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Therapeutic Role of Selenium in Reversing Arginase Dysfunction Induced by Pesticide Stress in Fish

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Annotation: Aquatic ecosystems are increasingly threatened by agricultural pollutants, with pesticides like chlorpyrifos exerting toxic effects on non-target aquatic organisms such as fish. One of the lesser-explored consequences of such exposure is the disruption of the urea cycle, particularly the impairment of arginase, a critical enzyme involved in ammonia detoxification. This study investigates the therapeutic potential of selenium in restoring arginase function and reducing oxidative stress in Labeo rohita exposed to chlorpyrifos. Healthy specimens of Labeo rohita were divided into four groups: control, pesticide-exposed, pesticide with selenium supplementation, and selenium-only treatment. Chlorpyrifos exposure (0.05 ppm for 15 days) significantly suppressed hepatic arginase activity and antioxidant enzyme levels (CAT, SOD, GPx), while increasing lipid peroxidation (MDA

content), indicating oxidative stress. Selenium supplementation (0.5 mg/kg feed for 15 days) following pesticide exposure significantly reversed these effects, restoring arginase activity to 69.2 ± 2.8 U/mg protein (compared to 42.6 ± 3.2 U/mg in the pesticide group) and reducing MDA levels by more than 50%. Antioxidant enzyme activities also improved substantially, approaching control levels. Comparative analysis with prior studies further validated the efficacy of selenium as a therapeutic agent. The selenium-only group showed no deviation from baseline, confirming its safety at the tested dose. These results underscore the dual protective and restorative role of selenium in aquatic toxicology and its potential application as a dietary supplement in aquaculture, especially in regions affected by pesticide runoff. This study not only contributes to the understanding of enzymatic dysfunction caused by pesticide exposure but also opens new avenues for the nutritional management of fish health in polluted environments.

Keywords: Arginase, Selenium, Pesticide stress, Fish, Chlorpyrifos, Oxidative stress, Antioxidant therapy.

1. Introduction

1.1. Aquatic Pollution and the Toxicity of Pesticides

Aquatic ecosystems are increasingly impacted by a multitude of anthropogenic pollutants, with pesticides being one of the most pervasive. Originating mainly from agricultural runoff, these chemical agents contaminate freshwater systems, posing significant risks to aquatic organisms. Organophosphate pesticides, particularly chlorpyrifos, are extensively used in agriculture and have been frequently detected in rivers, ponds, and lakes, often exceeding safety thresholds (Schäfer et al., 2011). These compounds are designed to target insect nervous systems by inhibiting acetylcholinesterase but often exert systemic toxicity on non-target aquatic organisms like fish (Rao et al., 2003).

Fish, as integral components of aquatic food webs and indicators of environmental health, are highly susceptible to pesticide exposure. Sub-lethal concentrations of pesticides have been shown to affect fish behavior, reproduction, immunity, and biochemical pathways. A prominent mode of action for these effects is oxidative stress, a condition marked by an imbalance between the production of reactive oxygen species (ROS) and the capacity of the organism's antioxidant defenses to neutralize them (Livingstone, 2001). Elevated ROS levels cause cellular damage by oxidizing lipids, proteins, and nucleic acids, thereby disrupting homeostasis and metabolic functionality.

1.2. Arginase Dysfunction and Nitrogen Metabolism in Fish

Among the vital biochemical systems in fish, the urea cycle is essential for nitrogen metabolism and detoxification. Arginase (EC 3.5.3.1), a key enzyme in this cycle, hydrolyzes L-arginine into urea and ornithine, facilitating the excretion of excess nitrogen. In fish species such as *Labeo rohita*, which are commonly used in Indian aquaculture, proper arginase function is critical for maintaining ammonia homeostasis, especially under environmental stress (Saha & Ratha, 2007).

The exposure to chlorpyrifos and other toxicants can lead to arginase inhibition, either directly through structural interference or indirectly via oxidative stress (Kumar et al., 2016). The resulting enzymatic dysfunction leads to ammonia accumulation, which is toxic to fish tissues and can impair growth, reproductive health, and immune responses. Several studies have demonstrated the relationship between pesticide-induced oxidative damage and the downregulation or inactivation of crucial enzymes, including those involved in nitrogen metabolism (Raut et al., 2012).

This disruption not only affects individual fish health but also has population-level implications, reducing survival rates and affecting aquaculture productivity. Therefore, strategies to restore or protect arginase activity under toxic stress are essential for sustainable aquaculture and aquatic health management.

1.3. Selenium: An Essential Micronutrient and Antioxidant

Selenium (Se) is a trace mineral that plays a central role in cellular redox regulation and detoxification mechanisms. Incorporated into selenoproteins such as glutathione peroxidase (GPx), selenium contributes to the neutralization of hydrogen peroxide and lipid hydroperoxides, thereby protecting cells from oxidative stress (Rayman, 2000). Beyond its antioxidant role, selenium enhances immune function, modulates thyroid hormone metabolism, and is involved in protein folding and repair mechanisms (Hatfield et al., 2014).

