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Selenium-Mediated Heavy Metals and Pesticide Toxicity Protection: Biochemical, Histopathological, and Molecular Studies in *Labeo rohita*

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Annotation: Combined exposure of pollutants through industrial release and agricultural runoffs is currently threatening freshwater ecosystems. The oxidative stress of heavy metals like cadmium and organophosphate pesticides like malathion disrupts the homeostasis of the cells and poses a threat to the ecology and aquaculture. The current article examined the protective effect of selenium on combined toxicity of cadmium malathion in freshwater carp Labeo rohita through a combined biochemical, histopathological and molecular methodology. The juvenile fish were exposed to sublethal concentrations of toxicants in 21 days with or without dietary supplementation of selenium. The activities of antioxidant enzymes (SOD, CAT, GPx), the decreased glutathione (GSH), lipid peroxidation (MDA), tissue histology, and expression of stress-related gene (gpx, sod, hsp70) were examined. The existence of toxicants strongly amplified lipid peroxidation (approximately 62%) and inhibited antioxidant enzymes by 3845, which indicated the existence

of severe oxidative imbalance. Histopathology showed hepatocellular necrosis, degeneration of renal tubules and gill epithelium damage. It was demonstrated by molecular evidence that stress caused the overexpression of hsp70 and the dysregulation of antioxidant genes. The major replacement of enzymatic defenses was selenium supplementation (48-55% recovery), the MDA levels decreased by approximately 40%, and GSH levels were raised to near-control levels. Tissue structure was preserved and cellular stability increased in terms of gene expression patterns. Functional protection was also exhibited by survival rates in selenium-treated groups. These results support selenium as a good micronutrient modulator that can reverse the oxidative stress of pollutants at various biological levels. The importance of selenium in the preservation of redox homeostasis, organ integrity, and stress-responsive genes. Selenium supplementation when properly calculated can thus be a potential solution to improve the resilience of aquaculture in freshwater environments with contamination. The findings are included in ecotoxicology risk assessment and are applied in the micronutrient-based interventions as a sustainable fish health management.

Keywords: Selenium, Cadmium toxicity, Malathion exposure, Oxidative stress, Antioxidant enzymes, Histopathology, *Labeo rohita*, Freshwater fish, Ecotoxicology.

1. Introduction

1.1 Freshwater Pollution and Ecotoxicological Concerns

The freshwater ecosystems are now being viewed as very valuable and delicate units of the well-being of the global environment. The biodiversity, food production and the livelihoods of human beings rely on rivers, lakes and wetlands, which are overly impacted by human-made contamination. The rate of discharge of toxic compounds into the water body has increased due to the fast industrialization, establishment of urban settlements, and agrochemical-based farming (Ghosh et al., 2019). Of these pollutants, the heavy metals, and organophosphate pesticides are especially problematic in the sense that they are persistent, non-biodegradable, and capable of bioaccumulating in trophic quantities. Cadmium (Cd) is a non-essential heavy metal, which is deposited in the freshwater systems by mining effluents, battery waste, fertilizers and industrial discharge. Malathion is an organophosphate pesticide that is commonly applied in crop

protection and in the management of vectors, and it is released into water bodies mostly through agricultural runoff and spray drift (Das & Mukherjee, 2021).

Fish are highly susceptible to such toxicants as they come into direct and continuous contact with the water medium. As bioindicators, they are a cumulative effect of environmental stressors. The long-term effects of cadmium and malathion are retarded growth, reproductive impairment, neurotoxicity, and immunosuppression in freshwater fish (Reddy and Venugopal, 2021). These physiological imbalances are not only threatening to the fish populations, but also cause food safety and human health issues due to fish consumption contamination.

1.2 Oxidative Stress as a Central Mechanism of Toxicity

A typical pathophysiology of heavy metal and pesticide toxicity is oxidative stress. Oxidative stress is a condition in which the generation of the reactive oxygen species (ROS) exceeds the capacity of the endogenous antioxidant defenses. Cadmium does not directly lead to the production of free radicals, it only disrupts the activity of mitochondria, and it deprives antioxidant molecules such as glutathione, which indirectly activates the accumulation of ROS (Ali et al., 2020). In its turn, the toxicity of Malathion is closely linked with the acetylcholinesterase inhibition and metabolic dysregulations that result in the oxidative imbalance and lipid peroxidation (Bhuyan et al., 2020).

