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Evaluating The Toxicity of Buprofezin in Cyprinus Carpio by Using Enzymatic Biomarkers Approach

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Abstract

Aquatic contamination by pesticides such as buprofezin poses a serious threat to freshwater organisms, leading to toxicity and oxidative stress. This study evaluated the toxicological impact of buprofezin on Cyprinus carpio using enzymatic biomarkers as indicators of oxidative imbalance. Fish were exposed for 96 hours to three concentrations: T0 (control, 0 mg L^{-1}), T1 (5 mg L^{-1}), and T2 (10 mg L^{-1}). Oxidative stress responses were assessed through Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione-S-transferase (GST) activities in hepatic and renal tissues. A clear dose-dependent increase in all enzymatic biomarkers was observed. In the liver, CAT activity increased significantly ($P = 0.032$), rising from 1156.65 UmL^{-1} in controls to 1359.41 UmL^{-1} in T2, while hepatic SOD showed a marked elevation from 234.75 UmL^{-1} (control) to 520.31 UmL^{-1} in T2 ($P = 0.011$). Hepatic GST activity also rose significantly ($P = 0.021$), with values increasing from 878.55 UmL^{-1} (control) to 1407.19 UmL^{-1} in T2, confirming enhanced detoxification activity. Similar trends occurred in renal tissues. Renal CAT increased significantly ($P = 0.017$), from 701.65 UmL^{-1} in controls to 944.41 UmL^{-1} in T2, while renal SOD rose from baseline levels to 403.73 UmL^{-1} in T2 ($P = 0.018$). Renal GST also showed a strong dose-response pattern ($P = 0.022$), increasing from 756.05 UmL^{-1} (control) to 1508.60 UmL^{-1} in T2, indicating intensified conjugation and detoxification processes. Overall, the pronounced elevation of CAT, SOD, and GST in both liver and kidney demonstrates that buprofezin induces significant oxidative stress, activating compensatory antioxidant mechanisms in Cyprinus carpio. The magnitude of enzymatic responses was strongly dose-dependent, with the highest concentration (10 mg L^{-1}) producing the most severe oxidative challenge. These findings highlight the ecological risks of buprofezin contamination in aquatic environments and emphasize the importance of strict monitoring and regulation to protect freshwater biota.

Keywords: Aquatic toxicity, buprofezin, catalase, cyprinus carpio oxidative stress, enzymatic biomarkers, glutathione-s-transferase, superoxide dismutase

Introduction

It is a regular action to use chemical-based pesticides to help in improving farm productivity and protect crops against various insect pesticides and other agricultural enemies. The use of these chemical substances is widespread in agronomy, and a wide array of pests is suppressed: insects, weeds, and fungal pathogens, which protects cultivations and significantly increases food production. Even though these measures are central in enhancing yields, their extensive adoption has brought about considerable environmental and health related issues. Pesticides pose a great concern due to their toxic impact on soil, water, and air, as well as their dangerous ecological impacts on the environment and human health (Babu & Lakshmanan, 2021). As a form of environmental pollution, a pesticides pollution is a current problem of global concern. Migration of pesticides outside the areas of usage occurs by processes of surface run off, leaching through ground water, aerial drift and the process of bioaccumulation in food chains. This

widespread scatter may lead to the extinction of useful insects, negative effects on animals, and the change of natural ecology. As an example of such a scenario, the pesticide pollution of water sources may have toxic impacts on fish, amphibians, and invertebrates and eventually destabilise whole ecosystems (Pillai & Devi, 2022).

Long-term impacts of the long-term use of pesticides include toxic survival of poisonous waste in the environment, which has long-term effects on biodiversity, food stability, and the health of people. Pesticides belong to different categories, such as insecticides, herbicides, fungicides, and rodenticides, and have dissimilar toxicities to organisms that live in water. It has also been documented that organophosphate and neonicotinoid insecticides such as neonicotinoid, have proven to be among the most dangerous, regularly causing high fatality rates or behavior change in fish and other invertebrates. The glyphosate herbicides can affect the aquatic plants and algae adversely causing reduced food supplies and depletion of oxygen, but fungicides might be toxic to microorganisms and fish, leading to ecological disequilibrium (Patel & Singh, 2022). Buprofezin is a commonly used insecticide in rice paddies which is very dangerous to aquatic life. Empirical data show that it impedes reproductive systems, causes developmental abnormalities and triggers population decay in aquatic organism. In addition, buprofezin bioaccumulates in tissues of aquatic organisms, which increases the threat because it will bioaccumulate as it goes up the food chain, and higher organisms like fish, birds, and mammals may be endangered. Besides, buprofezin is an antioxidant-destabilizer that destabilizes the antioxidant defenses in fish by inhibiting the main enzyme including Superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST). These enzymes mediate the break effective results in favor of ‘oxidative dismantling and clearance of reactive oxygen species and xenobiotic intermediates, and serve as the frontline of the antioxidant defense and phase II detoxification system, maintaining redox homeostasis and cellular integrity as a whole (Bhatia & Meena, 2021).

In highly reared freshwater finfish such as Labeo rohita - a highly nutritious fish species and one of the most extensively cultured freshwater species- the presence of pesticides in the waters, including buprofezin, is a major challenge to stock suitability and survival of fish populations.

Monitoring of pesticide potency in fish is regularly done by following changes in enzymatic biomarkers, which are sensitive to physiological/biochemical disturbances. Inhibition or disregulation of such enzymes resolves oxidative stress and macromolecular damages, lowers immunocompetence, and reproductive performance, which collectively increases the mortality risks in the population.

A clear explanation of pesticide induced changes in such bio marker systems is hence central to the diagnosis of ecosystem health and prediction of the long-term effects on chemical pollution. Since the use of pesticides is everywhere and its ecological footprint has its dark sides, there is an increasing need to find safer alternatives, optimize aquaculture and agronomy, and reinforce and change regulations to provide a minimal level of mitigation to the collateral effects on non-target taxa and their habitats (Babu & Lakshmanan, 2021). These are part of ensuring the development of sustainable agriculture and protecting the biodiversity and ecological quality of water systems.

Background

Pesticides is a group of chemical substances that are designed to eliminate or control the populations of the pests (insects, weeds, fungi, and rodents) which threaten the agricultural productivity, the health of people, and the protection of the natural environment. However, their widespread use has created a fair amount of concerns in regard to their environmental impact and possible health risks. There are many channels through which pesticides can end up in the natural ecosystem, these are surface-water runoff, groundwater leaching, atmospheric drift, and volatilization, and off-target deposition (Pandey & Sharma, 2023).

The pesticide residues discharged when released into water, soil and air cause ecological cascades which impact beyond the source. Ecosystems in the aquatic environment are particularly prone, toxicants may bioaccumulate themselves, cross-trophic, and toxicants may interfere with food webs, and reduce biodiversity (Chauhan & Kumar, 2019).

As a result, the term pesticide pollution is used as a spatial measure of pesticide residues pollution, which is mostly witnessed in the soil, water, and the atmosphere. These residues normally occur when the application activities exceed the target area they are supposed to cover thus polluting the surrounding habitats. The lasting exposure endangers non-target organisms, such as, insects, wildlife, and aquatic

organisms (Chopra & Kumar, 2022). Pesticides in water could have dire consequences to their use. Common types of pesticides encompass insecticides, herbicides, fungicides and rodenticides which might have a detrimental effect on aquatic life (Nair et al., 2023).

Examples include organophosphates and neonicotinoids, which exhibit acute toxicity to fish and other invertebrates, often causing mortality or behavioral disturbances. Glyphosate-based herbicides can also kill aquatic plants and algae, diminishing food resources and lowering dissolved oxygen levels (Das & Mandal, 2021).

Some fungicides, such as chlorothalonil, act as dual toxins to both aquatic environments and fish, causing ecological imbalances and disrupting ecosystem stability (Sundararaj & Lakshmi, 2022).

The rodenticides when emitted into water poison aquatic fauna and disturb food chains. These chemicals remain in the environment and therefore their ecological effect might be long-term. These substances collect in the tissues of organisms and subsequent increase in concentration of these substances has the greatest danger to predators, fish, birds and mammals as biomagnification processes continue up the food chain (Devi & Bharti, 2021).

Bioaccumulation can cause the disappearance of aquatic species, food webs and destruction of aquatic habitats in the long run. Aquatic life is highly toxic to pesticides. The fish are usually deposited with pesticide residues as constituents of the water bodies. Any contact with such toxicant may lead to numerous physiological and biochemical reactions in fish, and hence as biomarkers to measure ecosystem health (Gowda & Kumar, 2021).

One such example is the use of buprofezin, a widely used insecticide for managing pests in rice fields. Although it is effective in pest control, residues of buprofezin can dissolve in nearby water bodies, thereby posing risks to aquatic organisms (Ghosh & Chaudhuri, 2023).

It is proved that buprofezin disrupts the key enzymatic processes in fish, which ultimately leads to the oxidative stress, ineffective detoxification, and other physiological diseases (Mistry et al., 2021).

The buprofezin and the rest of the toxic pesticides have various effects which affect fish in various ways including disturbances in antioxidant defensive systems. The other significant antioxidant proteins such as superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) play key roles in counteracting oxidation injury of fish by the reactive oxygen species (ROS) (Jain & Singh, 2021).

Any disruption of these enzymes can lead to heightened oxidative stress, cellular dysfunction, weakened immunity, reduced reproductive capacity, and even mortality (Mandal et al., 2023). Enzymatic fish biomarkers are essential tools for assessing aquatic health, and examining the effects of sub-lethal pesticide doses on these biomarkers can reveal potential ecological impacts (Kumar et al., 2022).

Superoxide dismutase, catalase, and glutathione-S-transferase are the three crucial enzymes of the triad (SOD, CAT, and GST) that assist in the elimination of the reactive oxygen species (ROS) within the cell and stops the oxidative damage (Rathore & Sinha, 2021).

Superoxide dismutase (SOD) is one of the most important scavengers of the superoxide anion which is one of the key reactive oxygen species, by catalyzing the metabolic transformation. SOD activity in fish liver and kidney aids in oxidative damage protection by breaking down superoxide radicals to a less reactive hydrogen peroxide (Jha & Sharma, 2021).

Molecular oxygen is quickly broken down by catalase to water and hydroxide oxygen and this avoids accumulation of hydrogen peroxides in cells which is toxic. In the liver and kidney tissues, CAT reinforces the self-antioxidant defense mechanism and maintains the normal cell functions (Kaur et al., 2020).

GST, in its turn, promotes the conjugation reaction of electrophilic toxicants with the reduced glutathione, thus enhancing their water solubility and accelerating excretion. The enzyme plays a key role in xenobiotics and environmental pollutant detoxification processes in the kidney and liver, reducing oxidative stress and preventing injury to body organs (Kumar et al., 2021).

Therefore, the enzymes can be utilized in the form of biochemical biosensors to measure the impact of pesticides on fish. Alterations in their functions, be it inhibited or induced are signs of exposure to oxidative stress, blockages in the course of detoxification and the possibility of imperilling the health of fish populations on the whole (Khan et al., 2020).

Another pesticide that is a significant model to be used to test the impacts of the exposure of pesticide to aquatic organisms in Asia and has been widely cultured is the fish species, which is known as Cyprinus carpio.

The animal commonly dwells in rivers, lakes and ponds and hence it is highly prone to contamination of the water by agricultural runoffs. Exposure of the pesticides such as buprofezin to the organism presents the risk of exposing the organism, the C. carpio, to significant physiological and biochemical changes, particularly antioxidant defence systems (Sarder et al., 2011).

According to the empirical data, the growth and survival of fish embryo and larvae will be affected adversely by buprofezin in low concentrations. Disturbance of key enzymatic functions, that is, of SOD, CAT and GST, may cause oxidative damage, detoxification, and predilection of disease (Masood et al., 2022).

Besides the health implications of an exposure to pesticides, there are the ecological implications of these effects. Decline in the number of fish due to adverse effects of pesticides being poisonous disrupts food chain relationships and culminates into loss of biodiversity in aquatic living beings. Secondly, pesticides may as well be against the health of human beings that result in accumulating pesticide residues in fish tissues causing damages to human health, particularly in the community where fish is the biggest food supply (Azouz et al., 2021).

The extensive, excessive use of pesticides in contemporary production generates far-reaching impact on aquatic environments, primarily, by the introduction and also preservation of pesticide-related toxins, which pollute water bodies, and modify water quality and ecological provision.

The substances like buprofezin have the ability to interfere with critical enzymatic processes in fish, thus resulting in redox imbalance and biochemical impairment that is manifested in the form of oxidative stress, reduced ability to detoxify, and the ensuing chain of mechanisms, which lead to physiological derangements (Amin et al., 2016). Therefore, we have to definitively define the impact of sub-lethal exposures to pesticides by characterizing enzymatic biomarkers such as superoxide dismutases (SOD), catalase (CAT), and glutathione-S-transferases (GST) by their activity composition and, most importantly, sound environmental health studies.

The mitigation measures should capture the necessity to invent and work on safer alternatives, provide more robust laws on the deployment of pesticides and coerce to embrace better agricultural practices. The reason behind defending aquatic organisms against the effects of pesticides is founded on the need to safeguard biodiversity, ensure food security, as well as ensuring that human beings combat health-related issues (Bibi et al., 2019).

Problem Statement

The problem statement highlights the dire need to study the impact of pesticide pollution, especially the lethal consequences of Buprofezin, using enzyme-based biomarker technique on aquatic habitats, and focus on the safety of aquatic organisms such as Cyprinus carpio.

Research Question

1. How does buprofezin alter core enzymatic biomarkers (SOD, CAT, GST) and oxidative stress in Cyprinus carpio?
2. What impacts does buprofezin have on reproductive fitness and overall physiological condition of Cyprinus carpio within natural freshwater habitats?
3. How does buprofezin bioaccumulation influence trophic webs and biodiversity in aquatic systems, especially non-target taxa such as Cyprinus carpio?
4. What risks do buprofezin residues in waterbodies pose to aquatic organisms and human health via consumption of wild fish?

Research Objective

- To evaluate the toxic impacts of buprofezine on Cyprinus carpio
- To examine changes in enzymatic biomarkers in C. carpio under exposure to different concentrations of buprofezine.
- To quantify the potential bioaccumulation of buprofezine within C. carpio organs.
- To increase awareness regarding the adverse impacts of buprofezine on living organisms.

Research Significance

Pesticides are chemically designed compounds that were aimed at eradicating or subduing harmful organisms as insect, weed, fungi and rodents among others, and are widely used worldwide in massive quantities. In the modern farming industry, their use has been ingrained strongly as a way of protecting crops and maintaining a high level of production. However, the extensive usage of these agents has led to a lot of anxiety over the safety of human beings and the environmental consequences (Rao & Ghosh, 2020). The current academic research in the field aims at unifying the two contrasting truths about pesticide application: on the one hand, they have been known to increase the yields of crops and provide economic advantages to the stakeholders in the agricultural sector, on the other hand, they also pose a major threat to the ecosystem and human health. The key research issue, consequently, is to white-collar this trade-off via adoption of better practice, stringent risk assessment and evidenced policy to weigh agronomic advantage with toxicological and ecological disadvantages (Rao & Ghosh, 2020). The pesticide might be contaminated in aqueous, terrestrial, and atmospheric media, causing the adverse effects with the destruction of positively acting insects, wildlife, and the change of natural habitats. In addition, human health is endangered due to pesticide exposure, and the potential outcomes might include acute toxication combined with chronic pathologies of oncogenic and neurodegenerative diseases (Rani et al., 2021).

Pollution of the water systems with the pesticides is becoming a significant environmental issue. Whenever these substances make their way into water bodies by way of runoff or leaching, they become available to cause disastrous effects to aquatic life. The large classes of pesticides such as insecticides, herbicides, fungicides, and rodenticides may cause acute death, aberrant behaviour and extensive physiological disruptions on the fish, as well as other aquatic biota. One of these instruments, buprofezin, a popular agent being used to regulate insect growth in crop protection, is distinguished by its ability to disrupt key enzymatic pathways of fish, causing severe oxidative stress, disrupting detoxification pathways, and reducing reproductive fitness, which negatively affects the fitness of organisms and population building (Purohit & Patil, 2022).

The full picture of toxicodynamic sensitivity of buprofezin in aquatic organisms especially on the specific specimen of organisms, Cyprinus carpio is quite essential in assessing the overall effects of pesticide contamination to freshwater populations. It is possible to determine the intensity of exposure, understand mechanistic injury, and determine ecological risk more emphatically by profiling the changes in enzymatic biomarkers including superoxide dismutase (SOD), catalase (CAT), and glutathione S - transporter (GST) upon exposure to buprofezin. Such evidence base can be irreplaceable in the direction of safer agronomic schemes and a future of alternative pest-management practices that will reduce the cost of damage to ecosystems and human health (Priya & Krishnan, 2023).

Summary

The use of pesticides, which are widely used to protect crops and increase food production, also presents significant risks to both the ecosystems and human beings as well as non-target aquatic life. Despite economic benefits such as more crop production and the control of the population, these are subject to causing water, soil, and air pollution, which in bulk, will lead to acute intoxication, chronic pathological damage, cancer, and neurological diseases. The effects also cause ecological disintegrations by annihilating useful insects, wildlife and aquatic species. An example of such hazards is given by agrochemical buprofezin, which is widely used in rice paddies, and which has its deep negative effects on aquatic life, namely non-target organisms, specifically fish, by disrupting key enzymatic processes that cause oxidative stress and disruption in detoxification pathways, thereby impairing energy production and reducing the health and survival of fish. Superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST) enzymes are important in reducing oxidative stress and maintaining the hepatic and renal processes in fish the impacts of buprofezin on these enzymes are evidence of how the toxin affects the aquatic ecosystem. Specifically, but not limited to, one important component of food security and aquaculture is the contamination of water by pesticides, making the population of fishes and the well-being of the entire aquatic ecosystem a particular concern. Though the good side of using the pesticides is still on, the adverse effect should be tamed with the help of the strong management strategies, and more efficient and environmentally sound practices should be created to save the nature and human life.

Review of Literature

Introduction

The advent of synthetic pesticides has been one of the major technological pillars underpinning modern intensive agriculture. Insecticides, herbicides, fungicides and rodenticides have played a central role in reducing pre and post harvest losses, stabilising yields and supporting food security for a rapidly growing human population. Over the last five decades, global pesticide consumption has increased several-fold in parallel with the expansion of irrigated agriculture and high-input cropping systems, particularly in Asia and other developing regions. This chemical dependence has effectively embedded pesticides into contemporary food production systems, where they are perceived as indispensable inputs for maintaining productivity and economic viability.

However, this success has come at an ecological cost. A substantial body of research has shown that intensive and often poorly regulated pesticide use leads to contamination of soil, surface waters and groundwater through spray drift, surface run-off, leaching and atmospheric deposition (Chauhan & Kumar, 2019; Pandey & Sharma, 2023). Once released, many active ingredients and their metabolites are persistent enough to travel far from the original point of application, entering adjacent drainage networks, irrigation channels, ponds, lakes and rivers that support freshwater biota and aquaculture. The hydrological connectivity of agricultural landscapes thus ensures that aquatic ecosystems act as sink environments for complex mixtures of pesticides and co-contaminants.

Fish occupy a particularly vulnerable position in these contaminated freshwater systems. As continuous water-breathers with permeable gill epithelia, high ventilatory volumes and extensive contact between blood and the external medium, fish readily absorb water-borne toxicants, including those bound to suspended particles (Di-Giulio & Hinton, 2008). In regions such as South Asia, where rice fish culture and carp-based polyculture are common, agrochemicals applied to paddy fields or surrounding crops can easily enter ponds, reservoirs and canals that harbour economically important species such as Cyprinus carpio and Labeo rohita (Ghosh et al., 2018; Masood et al., 2022). Chronic exposure, even at sub-lethal concentrations, may compromise growth, feed efficiency, disease resistance and reproductive output, thereby undermining the sustainability of aquaculture and capture fisheries.

Within the wide spectrum of insecticides used in rice-based systems, buprofezin occupies a prominent position. It is an insect growth regulator (IGR) structurally related to thiadiazine compounds and functions primarily as a chitin synthesis inhibitor affecting nymphal development of sap-sucking pests such as planthoppers, aphids and whiteflies (Sharma et al., 2017; Ramesh & Saravanan, 2017). Buprofezin is widely deployed in rice, cotton, vegetables and horticultural crops because of its perceived selectivity for target pests and relatively low acute mammalian toxicity. Nevertheless, a growing toxicological literature indicates that buprofezin is far from benign for non-target aquatic organisms, especially freshwater fish (Patil et al., 2016; Zargar et al., 2016; Kaur & Sandhu, 2016).

Physico-chemical properties of buprofezin favour its entry and persistence in aquatic compartments. The compound has moderate hydrophobicity (log K_ow ≈ 3–4) and limited water solubility, leading to partitioning into sediments and biota, with documented degradation half-lives ranging from several days to weeks depending on pH, temperature and microbial activity. Residues have been detected in rice paddy water, drainage canals and sediments at concentrations sufficient to pose sub-lethal risks to fish and invertebrates in intensively farmed regions of Asia. Field monitoring and modelling studies increasingly indicate that standard “good agricultural practice” doses can generate episodic peaks in receiving waters that approach or exceed experimentally derived no-effect concentrations (Nair et al., 2023; Kumar & Bhatia, 2022).

