International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

Methanol induced oxidative stress: modulation of Glutathione Peroxidase in Cirrhinus mrigala

Madhav Bhilave

Division of Fisheries Science
Department of Zoology
Shivaji University Kolhapur
+919822874909 mpb_zoo@unishivaji.ac.in

Abstract

Aquatic toxicology is a specialized discipline that investigates the effects of anthropogenic chemicals, natural substances, and associated human activities on aquatic organisms. Its scope spans multiple levels of biological organization, ranging from subcellular components to individual organisms, populations, communities, and entire ecosystems. Being inherently multidisciplinary, aquatic toxicology integrates principles of toxicology, aquatic ecology, and aquatic chemistry, and encompasses freshwater, marine, and sedimentary environments. Standard bioassays in this field typically assess endpoints such as survival, growth, and reproduction across a concentration gradient, alongside appropriate controls. These experiments generally employ test organisms with ecologically relevant sensitivity to toxicants, which can be conveniently cultured under laboratory conditions and are relatively easy to handle. Methanol, an industrial chemical in use since the early 19th century, is widely utilized in the production of biodiesel and as a denaturant in ethanol manufacturing industries. According to the American Methanol Institute, global methanol demand was projected to reach approximately 882 million gallons per year, necessitating expansion in its production, transportation, storage, and distribution facilities. Such large-scale handling inevitably increases the risk of accidental environmental release, raising concerns about its fate and potential impacts on aquatic ecosystems. Despite its industrial importance, limited information exists regarding the toxicological implications of methanol exposure on aquatic biota. In the present investigation, an attempt has been made to evaluate the toxicological effects of acute methanol exposure in the freshwater fish Cirrhinus mrigala, with particular emphasis on alterations in the antioxidant defense enzyme glutathione peroxidase (GPx) as a biomarker of oxidative stress.

Key words: Methanol toxicity, Cirrhinus mrigala, Glutathione peroxidase, oxidative stress, aquatic toxicology, biomarker

Introduction

Toxicological testing provides both qualitative and quantitative insights into the impacts of toxicants on living organisms. Such assessments are essential for evaluating the potential hazards posed to aquatic environments and for generating databases that support risk assessment of specific contaminants. Toxicity is broadly classified into two categories: direct toxicity and indirect toxicity. Direct toxicity arises when a toxicant interacts directly with a biological target site within or on the organism, thereby disrupting normal physiological functions. In contrast, indirect toxicity results from alterations in the surrounding physical,

chemical, or biological environment, which subsequently affect the organism's health and survival. Toxicological endpoints are typically distinguished as lethal and sub lethal. Lethal effects such as mortality are commonly used in acute toxicity tests to determine the immediate hazards of a toxicant. On the other hand, sub lethal effects serve as endpoints in chronic toxicity tests, providing insight into long-term impacts that may not cause immediate death but impair the organism's biological functions. These sub lethal responses can manifest as behavioral, biochemical, physiological, or histological changes, thereby offering sensitive indicators of early toxic stress before mortality occurs.

Chemical contamination represents one of the most pressing environmental challenges of the present century. Aquatic ecosystems such as rivers, lakes, and reservoirs are increasingly polluted by industrial effluents, household wastes, and agricultural run-off, which introduce a wide spectrum of toxicants into water bodies. The problem is further exacerbated by the rapid and often unregulated expansion of urban areas, where insufficient infrastructure for waste collection, transportation, treatment, and disposal leads to the direct discharge of untreated wastes into aquatic environments. Industrial activities, in particular, contribute to the release of persistent xenobiotic compounds into the environment. These contaminants are frequently detected at alarming spatial and temporal concentrations in both terrestrial and freshwater ecosystems (Brack et al., 2002; Diez et al., 2002). Depending on their chemical nature, some of these pollutants are biodegradable and can be broken down by natural processes over time. However, a large proportion are non-biodegradable, persisting in the environment for extended periods and posing long-term ecological and toxicological risks. Such compounds can bio accumulate in aquatic organisms, bio magnify through food webs, and ultimately threaten biodiversity, ecosystem stability, and even human health. Thus, the persistence and complexity of chemical pollutants highlight the urgent need for systematic monitoring, stringent regulation of waste discharge, and eco-toxicological studies to assess their impact on aquatic life.