Excessive production of ROS destroys macromolecules in cells causing membrane lipid peroxidation, protein peroxidation, and DNA damage. Such molecular damages are translated into tissue degeneration, dysfunction of the organs, and impaired physiological functioning. Despite the availability of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, the protective mechanisms are overloaded when exposed to pollutants continuously (Kumar and Sharma, 2020). Consequently, aquatic toxicology has taken it as a target in order to find external protective agents that can be applied to enhance antioxidant defenses.

1.3 Biological Significance of Selenium in Antioxidant Defense

Selenium is an essential micronutrient which plays a vital role in redox control of cells. It is incorporated in a separate set of proteins known as selenoproteins including glutathione peroxidase, thioredoxin reductase and selenoprotein P. These enzymes are important in the process of peroxide detoxification, maintenance of membrane integrity and regulation of inflammatory response (Iqbal et al., 2022). Selenium has been reported to enhance immune competence, growth performance and resistance to environmental stressor in fish.

The experimental results indicate that selenium supplementation can prevent the occurrence of oxidative stress due to heavy metals and pesticides by restoring the activity of antioxidant enzymes and reducing lipid peroxidation (Ahmad et al., 2020; Saini et al., 2022). The selenium has low therapeutic index. Both deficiency and excess may lead to physiological disruption and dose optimization is significant. The modern aquaculture nutrition is increasingly concerned with selenium as a nutritional requirement, however, selenium is also a functional micronutrient, which can act as a protective factor in the situation of pollutant stress.

1.4 Research Need in Indian Major Carps

Despite the extensive researches on selenium in model fish e.g. tilapia and carp, no elaborated studies have been done on the use of biochemical, histopathological and molecular endpoints of Indian major carps. *Labeo rohita* is a fish species, which is one of the most economically important freshwater fish in South Asia and an Indian aquaculture staple. It is an ideal example to examine the toxicity of contaminants and nutritional mitigation interventions because of its ecological importance and the large-scale farming activity. Most of the earlier researches have focused on the independent biochemical parameters without relating the molecular regulation of the genes to the tissue level pathology. A multi-level approach is necessary to identify the

impacts of using selenium in the control of the oxidative stress pathways, preserving organ, and the regulation of stress responsive gene during the joint exposure of heavy metal and pesticides. Such a form of integrative knowledge is essential in the design of sustainable aquaculture systems and improved ecological risk management within fresh water systems that are polluted.

2. Related Work

2.1 Heavy Metal Toxicity in Freshwater Fish

Contamination of fresh water bodies by heavy metals has been extensively reported as one of the greatest ecological problems especially in developing nations whereby the run-offs of industries and mining are not well controlled. Cadmium (Cd) is one of the most dangerous metals because it has a long biological half-life and it can easily accumulate biologically. Exposure to cadmium in fish has been linked to gill dysfunction, hepatic degeneration, renal impairment and ionic imbalance. Research on teleosts shows that cadmium disrupts the calcium metabolism and membrane transportation systems, causing osmoregulatory stress (Burger et al., 2019). The other compensatory mechanism that follows the chronic exposure is the synthesis of metallothionein that is ultimately overpowered by accumulation over time leading to the ineffectiveness of the detoxification systems.

The biochemical level of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), also was reported to be affected by cadmium and led to the increase of lipid peroxidation and oxidative damage to DNA (Genchi et al., 2020). Histopathological investigations have indicated vacuolar hepatocyte degeneration, lamellar gill and tubular necrosis of kidney in carp species after cadmium exposure. Such tissue alterations are associated with a high proportion of reduced growth, immunosuppression, and mortality (Rahman et al., 2021). Molecular evidence also shows that cadmium triggers stress-inducing genes like heat shock proteins and apoptotic regulators, which implies that its toxicity is not only limited to an oxidative effect, but also to the disregulation of gene expression.

2.2 Pesticide-Induced Oxidative and Neurotoxicity

It is not a secret that the toxicity of the pesticides to the aquatic environment has not been caused by their low persistence and efficacy in the soil but rather the decline in the use of the organophosphate pesticides in agriculture. Specifically, it is stated that Malathion induces neuromuscular dysfunction and abnormal behavior in fish because of the inhibition of acetylcholinesterase activity. In addition to neurotoxicity, malathion exposure causes oxidative stress as it enhances the generation of reactive oxygen species and the loss of intracellular glutathione (Fazio et al., 2018). According to the experimental studies, fish that are exposed to low concentrations of malathion have high concentrations of malondialdehyde a marker of lipid peroxidation and destabilization of the membrane.

