separate control group without herbicide exposure was also maintained under identical laboratory conditions. Initially, a preliminary range-finding test was conducted to identify suitable concentrations for the definitive toxicity test. The concentrations selected for the final experiment ranged from the highest concentration that produced no mortality to the lowest concentration that resulted in complete mortality of the fish.

During the exposure period, the test medium was renewed every 24 hours to maintain the required concentration of the herbicide and to ensure stable water quality conditions. After renewal of the water, freshly prepared concentrations of Isoproturon were added to the aquaria. Fish mortality was monitored and recorded at 24-hour intervals up to 96 hours, and dead fish were removed immediately to prevent deterioration of the water quality. Based on the mortality data obtained during the 96-hour exposure period, the median lethal concentration (LC50) value of Isoproturon for *Cyprinus carpio* was determined.

Catalase Activity (EC 1.11.1.6)

Catalase activity in the experimental tissues was determined following the method described by (Luck, 1974) [31]. Fresh tissue samples were homogenized (4 %) in ice-cold phosphate buffer (50 mM, pH 7.0) using a glass–Teflon homogenizer to obtain a uniform tissue extract. The homogenates were then centrifuged at 3,000 rpm for 15 minutes, and the clear supernatant obtained was used for enzyme analysis.

For the assay, 1 ml of the supernatant was mixed with 2 ml of phosphate buffer containing 10 mM hydrogen peroxide, which served as the substrate for catalase activity. The reaction was initiated immediately after the addition of hydrogen peroxide, and the decomposition of hydrogen peroxide was monitored by measuring the decrease in absorbance at 240 nm using a UV–Visible spectrophotometer (Hitachi, Model U-3310). Catalase activity was expressed as millimoles of hydrogen peroxide decomposed per milligram of protein per minute.

Hydrogen Peroxide Levels

The hydrogen peroxide content in different tissues was estimated according to the method of (Pick and Keisari, 1981) [36] using horseradish peroxidase (HRP) and phenol red reagent. Tissue homogenates prepared for the assay were processed under controlled conditions, and the absorbance was measured spectrophotometrically. The hydrogen peroxide levels were calculated and expressed as nanomoles of hydrogen peroxide per milligram of protein.

Lipid Peroxidation Estimation

Lipid peroxidation was determined by estimating malondialdehyde (MDA), which is one of the major secondary products formed during oxidative degradation of membrane lipids. The estimation was carried out according to the method described by (Placer *et al*., 1966) [37] based on the reaction of thiobarbituric acid (TBA) with lipid peroxidation products. Since the assay measures thiobarbituric acid reactive substances (TBARS), it is commonly known as the TBARS assay and is widely used as an indicator of oxidative stress and membrane damage in biological tissues.

For the assay, 16 % tissue homogenates were prepared in ice-cold 50 mM Tris–HCl buffer (pH 6.8). From the homogenate, 0.8 ml was mixed with 2 ml of 15 % trichloroacetic acid (TCA) and centrifuged at 5,000 rpm for 15 minutes. The supernatant obtained after centrifugation was collected carefully, and 0.7 ml of 1 % TBA reagent was added to it. The reaction mixture was covered with aluminum foil and incubated in a boiling water bath at 100 °C for 60 minutes with continuous shaking.

After incubation, the test tubes were immediately cooled in ice-cold water for 10 minutes to stop the reaction. The absorbance of the developed pink-colored complex was measured at 532 nm using a UV–Visible spectrophotometer against a reagent blank. The level of lipid peroxidation was expressed as nanomoles of TBARS formed per milligram of protein using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Determination of Protein Carbonyls

Protein carbonyl content, an important indicator of oxidative protein damage, was estimated using the method described by (Quinlan *et al.,* 1994) [38] based on derivatization with 2,4-dinitrophenylhydrazine (DNPH). This method is widely used for evaluating oxidative stress-induced protein oxidation in biological tissues.