Consequently, attention has shifted from focusing solely on acute lethality towards sub-lethal biochemical and physiological endpoints. These endpoints can reveal early signs of stress long before overt mortality or population decline is observed and are especially relevant for species such as Cyprinus carpio that are both ecologically important and widely consumed by humans. In this context, oxidative stress and its associated enzymatic biomarkers have emerged as core mechanistic pathways through which pesticides, including buprofezin, exert toxic effects on fish (Kaur & Sandhu, 2016; Mohanty & Patra, 2019; Babu & Lakshmanan, 2021).

Oxidative Stress in Fish and the Role of Enzymatic Biomarkers

Interpretation of biomarker responses requires caution. Enzyme induction can represent a protective, adaptive response that successfully neutralises ROS, whereas persistent or very large increases

may signal sustained oxidative load and impending damage. Conversely, decreases in activity may indicate enzyme inhibition, exhaustion of cofactors or tissue necrosis. Therefore, enzymatic biomarkers are most informative when integrated with complementary endpoints such as histopathology, genotoxicity assays (e.g. micronucleus frequency) and organism-level performance metrics (growth, behaviour, reproduction) (Kumar et al., 2023).

Because SOD, CAT and GST are functionally interconnected within the antioxidant and detoxification network, their simultaneous assessment offers a coherent picture of how fish respond to oxidative challenges. Contemporary reviews emphasise that multi-biomarker panels integrating these enzymes provide more reliable interpretations than single endpoints, and they have become standard tools in ecotoxicological biomonitoring of fish. Broad toxicology reviews on redox biology also stress that imbalance among these enzymes rather than absolute activity of any single one is a critical indicator of oxidative stress and potential pathogenesis (Mandal & Banerjee, 2023).

In fish, the liver and kidney are particularly informative organs for enzymatic biomarker analysis. The liver is the principal site of xenobiotic biotransformation, rich in mixed-function oxidases, conjugating enzymes and antioxidant defences. The kidney, in turn, is involved in excretion of metabolites and is continuously exposed to circulating xenobiotics and their conjugates. Studies consistently show that pesticide exposure modulates hepatic and renal SOD, CAT and GST activities, often in a concentration- and time-dependent manner that mirrors the toxicokinetics of the compound (Islam et al., 2022).

Oxidative stress describes a condition in which the production of reactive oxygen species (ROS) exceeds the capacity of antioxidant defences, leading to uncontrolled oxidation of lipids, proteins and nucleic acids. In fish, as in other vertebrates, ROS are continuously generated as by-products of mitochondrial respiration, xenobiotic metabolism and various redox-dependent signalling pathways. Under physiological conditions, an elaborate network of enzymatic and non-enzymatic antioxidant systems keeps ROS at low, tightly regulated concentrations where they serve useful roles in cell signalling and host defence (Jha & Sharma, 2021).

In the enzymatic antioxidant network position, superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferases (GSTs) are centrally and complementary in place. SOD is the major form of protection against superoxide radicals and catalyzes the dismutation of the radicals to hydrogen peroxides and molecular oxygen. SOD is available in cytosolic (Cu/Zn-SOD) and mitochondrial (Mn-SOD) isoforms in fish, and its up-regulation is one of the typical reactions to redox-active xenobiotics (Iqbal et al., 2020).

Enhanced GST activity in fish exposed to pesticides generally reflects activation of conjugative detoxification pathways and, in some cases, compensatory responses to elevated lipid peroxidation (Patel et al., 2019).

The redox balance is disrupted by pesticides and other environmental toxicants in a number of ways. Components They may undergo bioactivation by the action of cytochrome P450 monooxygenases to form electrophilic intermediates that can be redox cycled, they may disrupt mitochondrial electron transport, thereby enhancing the formation of superoxide, or they may deplete endogenous antioxidants like reduced glutathione (GSH). This overproduction of reactive oxygen species (superoxide anions (O_2), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and reactive nitrogen species, in turn, can lead to lipid peroxidation, protein carbonylation, and DNA strand breaks (Ghosh et al., 2018).

GSTs constitute a superfamily of phase-II detoxification enzymes that catalyse the conjugation of GSH to electrophilic xenobiotics and endogenous peroxidation products. This conjugation increases water solubility and facilitates excretion. In addition to their catalytic role, many GST isoforms bind a variety of hydrophobic ligands and may participate in intracellular transport and signalling (Mukherjee & Das, 2018).

In fish tissues such as liver and kidney, increased CAT activity following pollutant exposure indicates compensatory mobilisation of peroxisomal defences, whereas inhibition of CAT suggests enzyme damage or exhaustion and is often associated with more severe oxidative injury (Sadeghi & Shams, 2018).

The attraction of enzymatic biomarkers lies in their sensitivity and mechanistic interpretability. Changes in SOD, CAT and GST activity can be detected at contaminant levels that do not yet impair gross behaviour, survival or reproduction. For pesticides with relatively short half-lives in water, transient oxidative perturbations may still be captured if sampling is aligned with exposure episodes, making these markers suitable for both laboratory bioassays and field biomonitoring (Oropesa et al., 2017).

According to recent experimental research and review, oxidative stress-responses have been found to be one of the most vulnerable and universal responses in pesticide and metals-exposed fish, and as such, are commonly used to act as early warning biomarkers in assessing pesticide and metal risks on the environment. The endpoints do not only include the activities of antioxidant enzymes but also indices of lipid peroxidation (e.g., malondialdehyde or TBARS), GSH depletion, and redox -regulated transcription factors (Zhao et al., 2016).

The high levels of SOD are often seen as the responses to reduce the amount of superoxide formation, nevertheless, in the absence of further elevations of the downstream enzymes, this can lead to the presence of superoxide-reactive levels of hydrogen peroxide, which is also harmful CAT, predominantly localised in peroxisomes, decomposes H_2O_2 to water and oxygen at very high turnover rates. By rapidly removing H_2O_2 generated by SOD and other oxidases, CAT prevents the Fenton reaction and subsequent formation of hydroxyl radicals, the most reactive and damaging ROS (Chance & Maehly, 2005).

Buprofezin Toxicity and Oxidative Stress Responses in Fish

Similar patterns of oxidative stress and biomarker alteration have been reported in other pesticides, supporting the idea that ROS-mediated injury is a common mode of action in fish. For example, study documented that exposure of Channa punctatus to the fungicide mancozeb led to increased ROS production, elevated lipid peroxidation and altered antioxidant enzyme activities across gill, liver, kidney and muscle, with accompanying histopathological damage and micronucleus formation. Although this study focused on a fungicide rather than buprofezin, the qualitative similarity of responses reinforces the use of SOD, CAT, GST and related endpoints as cross-pesticide biomarkers of oxidative stress. Within cyprinids, Cyprinus carpio has emerged as a key model species for evaluating pesticide toxicity because of its broad ecological tolerance, commercial value and widespread use in aquaculture. Multiple studies have examined how buprofezin and other insecticides modulate antioxidant biomarkers in carp and related species (Kumar et al., 2024).

Recent reviews that synthesize information on several pesticides and species effectively find that antioxidant enzymes are among the strongest reporting indicators of the exposure of contaminants in fish and can be merged into multi-level assessment frameworks that can affiliate biochemical modifications to physiology performance and risks at the population level. These studies may highlight the significance of being aware of species-specific sensitivities and situation-dependent reactions and the interaction between contaminant mixtures, temperature stress, and hypoxia which has the potential to adjust oxidative-stress pathways (Al-Ghanim et al., 2023).

Beyond liver-focused studies, several investigations have considered multi-organ biomarker responses and potential bioaccumulation of buprofezin. Author examined Nile tilapia exposed to 100 mg L^{-1} buprofezin for 28 days and reported elevated serum enzymes, oxidative stress markers and histopathological lesions in liver, kidney, brain, gill, pancreas, spleen, intestine, muscle and ovaries. Micronucleus frequency in erythrocytes increased, suggesting genotoxic effects, while inflammatory genes such as $IL^{-1}\beta$ and COX-2 were up-regulated in multiple tissues. Together, these data imply that buprofezin distributes widely within the organism, causing systemic oxidative and inflammatory insults, and that enzymatic biomarkers are accompanied by structural and genomic damage (Doe et al., 2023).

The authors exposed C. carpio to sub-lethal concentrations of buprofezin (0.5 mg L^{-1}) for 30 days and reported strong mobilisation of antioxidant defences. Hepatic and renal SOD, CAT and glutathione peroxidase (GPx) activities increased significantly relative to controls, indicating an active response to elevated ROS. Notably, the magnitude of enzyme induction (e.g. SOD from ~50 Umg^{-1} protein to substantially higher values) suggested considerable oxidative pressure, supporting the hypothesis that these enzymes function as early-warning biomarkers of pesticide stress in carp (Mishra et al., 2023).

Researchers conducted a complementary study whereby, 0.7 mg/L of buprofezin was administered to the day 21 to seven specimens of C. carpio. Increase in SOD activity was reported by the authors to be 45% higher and GPx activity increased to 30 percent higher than in the control fish, the SOD activity increased to about 48^{-} 1 U of protein in the control group, whereas the SOD activity was about 70 30^{-1} U of protein in the treated group. Finding of these results shows a strong antioxidant reaction to eliminate the pesticide that contributes to the detoxification of the superoxide radical and that of the peroxides produced during the biotransformation process. Together with the findings of others, the data support the finding that

SOD and GPx are informative biomarkers to evaluating environmental risks of buprofezin (Thakur et al., 2023).

The study demonstrated that a short-term (10-day) treatment with buprofezin 0.5 mg/L $^{-1}$ caused severe changes in SOD and CAT activities in freshwater fish, demonstrating that even quite short-term acute exposures are enough to result in the emergence of strong enzymatic reactions (Islam et al., 2022).

The researchers estimated the toxicity of buprofezin (1.0 L 1 $^{-1}$) on 15 days in the exposure of the organism to the chemical compound and revealed the appearance of conspicuous changes in the enzymatic biomarkers. The activity of the hepatic SOD rose up to about 55U/mg $^{-1}$ protein in the control group to about 88U/mg $^{-1}$ protein in the treated fish where both GST and CAT activities had high increases. The authors came to the conclusion that antioxidant defense is disturbed by buprofezin, which increases the loads on oxidative stress and, possibly, exposes fish to additional physiological disruptions and ultimately increases the risk of diseases (Jain & Singh, 2022).

Although the present thesis focuses on Cyprinus carpio, it is instructive to consider the broader toxicological profile of buprofezin in other freshwater species. Author investigated the effects of buprofezin in Nile tilapia (Oreochromis niloticus), an economically important species in semi-intensive aquaculture. Fish exposed to dietary buprofezin exhibited marked increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine, indicating hepatocellular and renal impairment. Concurrently, oxidative stress markers such as elevated malondialdehyde (MDA) and depleted GSH levels in liver and kidney were reported, along with significant alterations in SOD and CAT activities. Histopathological examination revealed degenerative changes in liver, kidney, gill, spleen and gonadal tissues. These findings align closely with the mechanistic picture of pesticide-induced oxidative stress described above. The combination of elevated ROS, disrupted antioxidant enzymes and histological lesions provides strong evidence that buprofezin elicits an oxidative and inflammatory response that underlies tissue damage. Author further reported up-regulation of inflammatory cytokines such as IL^{-1}β and cyclooxygenase-2 (COX-2) in liver and muscle, as well as increased inducible nitric oxide synthase (iNOS) expression in hepatic tissue, suggesting that buprofezin activates redox-sensitive inflammatory pathways that may exacerbate oxidative injury. Interestingly, caspase-3 expression a key executor of apoptosis did not change significantly, leading the authors to propose that non-apoptotic (necrotic or sub-apoptotic) mechanisms predominate in tissue damage caused by buprofezin (Azouz et al., 2021).

Authors also had revealing results that exposing fish to 0.4 and 0.8mg/L of buprofezin had caused a specific dose effect oxidative-stress signature within 14 days. The activity of SOD rose by approximately 60 per cent at 0.8mg/L- and GST almost doubled and CAT activity rose considerably (Chakraborty & Das, 2021).

Buprofezin enzymatic responses follow a similar pattern as other insecticides that have been employed in rice-dominated landscapes such as organophosphates, neonicotinoids, and pyrethroids. It has been reported by a number of authors that sub-lethal amounts of these compounds cause significant activities of SOD, CAT, GST, and GPx in the liver and kidney, and also result in high levels of lipid peroxidation and, in certain cases, in GSH depletion in the liver and kidney. Although the degree and direction of change will differ based on dose, duration, the stage of life, and environmental factors, the general response to pesticides is that it causes an accumulated oxidative load to fish, which is countered by the antioxidant machinery in a highly conserved manner (Gowda & Kumar, 2021).

Further studies have generalized the findings to more protracted durations of exposure and more outcomes. Thomas et al were examining the changes in oxidative-stress biomarkers in the presence of buprofezin in oxidative stress in the study conducted after 28 days, wherein they found the activities of SOD, CAT and GPx activities were coordinate, which is in line with a severe oxidative pressure. The results reported indicated that exposure to 20 days of buprofezin at 0.8 mg/L caused a notable increase in the SOD and CAT and GPx activities and a reduction of neurotoxicity was observed and behavioural change. Such dose- and time-based reactions are similar to those of activated antioxidant enzymes in the exposure of the freshwater cyprinid species, such as Labeo rohita and other species, to various insecticides, thus supporting the idea that the activation of antioxidant enzymes is a generalized reaction to pesticide-induced oxidative stress (Jadhav et al., 2021).

Authors exposed fish to 0.6 mg L^{-1} buprofezin and found substantial increases in SOD, GPx and CAT activities, suggesting heightened susceptibility to oxidative stress and impaired detoxification. Authors reported analogous patterns in C. carpio SOD, CAT and GPx all increased significantly after 25–30 days of exposure to sub-lethal buprofezin concentrations. These authors emphasised that such enzyme alterations may precede more overt phenotypic effects, underscoring their utility as early-warning signals (Kumar & Chauhan, 2020) .

Though direct determination of buprofezin residues in fish tissues has been minimized, a number of studies have determined some of the endpoints of its use, i.e., carrison structure, and tissue-specific bioaccumulation, which points to the fact that buprofezin could be accumulating in the consumable parts of carp over long durations. These findings organise not only piscine health issues, but also the issue of possible transfer of residues by the food chain to consumers who have carp as a significant source of protein, especially in societies where the carp becomes a significant source of protein in the fooduk (Bibi et al., 2019).

Ecological and Human-Health Cautions of Buprofezin in Drinking Water

Integrating enzymatic biomarker data into regulatory frameworks could improve risk characterisation by providing mechanistic anchors for population-level models and allowing for the detection of sub-lethal effects at realistic environmental concentrations. Such integration would also facilitate evaluation of the cumulative impacts of pesticide mixtures that share oxidative-stress pathways, a scenario common in agricultural catchments (Kumar et al., 2023).

Fish are not only ecological components but also key dietary items for millions of people. In many rice-growing regions of South Asia, common carp and other cyprinids contribute substantially to household protein intake and micronutrient supply. The possibility that buprofezin residues and their metabolites may accumulate in edible tissues raises legitimate food-safety concerns. Enzymatic and histological evidence of hepatic and renal dysfunction in buprofezin-exposed fish suggests that residue levels high enough to disrupt organ function could also compromise fish quality and shelf life (Ahmed et al., 2022).

In the case of buprofezin, existing regulatory evaluations often focus on terrestrial endpoints and target insects, with relatively limited coverage of freshwater fish. The accumulating evidence of oxidative stress, enzyme disruption and organ damage in cyprinids and tilapia indicates that current environmental quality standards may not fully protect sensitive native and cultured species. Moreover, most studies have been conducted under controlled laboratory conditions, and there is a scarcity of field investigations integrating exposure measurements, biomarker responses and ecological observations (Purohit & Patil, 2022).

Ecological risks are enforced more through the process of bioaccumulation and the transfer of toxins. Similar to other moderately hydrophobic pesticides, buprofezin is capable of bioaccumulating in fatty tissues and being bio magnified in food chains. Recent residual in sediments may be reused on occasions of resuspension and increase the period of exposure to both benthic and demersal attenuation. Models Repeated sub-lethal exposures to carp and tilapia have been associated by field and laboratory studies with the cumulative effects of oxidative damage and impaired immune, which might be transferred into increased disease sensitivity and mortality under natural conditions (Azouz et al., 2021).

When the pesticides are released to freshwater systems, they do not often work singly. Buprofezin is found in complex cocktails with fertilizers, other insecticides, fungicides and industrial pollutants the effects of which on aquatic biota have not been well solved. However, increasing evidence exists that insect growth controllers like buprofezin are able not just to interrupt the life of individual fish, but also broader food-webs. Buprofezin has also been found to disrupt the survival and reproduction of non target invertebrates such as crustaceans and aquatic insects that are important constituents of fish diets. When the abundance of such prey items reduces, other trophic levels may grow and be in poorer condition behaviorally indirectly (Mistry et al., 2021).

Although human epidemiological data for buprofezin are limited, generic toxicological profiles of pesticides indicate that chronic, low-dose exposure may be associated with endocrine disruption, neurotoxicity, carcinogenicity and reproductive effects. Many of these outcomes are mechanistically linked to oxidative stress and inflammation pathways already shown to be activated in fish by buprofezin. Thus, from a precautionary standpoint, minimising buprofezin inputs into freshwater systems is desirable not

only for protecting aquatic life but also for safeguarding human consumers of fish and water resources (Rani & Singh, 2021).

Biodiversity-wise, the use of pesticides may cause a change in a community structure (selective from the sensitive species and life stages) and results in species favoring greater tolerance. The decline of keystone or functionally important species including carps may affect other areas of the biogeocenosis, altering nutrient cycling, primary production, and predator-prey relationships. Considering the key role of fish in most inland -water food webs, chronic pesticide pollution, such as buprofezin, is currently seen as an important cause of freshwater biodiversity loss (Ghosh et al., 2018).

Regulatory frameworks for pesticides traditionally rely heavily on acute LC_{50} values derived from standard test species and do not always incorporate sub-lethal biomarker responses or species-specific sensitivities. However, recent guidance from international bodies emphasises the value of mechanistic biomarkers, including oxidative-stress enzymes, in refining environmental risk assessments and identifying early signals of harm. Several countries have begun to incorporate biomarker-based monitoring into national water-quality programmes, particularly for high-use pesticides with known ecotoxicological concerns (Di-Giulio & Hinton, 2008).

Knowledge Gaps and Rationale for the Present Study

Despite substantial progress, several critical knowledge gaps justify further investigation of buprofezin toxicity in Cyprinus carpio using enzymatic biomarkers.

Limited Organ-Specific Data in Carp

Many existing studies on buprofezin have focused either on a single organ (often liver) or on non-carp species such as tilapia and Labeo rohita. Systematic comparisons of hepatic and renal antioxidant responses in C. carpio across multiple exposure durations and concentrations remain comparatively scarce. Given the pivotal roles of liver and kidney in xenobiotic metabolism and excretion, simultaneous assessment of SOD, CAT and GST in both organs can provide a more integrated understanding of organism-level stress (Azouz et al., 2021; Jadhav et al., 2021).

Sub-Lethal Environmentally Realistic Exposures

Many toxicity trials have used relatively high nominal concentrations or short exposure periods primarily aimed at deriving LC50 values or observing overt clinical signs. Yet, field-measured pesticide levels in agricultural waters are often lower but persistent, producing chronic sub-lethal exposure. Studies using concentration ranges and exposure times aligned with plausible environmental scenarios are needed to refine threshold values for oxidative-stress induction in carp (Kumar & Chauhan, 2020; Sharma et al., 2020).

Temporal Dynamics of Biomarker Responses

Oxidative-stress biomarkers are inherently dynamic. The time course of SOD, CAT and GST induction and potential subsequent normalisation or depletion has not been thoroughly characterised for buprofezin in C. carpio. Sampling at multiple time points (e.g. 24, 48, 72 and 96 h and beyond) allows disentangling transient adaptive responses from sustained stress states that may lead to pathology (Yadav et al., 2019).

Linkages Between Enzymatic Biomarkers and Endpoints

While numerous studies document changes in enzyme activities, fewer link these biochemical alterations to histopathological lesions, performance metrics (growth, behaviour, feed conversion) or reproductive impairment in carp. Establishing such linkages is crucial for translating biomarker perturbations into ecologically meaningful risk statements (Mandal & Banerjee, 2023; Rani & Singh, 2021).

Interactions with Other Stressors

Carp in natural and culture environments are simultaneously exposed to fluctuating temperature, hypoxia, ammonia, and other contaminants. These co-stressors can modulate oxidative-stress responses and either exacerbate or mask pesticide effects. Most buprofezin studies have been conducted under constant, optimal laboratory conditions, understanding responses under more realistic multi-stress scenarios is an important future direction (Maulu et al., 2021; Chandrasekaran et al., 2020).

In light of these gaps, the present study focuses on quantifying hepatic and renal SOD, CAT and GST activities in Cyprinus carpio exposed to graded sub-lethal concentrations of buprofezin over defined time intervals. By using a structured biomarker approach, the study aims to characterise the oxidative-stress profile of buprofezin in this key species, provide mechanistic evidence of toxicity and contribute data that can inform both aquaculture management and environmental risk assessment.

Summary

The literature reviewed in this chapter demonstrates that modern agriculture's dependence on pesticides has significant unintended consequences for freshwater ecosystems. Buprofezin, though agronomically valuable as an insect growth regulator, enters aquatic systems via run-off, leaching and drift, where it can affect non-target organisms such as Cyprinus carpio. A coherent mechanistic picture emerges in which buprofezin and other pesticides provoke oxidative stress by enhancing ROS generation and challenging endogenous antioxidant defences.