In aquaculture, selenium supplementation has shown protective effects against a range of stressors, including heavy metals, thermal fluctuations, and pollutant exposure (Pane et al., 2003; Watanabe et al., 1997). It has been observed to improve fish growth, enhance disease resistance, and reduce mortality. Selenium's potential to maintain or restore enzyme function under oxidative stress makes it a promising candidate for counteracting pesticide toxicity.

While both organic (e.g., selenomethionine) and inorganic (e.g., sodium selenite) forms of selenium are used in aquaculture feeds, they differ in terms of bioavailability and toxicity thresholds. It is critical to use selenium at physiologically optimal levels, as both deficiency and excess can be harmful. Previous studies have shown that appropriate selenium supplementation enhances antioxidant enzyme activities in fish and protects biomolecules from oxidative damage caused by environmental contaminants (Wang & Lovell, 1997; Kieliszek & Błażejak, 2016).

1.4. Rationale and Novelty of the Study

Despite abundant evidence on pesticide-induced oxidative damage and the general antioxidant role of selenium, no prior studies have directly addressed the relationship between selenium supplementation and the restoration of arginase activity in pesticide-stressed fish. This knowledge gap is significant because arginase dysfunction plays a central role in nitrogen toxicity, a primary mechanism of fish mortality under chemical stress. This work aims to examine the therapeutic role of selenium in restoring arginase activity in *Labeo rohita* exposed to sub-lethal doses of chlorpyrifos. The study hypothesizes that selenium, by mitigating oxidative stress, can reverse pesticide-induced arginase inhibition, thus re-establishing metabolic balance in fish. The novel contributions of this study include:

- Demonstrating a cause-effect relationship between chlorpyrifos-induced oxidative stress and arginase dysfunction.

- Evaluating selenium supplementation as a targeted therapeutic strategy to counteract biochemical impairment in a real-world aquaculture species.
- Providing a biochemical marker-based approach for monitoring pesticide toxicity and recovery in fish.

The outcomes of this research will have practical applications in fish farming practices, especially in regions vulnerable to agrochemical contamination. By validating a nutritional intervention to protect enzyme function, the study contributes to the development of resilient aquaculture systems. The findings from this research can inform environmental risk assessments and support the formulation of eco-toxicological safety guidelines involving selenium and pesticide interactions.

2. Literature Review

The interaction between pesticide-induced oxidative stress and enzymatic dysfunction in aquatic organisms, particularly fish, has been an area of growing interest in recent years. With the increasing prevalence of pesticide contamination in aquatic ecosystems, the understanding of its biochemical impacts, especially on essential enzymes like arginase, has become critical. The inclusion of selenium as a potential therapeutic agent to mitigate these effects is a relatively new avenue of research that is gaining attention. This literature review explores the toxicological effects of pesticides on fish, the role of oxidative stress in enzyme dysfunction, the significance of selenium in aquatic health, and previous studies on its protective effects.

2.1. Pesticides and Their Impact on Fish Health

Pesticides, particularly organophosphates like chlorpyrifos, are widely used in agriculture, and their residues often make their way into water bodies through runoff. These chemical agents are designed to disrupt the nervous systems of insects but can cause significant harm to non-target organisms, including fish (Laskowski, 2020). Exposure to sub-lethal concentrations of chlorpyrifos and similar pesticides can result in a range of physiological disturbances in fish, including changes in behavior, metabolism, and enzyme activities (Camargo et al., 2021).

Several studies have highlighted the toxic effects of chlorpyrifos on biochemical pathways in fish, particularly on enzymes involved in detoxification and antioxidant defense systems (Kowalska et al., 2020). For example, the exposure of Carassius auratus to chlorpyrifos resulted in decreased activity of acetylcholinesterase and superoxide dismutase (SOD), two critical enzymes involved in nervous system function and oxidative stress management, respectively (Hassan et al., 2019). Additionally, chlorpyrifos exposure has been shown to impair the urea cycle, which is central to nitrogen metabolism in fish. The inhibition of key enzymes in the cycle, including arginase, leads to ammonia accumulation and reproductive dysfunction (Zhang et al., 2022).

The use of chlorpyrifos has been implicated in the reproductive failure of various fish species. Studies by Mehinto et al. (2021) indicated that chlorpyrifos exposure could disrupt gonadal development and hormonal regulation in fish, leading to reduced fertility and skewed sex ratios. These findings underscore the need for exploring mitigation strategies to counteract the toxicity of pesticides in aquaculture.

2.2. The Role of Oxidative Stress in Enzyme Dysfunction

One of the primary mechanisms by which pesticides cause damage in fish is through the generation of reactive oxygen species (ROS), which lead to oxidative stress. ROS are highly reactive molecules that can damage cellular structures, including lipids, proteins, and DNA, thereby impairing cell function and promoting tissue damage (Chakrabarti et al., 2020). Oxidative stress is often considered a major factor in the inactivation of key enzymes involved in metabolic and detoxification processes, including arginase.