For the assay, tissue homogenates (16 %) were prepared in ice-cold 50 mM Tris–HCl buffer (pH 6.8). Two aliquots of 40 µl from each homogenate were taken separately. To the first aliquot, 1 ml of freshly prepared 10 mM DNPH dissolved in 2 M HCl was added, while the second aliquot received 1 ml of 2 M HCl and served as the corresponding blank for each sample. The reaction mixtures were incubated at 37 °C for 90 minutes with intermittent gentle shaking to allow complete derivatization of protein carbonyl groups.

Following incubation, 2 ml of 15 % trichloroacetic acid (TCA) was added to each tube to precipitate the proteins. The tubes were then kept on ice for 5 minutes and centrifuged at 5,000 rpm for 10 minutes. After centrifugation, the supernatant was discarded carefully, and the resulting protein pellet was washed with 5 ml of a solution containing equal volumes of ethanol and ethyl acetate (50:50). The mixture was centrifuged again at 4,000 rpm for 5 minutes, and the washing procedure was repeated to remove excess reagent and impurities.

After the final wash, the tubes were inverted for a few minutes to drain excess liquid, and the protein pellet was dissolved in 1 ml of 6 M guanidine hydrochloride prepared in 2 M HCl. The samples were then kept in the dark for 60 minutes to facilitate complete color development. The absorbance was measured at 360 nm using a UV–Visible spectrophotometer. Protein carbonyl concentration was calculated by subtracting the absorbance of the blank from the DNPH-treated sample and using an extinction coefficient of 21,000 M^{-1} cm^{-1} for the protein carbonyl–DNPH complex.

Estimation of Protease Activity

Protease activity in the experimental tissues was estimated by the ninhydrin method described by (Davis and Smith, 1955) [10]. Fresh tissue homogenates (4 %) were prepared in ice-cold phosphate buffer (50 mM, pH 7.0) using a homogenizer and then centrifuged at 3,000 rpm for 15 minutes. The clear supernatant obtained after centrifugation was used for the enzyme assay.

For the determination of protease activity, 2 ml of the supernatant was mixed with 0.5 ml of 1 % casein solution and 2 ml of 0.1 M phosphate buffer (pH 5.0). The reaction mixture was mixed thoroughly and incubated at 37 °C for 30 minutes to allow enzymatic digestion of casein. After incubation, the reaction was terminated by adding 2 ml of 2 % ninhydrin reagent. The contents were again mixed properly and heated in a boiling water bath for 20 minutes for color development.

The reaction mixture was then cooled, and the final volume was made up to 10 ml using a diluent containing distilled water and n-propanol in a 1:1 ratio. The intensity of the developed color was measured at 570 nm using a UV–Visible spectrophotometer against a reagent blank. Protease activity was expressed as micromoles of tyrosine equivalents released per milligram of protein per hour.

Estimation of Free Amino Acids

Free amino acid levels in the tissues were estimated following the ninhydrin method of (Moore and Stein, 1954) [33]. Tissue homogenates (4 %) were prepared in ice-cold phosphate buffer (50 mM, pH 7.0). To 0.2 ml of the homogenate, 2 ml of 15 % trichloroacetic acid (TCA) was added for protein precipitation, and the mixture was centrifuged at 3,000 rpm for 15 minutes.

The supernatant obtained after centrifugation was collected carefully, and 2 ml of ninhydrin reagent was added to the entire supernatant (2.2 ml). The contents were mixed thoroughly and heated in a boiling water bath for exactly 5 minutes to allow color development. After heating, the tubes were cooled immediately in ice-cold water, and the final volume was adjusted to 10 ml using a mixture of distilled water and n-propanol in a 1:1 ratio.

The optical density of the developed color was measured at 570 nm using a UV–Visible spectrophotometer against a reagent blank. The free amino acid content was expressed as micromoles of tyrosine equivalents per gram wet weight of tissue.

Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) of six replicates. The obtained results were statistically analyzed to determine the significance of variations among control and treatment groups. Statistical comparisons were carried out using Duncan's Multiple Range Test (DMRT) as described by (Duncan, 1955) [11]. Differences between the groups were considered significant at the appropriate probability level.