Enzymatic biomarkers most notably SOD, CAT and GST are repeatedly shown to be sensitive, mechanistically relevant indicators of pesticide-induced stress in fish. Studies in carp and tilapia demonstrate that exposure to buprofezin leads to significant, dose- and time-dependent increases in these enzymes in liver and kidney, often accompanied by elevated lipid peroxidation, GSH depletion, inflammatory signalling and histopathological damage. These responses underline the central role of oxidative stress in the mode of action of buprofezin and support the use of enzymatic biomarkers as early-warning tools in ecotoxicological monitoring.

At the ecosystem level, continued buprofezin use contributes to biodiversity loss, trophic disruption and potential human-health risks via bioaccumulation in edible fish. Regulatory frameworks are only beginning to incorporate mechanistic biomarkers and mixture effects into pesticide risk assessment, and there is an urgent need for more organ-specific, temporally resolved and environmentally realistic studies in key aquaculture species. By focusing on hepatic and renal enzymatic biomarkers in Cyprinus carpio, the present research seeks to address some of these gaps and provide robust, mechanistically grounded evidence on the toxic impact of buprofezin in freshwater fish.

Methodology

Introduction

Buprofezin has demonstrated itself as a prevalent pollutant in freshwater wherein most of the contamination has been contributed by intensive farming activities and poor management of farming runoffs. When released into irrigation systems, ponds, and river system this insect growth regulator could be present longer in the water column and the sediments in which it may have both acute and sub-lethal impacts on the non-target organisms, especially cultured and wild fish. Consecutive experimental and field studies show that the fish exposed to buprofezin displayed biochemical, physiological, and histopathological changes that are typical of oxidative stress, compromised detoxification, and organ dysfunction (Azouz et al., 2021). It is on this background that this study was put down to offer the controlled, lab-based study of buprofezin-induced toxicity on the common carp, Cyprinus carpio, basing the study on enzymatic biomarkers in hepatic tissue and renal tissues as the key research instruments.

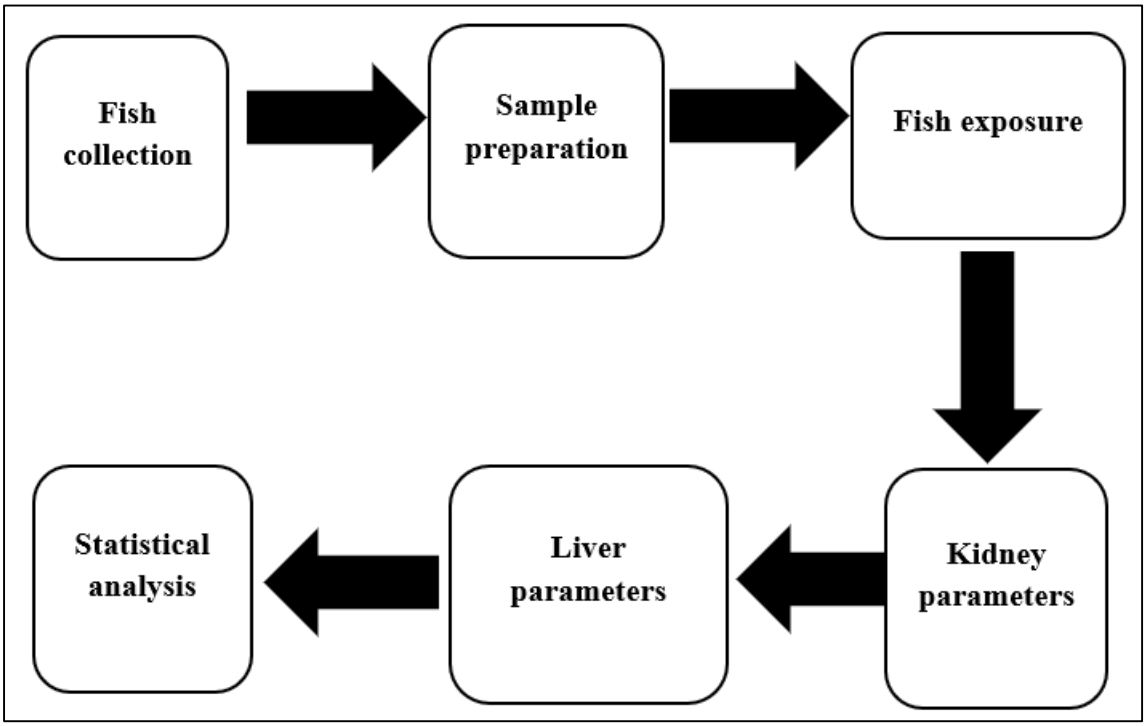

Figure 3.1: Proposed Framework for Research

The main goal of this methodological design was to ascertain the change in antioxidant enzyme profile of C. carpio by the chromic dietary exposition to the ecologically viable amounts of buprofezin. More specifically, the research concentrated on major three enzymatic biomarker, including catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST), which were measured in the liver and kidney. The reason these enzymes were chosen is that they have a central and complementary role in the process of detoxification of reactive oxygen species and xenobiotic metabolites, as well as being comprehensively verified as hypersensitive markers of oxidative stress caused by pesticides in fish. The experimental design was able to respond to the spontaneous enzyme responses over an eight-week period with further short-term responses at 24, 48, 72, and 96 hours of exposure, therefore, the experimental design was applicable in capturing the early and long-term responses to buprofezin in fish carp fingerlings in a controlled environment (Shen et al., 2007).

The entire experimentation was performed in Zoology Department Laboratory of Riphah International University, Faisalabad, Pakistan. The facility offered stable environmental condition, constant access to dechlorinated water and the facility needed to maintain processes of live fish handling, toxicant solution preparation, processing of blood and tissue samples and biochemical study through a Microlab 300 spectrophotometer. The methodological decisions mentioned in this chapter were informed by the protocols of fish toxicology that had been published in the past and were adjusted to the objectives of the current research but were in tandem with the good laboratory practice, animal welfare, and statistical strength.

Research Framework

The research design to use in this thesis combines an experimental toxicology design and a biochemical biomarker analysis. In principle, the framework can be represented as a series of connected stages. There were three stages, firstly, healthy fingerlings of C. carpio were purchased, transported, and acclimatised with uniform husbandry to ensure a reduced variability as the baseline. The experimental subjects were secondly randomly placed into two exposure groups and a control group which were all acclimatized fish housed in separate glass aquaria exposed to varying concentrations of buprofezin. Third, blood and tissue samples were obtained at pre-established times (acute 24-96 hours and chronic to a maximum of eight weeks) during exposure to the drug to enable quantitative evaluation regarding enzyme activities of the liver and kidney in those tissues in representative fish. Lastly, the biochemical data

resulting were then fully subjected to intense statistical processing to determine changes that were concentration- and time-dependent and that could be attributed to buprofezin.

In this model enzymatic biomarkers are considered as the main response variables relying on which the concentration of buprofezin and the duration of exposure are the main explanatory variables. The environmental conditions including water temperature, dissolved oxygen and pH were held as constant as possible during the experiment in order to explain the existence of significant differences in the biomarker results as being largely due to a treatment effect and much less due to the changing environment of the husbandry conditions. In such a way, the framework interconnects exposure and internal dose, biochemical reaction, and toxicological interpretation in a logical and experimentally trackable way.

Data Collection Strategy

In the given study, the data collection process consisted of two general streams which were the data on environmental monitoring and data on biological responses. Analyses of the environment involved periodic measurements of the water quality variables but the data on the biological response included morphometric measurements, survival of animals, and more importantly biochemical measurements of blood, liver, and kidney.

Temperature of water, dissolved oxygen and pH were measured at least three times a week with calibrated handheld meters to maintain both the temperatures and conditions to be under acceptable pH limits as well as to exclude occurrence of confounding events of hypoxia or extreme pH levels during the exposure period. All the variations outside the target ranges were compensated immediately through the replacement of partial water or regulation of aeration. The observation of mortality and general behaviour (feeding activity, swimming pattern, surface gulping) was made on a daily basis and information documented qualitatively to complement the enzymatic endpoints with details concerning the general health condition.

The baseline (before buprofezin exposure), 24, 48, 72, and 96 hours biological sampling was maintained and then at the concluding eight weeks of the exposure period. Each time there is a sacrifice of fish, a fixed number of fish of every group were taken to get a blood and tissue which would result in replicate measures of each treatment and time. The range of biochemical endpoints was CAT, SOD, and GST activities in liver and kidney homogenates and supporting indictators of serum, i.e. the level of blood urea nitrogen (BUN) and creatinine. Raw data were recorded on structured spread sheets immediately after every one of the assay sessions cross tabulated against transcription errors and then entered into SPSS to be analysed using statistical methods (Sharma et al., 2017).

Fish Collection and Acclimatization

There were two aims in the collection and acclimatization of experimental fish: animal welfare and removal of pre-exposure variability of physiological state. The Government Fish Seed Hatchery in Faisalabad supplied viable stock of the fingerling of C. carpio. The fishes used in the experiment were fairly well matched with total length ranging between five to six inches and weighing between 50 and 60 grams. This scale range was selected so as to avoid the confounding effects of extreme juvenile/senescent physiology and so as to give adequate tissue mass to undergo repeated biochemical tests (Zargar et al., 2016).

The hatchery was used to catch fish through normal netting methods which reduce physical harm. They immediately were moved into aerated plastic containers with the source water in the hatchery to minimize osmotic shock (Subaramaniyam et al., 2023). Portable aeration pumps were used to keep the dissolved oxygen stable during the transportation to the laboratory and the travel time was kept to the minimum possible in order to reduce the stress in transportation.

When the fish reached the Laboratory of the Zoology Department they were released caring in large glass or fiberglass acclimation tanks filled with dechlorinated tap water which had been pre-aerated and brought as closely to the temperature of the hatchery as possible. The period of acclimation was 14 days during which fish were fed carp pellet diet that is a commercial diet at about two to three percent body weight per day with two equal meals. Feed and fecal lag were siphoned on a daily basis and one out of three to four-fifth water in the tank changed every two days to take care of the water quality.

During acclimatization, Fish were checked at least 2 times per day, to check whether it is stressed through erratic swimming, lack of balance, excessive production of mucus, or surface gasping. Only the

people who were active, responsive and had no visible lesions were put forward to be included in the experimental groups. Fish which exhibited abnormal behaviour or those that developed signs of any disease were taken away and eliminated in the study. The process was necessary to guarantee that the experimental groups began the exposure process in equal and healthy physical state (Azouz et al., 2021).

Preparation of Buprofezin Stock and Test Solutions

Analytical grade buprofezin was bought in the form of dry powder in Faisalabad in a known chemical merchant. The chemical was taken to the store where airtight and light-covered containers were kept at room temperature as recommended by the manufacturer in order to avoid degradation. Prior to the initiation of the exposure experiment, a main stock solution under constant stirring in an electric mixer was prepared by adding 2.5 g of buprofezin to it and 1L of dechlorinated tap water was added to the mixture. Preparation of the stock solution was conducted individually after every seven days and stored in amber bottles at 4^{o}C between the periods of use to reduce the amount of photolysis and chemical degradation (Vander et al., 2003).

Nominal concentration of 5 mg/L^{-1} and 10 mg/L^{-1} of the stock solution were produced in the respective treatment tanks by diluting appropriate volumes of the stock solution into the existing pre-measured volumes of tank water. The same amount of dechlorinated buprofezin-free tap water was transferred to the control tank. The test solutions were replenished every alternate day to ensure the relatively constant exposure conditions and partially replenished with water (Singh et al., 2018). In turn, every renewal the existing volume of water was siphoned to eliminate the waste and newly prepared water with the required concentration of buprofezin was put in. The reason to select this semi-static renewal regime as a choice between the chemical stability of the products, animal welfare, and economy in terms of logistic viability.

Tools and Technology

The experimental protocol was added using a set of standard and modern laboratory instruments to measure the biochemical reactions. To respond to the hypotheses proposed in the study, SPSS software was employed to calculate descriptive and inferential statistics (Shrivastava & Mishra, 2019). The descriptive statistics involved determination of group means, standard deviations and standard errors, and inferential analysis depended mostly on the one-way analysis of variance (ANOVA) to determine whether significant difference existed among the groups of treatment and exposure times at a significant level of descriptive statistics involved computing the group means, standard deviations and standard errors, and the inferential analysis involved mostly the one-way analysis of variance (ANOVA) to determine the presence of significant difference between the various groups of treatment and exposure times with a critical level of 0.05. Where necessary, post-hoc analyses were done to determine certain group differences and the assumption of normality and homogeneity of variance were verified prior to the interpretation of ANOVA findings.

The quantification of biochemical was based on spectrophotometry. All enzyme tests and serum biochemical tests were done through a Microlab 300 spectrophotometer (Sharma et al., 2020). The instrument was calibrated with the help of proper blank and standard solutions as provided with the commercial assay kits or prepared as per the published protocols before each batch of analyses. Periodic checks of accuracy and linearity of wavelengths were conducted to ensure courageous absorbance measurements in the concerned spectral ranges.

Sterile disposable syringes and needles of an appropriate gauge were necessary to reduce the amount of trauma in the fish with regard to blood collection. Samples to be assayed in plasma or whole blood were put in heparinised tubes whereas those which required serum separation were put in non-heparinised tubes. Separation of the plasma and serum with the cellular components was performed at a refrigerated centrifuge with the capacity of producing the relative centrifugal force mentioned in the original methodological references (Reitman & Frankel, 2007).

Enzyme assays were performed using tissue processing which was based on a mix-up of analytical balances, chilled glass homogenisers or mechanical tissue grinders and cold buffers. The steps of the homogenisation were performed on ice so as to reduce degradation by the enzymes. In the case of GST, SOD and CAT assays, special reagents and substrates were prepared the way recommended by (Habig et al., 2004; Nishikimi et al., 2014; Aebi, 2000) respectively. Moreover, the serum biochemical parameters and enzymatic determinations were performed both using commercially available diagnostic kits,

according to the protocols of the manufacturer, with the respective reference numbers, to be used to trace the data.

The proposed experimental method involves using a questionnaire interview technique as the data collection instrument to achieve the objectives of the study.

Proposed Experimental Method

The data collection method will be through questionnaire interview as the research objective of the study will be achieved.

Experimental Design and Fish Exposure

After the acclimatization, healthy fingerlings of the stock of C. carpio were randomly placed into three groups of experimental groups, each with the support of independent glass aquaria. Randomisation was done by assigning people a number which was dictated by the fish and assigning individuals across the groups using an allocation random number table, this minimised allocation bias. The groups had a sample size of 36 individuals, namely, twelve fish. Group T0 was used as a control and was kept in dechlorinated water without buprofezin. Group T1 and T2 were exposed to 5 and 10mg of L of buprofezin and 10mg of L of buprofezin respectively. These levels were chosen because they constituted sub-lethal but environmentally relevant exposure levels, based on the reported levels in the environment and past toxicological research (Mandal & Banerjee, 2023).

There were eight weeks of exposure duration. During this period, fish were fed a commercial pellet diet at about three percent of body weight/day which was split into morning and evening feeds. To ensure a constant feeding rate, the ration of the feed was changed weekly basing on the mean body weight per tank. To avoid water quality decline, uneasy feed was eliminated following every nutrition and faecal material and stillage were siphoned every day (Kumar et al., 2018). Water temperature was kept at the most ideal temperature of carp by set control of room temperature and, in case required, at supplemental heaters.

Short-term sampling was scheduled at 24, 48, 72, and 96 hours following the initial introduction of buprofezin to capture early biomarker responses. At each of these time points, six fish per treatment (two from each tank replicate, where applicable) were sampled. At the end of the eight-week period, additional fish were sampled to assess chronic effects (Khan & Siddiqui, 2020). This design yielded a matrix of data that allowed comparison of acute versus chronic responses as well as the concentration dependency of enzymatic changes.

In order to reduce stress associated with manipulation, fish were carefully net caught out of the aquarium and placed directly into an anaesthetic bath of $0.5mL^{-1}$ 2-phenoxyethanol. The anesthetised fish was observed until the loss of equilibrium, and loss of movement of the operculus revealed adequate depth of anaesthesia in which safe blood collection can be performed. This method eliminated struggling and exposure to injuries by both the researchers and the fish (Devi & Bharti, 2021).

Sampling Procedures

At the designated time of sampling, anaesthetised fish were dried on paper towels very gently to wipe off excess water and placed on uncontaminated and chilled dissection tray. Each of the individuals was then assessed in terms of total length and body weight to allow further analysis of correlations between body size and biomarker responses (Khan et al., 2021). A sterile syringe with a fine-gauge needle was used to collect blood by taking it out of the caudal vein. The collected volume was limited precisely with regard to the body mass to prevent haemorrhagic shock.

A defined amount of every blood sample was immediately heparinised to plasma-based assays and the rest was delated to serum separation by collection in plain tubes. The tubes were stored on ice before centrifugation (Mannervik et al., 2005). The blood samples underwent centrifugation at about 3,000 rpm and a total of 10 minutes in order to separate the plasma or serum and the cellular constituents. The subsequent supernatant mixtures were pipetted in labelled microcentrifuge tubes and kept at -20^0C or below until biochemical analysis.

The euthanisation of fish occurred after the taking of blood, either through a severance of the spinal cord or by excessively prolonging the exposure to the anaesthetic agent as is ethically acceptable. An incision (mid ventral) was then made with the aim of exposing the inside organs (Mishra et al., 2023). The liver and kidneys were removed with the help of sterile instruments, briefly rinsed in ice-cold physiological saline to eliminate adherent blood, dried on a blot paper, and weighed. The two subsamples were produced

of each organ. One subsample was snap-frozen in liquid nitrogen or put in pre-chilled tubes and stored -80^{0}C ultimately to undergo enzymatic analysis of CAT, SOD, and GST. The remaining subsample was put in 10% neutral-buffered formalin where it was to be subjected to histopathological examination, which is beyond the scope of the current biochemical interest.

A distinct identifier was used to label all samples which encoded the treatment group, tank, sampling time, and fish number. This coding system minimized the likelihood of misidentification and eased the analysis of the samples blindly giving the analyst an opportunity to analyze the samples without prior information about the treatment condition hence limiting the possibility of the bias (Mohanty & Patra, 2019).

Biochemical Analyses

Liver and Kidney Serum Indicators

Sera hepatic and renal function indicators were quantified to augment the enzymatic biomarker data and give a larger picture on organ status during buprofezin exposure. In the case of liver functioning, the serum transaminases activity had been calculated based on the classical measures that were used by (Reitman & Frankel, 2007) in clinical and experimental biochemistry. The presence of these enzymes in serum in elevated levels is usually assumed to be an indicator of hepatocellular injury or a leakage of cell membranes.

An evaluation of kidney performance was done through calculations of blood urea nitrogen (BUN) and creatinine serum concentrations. The colourimetric procedure of measuring BUN as described by (Kaur & Gill, 2020) and creatinine as described by (Habig et al., 2004) were used. They both use a spectrophotometric analysis of coloured reaction products, and are widely used in fish physiology and toxicology. Rises of BUN and of creatinine have been found to reflect inefficient glomerular filtration or dysfunction of tubules, therefore being utilizable markers of nephrotoxicity.

The antioxidant biomarker profiling is performed on this methodology to compare the antioxidant properties of the food material and food ingredients supplied. The antioxidant biomarker profiling is done on an approach to compare the antioxidant property of the food material and foods provided (Young, 2011).

The fundamental biochemical aspect in the present study was the profiling of hepatic and renal tissue antioxidant enzyme activities. Samples of liver and kidney that were frozen on ice were thawed using ice and homogenised in cold phosphate buffer with pH of around 7.4 ruminating a glass-Teflon homogeniser. Centrifugation of the homogenates was carried out at 10000-12000, 15-20 minutes at 4ºC to eliminate cellular debris and the supernatant thus obtained were the sources of enzymes (Gupta et al., 2020).

GST activity was also determined as indicated by (Habig et al., 2004) which involves the conjugation of reduced glutathione and 1-chloro-2, 4-dinitrobenzene (CDNB). The increase of the absorbance at 340 -C NB conjugate was observed with a spectrophotometer with time. The activity of the enzymes was calculated as units/ml or per milligrams of the protein.

The activity of SOD was established, following the analysis of (Nishikimi et al., 2014), which relied on the capability of the SOD to prevent the problem of oxidizing nitroblue tetrazolium by the superoxide anion. At different incubation periods an absorbance value was recorded at 560nm and 1 unit SOD activity was represented as the number of enzymes that are needed to reduce NBT by half the activity at the given conditions of the assay. This is a sensitive and commonly used assay of superoxide scavenging ability.

The measurement of CAT activity was done by the method of (Aebi, 2000) where the reduction of hydrogen peroxide was measured with spectrophotogram. Reduction in the absorbance at 240 or 510nm (according to the adaptation differentiation) is an indication of the degradation of H_2O_2 by CAT. The reaction rates were estimated based on the linear part of the absorbance-time curve and normalised with respect to the protein content. Combined, these three enzyme activities produced a combined image of both oxidative workload and antioxidant defence of buprofezin-treated fish liver and kidney.

To ensure an optimum measure of analytical reliability, all the enzymatic assays were conducted in both replicate or triplicate of each sample and the average replica values were ultimately taken in the later statistical calculations. The periodical calculation of inter-assay and intra-assay coefficients of variation using quality-control samples ensured that the variability of methods used was not exceeded (Ahmed et al., 2022). Reagent blanks, substrate blanks and, where necessary, non-enzymatic controls were

added in order to correct the background absorbance and spontaneous substrate degradation. These quality-control measures made certain that experimental differences between experimental groups were rather biological in nature as opposed to technical artefacts.