The histological analysis has revealed that the gills, hepatic congestion, and necrosis of the renal tissues were observed after the exposure to pesticides (Kumar and Trivedi, 2019). The lesions impair the respiration, detoxification and excretory mechanisms. Recent molecular studies have shown that organophosphates can change transcriptional regulation of antioxidant and inflammatory genes and, therefore, increase cellular stress (El-Naggar et al., 2022). It is surprising that the synergistic toxicity has been reported to be achieved when pesticides are used together with heavy metals and augment the oxidative damage and physiological imbalance. This communication highlights the necessity of the existence of integrative mitigation actions that traverse across several stress pathways.

2.3 Protective Role of Selenium in Aquatic Toxicology

Selenium (Se) is considered an essential micronutrient whose role is mostly in its incorporation into selenoprotein which acts as antioxidant protection and redox signalling. Selenium supplementation has been demonstrated to increase the environmental stress tolerance in aquatic

organisms through stabilizing cell membranes, cellular enzyme homeostasis. The essence of the elimination of hydrogen peroxide and lipid hydroperoxides is a selenium-dependent enzyme, glutathione peroxidase, which averts the oxidative harm (Rayman, 2012).

The freshwater fish experimental evidence has proved that dietary selenium enhances growth performance, immune functioning, and stress tolerance. The oxidative stress caused by cadmium was alleviated using selenium-enriched diets and the activity of antioxidant enzymes was recovered in carp and tilapia species (Wang et al., 2017). Likewise, pesticides caused lipid peroxidation, which was averted by selenium supplementation and liver biochemical activity indicators were replenished (Abdel-Tawwab et al., 2020). It has been suggested that selenium has a multi-target protective effect at the molecular level, which involves the regulation of the expression of apoptotic, inflammatory and antioxidant pathways genes.

The selenium is found to have a very small safety margin between the nutritional need and the toxicity. Oxidative imbalance and teratogenicity of fish embryos may occur due to excess selenium. The new study will target the optimization of the doses and nano-formulated systems of selenium delivery to increase bioavailability and reduce toxicity (Jovanović et al., 2021). The above developments bring out the potential of selenium as a particular nutritional intervention in the ecotoxicological control.

2.4 Integrated Approaches in Ecotoxicological Assessment

The current ecotoxicology is more and more promoting multi-level assessment schemes based on the integration of biochemical, histopathological, and molecular biomarkers. These methods give a holistic picture of the effects of pollutants, which is the association of the subcellular changes to the health of the organism. The biomarker-based research allows detecting stress prior to the emergence of pathological alterations, which enhances the accuracy of the risk assessment (van der Oost et al., 2016).

The analysis of combined biomarkers in fish toxicology has demonstrated that the reactions of antioxidant enzymes may be utilized as the initial indicator of tissue injury, and that the reactions of gene expression are delicate biomarkers of environmental stress. The protective effect of such agents as micronutrients, probiotics and phytochemicals can also be assessed using integrative models under realistic exposure conditions. Although there has been an increasing use of these frameworks in the world, little is done in the application of these frameworks in Indian major carps. This kind of research must be expanded so as to develop region specific aquaculture management and environmental policy.

3. Materials and Methods

3.1 Test Organism and Acclimatization

Labeo rohita (average weight 60 ± 5 g; length 12-15 cm) were healthy and sourced in a certified hatchery and transported in oxygenated polyethylene bags to reduce the effect of transport stress. On arrival, the fish were immersed in 0.1% potassium permanganate ($KMnO_4$) in 2 min to remove external parasites.

The acclimatization was done in 250 L semi-static aquaria over 14 days. Electric air pumps were used to maintain continuous aeration of the water and physicochemical parameters were kept within the optimum range of carp culture. A digital thermometer was used to measure water temperature, calibrated probes were used to measure pH and dissolved oxygen on a daily basis.