Results and Discussion

The present study revealed significant biochemical alterations in the gill, liver, and kidney tissues of *Cyprinus carpio* exposed to lethal and sublethal concentrations of Isoproturon. The activities of catalase and protease, along with the levels of hydrogen peroxide, malondialdehyde (MDA), protein carbonyls, and free amino acids, were found to increase considerably in the treated fish when compared to the control group. In contrast, total protein content showed a noticeable decrease in all the examined tissues (Figs. 1–7).

The magnitude of these alterations varied depending on both exposure duration and concentration of the herbicide. Higher concentrations and longer exposure periods resulted in more pronounced biochemical changes in the tissues of *Cyprinus carpio*. Among the different organs studied, the liver exhibited greater changes in oxidative stress parameters, possibly because of its major role in detoxification and metabolism of toxic substances. Significant alterations were also observed in the gills and kidneys, which are directly involved in respiration, osmoregulation, and excretion. The overall findings indicate that exposure to Isoproturon induces oxidative stress and disturbs the antioxidant defense system of the fish.

Hydrogen peroxide is a normal metabolic byproduct formed during aerobic respiration in living organisms. Under normal physiological conditions, its levels are regulated by antioxidant defense systems. However, exposure to toxicants such as Isoproturon can disturb the balance between reactive oxygen species (ROS) generation and antioxidant protection, resulting in excessive accumulation of H_2O_2 and oxidative stress. In the present study, elevated hydrogen peroxide levels recorded in the tissues of exposed fish indicate enhanced ROS generation and impairment of antioxidant defense mechanisms. Similar observations were reported by (Fantone and Ward, 1982; Freeman and Crapo, 1982; and Cerutti, 1985) [8, 12, 13] who demonstrated that

excessive ROS production contributes significantly to cellular injury and pathological conditions.

Although hydrogen peroxide itself is comparatively less reactive, it becomes highly toxic through the formation of hydroxyl radicals in the presence of transition metal ions and catalytic reactions (Halliwell *et al.,* 1992) [17]. These highly reactive radicals can attack cellular macromolecules such as lipids, proteins, carbohydrates, and nucleic acids, leading to oxidative injury and cellular dysfunction. Reactive intermediates including superoxide anions, hydroxyl radicals, and hypochlorous acid are capable of causing irreversible damage to cellular structures and disturbing normal physiological activities (Husain *et al.,* 2001[20]; Talas *et al.,* 2008). Excessive ROS accumulation therefore results in oxidative stress, tissue degeneration, and metabolic impairment in exposed organisms.

The increased catalase activity observed in the present study appears to be an adaptive protective response against excessive hydrogen peroxide accumulation. Catalase is an important antioxidant enzyme that converts hydrogen peroxide into water and oxygen, thereby minimizing oxidative injury within cells. The elevation in catalase activity during experimental exposure indicates activation of antioxidant defense mechanisms in response to herbicide-induced oxidative stress. Similar findings were reported by (John *et al.,* 2001) [22], who suggested that enhanced catalase activity acts as a protective mechanism against increased ROS production under toxic stress conditions. Comparable observations were also made by (Zhi-Hua *et al.,* 2010) in rainbow trout (*Oncorhynchus mykiss*) exposed to carbamazepine and by (Verlecar *et al.,* 2008) in *Perna viridis* exposed to mercury contamination. However, prolonged exposure to Isoproturon appears to overwhelm the antioxidant defense system, resulting in oxidative damage and tissue injury.

Lipid peroxidation is considered one of the major consequences of oxidative stress and an important indicator of membrane damage in aquatic organisms. Fish tissues contain high levels of polyunsaturated fatty acids, which are highly susceptible to oxidative attack by reactive oxygen species (Huang *et al.,* 2003) [19]. In the present study, increased levels of malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) in the gill, liver, and kidney tissues clearly indicate enhanced lipid peroxidation following exposure to lethal and sublethal concentrations of Isoproturon. Increased lipid peroxidation may alter membrane permeability, enzyme activity, ion transport systems, and overall cellular metabolism. According to (Storey, 1996), lipid peroxidation is one of the major causes of loss of cellular function under oxidative stress conditions. Similar observations were reported by (Oakes and Van der Kraak, 2003) [29], who identified TBARS as reliable indicators of oxidative damage in fish tissues.