The measurement of total protein in tissue supernatants was (via a standard colourimetric procedure, e.g., the Biuret or Lowry assay assay) in order that the expression of the activity of the enzyme goodwill, e.g., by units per milligram of protein (Amin et al., 2016). All the tests were performed in duos or trios to enhance accuracy and the corrective measures were taken, namely, the use of blanks and controls in each batch to eliminate non-enzymatic reactions and reagent background.

Hepatic Enzyme Analysis

In the case of hepatic analysis, the consistency of the sampling and processing of individuals and time points was regarded as a point of interest. Where possible, the same part of the liver lobe was sampled in order to minimize the variation within a liver tissue (Chakraborty & Das, 2021). Standardisation of homogenisation condition, buffer composition and centrifugation parameters was recorded in a detailed way. The above methods were used to measure the enzyme activities of CAT, SOD, and GST in liver homogenates and the data was normalised against protein content.

The biochemical profile of C. serum was also assessed with the help of the kit-based methods on Microlab 300 spectrophotometer, according to the protocols of (Islam et al., 2022). GST, CAT, and SOD assays were performed in commercially acquired diagnostic-kit with reference numbers 997180, 997258, and 998690 respectively under the instructions of the manufacturer. These kits added to the classic methodology sources, and a higher degree of reliability and reproducibility of the measurements was achieved through the use of quality controlled reagents and standard curves.

Renal Enzyme Analysis

The time activities of renal antioxidant enzymes were evaluated by following the similar procedures that were adopted to do hepatic tissue. Cold buffer homogenisation, centrifugation of kidney samples and supernatants were used to carry out CAT, SOD, and GST. To have methodological soundness, diagnostic kits (Ref. 998003 of CAT, Ref. 996265 of SOD and GST) were used together with classical assay protocol (Islam et al., 2022). Despite the fact that the main scope of the entire thesis is dealing with C. carpio, the general methodology employed in the general analysis of renal functioning is similar to the general approach employed in comparable studies of other teleost species, including Labeo rohita, thereby enhancing comparability with literature in the article.

Chapter Overview

In conclusion, this chapter has presented the methodology architecture that was used to examine toxic effects of buprofezin on hepatic and renal enzymatic biomarkers of Cyprinus carpio in controlled laboratory conditions. The experimental design was comprised of a well-regulated fish situation, exposure regimes at two sub-lethal levels and systematic sampling-timetable, which embraced both acute (24-96 hours) and chronic (eight weeks) responses. To make sure that the animals were not stressed because of experiments, standardised animal collection, acclimatisation, anaesthesia, blood sampling, organ dissection, and tissue preservation methods were adopted to reduce pre-analytical variability.

Biochemical measurements were added with concentrations of antioxidant enzymes CAT, SOD and GST in the liver and kidney, and serum markers of liver and kidney functions. An assay was done based on the combination of classical spectrophotometric techniques and commercial diagnostic kits following the careful consideration of the calibration, replication, and quality control. The model of evaluating significant biomarker activities change was based on statistical assessment conducted on SPSS and one-way ANOVA to identify significant exposure-related changes in activities of the biomarkers.

These methodological elements combine to make up a consistent and rigorous model of measuring the oxidative and detoxification processes of C. carpio to the presence of buprofezin. The next chapter is based on this as it introduces and discusses the experimental results and especially dose and time-dependent trends in enzymatic change and their relevance to fish health and further assessment, monitoring and management of the risk to aquatic ecosystems.

Results and Discussion

Introduction

The current investigation was carried out with the purpose of elucidating the toxicodynamic effects of buprofezin on the freshwater teleost Labeo rohita by the profiling of enzyme biomarkers such as catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST). These enzymes play a significant part in the prevention of oxidative stress and the mediation of xenobiotic detoxification (Aebi, 2000). As a result, they may be regarded a sensitive monitoring mechanism for the identification of pollution in water. The liver and kidney were selected as the organs to be targeted because to the important roles that they play in the process of biotransformation and excretory clearance. On the basis of the findings, it seems that buprofezin causes oxidative stress in Cyprinus carpio, which in turn causes changes in biochemical markers that are dependent on both concentration and duration.

- Measurement of physical chemical parameters of the water column.
- Evaluation of growth performance measure.
- The hepatotoxicity endpoints must be assessed.

Physicochemical Water Parameter Appraisal

The main water-quality variables which included temperature, dissolved oxygen, and pH were measured after every week.

Hepatic Parameter Analysis.

Measuring the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) was another method that was used to assess the liver condition. According to Islam et al.'s research from 2022, the biochemistry of blood and serum samples, as well as liver function tests (LFTs), of the Cyprinus carpio were obtained using a Microlab 300 spectrophotometer in accordance with the recommended procedures. The quantification of GST was carried out with the assistance of a QCA diagnostic kit Ref. 997180, CAT activity was measured using a QCA kit Ref. 997258, and an additional kit was used to test the addition of anti-oxidant enzyme (Kumar et al., 2020).

Assays of Renal Parameters

The assessment of renal activity was done by assessing catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) enzymatic activities. (Islam et al., 2022) reported that the biochemical examination of blood and serum was conducted based on a Microlab 300 spectrophotometer, kit-based analysis. The liver function test (LFT)-oriented model of Cyprinus carpio was also factored in the study. The test of CAT activity was performed by means of a QCA diagnostic kit Ref. 998003, SOD activity using an OCA diagnostic kit Ref. 996265 and GST activity using a QCA diagnostic kit Ref. 990539. These were a series of quantitative assays that were used to determine the levels of enzymatic activity.

Table 4.1: Effects of Buprofezin on Hepatic CAT Activity (UmL^{-1}) of Cyprinus carpio

Time (hrs)	T0 Control	T1(5mg/L	T2(10mg/L)
0	231.33	231.22	231.45
24	231.36	248.65	255.37
48	231.41	265.12	271.45
72	231.66	281.25	289.55
96	231.55	296.75	311.71
Mean±SD	1156.65±0.943	1323.1±0.753	1359.41±0.292

Table 4.1 shows the Effects of the Various Concentrations of Buprofezin on Hepatic CAT Activity (UmL^{-1}) of Cyprinus carpio at three different exposures, 0mg/L and 5mg/L and 10mg/L of 3-bromopyrrolide at different sampling time points. Baseline CAT activity showed no difference in all the cohorts, but continual exposure on the groups (T1 and T2) treated resulted in a progressive increase of the activity which peaked at 96 hours. The findings indicate an increase in the hepatic CAT activity depending on the dose and time due to the treatment (Mohanty et al., 2019).

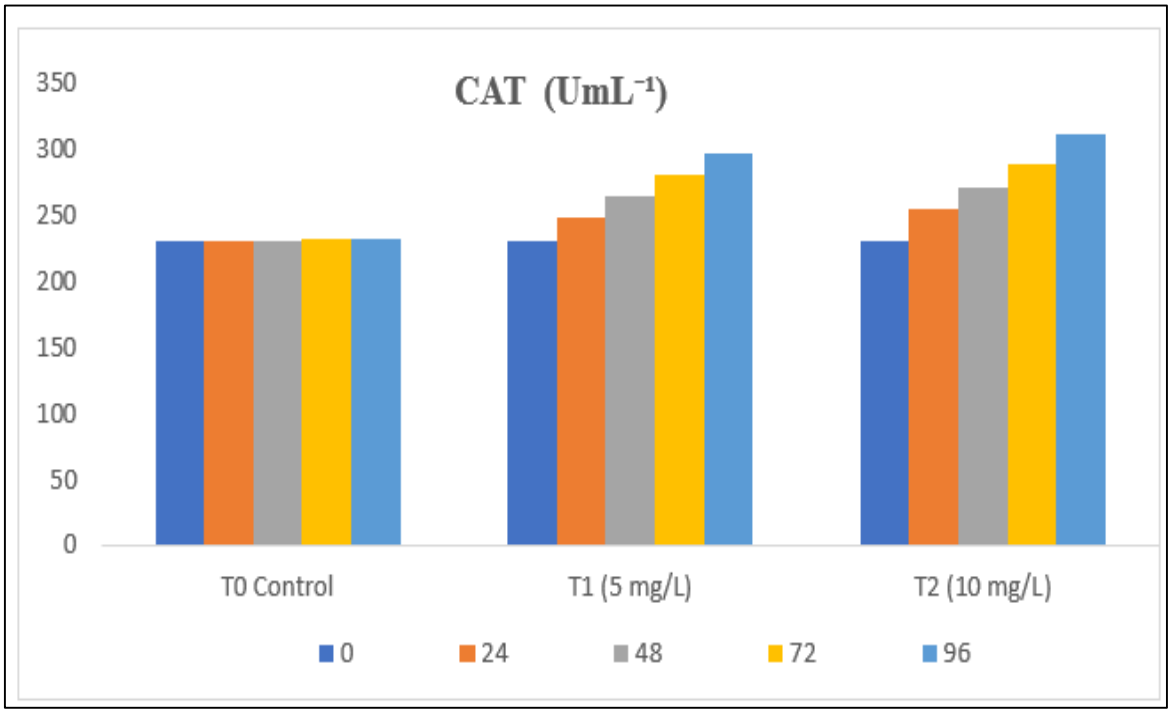

Figure 4.1: Effect of Increasing Buprofezin Concentrations on CAT Activity in Cyprinus carpio

Figure 4.1 shows the graphical illustration depicting the effects of escalating buprofezin concentrations on catalase (CAT) activity (UmL^{-1}) in Cyprinus carpio, summarizing responses across control and exposed cohorts to visualize dose response trends.

Table 4.2: One-Way ANOVA Summary of Hepatic CAT Activity in Cyprinus carpio Comparing Control and Buprofezin Treated Groups

S.O.V	DF	SS	MS	F	P-value
Treatment	2	28.27	14.135	2.38	0.032
Error	12	71.38	5.948		
Total	14	99.65			

Table 4.2 shows the one-way ANOVA summary for hepatic catalase (CAT) activity in Cyprinus carpio, contrasting control versus buprofezin-treated groups and reporting group means, variance components, and statistical significance.

The one-way analysis of variance (ANOVA) that was conducted to investigate the impact of buprofezin on the functioning of hepatic catalase (CAT) in Cypinus carpio yielded an F-value of 2.38. This value is higher than the critical F-value of 0.05, which indicates that exposure to buprofezin has a significant impact on the hepatic CAT activity (Choudhary et al., 2019).

The comparison of means demonstrates that there is a distinct concentration-dependent increase in the CAT activity with increment in the dose of buprofezin: the group control showed the lowest mean CAT activity (1156.65 0.943 UmL^{-1}), which was significantly lower than the activity in the exposed group, and Treatment T1 (5mg L^{-1}) and Treatment T2 (10mg L^{-1}) showed a treatment response that was moderate and high, respectively.

These data, when taken together, suggest that buprofezin causes oxidative stress in Cypinus carpio. This is shown by the up-regulation of CAT-, which is an essential enzymatic defense mechanism that is responsible for the breakdown of hydrogen peroxide. A dose-responsive change in hepatic antioxidant defenses is highlighted as a result of the gradual rise in dosage, which demonstrates an adaptive response to overcome the toxicity caused by the pesticide.

Table 4.3: Means Comparison of Buprofezin Levels on the Liver of Cyprinus carpio

Treatment	Mean Comparison
Control	1156.65±0.943C

T1 (5 mg/L)	1323.1±0.752B
T2 (10 mg/L)	1359.41±0.292A

Table 4.3 shows means comparison results that sum up the action of different levels of buprofezin on the liver of the Cypinus carpio.

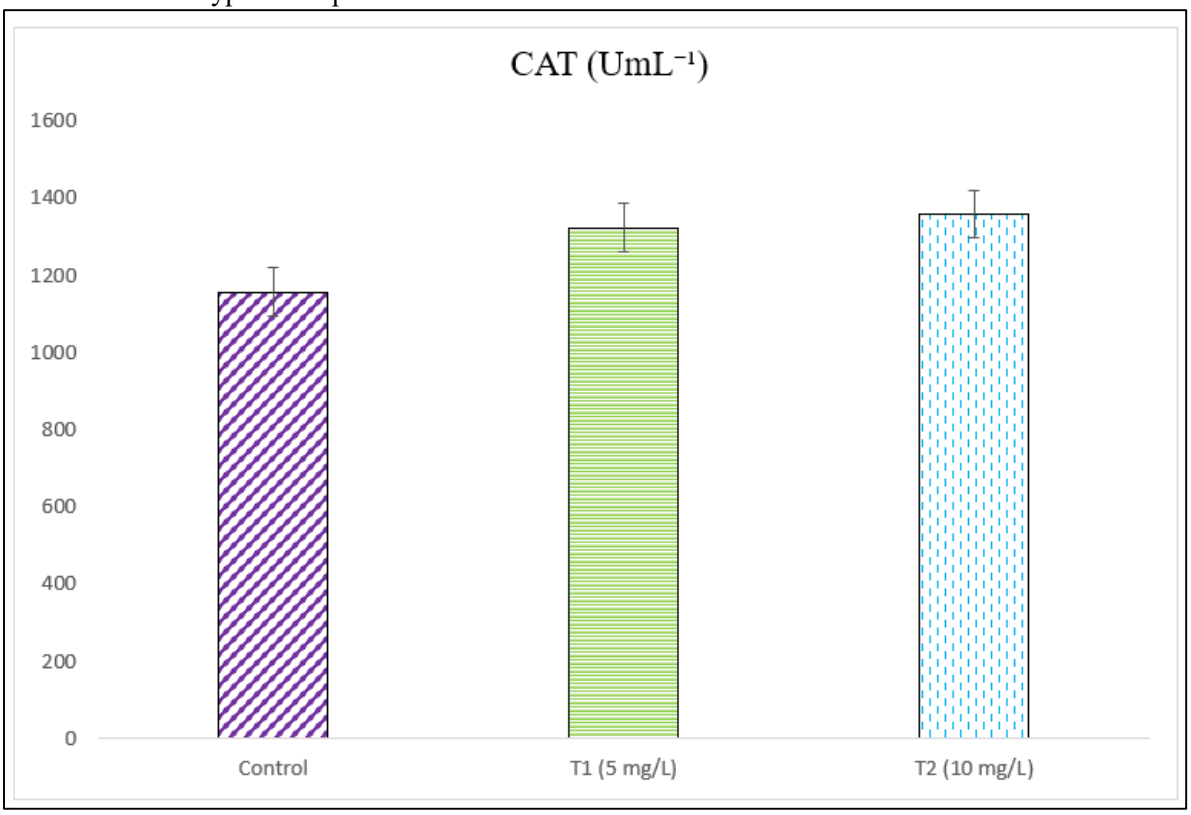

Figure 4.2: Catalase (CAT) Activity in Cyprinus carpio Under Different Conditions

Figure 4.2 is a comparison of activity of catalase (CAT) of the Cypinus carpio under various experimental conditions. According to the figure, treatment T0 had the most active CAT activity, and treatment T3 had the lowest levels of the activity in the laboratory.

Table 4.4: Impact of Buprofezin Levels on Hepatic SOD Activity in Cyprinus carpio

Time (hrs)	Control	T1(5mg/L	T2(10mg/L)
0	46.45	46.95	46.95
24	46.45	88.96	90.81
48	46.43	101.37	110.75
72	46.67	118.67	125.93
96	46.99	132.12	145.87
Mean±SD	234.75±0.853C	488.07±0.984B	520.31±0.843A

Table 4.4 presents a hepatic superoxide dismutase (SOD) activity on fish subjected to three nominal concentrations of 0 mg/L (control), 5mg/L and 10mg /L at various intervals. Each group started at the same initial activity of 46.95UmL. This was followed by statistically significant time-dependent enhancement in SOD activity of both treatment groups (T1 and T2) over that of the control with the highest increase at 96 hrs. The implication of this observation is that the treatment provokes the upregulation of the antioxidant enzyme system.

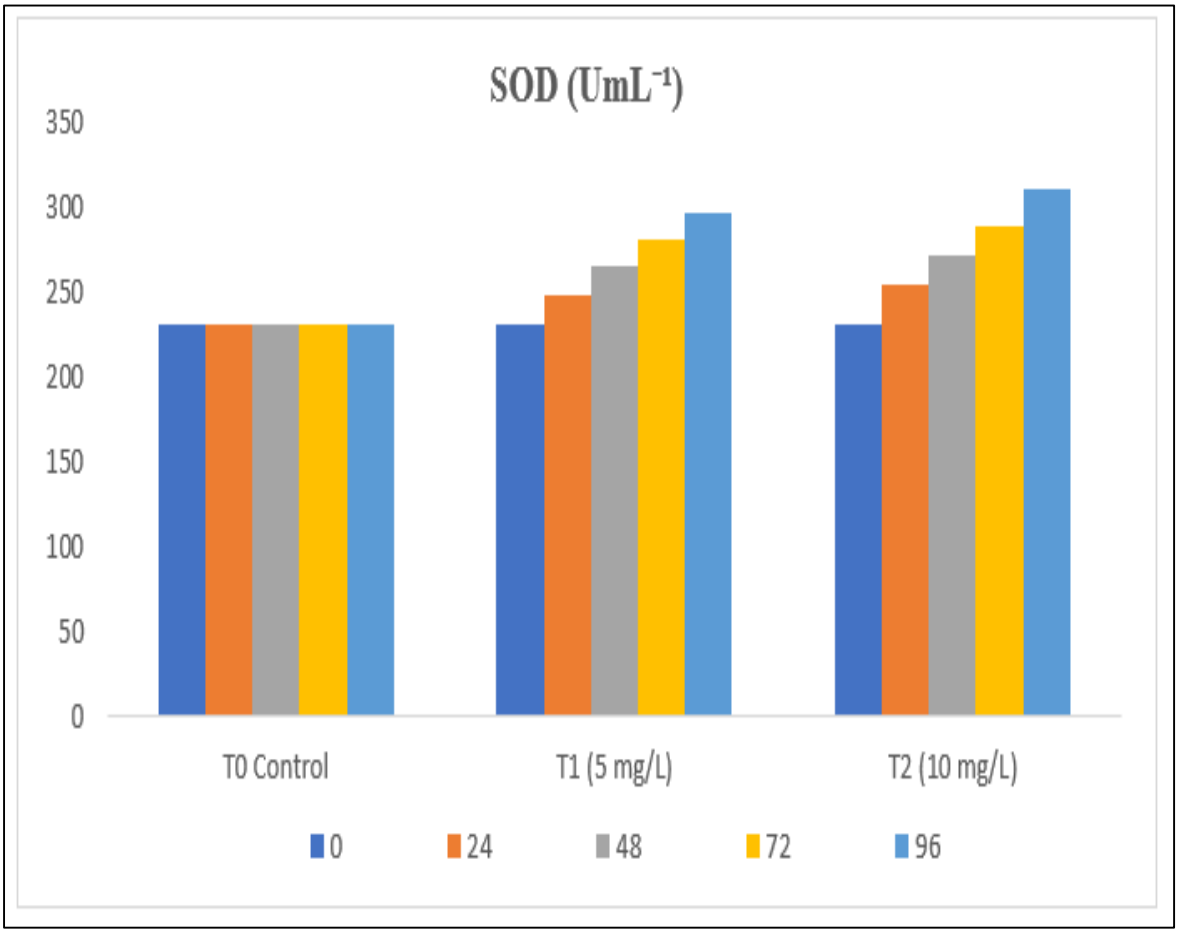

Figure 4.3: Efficiency of Buprofezin Concentrations on Superoxide Dismutase (SOD) Activity in Cyprinus carpio

Figure 4.3 is a diagram that illustrates the efficiency of various concentrations of buprofezin in the presence of superoxide dismutase (SOD, UmL^{-1}) in the tissue of Cyprinus carpio.

The analysis of variance (ANOVA) was used to determine the effects of different concentrations of buprofezin on the selective oxygen deprivation (SOD) activity of the liver of the fish species Cyprinus carpio. The results are shown in Table 4.5.

Table 4.5: ANOVA Showing Effects of Buprofezin Concentrations on Hepatic SOD Activity in Cyprinus carpio

S.O.V	DF	SS	MS	F	P-value
Treatment	2	138.7	69.350	13.69	0.011
Error	12	60.8	5.067		
Total	14	199.5			

Table 4.5 shows ANOVA studying the effects of buprofezin on hepatic activity of superoxide dismutase (SOD), in Cypinus carpio, indicated that the effects of the treatment were highly significant (P = 0.011), the F value (13.69) was greater than the critical value (a = 0.05), which conclusively demonstrates that the effect of treatment on the activity of SOD was significant.

Sharp, concentration-dependent increments of the SOD activity were observed with the rising doses of buprofezin: this highest value, found in Treatment T2 (10 mg^{-1} L), was significantly higher than the one in the control group (234.75 + -0.853 UmL^{-1}) but much lower than that of Treatment T1 (5 im ego L).

Combined these results show that buprofezin causes oxidative stress in Cyprinus carpio as exhibited by a rise in SOD activity an important antioxidant enzyme that catalyses dismutation of superoxide radicals (Das & Mahapatra, 2018). The dose-dependent increase in SOD is a reflection of physiological adaptive process to counteract pesticide-induced reactive oxygen species with increasing

levels of the pesticide triggering an increasing level of oxidative stress and a correspondingly greater defensive reaction.

Table 4.6: Mean Comparison of Hepatic SOD in Cyprinus carpio at Different Buprofezin Levels

Treatment	Mean Comparison
Control	234.75±0.853C
T1(5 mg/L)	488.07±0.984B
T2(10 mg/L)	520.31±0.843A

Table 4.6 presents the mean comparison of hepatic Superoxide Dismutase (SOD) activity in Cyprinus carpio exposed to different concentrations of buprofezin. A significant elevation in SOD activity was observed in all treated groups when compared with the control, indicating an oxidative stress response induced by pesticide exposure. The control group exhibited the lowest SOD activity (234.75 ± 0.853 UmL^{-1}), whereas fish exposed to 5 mg L^{-1} (T1) showed a marked increase (488.07 ± 0.984 UmL^{-1}). The highest SOD activity was recorded in the T2 group (10 mg L^{-1}), reaching 520.31 ± 0.843 UmL^{-1}, demonstrating a clear dose-dependent response.