Methanol (CH_3OH), the compound selected for the present investigation, is a colorless, volatile, and flammable liquid widely recognized as an important industrial chemical since the early 19th century. It has diverse applications, being used as a solvent, antifreeze agent, and fuel, as well as a feedstock in numerous chemical industries. Historically, methanol was produced through the destructive distillation of wood, which is why it was once referred to as wood alcohol. In modern times, it is primarily synthesized from carbon monoxide and hydrogen through catalytic processes, allowing for large-scale commercial production. Despite its industrial value, methanol is highly toxic to humans and other organisms. Even small accidental ingestions can have severe health consequences: approximately 10 mL of methanol may cause irreversible blindness, while ingestion of about 100 mL can be fatal due to its metabolism to formaldehyde and formic acid, both of which exert toxic effects on the central nervous system and ocular tissues.

In addition to its traditional uses, methanol plays a crucial role in biodiesel production as a transesterification agent and is commonly employed in the ethanol manufacturing industry as a denaturant additive, rendering ethanol unsuitable for human consumption. These large-scale applications, coupled with growing industrial demand, increase the likelihood of environmental exposure and accidental release, underscoring the importance of toxicological investigations on aquatic organisms. Fish are widely regarded as vital bio indicators in aquatic ecosystems due to their sensitivity to environmental fluctuations

and contaminant exposure. Because they live in continuous and direct contact with the surrounding water, even minor changes in water quality can exert immediate and measurable effects on their health and physiology. This makes fish an excellent model for assessing the eco toxicological impacts of pollutants. Among fish organs, the liver and kidney play particularly crucial roles in coping with xenobiotic. The liver serves as the primary site for detoxification, biotransformation, and metabolism of toxicants, while the kidney is integral to osmoregulation, excretion, and maintenance of ionic balance (Vesey, 2010). Because these organs are directly involved in the processing and elimination of contaminants, they are highly vulnerable to damage under polluted conditions.

Fish are exceptionally sensitive to chemical contamination of aquatic environments, and exposure to toxicants can significantly disrupt their biochemical, physiological, and metabolic processes. Such disruptions may manifest in altered enzyme activities, oxidative stress responses, impaired growth, reproductive failure, or histopathological damage to vital tissues (Banaee et al., 2011; Shrivastava et al., 2004). Therefore, monitoring biochemical and physiological alterations in fish not only provides insights into the toxic mechanisms of pollutants but also serves as an early-warning tool for environmental health assessment.

The Indian Major Carps are highly valued as table fish when compared with other freshwater species, owing to their favourable taste, nutritional quality, and cultural importance in the Indian subcontinent. Among them, Cirrhinus mrigala (Hamilton, 1822), the species selected for the present investigation, is one of the most important carp species in aquaculture and capture fisheries. Cirrhinus mrigala is a benthopelagic and potamodromous fish, predominantly feeding on plankton and detritus, and is characterized by a fast growth rate, making it suitable for large-scale culture practices. In its natural habitat, it thrives in fast-flowing rivers and streams, where it finds optimal ecological conditions for feeding and growth. Spawning occurs during the monsoon season, typically in shallow marginal areas of rivers and water bodies at depths ranging from 50 to 100 cm, usually over sandy or clayey substrates. However, in confined or managed aquatic environments such as lakes, tanks, and reservoirs, Cirrhinus mrigala is unable to reproduce naturally. Consequently, it is widely subjected to induced breeding techniques to sustain seed production for aquaculture practices (Rema Devi et al., 2011).

Acute toxicity refers to the harmful effects of a chemical or substance that arise either from a single exposure or from multiple exposures within a short time frame, typically less than 24 hours. To qualify as acute toxicity, the resulting adverse effects are generally observed within 14 days following administration of the substance. Acute toxicity is conceptually distinct from chronic toxicity, which encompasses the adverse health effects arising from repeated or continuous exposure to a substance over a prolonged period, often spanning months or years, usually at lower doses. Most acute toxicity data are derived from animal testing, with fish often serving as an ecologically and biologically relevant model, or through in vitro testing approaches. In some cases, toxicity is inferred from data obtained on structurally or functionally similar compounds.