The elevated MDA production observed during both acute and subacute exposure periods strongly suggests the involvement of free radical-mediated cellular injury in Isoproturon toxicity. Similar findings were reported by (Li *et al.,* 2003), who observed increased MDA levels in the liver tissues of *Carassius auratus* exposed to 3,4-dichloroaniline, an intermediate used in herbicide synthesis. (Ahmad *et al.,* 2004) [1] also documented tissue-specific lipid peroxidation damage in the gill, kidney, and liver of *Anguilla anguilla* exposed to polluted water. These studies support the present findings and indicate that oxidative membrane damage is a common response to environmental pollutants in aquatic organisms.

Protein oxidation is another major consequence of oxidative stress. Reactive oxygen species directly attack proteins and induce the formation of carbonyl groups, which serve as reliable indicators of oxidative protein damage (Bainy *et al.,* 1996) [4]. In the present investigation, protein carbonyl levels increased significantly in both lethal and sublethal exposure groups, indicating oxidative modification of cellular proteins due to Isoproturon intoxication. Oxidative protein damage may lead to conformational alterations, loss of enzymatic activity, impaired cellular metabolism, and increased susceptibility of proteins to proteolytic degradation (Zhang *et al*., 2008). The increased protein carbonyl content observed in the present study therefore confirms severe oxidative injury and disruption of normal protein metabolism in *Cyprinus carpio*.

The reduction in total protein content recorded in the exposed fish may represent an adaptive physiological response to herbicide-induced stress. Depletion of tissue proteins generally indicates enhanced proteolysis and utilization of degraded protein products for metabolic and energy-generating processes. According to (Klassan, 1991) [27], decreased protein levels under toxic stress conditions may occur due to increased breakdown of proteins to provide intermediates for the Krebs cycle and to maintain osmotic balance within cells. Earlier reports by (Rafat, 1986 and Rajeshwari, 1986) also suggested that amino acids released during protein degradation help compensate for osmoregulatory disturbances caused by pesticide stress. These amino acids may subsequently enter the tricarboxylic acid cycle through transamination reactions and provide additional energy required for detoxification and elimination of toxicants from the body.

In the present study, decreased protein content along with increased protease activity and elevated free amino acid levels clearly indicate enhanced protein degradation in the gill, liver, and kidney tissues of *Cyprinus carpio* exposed to isoproturon. Oxidative stress generated by the herbicide may have damaged structural and functional proteins, resulting in their breakdown by proteolytic enzymes. Increased protease activity suggests that protein degradation was more active than protein synthesis during the experimental period. Similar findings were reported by (Kabeer *et al.,* 1984) [23] and (Rajamannar and Manohar, 1998), who observed enhanced proteolysis and elevated free amino acid levels in stressed fish tissues. (Ganeshan *et al.,* 1989) and (Jha and Verma, 2002) [14, 21] also reported activation of gluconeogenesis as an adaptive response under toxic stress conditions.

Elevated free amino acid levels observed in the present study may be associated with intensified proteolysis and altered transaminase activity under herbicide-induced stress. (Bell, 1968) [7] reported that increased amino acid levels act as indicators of protein degradation in salmonoid fishes, while (Gingerich and Weber, 1976) [15] documented similar alterations during liver intoxication in rainbow trout. Increased amino acid accumulation may also result from reduced utilization of amino acids, as suggested by (Seshagiri *et al*., 1987), or enhanced transamination and catabolism of keto acids as reported by (Shakoori *et al.,* 1976). The amino acids released during protein degradation may enter the Krebs cycle through oxidative deamination

and transamination pathways to meet the increased energy demand during stress conditions. Similar reductions in total protein content and elevations in free amino acid levels were also reported by (Tripathi *et al.*, 2003) in fish exposed to sublethal concentrations of dimethoate.