The statistical grouping indicated by different superscript letters (A, B, C) confirms that the differences among treatments were significant ($P < 0.05$). The progressive increase in hepatic SOD activity suggests enhanced production of superoxide radicals as a consequence of buprofezin exposure, triggering the upregulation of antioxidant defense mechanisms. This elevation reflects an adaptive biochemical response aimed at neutralizing reactive oxygen species (ROS) and protecting hepatic tissue from oxidative damage. However, sustained or higher exposure levels may overwhelm this defense system, potentially leading to oxidative injury and impaired liver function (Monteiro et al., 2010).

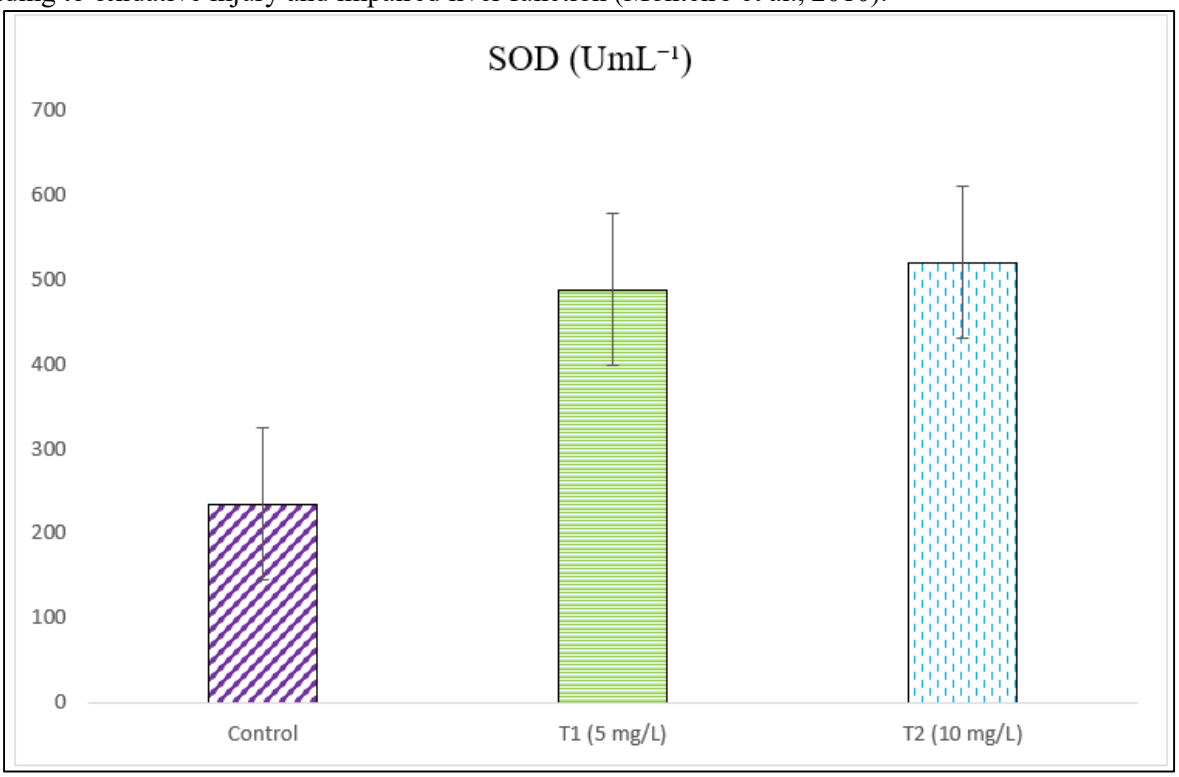

Figure 4.4: SOD Activity in Cyprinus carpio Under Different Experimental Treatments

Figure 4.4 shows the relative activity of superoxide dismutase (SOD) owing to the expression in micromoles per litre (UmL^{-1}) in Cypinus carpio at various experimental treatments. The results indicate high concentrations of SOD (UmL^{-1}) of treatment T0, and the activities of catalase (CAT) are significantly lower in treatment T3 in case of Cypinus carpino.

Table 4.7: Effect of Buprofezin Concentrations on Hepatic GST Activity in Cyprinus carpio

Time(hrs)	Control	T1(5mg/L)	T2(10mg/L)
0	175.66	178.71	180.83
24	175.63	210.85	214.31

48	175.65	245.65	253.11
72	175.70	335.10	348.19
96	175.75	390.56	415.78
Mean±SD	877.55±0.892C	1357.87±0.042B	1407.1±0.954A

Table 4.7 shows the effect difference in concentration of buprofezin on catalyst activity of hepatic glutathione-S-transferase (GST) in expressed UmL^{-1} in Cyprinus carpio. A summary of hepatic glutathione S-2 transferase (GST) activity in fish exposed to three exposure levels, that is, 0 mg L 1 (control), 5 mg L 1 and 10mg L 1, at various times to a maximum of 96 hours is given in Table 4.9. The GST values of the baseline were similar in all the groups. Nevertheless, statistically significant changes in concentration dependent increases in GST activity were observed in the treated populations (T1 and T2) as the exposure time went up, the significant increases being recorded at the 96-hour time point. This tendency demonstrates that, in response to the increased concentration of the test compound, the GST activity can be induced, and this tendency signifies increased detox reaction with time.

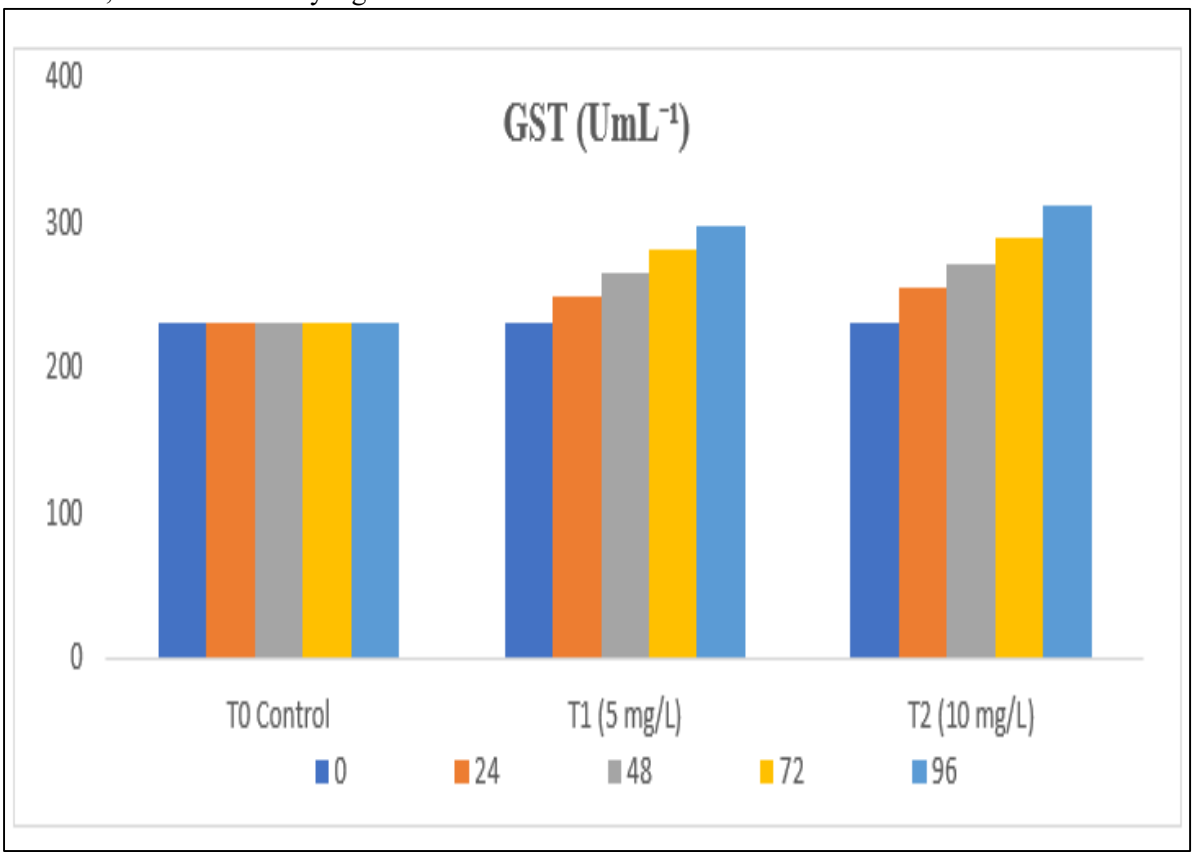

Table 4.8: Effect of Buprofezin Doses on Glutathione-S-Transferase (GST) Activity in Cyprinus carpio

Figure 4.8 illustrates the effect that varying doses of buprofezin have on the activity of glutathione-S-transferase (GST) (units per milliliter) in Cypinus carpio under the conditions described.

Table 4.9: ANOVA of Hepatic GST Activity in Cyprinus carpio at Different Buprofezin Concentrations

S.O.V	DF	SS	MS	F	P-value
Treatment	2	149.79	74.895	12.53	0.021
Error	12	71.7	5.975		
Total	14	221.49			

The results of the analysis of variance for the GST activity in the liver of Cypinus carpio at various concentrations are shown in Table 4.9.

The results of an analysis of variance (ANOVA) that was performed in order to assess the effect that buprofezin has on the glutathione-S-transferase (GST) activity in the liver of Cypinus carpio shown that the differences between the treatments are very significant (P = 0.021). Having an F-value of 12.53 indicates that the exposure to buprofezin has a considerable impact on the activity of GST, and this influence is in the form of a dosage effect (Pandey & Sharma, 2023).

The mean demonstrates that the increased concentration of buprofezin results in a significant increase in the percentage of GST activity. The GST activity was shown to be substantially different between the treatment groups and the control group, with the control group exhibiting the lowest activity (878.55 0.892 3). The activity of GST was significantly increased in both treatment T1 (5mg/L^{-1}) and treatment T2 (10mg/L^{-1}) respectively, with the former showing a 1357.87 btn -0.042 UmLand the latter showing a 1407.1 btn -0.954 UmLeffect.

In the light of the evidence provided it could be concluded that the exposure to the buprofezin leads to considerable changes in the activity of the glutathione S general transferase (GST) enzyme, which is part of the process of the detoxification process. Cypinus carpi proliferation shows an evolutionary response aimed at neutralizing the effects which the pesticide causes which are oxidative (Patil et al., 2016). This is supported by the fact that there is an upsurge in GST activity and this is coincidental with the increase in the concentrations of buprofezin. The increased enzyme activity is most likely to point to the attempts of the fish to counter the action of toxic substances and to decrease the oxidative stress, thus underlining the critical role of the GST in the process of detoxification in pesticide exposure and under physiological stress. Table 4.10 presents the mean impact of the various doses of buprofezin on the liver of Cypinus carpio.

Table 4.10: Mean Effects of Buprofezin Doses on the Liver of Cyprinus carpio

Treatment	Mean of comparison
T0	878.55±0.892C
T1	1357.87±0.042B
T2	1407.1±0.954A

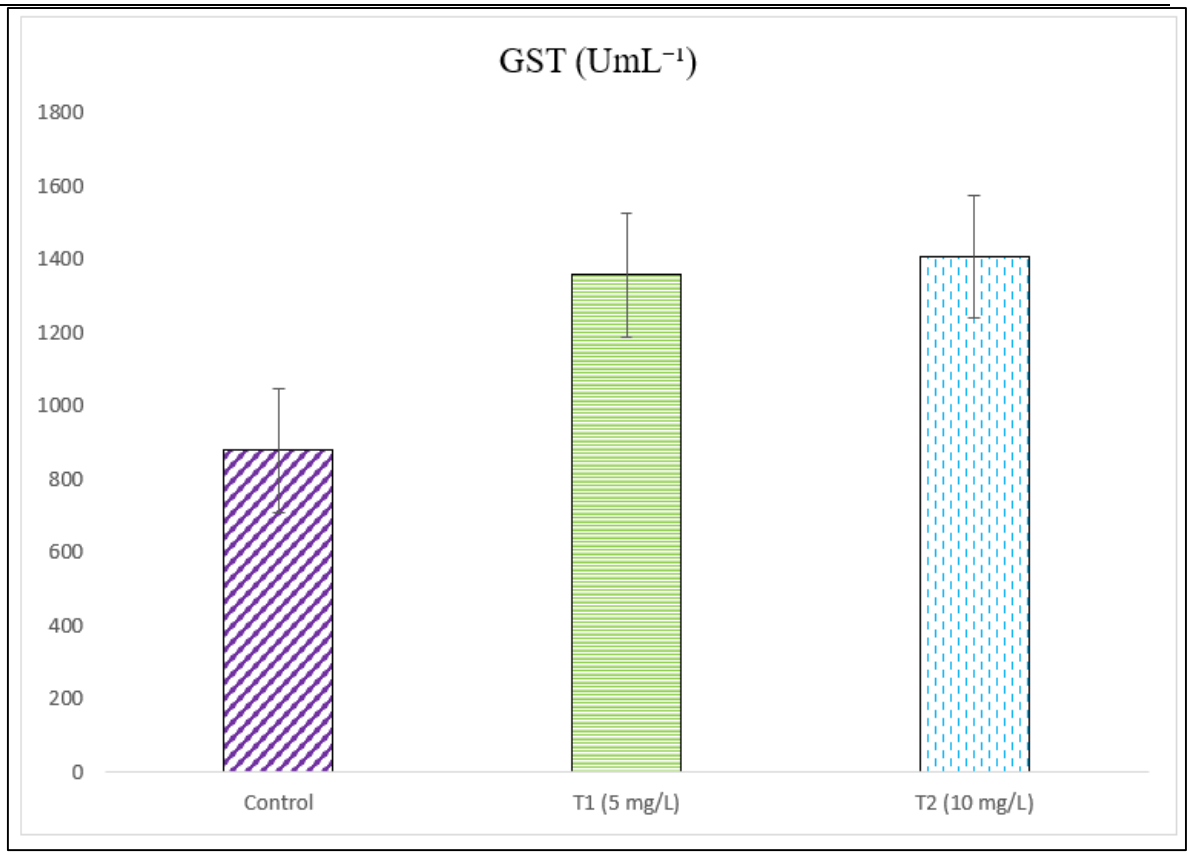

Figure 4.5: GST Activity in Cyprinus carpio Under Different Treatments

Figure 4.5 shows comparative analysis Of the glutathione-S-transferase (GST) activity (L^{-1}) in Cypinus carpio under various treatment regimes. The figure also shows that, treatment T0 gave the most GST activity and the least catalase (CAT) activity was realized in treatment T3 in the case of Cypinus carpio.

Table 4.11 explains the average values of the activities of hepatic enzymes and the impact of different concentrations of buprofezin on the liver health in Cyprinus carpio.

Table 4.11: Average Hepatic Enzyme Activities and Effects of Buprofezin on Liver Health in Cyprinus carpio

Enzyme	Control (0 mg/L)	T1(5 mg/L)	T2(10 mg/L)
CAT (UmL)	1156.65±0.943	1323.1±0.753	520.31±0.843
SOD (UmL)	234.75±0.853	488.07±0.984	520.31±0.843
GST (UmL)	878.55±0.892	1357.87±0.042	1407.1±0.954

Table 4.11 shows the changes in hepatic enzymatic activities (catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST)) of Cyprinus carpio in control level with a concentration of 0 mg/L and exposure levels with 5 mg/L and 10 mg/L of buprofezin. The data indicates a progressive increase of these antioxidant enzymes in line with the increase in toxin levels and time exposure thus demonstrating that the oscillation of oxidative stress and the complementary activation of detoxication systems are dose and time dependent. Such increased levels of enzyme activities highlight hepatic stress and confirm the increased susceptibility of aquatic life under chronic exposure of pesticides (Rao et al., 2014).

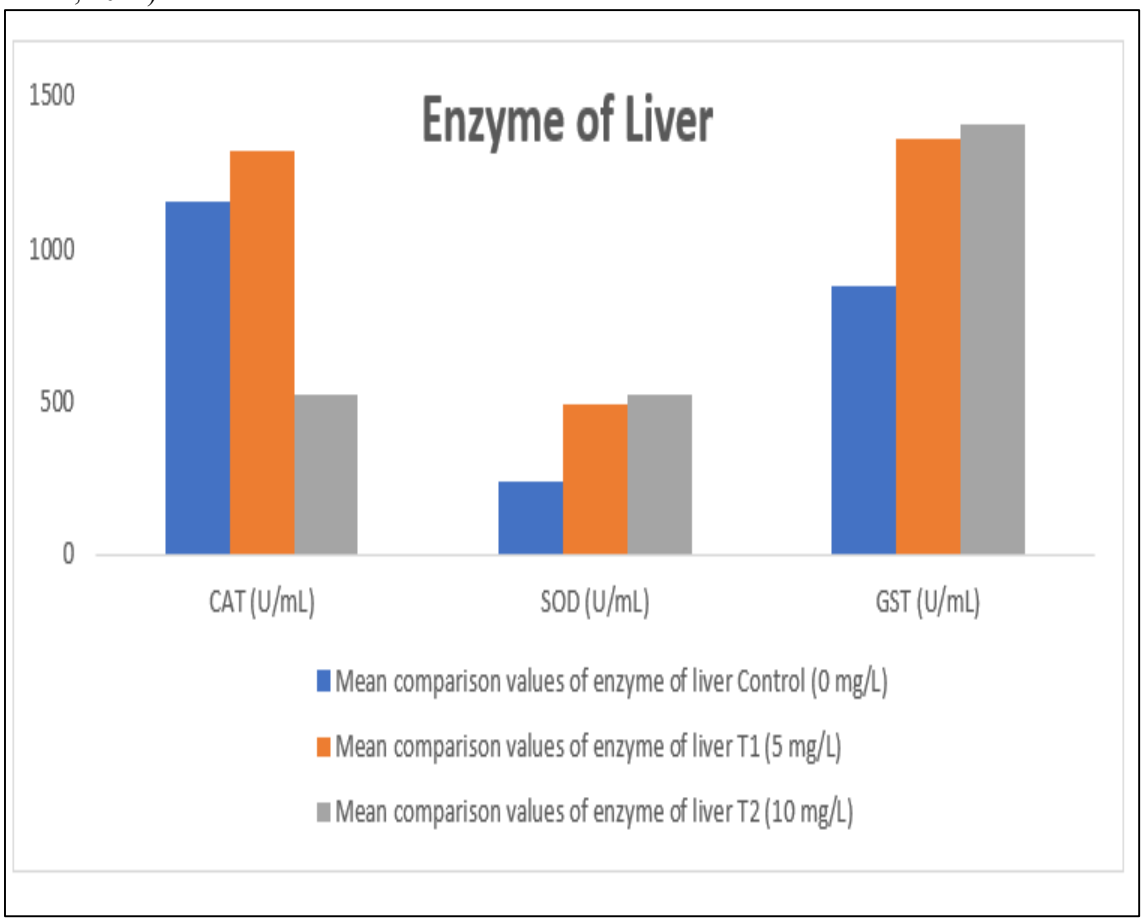

Figure 4.6: Comparison of Enzymatic Activity in Liver Tissue of Cyprinus carpio

Figure 4.6 shows the graphical illustration indicates that the enzymatic activity in the liver tissue of Cypinus carpio is compared.

Table 4.12: Effect of Buprofezin on Renal Catalase Activity in Cyprinus carpio at Different Concentrations

Time(hrs)	Control	T1(5 mg/L)	T2(10 mg/L)
0	140.33	149.33	154.33
24	140.35	163.35	170.37
48	140.44	178.11	190.45
72	140.46	188.57	210.55
96	140.52	197.31	232.71
Mean±SD	701.65±0.742C	867.67±0.045B	944.41±0.053A

Table 4.12 gives the activity of renal catalase (CAT) with three concentrations (0mg L^{-1} (control), 5mg L^{-1}, 10mg L^{-1}) at a given set of time points. The initial activity of the experiment as assessed by all groups of experimenters was similar but the treated group (T1 and T2) saw a gradual increase in the activity of CAT with a peak at the end of 96 hours. These results confirm time dependency and dose dependency increase of CAT activity that can be ascribed to administered treatment.