A chemical is considered to have high acute toxicity if even a small quantity can induce severe adverse effects or death. Acute toxic exposures can occur via different routes, including oral ingestion, dermal contact, or inhalation, and are sometimes collectively described as acute poisoning. In aquatic toxicology,

evaluating acute toxicity in fish provides critical insight into the hazard potential of pollutants, allowing for estimation of lethal concentrations LC_{50} and the early detection of biochemical, physiological, or behavioral disturbances induced by toxicants. Such studies are fundamental for risk assessment, environmental monitoring, and the development of regulatory guidelines for chemical usage and discharge.

Glutathione peroxidase (GPx) is a crucial antioxidant enzyme that provides protection against oxidative stress by utilizing glutathione (GSH) as a substrate. Glutathione itself serves as a substrate for various other detoxifying proteins, such as glutathione S-transferases, which help neutralize reactive oxygen species (ROS) and maintain cellular redox balance. GPx participates in multiple cellular protective mechanisms. It catalyzes the reduction of hydrogen peroxide H_2O_2 and lipid peroxides to water and corresponding alcohols, thereby detoxifying these potentially harmful oxidants. GPx also contributes indirectly to amino acid transport across plasma membranes and scavenges reactive species such as hydroxyl radicals and singlet oxygen. Additionally, glutathione helps regenerate other key antioxidants, including vitamins C and E, back to their active forms, enhancing the overall cellular antioxidant capacity.

The protective function of glutathione relies on its thiol -SH group present in cysteine, making it a vital intracellular, non-enzymatic antioxidant. Glutathione is highly concentrated in the cytosol, nucleus, and mitochondria, serving as a major soluble antioxidant within cellular compartments. The intracellular concentration of GSH reflects a dynamic balance between its synthesis and consumption, and can increase in response to exposure to ROS, reactive nitrogen species (RNS), or compounds that generate ROS, thereby enhancing cellular defense mechanisms. GPx operates in conjunction with other antioxidant enzymes, most notably catalase, which also detoxifies H_2O_2. While catalase becomes more critical under conditions of severe oxidative stress, GPx is considered the primary enzyme for H_2O_2 detoxification under mild to moderate oxidative stress, particularly in animal cells. In human erythrocytes, for example, GPx serves as the main enzymatic antioxidant for H_2O_2 detoxification because catalase exhibits lower substrate affinity for H_2O_2 compared to GPx.

Overall, the glutathione–GPx system is a central component of cellular defense against oxidative damage, maintaining redox homeostasis and protecting biomolecules from oxidative injury.

Materials and Methods

Methanol (CH_3OH), often abbreviated as MeOH, is a simple alcohol that historically earned the name “wood alcohol” because it was originally obtained as a by-product of the destructive distillation of wood. In modern industry, methanol is predominantly synthesized through catalytic processes using carbon monoxide, carbon dioxide, and hydrogen, enabling large-scale production for diverse applications. Methanol is highly toxic to humans, with adverse effects typically manifesting within a few hours after exposure. Its toxicity is particularly concerning because methanol is colorless and volatile and closely resembles ethanol, the alcohol present in beverages, making accidental ingestion difficult to detect.

The toxic effects of methanol occur via two primary mechanisms. First, methanol itself acts as a central nervous system depressant, which can be fatal when absorbed through ingestion, inhalation, or dermal contact. Second, methanol undergoes metabolic activation in the liver, catalyzed by the enzyme alcohol

dehydrogenase, forming formaldehyde, which is subsequently oxidized to formic acid present as formate ion. Formate exerts its toxicity by inhibiting mitochondrial cytochrome c oxidase, leading to cellular hypoxia, metabolic acidosis, and disruption of multiple biochemical pathways. Methanol poisoning has been reported worldwide, often resulting from contaminated or illicitly produced alcoholic beverages, with higher incidence in developing countries. The combination of its metabolic toxicity, rapid absorption, and difficulty in distinguishing it from ethanol underscores the need for careful handling and monitoring of methanol in both industrial and environmental contexts.