Although isoproturon belongs to the phenyl urea group of herbicides rather than organophosphate pesticides, the present findings indicate that it can induce severe oxidative stress and metabolic disturbances comparable to those caused by organophosphate compounds. Increased lipid peroxidation, elevated hydrogen peroxide levels, enhanced protease activity, and disturbed protein metabolism observed in the present study clearly suggest that oxidative damage is one of the major mechanisms involved in isoproturon toxicity. These biochemical alterations may significantly affect the physiological health, survival, and adaptive capacity of fish inhabiting herbicide-contaminated freshwater ecosystems.

Overall, the results of the present investigation clearly demonstrate that exposure to Isoproturon induces severe oxidative stress in *Cyprinus carpio*, leading to alterations in antioxidant defense systems and oxidative damage biomarkers. Parameters such as catalase activity, hydrogen peroxide levels, malondialdehyde, protein carbonyls, protease activity, free amino acids, and total protein content may therefore serve as sensitive biomarkers for assessing herbicide-induced toxicity and monitoring environmental contamination in freshwater ecosystems.

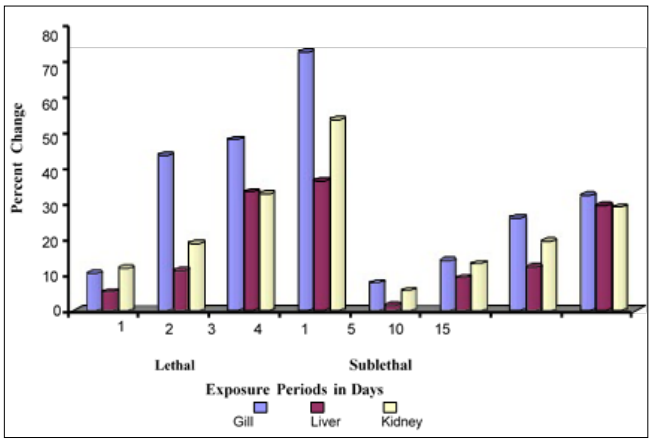

Fig 1: Percent increase over control in catalase activity in the tissues of *C. carpio*, following exposure to lethal and sublethal concentrations of Isoproturon.

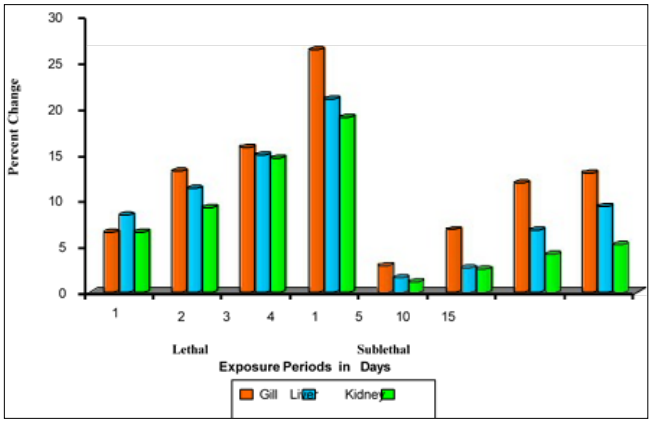

Fig 2: Percent increase over control in hydrogen peroxide levels in the tissues of *C. carpio* fingerlings, following exposure to lethal and sublethal concentrations of Isoproturon

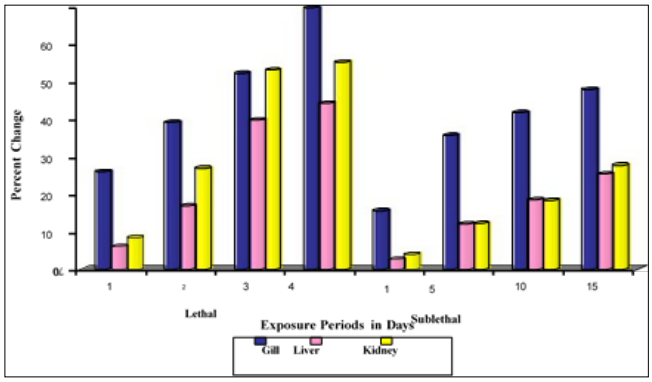

Fig 3: Percent increase over control in MDA levels in the tissues of *C. carpio* fingerlings, following exposure to lethal and sublethal concentrations of Isoproturon