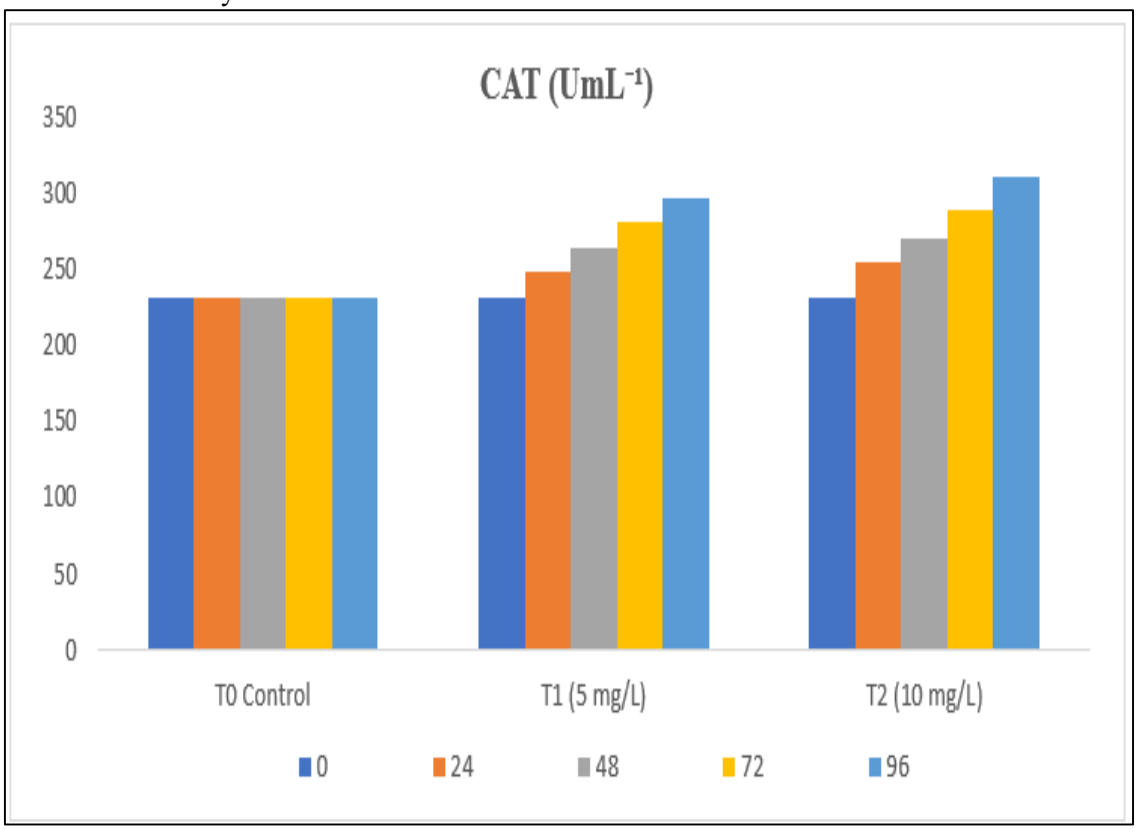

Figure 4.7: Effects of Buprofezin Doses on Catalase Activity in Cyprinus carpio

Figure 4.7 is a diagrammatic index that illustrates the observation of the effects of different doses of buprofezin on the catalase activity (units per milliliter) in Cypinus carpio.

Table 4.13: Statistical Analysis of Renal Catalase Activity in Cyprinus carpio at Different Buprofezin Concentrations

S.O.V	DF	SS	MS	F	P-value
Treatment	2	83.9	41.950	13.01	0.017
Error	12	38.7	3.225		
Total	14	122.6			

A statistical analysis of the differences in the catalase activity of the kidneys when exposed to varying concentrations of Cypinus carpio is shown in Table 4.13.

Table 4.14: ANOVA Summary Showing Treatment Effects on Catalase Activity

S.O.V	DF	SS	MS	F	P-value
Treatment	2	83.9	41.950	13.01	0.017
Error	12	38.7	3.225		
Total	14	122.6			

Table 4.14 shows Degrees of Freedom (DF), Sum of Squares (SS), Mean Squares (MS), F-Value, and P-Value. The results of an analysis of variance (ANOVA) that was performed to examine the impact of exposure to buprofezin on the activity of catalase (CAT) in the kidneys of Cyprinus carpio revealed that there were extremely significant differences between the groups (P = 0.017). A statistically significant impact of buprofezin on the activity of CAT is shown by the F-value of 13.01, which verifies this finding.

As the concentration of buprofezin increases, there is a clear dose-dependent improvement in CAT activity, as shown by the comparison of the mean, which demonstrates that there is a consistent improvement (Rani & Singh, 2021). In comparison to the treatment groups, the CAT activity of the control

group was substantially lower (701.65 - 0.742^{-1} UmL), making it the group with the lowest CAT activity among the groups. The CAT activity was shown to increase with treatment T1 (5mg/L^{-1}) (867.67 ± 0.045UmL), whereas treatment T2 (10mg/L^{-1}) exhibited the greatest level of activity (944.41 ± 0.053UmL).

It has been shown that exposure to buprofezin generates oxidative stress in the kidney of Cyprinus carpio, as evidenced by the elevated activity of CAT (Rani et al., 2021). This data is consistent with the results that have been presented. Catalase, an essential antioxidant enzyme, plays a crucial role in the biochemical process of reducing hydrogen peroxide to oxygen and water, therefore protecting the components of the cell from the damaging effects of oxidative stress. As a result of the dose-related increase in CAT activity, it is shown that the fish have adapted to the toxic medicine in order to protect themselves from increasing levels of oxidative stress brought on by the pesticide. This demonstrates that the true toxicity of buprofezin on the antioxidant defenses in the kidney is clearly demonstrated.

Table 4.15: Comparison of Mean Effects of Buprofezin Concentrations on the Liver of Cyprinus carpio

Treatment	Mean of comparison
T0	701.65 ±0.742C
T1	867.67±0.045B
T2	944.41±0.053A

Comparison data of the mean effects of different concentrations of buprofezin on the liver of Cyprinus carpio are shown in Table 4.15.

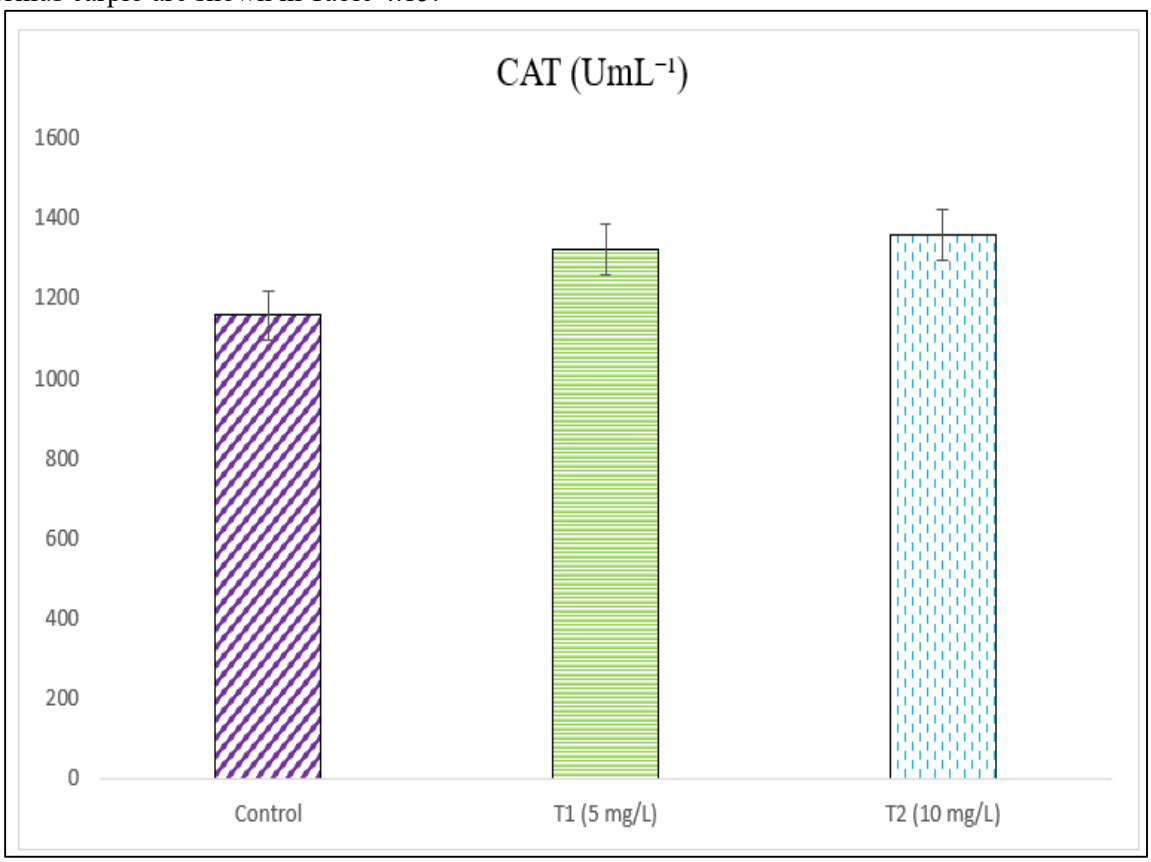

Figure 4.8: CAT Activity in Cyprinus carpio Across Different Treatment Groups

Figure 4.8 displays peak CAT values in the T0 cohort, while T3 exhibits the minimal CAT activity in Cyprinus carpio, underscoring divergent biomarker shifts across treatments.

Table 4.16: Effect of Buprofezin Concentrations on Renal SOD Activity in Cyprinus carpio

Time (hrs)	Control	T1 (5 mg/L)	T2 (10 mg/L)
0	26.77	29.87	33.66
24	26.66	53.81	58.41
48	26.54	77.39	79.75
72	26.85	102.41	99.93

96	26.91	131.12	138.87
Mean±SD	133.85±0.482C	391.5±0.743B	403.73±0.742A

Table 4.16 shows comparative summary of the temporal response to three exposure levels control (0 mg/L), 5 mg/L, and 10 mg/L showing that groups began with equivalent baseline values, after which the treated cohorts (T1 and T2) exhibited steadily rising levels relative to the control, culminating in the highest measurements at 96 hours, this trajectory indicates a clear dose- and time-dependent augmentation attributable to the treatment.

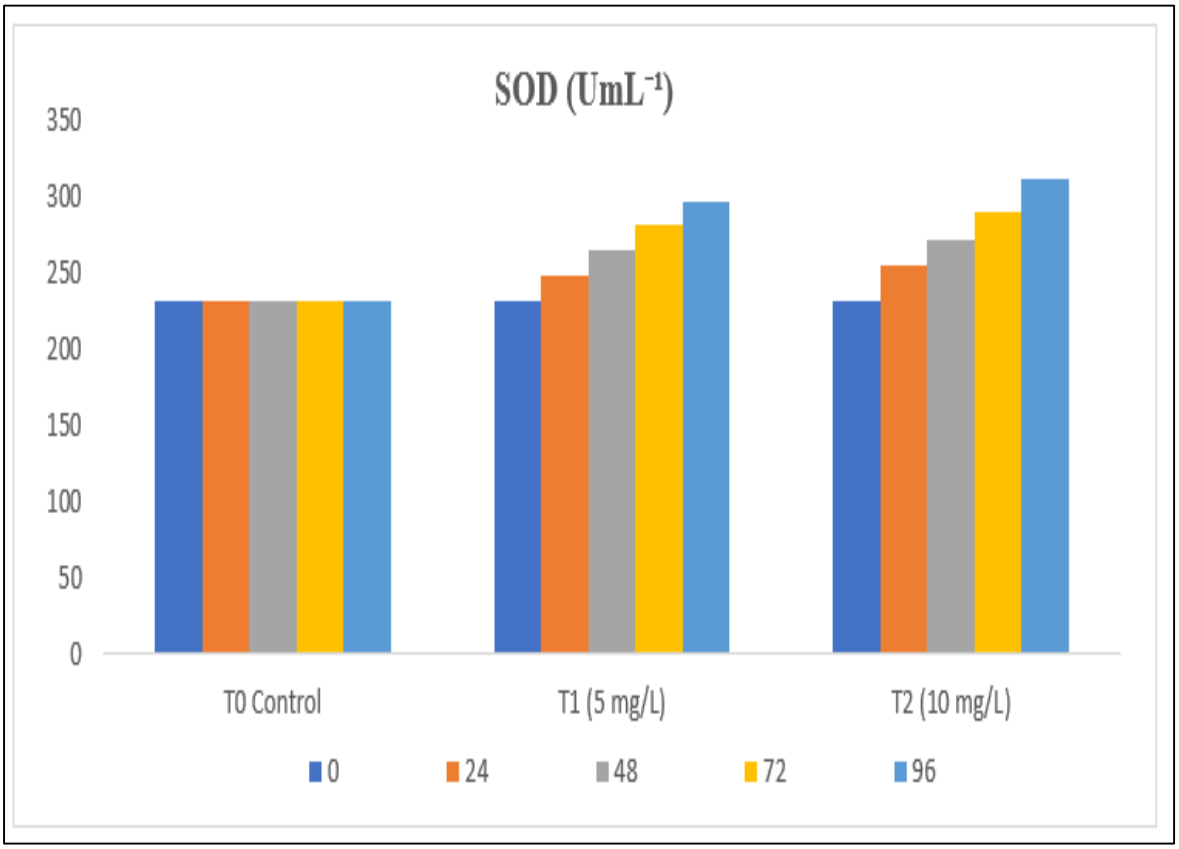

Figure 4.9: Effect of Increasing Buprofezin Concentrations on Superoxide Dismutase (SOD) Activity in Cyprinus carpio

Figure 4.9 shows graphical depiction showing how escalating buprofezin concentrations modulate superoxide dismutase (SOD) activity (UmL^{-1}) in Cypinus carpio, highlighting dose response patterns across control and exposed groups.

Table 4.17: One-Way ANOVA of Renal SOD Activity in Cyprinus carpio at Different Buprofezin Concentrations

S.O.V	DF	SS	MS	F	P-value
Treatment	2	127.8	63.900	13.29	0.018
Error	12	57.7	4.808		
Total	14	185.5			

Table 4.17 shows an analysis of variance (ANOVA) was conducted to determine the impact of Buprofezin on the activity of superoxide dismutase (SOD) in the kidneys of Cypinus carpio. The results showed that there were highly significant differences between the groups ($P = 0.018$), and the F-value was 13.29. This conclusively demonstrated that exposure to Buprofezin has a statistically significant impact on the dynamics of SOD.

The mean comparisons showed that there was a significant increase in SOD activity as the concentration of Buprofezin increased. The control group exhibited the lowest activity (133.85 ± 0.492 UmL^{-1}), which was significantly lower than both exposure cohorts. Treatment T1 (5 mg/L) demonstrated a significant increase (391.5 ± 0.743 UmL^{-1}), while Treatment T2 (10 mg/L) registered the highest response (403.73 ± 0.742 UmL^{-1}).

When all of these findings are considered together, they suggest that exposure to buprofezin causes an increase in oxidative pressure in the kidney of Cypinus carpio, which is represented by an increase in SOD activity (Rao & Ghosh, 2020). In its role as a leading antioxidant enzyme, superoxide dismutase is responsible for the elimination of superoxide radicals and the protection of cellular structures from oxidative damage. The dose-dependent increase in superoxide dismutase (SOD) that was found is thus indicative of an adaptive mobilization of redox defenses to combat pesticide-induced stress. This highlights the disruptive influence that buprofezin has on renal oxidative equilibrium and antioxidant protection mechanisms during the treatment of the pesticide.

Table 4.18: Pairwise Comparison of Buprofezin Effects on Renal Activity in Cyprinus carpio

Treatment	Mean of comparison
T0	133.85±0.492C
T1	391.5±0.743B
T2	403.73±0.742A

The pairwise mean comparison data that illustrate the effects of increasing Buprofezin concentrations on the kidney of laboratory Cypinus carpio are shown in Table 4.18.

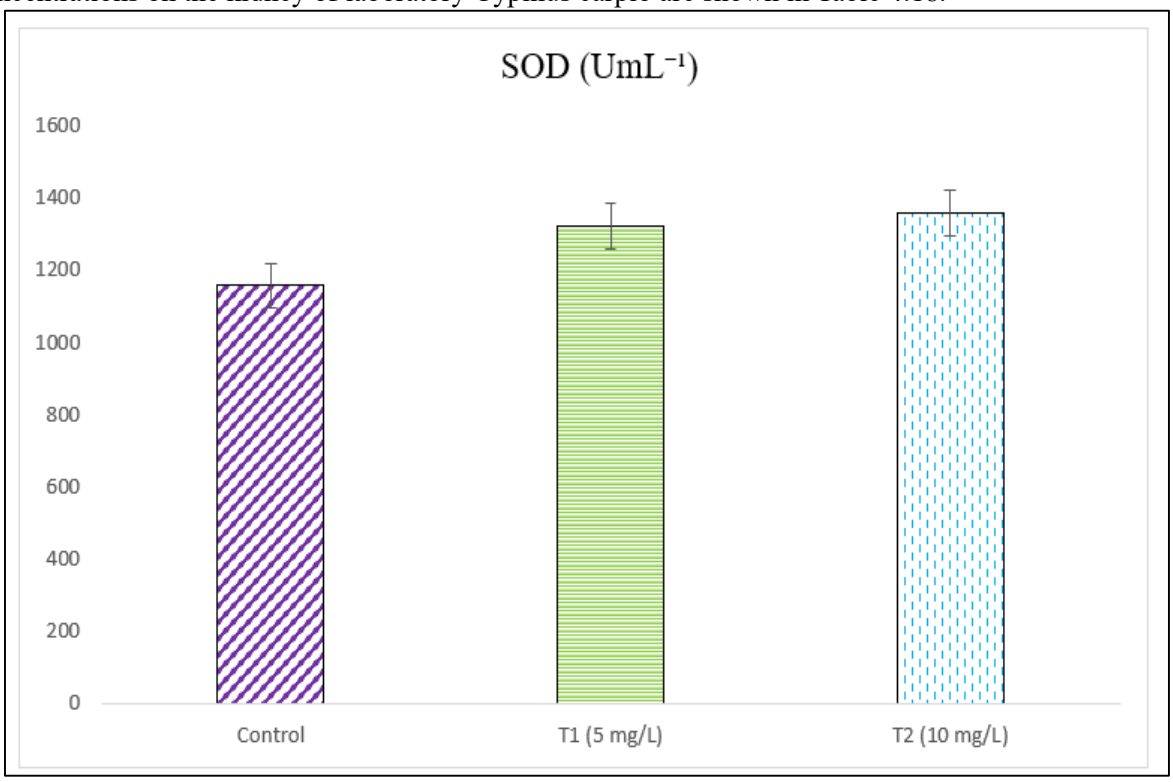

Figure 4.10: Comparative SOD Activity in Cyprinus carpio Across Different Treatments

Figure 4.10 shows comparative profile of superoxide dismutase (SOD) activity (UmL^{-1}) in Cyprinus carpio across the different treatment regimens, enabling side-by-side evaluation of responses under varying exposure levels.It Depicts peak SOD (UmL^{-1}) values in the T0 group, whereas T3 exhibits the lowest catalase (CAT) levels in Cyprinus carpio, highlighting divergent enzymatic trends among treatments.

Table 4.19: Comparative Effect of Buprofezin Concentrations on Renal Glutathione-S-Transferase (GST) Activity in Cyprinus carpio

Time (hrs)	Control	T1 (5mg/L)	T2(10mg/L)
0	151.08	161.21	154.21
24	151.33	244.75	260.31
48	151.11	287.13	294.11
72	151.15	350.32	387.19
96	151.22	398.44	415.78
Mean±SD	756.05±0.853C	1431.85±0.643B	1508.6±0.322A

Table 4.19 shows Comparative analysis of the influence of graded buprofezin concentrations on renal glutathione-S-transferase (GST) activity (UmL^{-1}) in Cyprinus carpio, summarizing dose-responsive enzymatic shifts across the experimental treatments. It shows how different levels of exposure (control 0 mg/L, 5 mg/L, and 10 mg/L) affected the activity of the enzyme glutathione-S-transferase (GST) in fish's kidneys over the course of the sampling period. It shows that all groups started with similar GST levels, but enzyme activity rose significantly in the treated groups (T1 and T2) compared to the control, most notably at 96 hours, showing a clear dose- and time-dependent response.

Overall, the sustained elevation of renal GST activity with increasing buprofezin concentration highlights the kidney's pivotal role in xenobiotic detoxification and antioxidant defense. The pronounced increase in the T2 group indicates intensified conjugation and elimination processes in response to higher toxic stress. This dose- and time-dependent enzymatic upregulation reflects an adaptive biochemical mechanism aimed at mitigating pesticide-induced oxidative damage (Saini & Kumar, 2020). However, prolonged stimulation of GST activity may signal metabolic overload and reduced detoxification efficiency. These findings emphasize the potential risk of chronic buprofezin exposure to renal health in aquatic organisms.