Live fingerlings of the freshwater fish Cirrhinus mrigala, weighing approximately 8–10 g and measuring 10–12 cm in length, were procured for the present study. Upon acquisition, the fish were acclimatized to laboratory conditions and housed in glass aquaria containing aerated freshwater. To prevent dermal infections and other pathogenic invasions, the fish were treated with 0.05% potassium permanganate for 4–5 minutes prior to experimentation. The fish were fed twice daily with groundnut oil cake at a rate of 2% of their body weight, and water in the aquaria was renewed every 24 hours to maintain optimal environmental conditions and ensure fish health throughout the experimental period.

Estimation of Glutathione Peroxidase (GPx) Activity:

The activity of glutathione peroxidase was determined following the method described by Beers and Sizer (1952). Fish tissues were homogenized in 0.067 M phosphate buffer (pH 6.8) and centrifuged at 10,000 rpm for 15 minutes. The resulting supernatant served as the enzyme source for GPx assays. For the assay, 3 mL of 0.067 M phosphate buffer containing H_2O_2 was pipetted into a cuvette, followed by the addition of 0.05 mL of tissue supernatant and 0.01 mL of 1 mM sodium azide. The mixture was gently homogenized using a glass rod. The change in absorbance was monitored from 30 seconds up to 3 minutes using a UV-1800 spectrophotometer. GPx activity was calculated, expressed, and percent changes were determined relative to control samples. All experimental results were subjected to statistical analysis, and the levels of significance were determined using Student's t-test, ensuring the reliability and validity of the observed changes in enzyme activity.

Results

The mean values and percent changes **in** glutathione peroxidase (GPx) activity in different tissues of Cirrhinus mrigala are presented in Table 1 and graphically illustrated in Figure 1. In control fish, the basal GPx activity followed the order: liver > muscle > brain > gill, indicating that the liver exhibited the highest enzymatic activity under normal physiological conditions. Upon exposure to methanol, a significant increase in GPx activity was observed in all examined tissues, maintaining the same tissue-specific order liver > muscle > brain > gill in both LC_0 and LC_{50} groups. During the exposure period, GPx activity ranged as follows: gill, 5.17–5.86 U/mg protein; liver, 8.20–10.98 U/mg protein; brain, 5.44–6.45 U/mg protein; muscle, 6.99–8.79 U/mg protein**.** In the LC_0 exposure group, GPx activity showed a highly significant increase ($P < 0.001$) in the liver 20.73% and muscle 16.3%, while a significant elevation was also noted in the brain 11.39% and gill 5.42%. Exposure to LC_{50} resulted in an even more pronounced enhancement of GPx activity, with the liver exhibiting the highest increase 33.9%**,** followed by muscle 25.75%**,** brain 18.56% and gill 13.35%**.** These results indicate a dose-dependent induction of GPx activity in response to methanol exposure, reflecting the activation of antioxidant defense mechanisms to counteract the oxidative

stress induced by the toxicant. The liver, being the primary detoxification organ, consistently showed the highest enzymatic response, whereas gill tissue, in direct contact with water, showed comparatively lower but significant activity changes.

In the LC_0 exposure group**,** the percent change in glutathione peroxidase (GPx) activity was highest in the liver 20.73% and lowest in the gill 5.42% relative to the control. Similarly, in the LC_{50} group, the liver again exhibited the maximum increase 33.9%, whereas the brain showed the minimum percent change 13.35% compared to control fish.

Overall, these findings demonstrate that acute methanol exposure induces a significant elevation of GPx activity across all examined tissues gill, liver, brain and muscle in Cirrhinus mrigala. The liver, as the primary organ of detoxification, consistently showed the strongest enzymatic response, while other tissues also displayed substantial but comparatively lower increases, reflecting tissue-specific activation of antioxidant defenses. These results highlight the role of GPx as a sensitive biomarker of oxidative stress in response to methanol-induced toxicity in freshwater fish.