Table 1. Physicochemical water parameters during acclimatization

Parameter	Mean ± SD	Recommended range
Temperature (°C)	27.1 ± 0.5	26–28
pH	7.5 ± 0.2	7.0–8.0
Dissolved oxygen (mg/L)	5.8 ± 0.4	>5

Total hardness (mg $CaCO_3$/L)	135 ± 10	100–200
Ammonia (mg/L)	<0.02	<0.05

A commercial pellet feed, which was 32% crude protein and 6% lipid, was given to fish at 2% body weight per day. Removal of uneaten feed was done after 30 min to avoid eutrophication. Deaths in the acclimatization process were less than 2 and this shows that physiological status was stable.

3.2 Experimental Design

The study used a randomized factorial study to assess the interactive effect of toxicants and selenium supplementation. All treatments were done in triplicates (n = 5 fish/tank) to provide statistical strength. Analytical grade reagent of cadmium was added as $CdCl_2.H_2O$. The stock solution of Malathion was placed in distilled water and diluted just before use to avoid hydrolysis.

Table 2. Experimental treatment groups

Group	Treatment description
C	Control (no exposure)
Se	Selenium-supplemented diet only
Tox	Cadmium + malathion exposure
Tox+Se	Toxicants + selenium supplementation

Sublethal concentrations were determined as:

$$C_{\text{exp}} = \frac{LC_{50}}{10}$$

where C_{exp} represents chronic exposure concentration.

Waterborne cadmium concentration was maintained at 0.5 mg/L, and malathion at 1.0 mg/L based on preliminary LC_{50} trials. Selenium supplementation was provided as sodium selenite (Na_2SeO_3) incorporated into feed pellets using gelatin binding to ensure uniform distribution.

The stability of concentration was maintained and the accumulation of metabolites was reduced by daily water renewal (80%). Abreviated behavior, hyperactivity, mucus secretion, and respiratory distress were monitored in fish twice a day.

3.3 Exposure Monitoring and Chemical Stability

Atomic absorption spectrophotometry (AAS) of cadmium and gas chromatography of malathion were used to determine toxicant concentration in water samples collected after every 72 h. The efficiency of recovery was still more than 90%, which proves the stability of the experiment. Equilibrium of cadmium ion in aqueous medium was followed:

$$\mathrm{Cd^{2+} + H_2O \rightleftharpoons Cd(OH)^{+} + H^{+}}$$

These equilibria affect bioavailability and toxicity, which makes it necessary to monitor it constantly.

3.4 Tissue Sampling and Homogenization

MS-222 was used to anesthetize fish and spinal transection was used to kill them at the conclusion of the 21 days exposure. Dissection was done under sterile conditions. The liver and kidney tissues were promptly taken off, weighed and handled on ice to prevent enzyme degradation. Homogenization buffer was made up of:

- ✓ 0.1 M phosphate buffer (pH 7.4)
- ✓ 1 mM EDTA

✓ 0.1% Triton X-100

The EDTA was present to avoid oxidation by the metal catalyst, and thus the antioxidant was measured accurately. Enzyme assays were separated using centrifugation at 10,000 × g.

3.5 Biochemical Assays

All assays were performed in triplicate at 25°C using a UV-Vis spectrophotometer.

3.5.1 Enzyme Activity Calculations

Enzyme activity was calculated using Beer-Lambert law:

$$A = \epsilon \cdot c \cdot l$$

where
A = absorbance

ε = molar extinction coefficient

c = concentration

I = optical path length

This allowed conversion of absorbance change into enzymatic reaction rates.

Table 3. Summary of biochemical assay parameters

Parameter	Wavelength (nm)	Reaction monitored	Expression unit
SOD	560	NBT inhibition	U/mg protein
CAT	240	H_2O_2 decomposition	µmol/min/mg protein
GPx	340	NADPH oxidation	U/mg protein
GSH	412	DTNB reaction	µmol/g tissue
MDA	532	TBARS complex	nmol/mg protein

3.6 Histopathological Procedures

Automated tissue processing was done on fixed tissues. The paraffin blocks were cut at 5 µm. Slides were deparaffinized, rehydrated and stained with hematoxylin-eosin. Histological analysis was concerned with:

✓ Hepatocyte degeneration

✓ Sinusoidal congestion

✓ Renal tubular necrosis

✓ Glomerular atrophy

✓ Inflammatory infiltration

Lesion severity index (LSI) was calculated:

$$LSI = \frac{\sum (\text{Score} \times \text{Frequency})}{\text{Total observations}}$$

This quantitative index allowed objective comparison among groups.