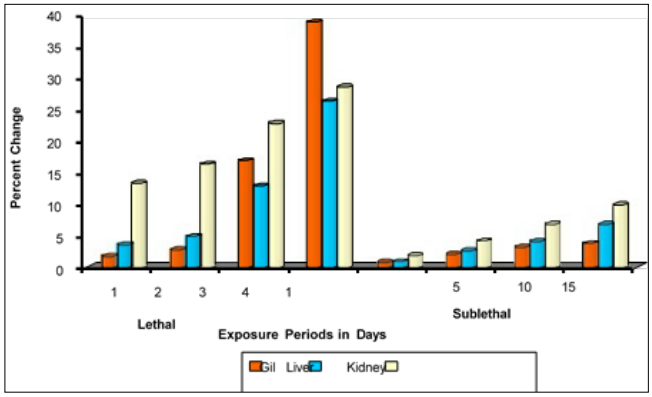

Fig 4: Percent increase over control in protein carbonyls in the tissues of *C. carpio*, following exposure to lethal and sublethal concentrations of Isoproturon

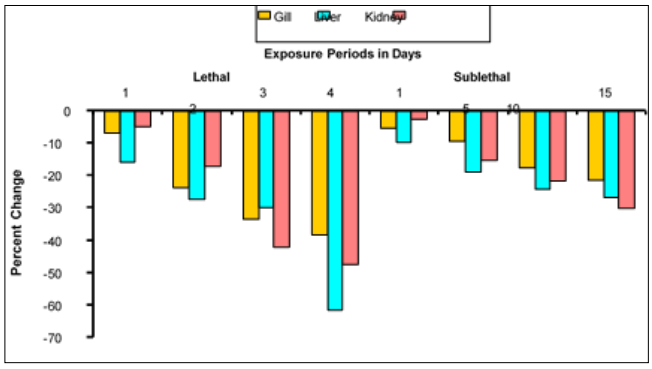

Fig 5: Percent decrease over control in protein contents in the tissues of fish, *C. carpio*, following exposure to lethal and sublethal concentrations of Isoproturon

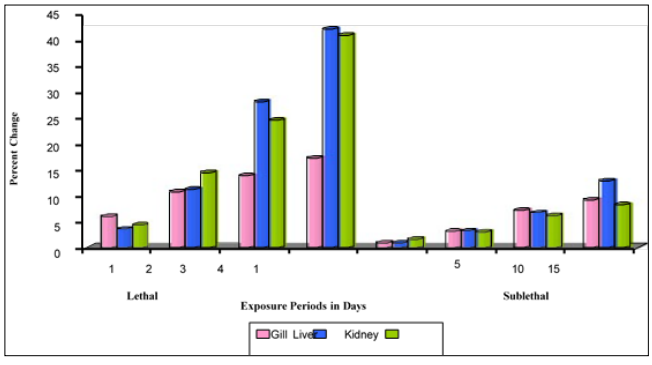

Fig 6: Percent increase over control in protease activity in the tissues of *C. carpio*, following exposure to lethal and sublethal concentrations of Isoproturon