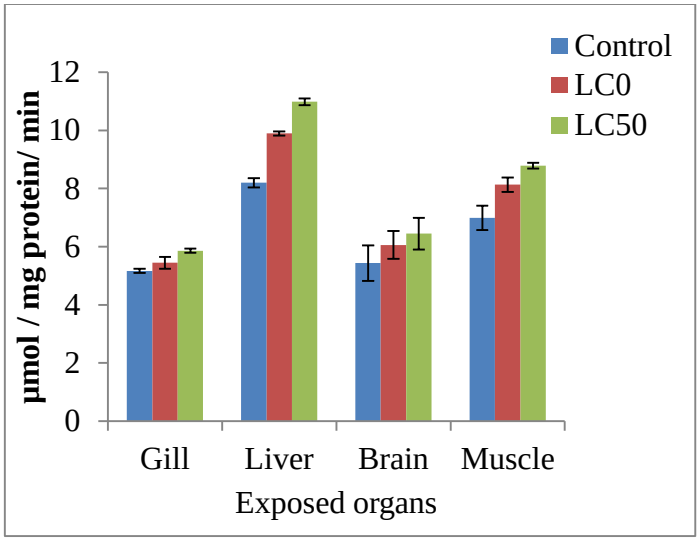
Table No 01: Effect of Methanol on the total Glutathione peroxidase (GPx) in various organs of the fish Cirrhinus mrigala after acute exposure

(µmol / mg protein/ min)

Sr. No.	Organs	Control	Exposure of Methanol	
			LC_0	LC_{50}
1.	Gill	5.17±0.074	5.45±0.20* (5.42)	5.86±0.07** (13.35)
2.	Liver	8.20±0.15	9.90±0.07*** (20.73)	10.98±0.12*** (33.9)
3.	Brain	5.44±0.61	6.06±0.48* (11.39)	6.45±0.55* (18.56)
4.	Muscle	6.99±0.42	8.13±0.25*** (16.3)	8.79±0.10*** (25.75)

Values are the mean of (n=5) ± SD *= P < 0.05; ** = P < 0.01; ***= P < 0.001

Figure 01: Effect on the Glutathione peroxidase of freshwater fish Cirrhinus mrigala after acute exposure to Methanol.



Discussion

Rapid human population growth, industrialization, intensive agriculture, and other anthropogenic activities have led to the contamination of aquatic ecosystems with a wide array of harmful pollutants. The physicochemical properties of water play a critical role in determining the impact of these toxicants on the growth, survival, and overall health of aquatic organisms. Key water parameters including pH, temperature, hardness, salinity, and dissolved oxygen significantly influence the bioavailability, toxicity, and uptake of pollutants in aquatic organisms. The accumulation and effects of toxicants in fish tissues are governed by a combination of biotic, abiotic, and environmental factors. Biotic factors include species, age, size, metabolic rate, and physiological condition of the fish, while abiotic factors encompass water chemistry, temperature, salinity, and dissolved oxygen levels. Environmental conditions such as habitat type and seasonal variations also modulate toxicant bioaccumulation and toxicity. Moreover, the concentration of the toxicant, duration of exposure, and the physiological status of the fish determine the degree of biochemical, physiological, and histological responses, ultimately influencing growth rates and mortality. Therefore, the interplay of these factors is critical for understanding toxicological outcomes in fish and for assessing the ecological risk posed by contaminants in freshwater ecosystems.

Glutathione peroxidase (GPx, EC 1.11.1.9) refers to a family of enzymes with peroxidase activity whose primary biological function is to protect cells and tissues from oxidative damage. The main biochemical role of GPx is to catalyze the reduction of hydrogen peroxide H_2O_2 to water and convert lipid hydro peroxides into their corresponding alcohols, thereby preventing oxidative stress and cellular injury. This enzyme family comprises multiple isozymes, each encoded by distinct genes, exhibiting variation in cellular localization and substrate specificity. Among these, Glutathione peroxidase 1 (GPx1) is the most abundant isozyme, present in the cytoplasm of nearly all mammalian tissues, with hydrogen peroxide as its preferred substrate. Glutathione peroxidase 4 (GPx4) shows high specificity for lipid hydro peroxides and is expressed ubiquitously in mammalian cells, albeit at lower levels than GPx1. Other isozymes include Glutathione peroxidase 2 (GPx2), which is predominantly found in the intestinal tract and extracellular fluids, and Glutathione peroxidase 3 (GPx3), an extracellular enzyme highly abundant in plasma. The differential distribution and substrate preferences of GPx isozymes allow for a coordinated

antioxidant defense system, protecting diverse cellular compartments and tissues from oxidative damage induced by reactive oxygen species (ROS).