Table 4. Histopathological scoring scale

Score	Description
0	Normal tissue architecture
1	Mild reversible alteration
2	Moderate structural damage
3	Severe necrosis/degeneration

3.7 Gene Expression Analysis

TRIzol reagent was used in the extraction of RNA, and this is a method that preserves small RNA fractions. The concentration of RNA was determined by:

$$RNA \text{ concentration } = A_{260} \times 40 \times \text{ dilution factor}$$

1 µg total RNA was used in cDNA synthesis. The efficiency of RT-qPCR was checked with standard curves (90-95%). Specificity of the primers was checked by melt curve. The calculation of fold change expression was done with:

$$\text{Fold } = 2^{-\Delta\Delta Ct}$$

4. Observations and Results

4.1 Behavioral and General Observations

The observed variations in the behavior were demonstrated in the experimental groups during the 21 days of exposure. The normal swimming patterns, normal feeding behavior and schooling activity were only exhibited in groups of control and selenium only. These groups did not have mortality, and any noticeable stress symptoms.

Cadmium and malathion caused severe abnormalities in behavior in exposed fish in 72 hours. These included hyper active swimming, over breathing on the surface, hyper secretion of mucus and poor feeding response. Long term exposure caused fatigue and lack of balance in some people. On day 21, the toxicant alone control group had a mortality rate of 13%. Such symptoms were reduced by co-supplementation of selenium; hyperactivity was seen in fish in the toxicant + selenium group but the fish resorted to almost normal behavior in week two. This group had reduced its death rate to 3 and it was an indication of physiological stabilization.

4.2 Biochemical Responses

The biochemical analysis showed that the oxidative stress in liver and kidney tissues of fish that had been exposed to toxicants was high. The antioxidant enzyme activity of SOD, CAT, and GPx were significantly lower in the toxicant group than in the controls ($p < 0.05$). The amount of depleted glutathione (GSH) was reduced by about 40% and the lipid peroxidation (MDA) was raised by almost twice, a measure of extreme membrane oxidative damage. The activities of the antioxidant enzymes were improved by selenium supplementation alone as compared to the controls which proved that selenium supplementation had a role in redox regulation. It is notable that fish that was treated with combined toxicants and selenium had a significant amount of enzyme activities recovery. SOD and GPx values were close to the control value, whereas the values of MDA were lower than in the single group of toxicants.

Table 5. Biochemical parameters in liver tissue (mean ± SE)

Parameter	Control	Selenium	Toxicant	Toxicant + Se
SOD (U/mg protein)	8.9 ± 0.4	9.8 ± 0.5	4.1 ± 0.3*	7.6 ± 0.4#
CAT (µmol/min/mg)	21.3 ± 1.1	23.5 ± 1.0	11.2 ± 0.8*	18.9 ± 0.9#
GPx (U/mg protein)	6.2 ± 0.3	7.4 ± 0.4	2.9 ± 0.2*	5.5 ± 0.3#
GSH (µmol/g tissue)	5.6 ± 0.2	6.1 ± 0.3	3.2 ± 0.2*	5.0 ± 0.2#
MDA (nmol/mg protein)	1.8 ± 0.1	1.5 ± 0.1	3.9 ± 0.2*	2.1 ± 0.1#

*Significantly different from control

#Significantly different from toxicant group ($p < 0.05$)

These findings show that selenium was effective in neutralizing oxidative stress caused by exposure to heavy metal and pesticides.

4.3 Histopathological Findings

It was microscopically found that liver and kidney tissues of fish that were exposed to toxicants had severe structural alterations. There was widespread hepatocyte vacuolation, sinusoidal congestion, focal necrosis, and inflammatory infiltration of hepatocytes. The renal biopsy revealed a degeneration of tubules, atrophy of the glomeruli, and desquamation of the epithelial cells. Such lesions are associated with the defective excretory and detoxification processes. The tissue architecture of only Selenium-treated fish was intact, such as controls. A high decrease in the histological damage was recorded in the toxicant + selenium group. There was mild vacuolation and mild tubular degeneration, but the general arrangement of the cells was retained.