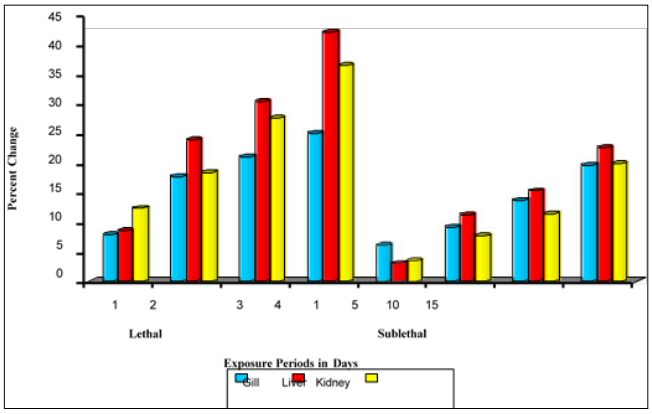

Fig 7: Percent increase over control in free amino acid levels in the tissues of *C. carpio* fingerlings, following exposure to lethal and sublethal concentrations of Isoproturon.

References

1. Ahmad I, Maria VL, Oliveira M, Pacheco M, Santos MA. Oxidative stress and genotoxic effects in gill, kidney and liver of European eel (*Anguilla anguilla*) from polluted environments. Mutation Research/Genetic Toxicology and Environmental Mutagenesis,2004:552(1–2):163–170. https://doi.org/10.1016/j.mrgentox.2004.06.021
2. Aluru N, Rao P, Kumar S, Patel R. Proteomic responses and oxidative stress alterations in zebrafish (*Danio rerio*) exposed to herbicides. Pesticide Biochemistry and Physiology,2024:204:106036. https://doi.org/10.1016/j.pestbp.2024.106036
3. American Public Health Association. Standard methods for the examination of water and wastewater (21st ed.). American Public Health Association, American Water Works Association, and Water Environment Federation, 2005.
4. Bainy ACD, Saito E, Carvalho PSM, Junqueira VBC. Oxidative stress in gill, erythrocytes, liver and kidney of Nile tilapia (*Oreochromis niloticus*) from polluted sites. Aquatic Toxicology,1996:34(2):151–162.
5. Barzilai A, Yamamoto K. DNA damage responses to oxidative stress. DNA Repair,2004:3(8–9):1109–1115. https://doi.org/10.1016/j.dnarep.2004.03.002
6. Beal MF. Aging, energy, and oxidative stress in neurodegenerative diseases. Annals of Neurology,1995:38(3):357–366. https://doi.org/10.1002/ana.410380304
7. Bell GR. Distribution of transaminases in Atlantic salmon with emphasis on their diagnostic use. Journal of the Fisheries Research Board of Canada,1968:25(6):1247–1268.
8. Cerutti PA. Prooxidant states and tumor promotion. Science,1985:227(4685):375–381. https://doi.org/10.1126/science.2981433
9. Cerutti PA. Prooxidant states and tumor promotion. Science,1985:227(4685):375–381. https://doi.org/10.1126/science.2981433
10. Davis JI, Smith EL. Proteolytic activity measurement using the ninhydrin method. Journal of Biological Chemistry,1955:224:261–275.
11. Duncan DB. Multiple range and multiple F tests. Biometrics,1955:11(1):1–42. https://doi.org/10.2307/3001478
12. Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. The American Journal of Pathology,1982:107(3):395–418.
13. Freeman BA, Crapo JD. Biology of disease: Free radicals and tissue injury. Laboratory Investigation,1982:47(5):412–426.
14. Ganeshan R, Thomas PC, Ramaswamy M. Biochemical changes induced by pesticide stress in freshwater fish. Indian Journal of Experimental Biology,1989:27:1052–1055.
15. Gingerich WH, Weber LJ. Hepatic indicators of sublethal toxicity in rainbow trout. Journal of the Fisheries Research Board of Canada,1976:33(3):586–592.
16. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species: Its mechanism and measurement in mammalian systems. FEBS Letters,1991:281(1–2):9–19. https://doi.org/10.1016/0014-5793(91)80347-6
17. Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human disease: Where are we now? The Journal of Laboratory and Clinical Medicine,1992:119(6):598–620.
18. Hu X, Zhang Y, Li H, Wang J. Environmental contaminant-induced oxidative and metabolic disturbances in fish tissues. Ecotoxicology and Environmental Safety, 2026.
19. Huang CH, Chu YL, Chen TH. Chronic exposure to copper induces oxidative stress in fish tissues. Aquatic Toxicology,2003:62(3):205–213.
20. Husain K, Feroz Z, Ahmad M. Modulation of pesticide-induced oxidative stress by antioxidant defense systems in fish tissues. Ecotoxicology and Environmental Safety,2001:49(2):174–181.
21. Jha BS, Verma SR. Biomarkers of exposure to pesticides in freshwater fish. Ecotoxicology and Environmental Safety,2002:52(2):145–152.
22. John S, Kale M, Bhide R. Response of antioxidant enzymes to oxidative stress in fish exposed to pollutants. Indian Journal of Experimental Biology,2001:39:123–127.
23. Kabeer AH, Begum G, Ramamurthi R. Effect of pesticide exposure on free amino acid metabolism in freshwater fish. Indian Journal of Comparative Animal Physiology,1984:2:12–18.
24. Kelly SA, Havrilla CM, Brumbaugh WG. Oxidative stress in aquatic organisms exposed to contaminants. Environmental Toxicology and Chemistry,1998:17(4):683–689.
25. Kelly SA, Havrilla CM, Brusick DJ, Troutman JA, Wang C. Oxidative stress in toxicology: Established mammalian and emerging piscine model systems. Environmental Health Perspectives,1998:106(7):375–384. https://doi.org/10.1289/ehp.98106375
26. Khan MZ. Toxic effects of pesticides on biochemical and physiological aspects of aquatic organisms. International Journal of Agriculture and Biology,2006:8(5):711–715.
27. Klassan CD. Principles of toxicology and environmental stress responses. Casarett and Doull's Toxicology, 4th ed., Pergamon Press, New York, 1991.
28. Krohn K, Rozman D, Suhadolnik L. Hydrogen peroxide in cellular metabolism and oxidative stress. Cell Biology International,2007:31(10):1230–1235.