In the present study, glutathione peroxidase (GPx) activity was found to be significantly increased ($p < 0.05$) in the gill, liver, brain, and muscle tissues of Cirrhinus mrigala following acute methanol exposure. This elevation reflects the activation of antioxidant defense mechanisms in response to oxidative stress induced by the toxicant. Similar findings have been reported in other fish species. Ahmad et al. (2000) observed a time-dependent increase in GPx activity in the freshwater catfish Channa punctatus exposed to paper mill effluents. Vinodhini and Narayanan (2009) reported a significant induction of GPx in the liver of Cyprinus carpio following exposure to heavy metals. Comparable increases in GPx activity were also noted in species such as Clarias gariepinus, Cyprinus carpio, and others, as documented by Farombi et al. (2007), Vinodhini and Narayanan (2009), and Jastrzębska (2010). Monteiro et al. (2010) reported enhanced GPx activity in the gill, liver, muscle, and heart of Brycon amazonicus subjected to mercury chloride stress. Likewise, Jastrzębska (2010) observed elevated peroxidase activity in lead-stressed Cyprinus carpio compared to unstressed controls. These results are further supported by Baysoy et al. (2012), who documented a significant increase in GPx activity in lead-exposed Oreochromis niloticus.

The increase in GPx activity is consistent with the enzyme's role as a scavenger of reactive oxygen species (ROS), catalyzing the conversion of hydrogen peroxide H_2O_2 into water and oxygen, thereby mitigating oxidative damage (Aruljothi and Samipillai, 2014). Similarly, Rahimikia (2017) reported enhanced GPx activity in the tissues of nickel-exposed goldfish, indicating that induction of this enzyme is a common protective response against metal and chemical-induced oxidative stress in fish. Collectively, these findings corroborate the present results, highlighting that acute exposure to toxicants such as methanol triggers upregulation of GPx activity, which serves as a key biomarker of oxidative stress in freshwater fish.

Previous studies have reported that exposure to environmental contaminants can induce the activity of antioxidant enzymes in fish. Li et al. (2003) observed elevated levels of glutathione peroxidase (GPx) in Cyprinus carpio exposed to microcystin. Similarly, Sanchez et al. (2005) reported increased GPx activity in the liver of Gasterosteus aculeatus following exposure to copper. The present results are also consistent with Farombi et al. (2007), who documented significantly higher GPx activity in lead-stressed Clarias gariepinus compared to control fish. Ruas et al. (2008) further demonstrated enhanced GPx activities in Oreochromis niloticus exposed to polluted river water, highlighting the role of antioxidant enzymes as biomarkers of environmental stress.

The effect of methanol on the antioxidant enzyme system of fishes remains one of the least explored areas in aquatic toxicology. In the present study, acute methanol exposure led to a significant increase in GPx activity across different tissues of Cirrhinus mrigala, with the liver showing the most pronounced response, followed by muscle, gill, and brain, at both sub lethal (LC_0) and lethal (LC_{50}) concentrations. The enhanced GPx activity in the liver, muscle, and intestine likely reflects a protective adaptive mechanism aimed at mitigating methanol-induced oxidative stress, by detoxifying hydrogen peroxide and lipid hydro peroxides generated during exposure. These findings reinforce the concept that upregulation of GPx is a key cellular

defense strategy in fish, allowing them to cope with oxidative challenges posed by chemical pollutants such as methanol, heavy metals, and industrial effluents.