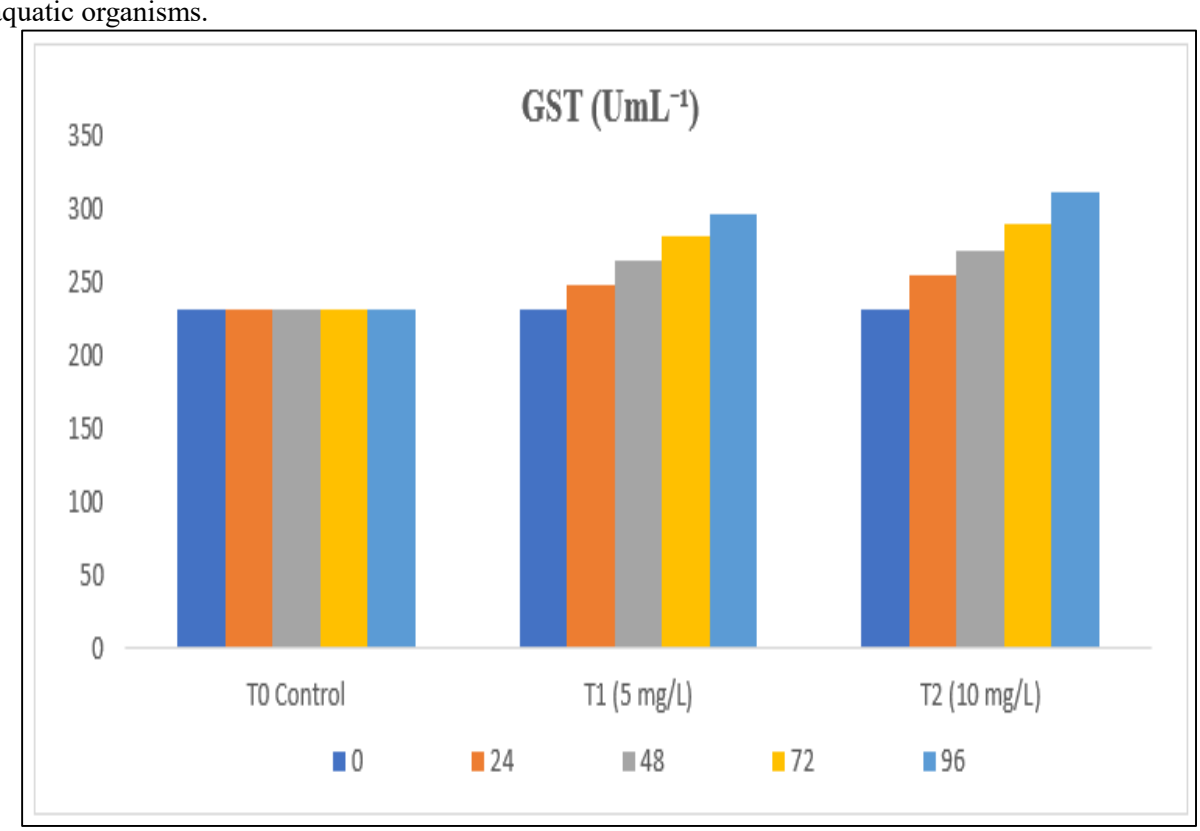

Figure 4.11: Effect of Buprofezin Concentrations on Glutathione-S-Transferase (GST) Activity in Cyprinus carpio

Figure 4.11 shows graphical depiction illustrating how varying concentrations of buprofezin affect glutathione-S-transferase (GST) activity (UmL^{-1}) in Cypinus carpio, enabling a clear visualization of dose-responsive shifts across control and treated groups.

Table 4.20: ANOVA of Renal GST Activity in Cyprinus carpio at Different Buprofezin Concentrations

S.O.V	DF	SS	MS	F	P-value
Treatment	2	82.94	41.470	10.33	0.022
Error	12	48.16	4.013		
Total	14	131.1			

Table 4.20 shows ANOVA summary evaluating renal glutathione-S-transferase (GST) activity in Cyprinus carpio across differing exposure concentrations, comparing treatment groups to quantify variance in enzymatic response. An ANOVA test that looked at how buprofezin affected kidney glutathione-S-transferase (GST) activity in Cyprinus carpio found very strong treatment effects ($P = 0.022$), the F-statistic of 10.33 shows that buprofezin had a big effect on GST modulation.

Average comparisons show that GST activity goes up as buprofezin concentration goes up: the control had the lowest value (756.05 ± 0.853 UmL^{-2}), which was much lower than the treated groups, Treatment T1 (5 mg/L) had a big increase (1431.85 ± 0.643 UmL^{-2}), and Treatment T2 (10 mg/L) had the highest activity (1508.6 ± 0.322 UmL^{-2}).

Collectively, these outcomes indicate that buprofezin exposure drives significant shifts in GST an enzyme central to detoxification and oxidative-stress mitigation. The graded increase reflects an adaptive upregulation of detoxification pathways in Cyprinus carpio, while also highlighting potential adverse consequences for renal function and the broader antioxidant defense apparatus under sustained pesticide challenge (Rao & Ghosh, 2020).

Table 4.21: Mean Effects of Buprofezin Concentrations on Renal Activity in Cyprinus carpio

Treatment	Mean of comparison
T0	756.05±0.853C
T1	1431.85±0.643B
T2	1508.6±0.322A

Compare the average effects of various Buprofezin amounts on the kidney of the Cyprinus carpio fish in Table 4.21.

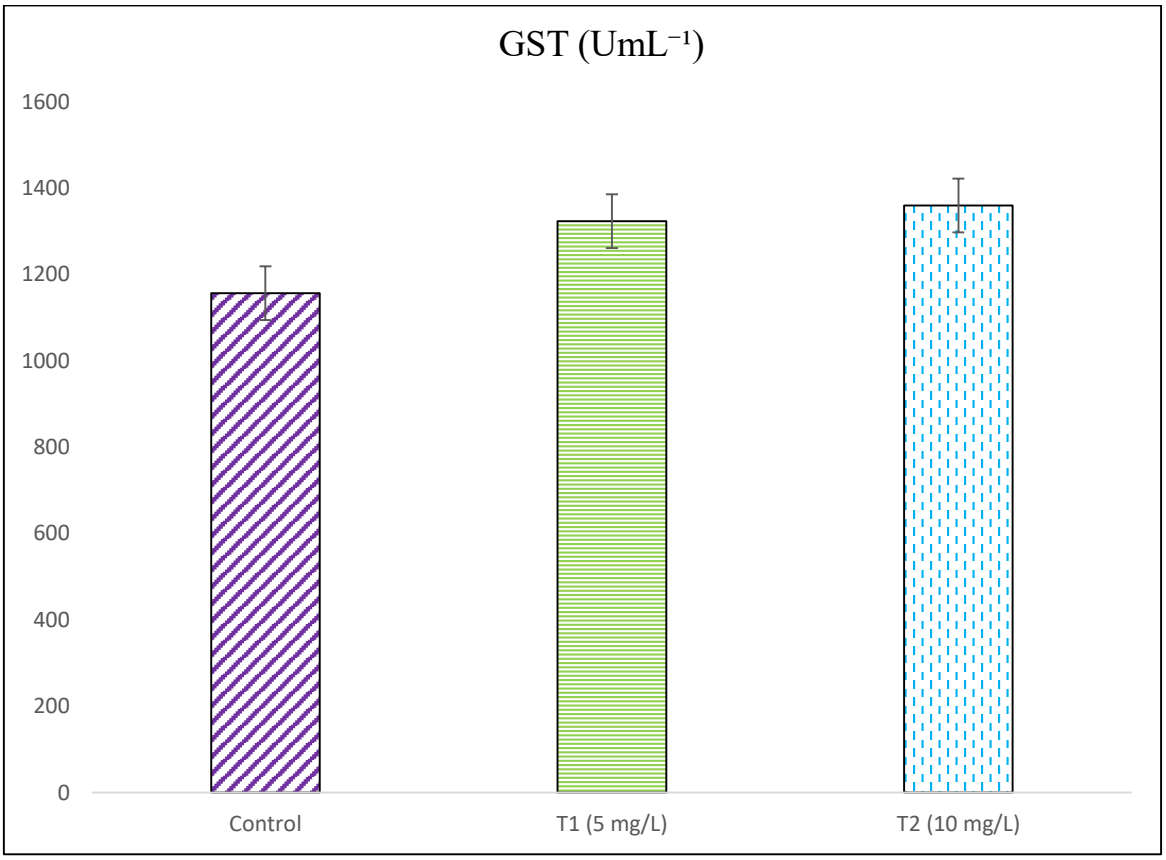

Figure 4.12: Comparative Glutathione-S-Transferase (GST) Activity in Cyprinus carpio Across Different Treatment Groups

Figur 4.12 Illustrates the highest GST (UmL^{-1}) values in T0, while T3 exhibits the lowest CAT levels in laboratory Cyprinus carpio, emphasizing the divergent biomarker shifts across treatments.

Table 4. 22: Comparative Assessment of Enzyme Activities in the Kidney of Cyprinus carpio

Enzyme	Control (0 mg/L)	T1 (5 mg/L)	T2 (10 mg/L)
CAT (UmL)	701.65±0.742	867.67±0.045	944.41±0.053
SOD (UmL)	133.85±0.492	391.5±0.743	403.73±0.742
GST (UmL)	756.05±0.853	1431.85±0.643	1508.6±0.322

Table 4.22 depicts a comparative profile of renal enzyme activities CAT, SOD, and GST in Cyprinus carpio subjected to buprofezin at Control (0 mg/L), 5 mg/L, and 10 mg/L, showing a graded, exposure-dependent elevation across treatments and further amplification with prolonged duration, this

trajectory is consistent with rising oxidative pressure and engagement of antioxidant and phase-II detoxification pathways, and it underscores the likelihood of adverse physiological consequences for fish health under sustained pesticide exposure.

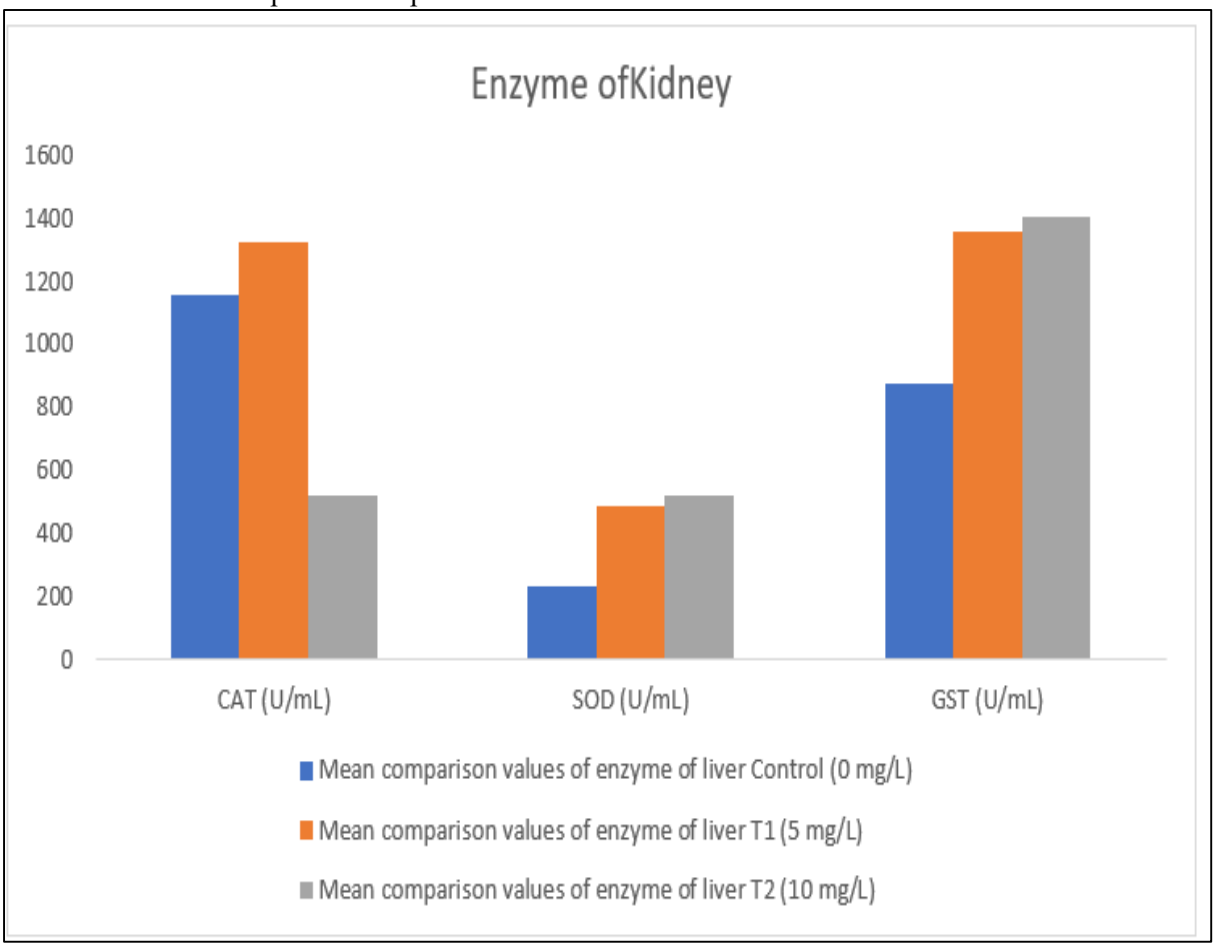

Figure 4.13: Comparative Renal Enzyme Activities (CAT, SOD, GST) in Cyprinus carpio Across Control and Buprofezin-Treated Groups

Figure 4.13 shows comparative visualization of renal enzyme activities in laboratory Cyprinus carpio, illustrating relative profiles (e.g., CAT, SOD, GST) across control and buprofezin-treated groups to highlight dose- and time-dependent trends.

Discussion

Pesticide toxicity in aquatic ecosystems should be assessed as there is a strong used of agrochemicals and they showed their harmful impact on non-target species especially fish. Buprofezin is an example of insect growth regulators that have been implemented widely in agriculture, it is known to be highly effective against pests of agricultural and food interest, it is persistent and toxic in nature which, together with its persistence, has raised strong ecological issues about its impact on aquatic organisms. Buprofezin is used as agrochemical, and it finds its way into freshwater through run-offs, effluent emissions, and direct agricultural application, thus decimating water quality and health (Chandrasekaran et al., 2020; Nwani et al., 2022). When these substances are introduced, they may cause significant disruption to the delicate state of aquatic communities and lead to changes in the behavior and observable alterations in fish physiology and biochemistry.

Fishes qualify as sensitive bioindicators of water quality and the risk of pollutants as keystone constituents of freshwater food webs. Their intensely sensitive response to xenobiotics makes them the appropriate model organisms in defining the effects of toxicants on biological systems. Interrogating discrete responses of biochemical endpoints, specifically, enzymes directly linked to antioxidant defense

and xenobiotic metabolism, researchers can deduce the health of the organism and outline hazards linked to exposure to contaminants (Kumar et al., 2023).

Common carp (Cyprinus carpio) was used in this study to test the toxicity of buprofezin and this species has commercial significance in a freshwater ecosystem. The relationship between the oxidation stress and the capacity to detoxify was explained by the determination of the quantity of enzyme biomarkers, such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-2 transferase (GST). All these indices are crucial in understanding physiological and biochemical responses to toxicants because they neutralize reactive oxygen species (ROS) and prevent oxidative stress to cell macromolecules (Rani et al., 2021). Liver and kidney were chosen as priority target organs due to its fundamental roles in biotransformation and excretory clearance functions which are critically needed to prevent diseases and ensure homeostasis in the state of stress.

Enzyme markers like CAT, SOD, and GST are thus inseparable in mapping the adaptive processes of environmental stressors on the aquatic organism. Such enzymes are the main defense mechanisms against ROS-induced damage, which is a widespread outcome of pesticides and other toxicants intake. An increase in their activity is usually a signal of a compensatory response to manage oxidative load and overcome cellular damage and disturbance of functioning, but such a response can be overly heated resulting in cell damage and dysfunction (Ali et al., 2019).

Hepatic Catalase (CAT) Activity

Another important antioxidant enzyme that helps protect the integrity of the cells is catalase (CAT), which breaks down hydrogen peroxide (H_2O_2) into water and oxygen. In this experimental research, there was an increase in the CAT activity of the hepatic tissue of Cyprinus carpio treated with buprofezin ($P = 0.032$). The control cohort exhibited the least activity (1156.65 ± 0.943 UmL^{-1}), and the best result was obtained with the T2 group (10 -1 UmL^{-1}) (1359.41-1 UmL^{-1}). These effects incriminate the exposure of buprofezin in facilitating the process of oxidative stress, leading to the compensatory increase of CAT to counteract the process of oxidative damage. Active hepatic detoxification of ROS, especially H_2O_2, by converting it to non-harmful products, is indicated by augmented CAT activity, therefore, preventing the progression of oxidative injury (Sadeghi et al., 2018). In line with the rest of the toxicology literature, this dose-related CAT enhancing effect is concordant to reports that CAT goes up in response to organophosphate exposure in Oreochromis mossambicus, which is influent to the activation of antioxidant-responses in response to oxidant challenge (Basha & Rani, 2003) and is analogous to the rises of hepatic CAT in fishes in response to pesticides as an adaptive response to oxidative insult. It is interesting to note that the consistent overproduction of H_2O_2 during buprofezin metabolism can eventually lead to the exhaustion of the CAT reserves with the resultant redox imbalance and increased susceptibility to oxidative damage (Slaninová et al., 2009).

Hepatic Super Oxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) is one of the leading defensive lines towards oxidative stress, which dismutes the superoxide radical into H_2O_2. The rate of Hepatic SOD also rose significantly and notably with the increase of buprofezin concentration ($P = 0.011$) to 520.31 UmL^{-1} in the T2 (10mg/L) as compared to 234.75 UmL^{-1} in controls. This pathway refers to an adaptive process of escalation in order to neutralise superoxide radicals- one of the major ROS that are formed in case of toxic stress. SOD reduces subsequent cell injury through the breakdown of the superoxide to less reactive intermediates (Iqbal et al., 2020). The dose-responsive SOD upshift corroborates the previous results on pesticide-stressed fishes (Rao et al., 2014) and confirms the hypothesis that buprofezin triggers the development of ROS and thus requires the strong mobilisation of antioxidants to prevent the negative effects on cells. The further evidence of Channa punctatus how it responds to stress involving pesticides also contributes to the main discovery that SOD helps to mitigate oxidative mechanisms (Ansari et al., 2011).

Hepatic Glutathione-S-transferase (GST) Activity

The role of glutathione-S-transferase (GST) is to confer conjugation of glutathione and xenobiotics to increase the solubility and the excretion of these compounds. In this study, the hepatic GST activity improved with exposure to buprofezin ($P = 0.021$) reaching a high point in T2 (10 mg^{-} L 1407.1 954 UmL) and a minimum in controls (878.55 952 UmL). Naked high levels: Elevated GST is an increase of detoxification throughput characterized by a response to buprofezin. GST performs the functional activity

of conjugating electrophilic toxicants with glutathione that is vital in terms of water solubility and elimination (Mukherjee & Das, 2018). Its dose-dependent nature is similar to previous studies in Cyprinus carpio treated with organophosphates in which GST was indicated to be a primary mediator of pesticide metabolite clearance (Prusty et al., 2015), and in the literature which shows GST causes alleviation of pesticide stress in aquatic animals (Elia et al., 2018). Although the increased GST activity in the current experimental indicates the active detoxification, prolonged exposure will cause the saturation of GST capacity with the consequent inefficient clearance and subsequent build up of harmful intermediates (Vander et al., 2003).

Renal Catalase (CAT) Activity

Buprofezin also caused a large response in renal CAT activity (P = 0.017), with the T2 (10 mg/L $^{-1}$) group having the highest (944.41 ± 0.053 UmL^{-1}) and controls the lowest (701.65 ± 742 UmL^{-1}). The response highlights the role of the kidney to balance the oxidative stress when facing the stress of toxicants. Increased CAT indicates renal support of antioxidant systems against buprofezin-induced ROS production (Rahman et al., 2022), which depicts the role played by the organ in the overall homeostasis of the body and countering the toxic effects of pesticides. These are consistent with the evidence that exposure to pesticides enhances renal CAT in fish, the mechanism of which is thought to be enhanced ROS generation during the processes of filtration and excretion, requiring strong antioxidant response (Sharma et al., 2012).

Renal Superoxide Dismutase (SOD) Activity

The activity of SOD showed a significant increase in the renal tissue (P = 0.018) and the highest response was seen with T2 cohort (10 mg/L $^{-1}$ 403.73 ± 0.742UM $^{-1}$), the dose-response profile resembled the one in the liver, which supports the central tendency of the role of SOD in extinction of oxidative pressure. These are consistent with the previous studies which utilized SOD as one of the sentinel biomarkers of pesticide-induced oxidative stress in fish (Jain & Tripathi, 2021; Ucar & Ozgul, 2021). Like it occurs in hepatic tissue, the kidney depends on SOD to counteract the effects of superoxide radicals and therefore reduce tissue damages. In complementary research, (Nwani et al., 2013; Herrera et al., 2016) reported a positive increase in SOD activity in Clarias gariepinus treated with organophosphate pesticides a trend that is associated with an adaptive upregulation mechanism aimed at extravagant production of ROS in the renal compartments.

Renal Glutathione-S-transferase (GST) Activity

The exposures to buprofezin (P = 0.022) had a significant effect of increasing the levels of renal GST, where the control group had the lowest levels of buprofezin (756.05 0.853 UmL^{-1}) and T2 group (10 mgL^{-1}) had the highest (1508.6 0.322 UmL^{-1}), increased GST is an indication of increased detoxification throughput that is particular to buprofezin. GST is functionally linked to conjugation of electrophilic toxicant with glutathione, as it enhances solubility of the compound in aqueous conditions and excretion (Mukherjee & Das, 2018). The noted rise in GST is thus a renal priority to amorphous pesticaste loads and protect the host organism against the toxic sequelae. The dose-dependent GST increment concurs with (Singh et al., 2016), who have registered similar renal retaliations to pesticides in fish, which further validates this fact as the kidney plays a central role in cleansing perilous buprofezin residues (Choudhary et al., 2019). The concomitant increases of GST in liver and kidney are consistent with an extensive biochemical mobilisation process to relieve buprofezin intoxication in line with an already undertaken detoxification programme to offset the oxidative stress and hasten the process of eliminating reactive metabolites.

Conclusion

In hepatic and renal tissues, catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) increased in an unmistakable dose-dependent pattern with exposure to buprofezin and indicate these detoxifying and antioxidant enzymes as essential elements in the Cyprinus carpio reaction to the oxidative load of pesticides. Increased enzymatic activities indicate a compensatory biochemical defence system neutralizing reactive oxygen species (ROS) and converting toxic substances, notwithstanding, with prolonged or greater exposure, the compensatory defense mechanisms are likely to be overwhelmed, leading to oxidative cell damage, immuno-suppressive effects, and dysfunction of internal organs (Al-Ghanim et al., 2023). A large literature of modern studies supports the presence of an

environment of oxidative disequilibrium induced by pesticides in aquatic organisms, and are no exception in the association of contact with pesticides with elevated levels of antioxidant defenses in the face of ROS. Taken together, these facts auger the ecological risks of buprofezin and predict the harm to the wellbeing of aquatic ecosystem in the long term. To reduce excessive runoff and create regulatory space to embrace best-practice agronomy (Khan et al., 2021), as well as enhance water-quality monitoring and implement other agronomically appropriate pest management practices, such as biopesticides, to curb bioaccumulation risk to human ultimately benefit beneficiaries of fishery products.