29. Li ZH, Velisek J, Randak T. Oxidative stress responses in goldfish exposed to herbicide intermediates. Chemosphere,2003:53(1):81–87.
30. Limon-Pacheco J, Gonsebatt ME. The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutation Research/Genetic Toxicology and Environmental Mutagenesis,2009:674(1–2):137–147. https://doi.org/10.1016/j.mrgentox.2008.09.015
31. Luck H. Catalase. In H. U. Bergmeyer (Ed.), Methods of enzymatic analysis (2nd ed., pp. 885–894). Academic Press, 1974.
32. Mates JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology,2000:153(1–3):83–104. https://doi.org/10.1016/S0300-483X(00)00306-1
33. Moore S, Stein WH. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. Journal of Biological Chemistry,1954:211(2):907–913.
34. Ozyurt H, Mukoyama M, Sahin K, Alici O, Sogut S. Oxidative stress and antioxidant defense in tissues affected by toxic compounds. Clinical Biochemistry,2004:37(10):908–915.
35. Pi J, Bai Y, Reilly CC, Liu F, Shi X. Reactive oxygen species as a signal in glucose-stimulated insulin secretion. Diabetes,2010:56(7):1783–1791. https://doi.org/10.2337/db06-1601
36. Pick E, Keisari Y. Superoxide anion and hydrogen peroxide production by chemically elicited peritoneal macrophages: Induction by multiple nonphagocytic stimuli. Cellular Immunology,1981:59(2):301–318. https://doi.org/10.1016/0008-8749(81)90405-1
37. Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analytical Biochemistry,1966:16(2):359–364. https://doi.org/10.1016/0003-2697(66)90167-9
38. Quinlan GJ, Gutteridge JMC, Halliwell B. Action of iron ions and hydrogen peroxide in the breakdown of DNA and deoxyribose. Methods in Enzymology,1994:233:289–299. https://doi.org/10.1016/S0076-6879(94)33033-7
39. Volodymyr IL. Lipid peroxidation in aquatic animals: Concepts and methods for oxidative stress assessment. Oxidative Stress and Diseases in Aquatic Organisms,2011:2:45–67.