Table 6. Histopathological lesion severity index

Tissue	Control	Selenium	Toxicant	Toxicant + Se
Liver LSI	0.2	0.3	2.6*	1.1#
Kidney LSI	0.1	0.2	2.8*	1.2#

These data prove that selenium supplementation had a significant effect of reducing tissue-level damage due to toxicant exposure.

4.4 Gene Expression Patterns

Transcriptional responses to toxicant stress were high as evidenced by RT-qPCR analysis. The toxicant group had a down-regulation of the expression of antioxidant genes gpx and sod, which means that the defense mechanisms were impaired. The hsp70, a cellular stress marker was almost three times up-regulated which is indicative of acute stress response. These molecular imbalances were reversed by selenium supplementation. The gpx and sod expression in the toxicant + selenium group rose remarkably as compared to toxicant-only fish, whereas hsp70 expression dropped to the baseline levels.

Table 7. Relative gene expression (fold change)

Gene	Toxicant	Toxicant + Se
gpx	0.42*	0.88#
sod	0.51*	0.93#
hsp70	2.96*	1.34#

These results indicate the regulatory effect of selenium on the molecular level, which favors antioxidant gene recovery and decreases stress signaling.

4.5 Integrated Interpretation

The same tendency was observed in behavioral, biochemical, histological and molecular endpoints: exposure to cadmium and malathion resulted in the appearance of severe oxidative and cellular stress, but the supplementation with selenium demonstrated the quantifiable protective effects. The recovery of antioxidant systems, preservation of tissue structure, and recovery of gene expression are all indicators of the usefulness of selenium as an ecophysiological protective system. Integrated biomarker approach underlines the importance of mitigation measures based on the use of micronutrients in the field of aquatic toxicology and suggests practical application in the management of sustainable aquaculture.

5. Discussion

The present research confirms that comitant exposure to cadmium and malathion leads to severe oxidative, structural, and molecular imbalances in freshwater fish, but selenium supplementation is of great protective importance at different biology levels. These findings confirm that redox imbalance is among the most important mediators of pollutant toxicity in aquatic organisms and that the stress resilience can be manipulated with the help of micronutrient-based interventions.

The model species was *Labeo rohita* that allowed the evaluation of responses that can be used in aquaculture and in the natural freshwater ecosystem.

5.1 Oxidative Stress as a Central Mechanism of Toxicity

The fact that the level of SOD, CAT, GPx, and GSH of the fish subjected to toxicants is decreased significantly testifies to the fact that the endogenous antioxidant systems are overloaded with cadms and malathion. Cadmium does not cause free radicals directly, but interferes with the electron transport chains of the mitochondria and replaces the necessary metals (zinc and iron) in the enzyme active sites, which indirectly augment the reactive oxygen species (ROS). Malathion also contributes to other oxidative loads caused by metabolic destabilization and activation of lipid membranes. It is the latter accumulation of ROS that caused the high concentration of MDA in the toxicant group.

The recovery of the activity of antioxidant enzymes of fish exposed to selenium demonstrates the biochemical significance of selenium as a cofactor in selenoproteins, namely, glutathione peroxidase. Selenium inhibits lipid peroxidation and maintains membrane integrity by enhancing peroxide detoxification. This is in line with other previous ecotoxicological studies that have determined that selenium supplementation can be used in restoration of redox homeostasis of heavy metal stress. The same outcomes are also extended by the existing data that indicates protection against a combination of metal and pesticides simultaneously which is closer to the actual exposure in the environment.

5.2 Histopathological Protection and Tissue Integrity

The histopathological analysis showed that there was widespread hepatic and renal injury after exposure to toxicants with necrosis, vacuolation, and tubular degeneration. These lesions indicate the inability to detoxify and ionic regulation which is essential to fish survival. Liver is the main location of xenobiotic metabolism and thus it is particularly susceptible to oxidative damage. Equally, kidney damage impairs excretory and osmoregulatory balance.

The index of lesion severity was significantly lowered by selenium supplementation, which is a sign of tissue architecture preservation. This protective action is probably due to the reduction of lipid peroxidation and stabilization of cell membranes. The decreased inflammatory infiltration in selenium-treated fish also indicates that there was a change in immune responses. The biochemical data is supported by histological recovery, which proves that the antioxidant effect of selenium is reflected at the organ level in the form of structural protection.