Summary

Acute exposure of Cirrhinus mrigala to methanol resulted in a significant increase in glutathione peroxidase (GPx) activity across all examined tissues, including the liver, muscle, gill and brain. The liver showed the highest induction, followed by muscle, brain, and gill, reflecting its central role in detoxification and antioxidant defence. The increase in GPx activity was dose-dependent, with higher elevations observed at lethal concentrations LC_{50} compared to sub lethal concentrations LC_0. This enhanced enzymatic activity indicates a protective response against oxidative stress, facilitating the detoxification of hydrogen peroxide and lipid hydro peroxides generated by methanol exposure. These results align with previous studies reporting similar GPx induction in fish exposed to heavy metals, microcrystals, and industrial pollutants, supporting the role of GPx as a sensitive biomarker of oxidative stress in aquatic organisms. Overall, methanol exposure triggers a tissue-specific antioxidant response**,** with the liver serving as the primary site of enzymatic defence, highlighting its critical role in maintaining cellular redox homeostasis under toxicant stress.

Conclusion

Acute exposure to methanol significantly elevates glutathione peroxidase (GPx) activity in the liver, muscle, gill, and brain of Cirrhinus mrigala, with the liver exhibiting the highest response. This dose-dependent increase reflects a tissue-specific antioxidant defense mechanism activated to counteract methanol-induced oxidative stress. The findings confirm that GPx serves as a sensitive biomarker of oxidative stress in freshwater fish, highlighting the liver's central role in detoxification and the organism's adaptive response to chemical toxicants.

Future avenues

Future studies could focus on the molecular mechanisms underlying methanol-induced oxidative stress in fish, including gene expression analysis of antioxidant enzymes like GPx, SOD, and catalase. Investigations on chronic and sub lethal exposures would provide insights into long-term physiological and biochemical effects. Additionally, exploring the protective roles of dietary antioxidants or environmental modulators could help mitigate toxicity. Comparative studies across different fish species and developmental stages may further establish GPx and related enzymes as reliable biomarkers for aquatic environmental monitoring.

References

1. Ahmad, I., Javed, M., & Usmani, K. (2000). Time-dependent induction of antioxidant enzymes in freshwater catfish Channa punctatus exposed to paper mill effluents. Environmental Toxicology and Chemistry, 19(3), 623–628.
2. Aruljothi, P., & Samipillai, S. (2014). Role of peroxidase enzymes in scavenging reactive oxygen species in fish. Journal of Aquatic Biology, 29(2), 45–52.

3. Baysoy, E., Yildiz, M., & Gokcimen, A. (2012). Effect of lead exposure on antioxidant enzymes in Oreochromis niloticus. Ecotoxicology and Environmental Safety, 84, 234–239.
4. Farombi, E. O., Adelowo, O. A., & Ajimoko, Y. R. (2007). Biomarkers of oxidative stress and heavy metal exposure in Clarias gariepinus. Ecotoxicology and Environmental Safety, 66(2), 246–252.
5. Jastrzębska, E. (2010). Lead-induced oxidative stress and antioxidant responses in Cyprinus carpio. Ecotoxicology, 19(5), 889–898.
6. Li, X., Wang, J., & Zhang, Q. (2003). Induction of antioxidant enzymes in Cyprinus carpio exposed to microcystin. Toxicology Letters, 143(2), 145–153.
7. Monteiro, S. M., Morsch, V. M., & Puntel, R. L. (2010). Mercury chloride-induced oxidative stress in Brycon amazonicus: Antioxidant enzyme responses. Aquatic Toxicology, 98(1), 66–72.
8. Rahimikia, S. (2017). Nickel exposure induces glutathione peroxidase activity in goldfish (Carassius auratus). Environmental Science and Pollution Research, 24, 15621–15630.
9. Ruas, A., Rodrigues, P., & Pereira, L. (2008). Antioxidant enzyme responses of Oreochromis niloticus exposed to polluted river water. Journal of Fish Biology, 72(7), 1725–1736.
10. Sanchez, W., Palluel, O., & Riso, R. (2005). Copper-induced antioxidant enzyme activity in Gasterosteus aculeatus. Comparative Biochemistry and Physiology Part C, 140(3), 205–212.
11. Vinodhini, R., & Narayanan, M. (2009). Heavy metal induced antioxidant responses in Cyprinus carpio. Journal of Environmental Biology, 30(2), 165–170.