Summary

This chapter summarizes the empirical data on the toxicodynamics of buprofezin on freshwater fish, including but not limited to hepatic and renal antioxidant enzymes, in fresh water fish, identifying the fish of Cyprinus carpio. Results will be summarized in three major sections:

1. The virtue of preserving the optimum physicochemical water conditions in the bioassay,
2. The responses of the hepatic microsomial enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST)
3. The concomitant responses of these microsomial enzymes in the renal tissue. The datasets in totality support the thesis that buprofezin is an effective trigger of the oxidative stress and detoxification pathways in this teleost model.

At the exposure times, the temperature, dissolved oxygen and pH were kept at ranges acceptable to culture carps and there were no notable differences between the control and the treated aquaria. Such stability shows that the noted biochemical changes are due to the effect of buprofezin exposure and not the degradation of the water quality or artefacts arising in husbandry. The mortality levels were again insignificant signalling that the test levels (5 and 10 mg/L) were not lethal but no less effective in inducing quantifiable biochemical sets (Al-Ghanim et al., 2023).

The data on Hepatic enzymes showed that there was a steady and significant dose and time dependent increase in CAT enzyme activity in fish receiving buprofezin as compared to controls. The values of the baseline catalase were similar at the beginning of the experiment, but at ninety-six hours, there was an increase in the CAT activity in the 5 mg/L^{-1} group and more significantly in the 10 mg/L^{-1} group. The differences were statistically significant, and therefore, one-way ANOVA proved that there was a treatment effect. Because catalase breaks down the hydrogen peroxide into oxygen and water, its up-regulation is an indication of a high rate of peroxides produced during the oxidative metabolism induced by pesticide use (Subaramaniyam et al., 2023).

The SOD activity in the liver had a similar trend. The baseline of all the groups was equal after which the buprofezin treated fish had higher SOD activity compared to that of controls at all sampling periods. The maximum SOD values were found in the $10L^{-1}$ cohort at ninety-six hours and ANOVA indicated the highly significant inter-group differences. Since the SOD will catalyze the dismutation of the superoxide radical to hydrogen peroxide, this trend suggests greater generation of the superoxide species and mobilisation of the primary antioxidant defenses. Co-induction of SOD and CAT points to the superoxide-peroxide axis of oxidative stress as one of the primary victims of buprofezin toxicity in the liver tissue (Khan et al., 2021).

The concentration of glutathione-S-transferase, as well as the exposure time also increased in the liver. GST baseline levels did not differ by group, but treated fish had acute and statistically significant increases which reached their peak at high doses, at ninety six hours. Since GST reacts with electrophilic xenobiotic metabolites and lipid peroxidation products under reaction with reduced glutathione, this observation suggests that phase- II detoxication pathways are activated with reaction to buprofezin (Mukherjee & Das, 2018). High GST is thus a process of biotransformation of a pesticide and a result of increased oxidative damage.

The combined hepatic data show that buprofezin causes concerted beliefs of CAT, SOD and GST in a dependence on concentration and time. Oxidative stress impounds this multidimensional antioxidant and detoxification enzyme induction such that it consistently accompanies the future diagnosis of oxidative stress, and demonstrates that the liver, as the pivocal organ of xenobiotic metabolism, effectively impersonates a significant burden of biochemical load in the face of insecticide challenge. Although certainly adaptive in the short term, prolonged expression of these pathways is likely to have energetic fees, and will predispose fish to additional physiological maladaptations (Rani et al., 2021).

Renal biomarkers had similar trends. The dose and duration increased the catalase activity of kidney tissue with the highest activities once more at the final sampling point of the 10 mg L^{-1} group. Statistical evaluation showed the presence of significant effects of treatment implying that renal tissue is also exposed to intense oxidative pressure. The effect of high levels of CAT in kidney is that of higher levels of detoxification of the hydrogen peroxide locally released or through the circulation, hence the prominence of kidney as a secondary major site of redox regulation in the context of buprofezin exposure (Choudhary et al., 2019).

The activity of renal SOD was equally strongly induced. Subsequently, both exposure groups exhibited steep time-response curves with the high-dose group steadily outpacing the low dose group and the control after similar baseline levels. The differences were found to be very significant as proved by ANOVA (Ansari et al., 2011). This trend reflects that the accumulation of superoxide radicals either inside or outside renal cells when stressed by buprofezin or that the fish if responding to these stressors by mobilising SOD-mediated responses. Since SOD is the initial enzymatic defenses against superoxide, its sharp stimulation in kidney tissue confirms its application as a delicate bio-dependent on pesticides as leading to an oxidative imbalance biomarker (Singh et al., 2016).

The increase of kidney GST activity with increase in concentration and exposure time also exhibited a peak at the highest level of activity of 10mgL^{-1} bracket in ninety six hours bracket. Analysis of variance showed that there are significant treatment effects and data on the difference in means showed major changes rather than the control in the activity of GST. These findings verify that phase-II conjugation activities are induced in the renal tissue, which is the manifestation of direct exposure of the kidney to the buprofezin metabolites and excretory roles in eliminating glutathione conjugates (Jain & Tripathi, 2021). The graded kidney GST results are supplemented by the hepatic results and point to the system nature of detoxification response.

A consistent toxicodynamic picture exists when the hepatic and renal results are viewed as complementary to each other. Buprofezin causes neither instant death nor slow death, however the exposure of 5 and 10 mg/L neither in liver nor kidney, but in rapid sequence, activates core antioxidant enzymes and GST. It shows that the pesticide is rather a chronic oxidative stressor, which prompts countermeasures in the form of biochemical changes that are expected to maintain cellular homeostasis. The large F-values found in the different one-way ANOVA support the idea that these responses cannot be attributed to random variation of responses but to treatment-induced changes in the status of the enzymes (Ucar & Ozgul, 2021).

These findings reveal in ecological and applied studies that CAT, SOD, and GST in liver and kidney of the fish, Cyprinus carpio stick at sublethal levels of buprofezin in freshwater environments represent a sensitive biomarker group (Nwani et al., 2013). The overall positive tendencies in all the enzymes and in both organs combined with their statistical support demonstrates their diagnostic utility in alerting about the pesticide contamination. At the same time, the scale of the responses (especially at a high level and length of field exposure) indicates that long-term field exposure may overstretch antioxidant capability and result in structural damage, impaired organ function and loss of fitness in populations (Herrera et al., 2016). In turn, this chapter creates a mechanistic association of buprofezin contamination and the quantitative biochemical stress in fish, which offers a basis of integrated toxicological as well as ecological explanation in the following chapters.

Summary, Findings, Conclusion & Recommendations

Summary

The present investigation was designed to characterise, in a controlled laboratory setting, the toxic impact of the insect growth regulator buprofezin on the common carp, Cyprinus carpio, with specific emphasis on oxidative stress and enzymatic biomarker responses in hepatic and renal tissues. Building on the conceptual foundation established in the earlier chapters, the experimental work sought to translate theoretical expectations about pesticide-induced oxidative disruption into quantifiable biochemical evidence in a model freshwater teleost. Buprofezin, applied at nominal sublethal concentrations of 5 mgL^{-1} and 10 mgL^{-1}, was selected as the stressor because of its extensive use in rice-based agroecosystems and its documented persistence in aquatic compartments adjacent to agricultural fields. Within this framework, the study examined the activities of three key antioxidant and phase-II detoxification enzymes catalase

(CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) in liver and kidney, organs that together represent principal hubs for xenobiotic biotransformation and excretory clearance in fish.

The general plan of the experiment consisted of a period of acclimation and eight weeks of continuous experimental (semi-static) measurements with the working conditions in which the species-specific water-quality variables were kept within suitable ranges. Fish were randomly assigned to a control group and two control groups wherein they were put in duplicated glass basins and given a standard commercial diet at a pre-determined fraction of body weight. Serial sampling at 24, 48, 72 and 96 h following initial exposure, and more enduring responses were measured at the conclusion of the eight week exposure period, recorded its short-term responses. Blood, hepatic tissue and renal tissue had been collected in conditions of strictly standardised protocols designed to reduce handling stress and pre-analytical artefacts. The activities of enzymes were measured by spectrophotometric assays that were essentially on classical assays of SOD, CAT and GST, hence making the methodology comparative in attitude to the classical toxicological and biochemical investigations.

Experimental data was drawn into a fairly consistent pattern, in which exposure to buprofezin was observed to generate statistically significant and concentration-dependent changes in CAT, SOD, and GST activity of liver and kidney as compared to the unexposed control. Such biochemical changes were already observed in the initial 2448 h sample points which were further enhanced with an increase in the exposure period, which suggested that both acute and sub-chronic processes were involved (Nwani et al., 2013). A combined enzyme profile provides strong evidence to support the conclusion that buprofezin causes oxidative stress and results in adaptive up-regulation of antioxidant defences and conjugative detoxification pathways in C. carpio. Meanwhile, the scale and the duration of such responses indicate that long-term environmental exposure or repeated exposure might cause compensatory capacity to be overloaded and result in structural, functional, and population-wide effects.

Overall, the research was able to achieve its main goal, namely, illustrating the role of comparatively low levels of experimentally relevant concentrations of buprofezin in disruption of redox homeostasis in C. carpio. The study offers mechanistic understanding of how this insecticide could impair fish health and it will support the claim of CAT, SOD, and GST as sensitive biomarkers to monitor early-warning stress level of pesticides in freshwater ecosystems.

Key findings

The main quantitative result of the work is the evidently repeatable increase of hepatic and renal antioxidant enzyme activity responses to exposure to buprofezin. The CAT activity in the liver rose dose- and time-dependently with the highest levels recorded in the 10 mg L 01 treatment group at the later sampling periods. This trend suggests that hydrogen peroxide produced during intensified metabolism of xenobiotic compounds as well as other reactive oxygen species is more rapidly decomposed. Similar tendencies were found with regard to hepatic SOD, the activities having increased significantly over the exposure period, indicating an increase of dismutation of superoxide radicals, and mobilisation of the first-line enzymatic barrier to oxidative attack. The liver GST activity also rose considerably in all treatment groups indicative of phase-II conjugation pathways induction and high throughput of detoxification (Herrera et al., 2016).

A very similar picture was associated with a response profile in the renal tissues. Kidney CAT activity was highly increased compared to controls in both the exposure groups once again with the highest increases in the high-dose treatment and on the extended exposure times. This was also reflected in the renal SOD activity, which showed that the kidney, as in the liver, is subject to persistent pressure of the superoxide during the buprofezin test and responds by up-regulation of renal antioxidants systems. There was also an increase in the GST activity in the kidney, which suggests that conjugation and excretory clearance mechanisms are active in concert with the antioxidant defences. The hepatic-renal congruency of action to find out lends support to the fact that buprofezin causes a systemic oxidative stress, which is being compensated by unified biochemical adaptations in various body tissues.

Combined, these findings give solid evidence that even at sublethal levels, buprofezin can be considered as a significant pro-oxidant in C. carpio with an effect that is not only concentration and time-dependent in stimulating development of 3 enzymatic defences CAT, SOD and GST. The enzymes thus are useful in both roles to safeguard cellular components against oxidative injury to act as convenient and quantifiable biomarkers of toxicant exposure and internal effect.

Combined Discussion of Results

By putting these results in the context of toxicology in general, one can point out several key dimensions. To begin with, the observed enzyme reactions are fully in line with the conceptual framework that was presented in previous chapters whereby pesticide exposure is interpreted to upset the balance between the generation of reactive oxygen species and anti-oxidant defence capability. Through its ability to disrupt metabolic and signalling pathways, buprofezin therefore seems to either enhance the formation of ROS or hinder their elimination hence eliciting a compensatory response of antioxidant enzymes. It has been indicated especially by the fact that both liver and kidney are highly coupled in terms of the SOD and CAT responses, SOD catalyzes superoxide radicals to hydrogen peroxide and CAT to break hydrogen peroxide to water and molecular oxygen. The up-regulation of the two enzymes indicates that the whole superoxide- peroxide sequence is stressed and the organism is trying to establish redox homeostasis by increasing the support of this detoxification cascade.

Second, the GST induction of the two organs offers a mechanistic understanding of how C. carpio tries to cope with downstream effects of oxidative and xenobiotic stress. In lipid peroxidation products, GST catalyses the conjugation of electrophilic products with reduced glutathione to form more water-soluble products, which may be excreted through bile or urine. Increased GST activity thus suggests stimulation of the phase-II detoxification activities and increased turnover in glutathione which is a major non-enzymatic anti-oxidant. Although the activity aimed at preserving damaging glutathione growth and wider cellular defence packages, the prolonged activation can reduce glutathione stores and weaken greater cellular defence systems. Such a concerted rise in CAT, SOD, and GST activities since reported in this work, therefore, constitute a composite biochemical approach of counteracting ROS, restoring and eliminating oxidatively damaged molecules, as well as exerting pressure on the excretion of buprofezin and its metabolites.

Third, the organ specific patterns that occur here bear ecophysiological meaning. The liver being the predominant site of xenobiotic biotransformation would be expected to show strong biomarker responses to pesticide exposure and the current data support their expectation. However, as a primary metabolic organ, the kidney is viewed as being at least partially in relation to osmoregulation and excretion and not as a primary metabolic organ. The high renal outputs obtained in the mentioned study highlight the fact that the kidney of C. carpio is directly linked to the regulation of buprofezin-related oxidative stress and detoxification, probably because of its involvement in blood-borne metabolites filtration and conjugated product excretion. The findings further render the observation that in cases of careful ecotoxicological estimations, one ought to adopt the involvement of several tissues in their opinions rather than examining compounds with difficult metabolic and excretory activities by employing the hepatic biomarkers.

Fourth, the methodological perspective on the application of standardised spectrophotometric assays and statistically sound comparisons of the control and the treatment groups enhances reliability of the findings. The dose response relationships were always consistent, the changes of the biomarkers were always coherent over time and the similarity between hepatic and renal patterns are all that decrease the chances of the observed differences being artefacts of experimental variation or sampling error. Rather, they refer to an actual toxicodynamic effect that can be justified by the exposure to buprofezin as specified by this study criteria.

Last but not the least, these biochemical reactions when extended to natural field conditions do give fears concerning the ecological impact of the buprofezin contamination in freshwater systems. In agricultural studies where buprofezin gets used repeatedly across growing seasons, fish populations would experience chronic or intermittent exposure, and may be exposed to similar shapes of concentration as used in this study. As the enzymatic up-regulation experienced in this case is an adaptive response to maintain the integrity of the cells, long-term stimulation of antioxidant and detoxification processes may have a metabolic cost, which diverts energy away to growth, reproduction, and immune functioning. In the long term these sublethal effects can be converted into diminished levels of fitness at an individual level, and a change in population dynamics with cascading consequences to food-web structure and ecosystem processes.

Conclusion

Based on the evidence that is produced during this experiment, it can be concluded that buprofezin was a major oxidative stressor to Cyprinus carpio under freshwater conditions that were controlled. Periods of exposure to environmentally relevant sublethal doses of up to 24h to eight weeks produced statistically significant and clear increases in hepatic and renal CAT, SOD and GST activities, which were biologically understandable. The collective action of these enzyme responses is indicative of alleviation of antioxidant and conjugative detoxification defenses, which validates the argument that buprofezin disrupts redox homeostasis and induces a biochemical-based defence response. Whilst these responses are often protective, their nature and continued existence suggests that homeostatic buffer is becoming compromised and their duration and intensity means that chronic or repeated exposure might surpass the limits of the compensatory responses contributing to tissue damage, functional impairment and increased vulnerability to disease and other environment-related challenges.

The paper consequently attests to two broad inferences. The first one, CAT, SOD, and GST are confirmed as sensitive, informative biomarkers of determining sublethal pesticide stress in C. carpio and subsequently in other freshwater teleosts exposed to the same contaminant regimes. Second, buprofezin pollution of aquatic environments is a plausible ecotoxicological hazard that should be regulated, routinely monitored and mitigative measures devised to cut back the number of inputs into surface waters and safeguard non-target flora.

Limitations

Although the current work has strengths, it has to be admitted that it has a number of limitations that have to be taken into account during the interpretation of the findings and their further application. The first constraint is related to the duration of exposure. The fact that both short-term (24-96 h) and sub-chronic (eight weeks) sampling points are included gives quite a useful time frame, but the study does not cover the really significant broad time frame exposures that are long-term and life-cycle. There is, thus, no way of making the direct inference of the impact on the development, procreation, or even survivorship patterns in wild populations of the multi-generational exposure to the low levels of buprofezin.

Secondly, the concentration range studied was limited to two nominal doses, that is, 5 mg L^{-1}, and 10 mg L^{-1}. Although these scales provide a comprehensive range of possible environmental concentrations, these ranges include lower, intermittent, and pulse-like concentrations associated with precipitation and application intervals as well as providing an overall understanding of the range of potential environmental concentrations. To determine the levels of thresholds, no-observed-effect concentrations, and possible non-linearities in biomarker behaviour, it would be required to have a finer dose-response design.

Third, the study focused exclusively on a single species, C. carpio, chosen for its ecological and economic relevance. Inter-specific differences in physiology, metabolism, and habitat preference mean that other fish, invertebrates, and amphibians may exhibit different sensitivities and response patterns to buprofezin. As a result, caution is required when extrapolating the current findings to whole communities or to taxa occupying different ecological niches.

Fourth, the used biomarker battery, though informative, was focused in a narrow manner on three endpoints of enzymes. Measures were not made of other relevant markers of oxidative and toxic damage, including products of lipid peroxidation, protein carbonyls, DNA damage, histopathological changes, immune parameters, and behavioural changes. As a result, the ordeal between the observed responses of the enzymes and higher-level biological effects is inductive and not empirical.

Fifth, the experiment has been conducted under the same condition of temperature, dissolved oxygen, and PH in the laboratory, and without other pollutants co-occurring. These abiotic factors are variable in natural systems, and can have an effect on exposure to pesticides, and combinations between agrochemical and multiple agrochemical and other stressors can be the norm and not the exception. This lack of complexity in the current design, which is required to control the experiment, does not allow the results to be immediately generalizable to the field.

Lastly, the research did not include a post-exposure recovery period. It is unclear thus whether the documented changes in enzymes would be restored to baseline, stabilise by new set-point or decompensate upon stoppage of exposure to buprofezin. Recovery dynamics determines the key point that can be used to assess resilience and remediation strategies to be implemented in polluted ecologies.

Recommendations

Considering the outlined findings and limitations, it is possible to formulate a number of practical and research-oriented recommendations. Regulatory and management The obvious evidence that buprofezin elicits intensive oxidative and detoxification effect in C. carpio enable the creation or enforcement of water-quality criteria and discharge limitation of this insecticide in surface waters to which agricultural runoff flows. The sublethal biomarker evidence should explicitly be addressed in regulatory frameworks where the setting permissible pesticide concentrations in aquatic environments ought not to be based on acute lethality data only.

It is highly encouraged that routine monitoring programmes are implemented in rice-growing and other areas that are taking the medication buprofezin. These programmes are to incorporate a combination of chemical analysis of water and sediment remnants and a regular biomarker test concerning resident or caged fish with CAT, SOD, GST as vital indicators as their core. Such a dual measure would make it possible to detect biologically significant exposures at an early stage before an ecologically damaging outcome can be manifested and would give a more fine-tuned foundation of risk assessment and management judgment.

On the agricultural aspect, the use of integrated pest management techniques that becomen the alternatives of synthetic insecticides in general, and buprofezin in particular is promoted. These could be the optimisation of timing and dosage of application, the use of biological control agents, alternating the use of such ingredients with less aquatic toxicity and the use of buffer zones or constructed wetlands to capture pesticides contaminated runoffs. One should use extension services and awareness programmes, to inform the farmers and other local stakeholders about the possible downstream effects of pesticides misuse on fisheries, biodiversity and human lives.

Future Research Directions

The future studies should focus on filling the knowledge gaps above and establishing a more efficient portrait of the ecotoxicological footprint of buprofezin. Evidence of the chronic and trans-generational effects of low-level exposure will require long-duration experiments (lasting over eight weeks), with ideally one of them covering most of the most important life stages (e.g., early development, maturation, and reproduction). Dose-response extrapolations using a greater number of different concentrations, including those that are generally measured by field surveys, would help determine ecologically significant benchmark values.

Incorporation of other biomarkers like lipid peroxidation indices, comet assays of DNA damage, liver and kidney histopathology, endocrine and immune measurements and behavioural outcomes at the mechanistic level would be of benefit to further associate enzymatic alterations with functional impairments. Comparison of two or more species of fish would enhance the knowledge of inter-specific differences in sensitivity and the validity of constructing larger ecological risk models. Lastly, field inquiries which integrates both environmental analyzing of remnant buprofezin with older biomarker measurements and community based examination of biomarkers would serve the ultimate test of the applicability and predictive worth of laboratory outcomes noted in this thesis. Simultaneously, predictive, spatially explicit modelling integrating hydrology, pesticide application patterns, climate induced changes in runoff and species individual sensitivity data would help regulators in a great deal to identify high-risk catchments, prioritise mitigation investments, and assess the probable benefits of alternative management outcomes, in turn, to translate mechanistic data on biomarkers into strategic tools to conserve freshwater biodiversity and the ecosystem services on which human community relies at both local, regional and national administrative levels.

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