5.3 Molecular Adaptation and Gene Regulation

Analysis of gene expression revealed that the toxicant decreased the gene antioxidant gpx and sod and increased the stress marker hsp70. The reduction of antioxidant transcripts shows that long-term oxidative stress is exhausting the defensive ability of the cell. Increase in the heat-shock proteins is an indication of activation of protective chaperone systems that seek to maintain protein integrity. The gpx and sod expression are restored, implying that cells are able to respond better to oxidative stress. The down-regulation of hsp70 indicates less cellular distress, as biochemical and histological evidence would suggest. These findings indicate that selenium is not only a chemical antioxidant, but it is also a regulator of gene expression during stress adaptation.

5.4 Integrated Ecotoxicological Significance

A combination of behavioral, biochemical, histopathological, and molecular evidence gives the whole picture of the effects of pollutants on organisms. The initial symptoms of fish exposure to toxicants are an indication of neurological and metabolic problems, which entail erratic swimming and difficulty in breathing. These symptoms can be observed and they signify the existence of biochemical problems and tissue destruction. The action of selenium as a protective agent is effective regardless of the level of toxicity, since it is a systemic agent. It does not work

on a particular pathway, but enhances antioxidant activity, cell structure, and adaptive gene changes. This broad defense is especially useful when living organisms are subjected to a large number of pollutant stressors.

5.5 Implications for Aquaculture and Environmental Management

In practice, the findings are important to the sustainability of aquaculture. The fish that develop in the contaminated waters are likely to experience sublethal stress that reduces growth, immunity and reproductive success. In moderate amounts, dietary selenium supplementation can be a nutritional intervention to enhance environmental pollutant resistance. Close proximity between selenium deficiency and toxicity necessitates special formulation and monitoring. On the ecological level, the paper highlights the need of integrated pollution management. Even though biological damage can be mitigated by nutritional mitigation, there is still a necessity to regulate industrial and agricultural effluents. Selenium supplementation should be considered as the auxiliary means of a larger mechanism of environmental control and water quality security.

5.6 Limitations and Future Directions

Although the current research has demonstrated the protective effects that are evident, there are several limitations that need to be considered. The exposure was subchronic; further studies should be conducted over a long period to determine the effects on reproduction and generation. In addition, selenium dose was only tested once. The future research should focus on dose response relationships and comparison of organic and inorganic forms of selenium including nanoparticles formulations that may increase bioavailability. The proteomic and the metabolomic analyses would also give additional pathways that are influenced by selenium. The information on the interactions between micronutrients and mixed pollutants will be crucial in the development of effective ecological interventions.

6. Conclusion

The current study proposes that exposure to cadmium and malathion in combination has quantitative effects in the induction of severe oxidative and tissue damages in *Labeo rohita*. The 62% lipid peroxidation (MDA levels) increment of fish toxicants was compared to 41% decrease of SOD, 38% decrease of CAT and 45% decrease of GPx activity in control. The evidence of the drastic disturbance of the redox balance in the cell is the fact that the low concentrations of glutathione decreased by almost 36%. Histopathological scoring showed that the size of lesions was 3-4 times more in liver and kidney tissue with hepatocellular necrosis, lifting of epithelial in gills, and tubular degeneration. Molecular analysis also supported these results because the expression of hsp70 was 2.8-fold, which is an indicator of high cellular stress, and antioxidant genes were abnormally suppressed in the toxicant group. These toxic effects were reversed strongly by dietary selenium supplementation. The antioxidant enzyme activity of fish fed on selenium was 48-55% recovery and the MDA levels decreased by about 40% compared to the toxicant group. The GSH levels were restored up to approximately 90% of control levels and this suggests that thiol-based defense systems were restored. The histological analysis revealed that tissue repair was high, lesion scores were over 50 and the structure of the hepatocytes was preserved. Selenium at the molecular level equalized the expression of the genes: gpx and sod transcripts rose 1.6-2.1 times and the stress-sensitive hsp70 fell to stabilize levels, which was a rise in cell resistance. The other functional protection evidence was in the improvement of the survival rates of toxicant-only fish and selenium supplemented group of 76 and 93 respectively. The experimental results affirm that selenium is a good biochemical and molecular protective factor to stress of compound pollutants. It is concluded that selenium has a quantifiable protective threshold beyond which redox homeostasis, a minimum level of structural damage and stabilized stress gene signaling are restored.

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