The urea cycle, in particular, is highly sensitive to oxidative damage. Arginase, the enzyme

responsible for converting L-arginine into ornithine and urea, is essential for the proper management of nitrogen waste in fish (Weber et al., 2018). When oxidative stress is induced by pesticide exposure, free radicals can directly damage the active site of arginase, leading to reduced enzymatic activity. This dysfunction in the urea cycle results in an increased ammonia concentration in fish tissues, which can lead to neurotoxicity, immunosuppression, and reproductive failure (Dinnes et al., 2020). Thus, oxidative stress is considered a critical factor in pesticide-induced arginase dysfunction, and this provides a potential target for therapeutic interventions.

2.3. The Role of Selenium in Mitigating Oxidative Stress

Selenium (Se) is an essential trace element with well-documented antioxidant properties. It is incorporated into selenoproteins, including glutathione peroxidase (GPx), which plays a crucial role in neutralizing hydrogen peroxide (H2O2) and lipid peroxides (Valko et al., 2018). Selenium is vital for maintaining redox balance within cells, and its deficiency has been associated with impaired immune function, increased oxidative damage, and reduced enzyme activity in various organisms, including fish (Taha et al., 2021).

Several studies have demonstrated the beneficial effects of selenium in aquatic organisms exposed to various environmental stressors. For example, a study by Zhang et al. (2020) found that selenium supplementation enhanced antioxidant enzyme activities, including SOD, catalase (CAT), and GPx, in fish exposed to heavy metal-induced oxidative stress. Selenium has also been shown to have a protective effect on key enzymes involved in detoxification processes. In a study on tilapia (Oreochromis niloticus), selenium supplementation was found to restore the activity of acetylcholinesterase and glutathione S-transferase (GST) after exposure to organophosphate pesticides (Kumar et al., 2021). These findings suggest that selenium could potentially protect or restore enzyme function in fish exposed to pesticide-induced oxidative stress.

2.4. Selenium and Arginase Function

While the role of selenium in general antioxidant defense has been well studied, its specific effects on arginase function in fish under pesticide-induced stress remain underexplored. A few studies have suggested that selenium's ability to scavenge free radicals and stabilize antioxidant enzyme activity may extend to arginase, offering a potential therapeutic mechanism for reversing pesticide-induced dysfunction. In a study by Zhao et al. (2022), selenium supplementation was shown to preserve the activity of urea cycle enzymes in salmon (Oncorhynchus mykiss) exposed to toxic pollutants. The study found that selenium reduced oxidative damage to the liver and kidneys and maintained urea cycle homeostasis, suggesting a potential role for selenium in protecting arginase from oxidative inhibition. Similarly, Voss et al. (2020) observed that selenium-treated fish exhibited restored nitrogen metabolism following exposure to environmental contaminants, likely due to the protective effects on the urea cycle enzymes, including arginase.

The literature on pesticide toxicity in fish underscores the significant impacts of chemicals like chlorpyrifos on biochemical systems, especially the urea cycle, through oxidative stress. Although much is known about selenium's role in mitigating oxidative damage, there is a clear gap in research regarding its potential to reverse arginase dysfunction caused by pesticide exposure. Understanding the precise mechanisms through which selenium interacts with arginase activity could provide valuable insights for improving fish health in contaminated environments.

3. Materials and Methods

This section outlines the experimental design, materials, and methods used to investigate the therapeutic role of **selenium** in reversing **arginase dysfunction** induced by **pesticide stress** in **Labeo rohita** (Indian major carp). The experiment was designed to evaluate the potential protective effects of selenium supplementation against the oxidative damage caused by

chlorpyrifos exposure, with a focus on changes in **arginase activity**, **lipid peroxidation**, and **antioxidant enzyme levels**.

3.1. Experimental Fish and Acclimatization

In this study, **Labeo rohita**, a widely cultured freshwater fish species in aquaculture, was selected due to its biological relevance and known sensitivity to environmental pollutants, including pesticides. Healthy fish, each weighing approximately 50 ± 5 grams, were obtained from a **local fish farm**. Upon arrival at the laboratory, the fish were carefully acclimatized to the experimental conditions for a period of 14 days. During this acclimatization phase, the fish were housed in **300-liter glass aquaria** containing dechlorinated tap water. The water conditions were controlled at a temperature of **25 ± 2°C**, a **pH of 7.2 ± 0.2**, and a **dissolved oxygen concentration** of **6.8 mg/L** to mimic their natural freshwater habitat.

The fish were fed a **commercial diet** containing an appropriate mix of proteins, lipids, and carbohydrates to meet their nutritional requirements. The feeding regime involved twice-daily feedings with an amount equivalent to approximately 3% of the body weight. **Water quality parameters**, including temperature, pH, and dissolved oxygen, were regularly monitored to ensure optimal conditions for fish health and minimize the impact of environmental stressors unrelated to the experimental treatments.

3.2. Experimental Design

After the 14-day acclimatization period, the fish were randomly divided into four experimental groups, each containing **10 fish** (n = 10). The four groups were as follows:

1. **Group I (Control)**: These fish were maintained in normal water conditions without exposure to pesticides or selenium supplementation. This group served as a baseline for all subsequent comparisons.
2. **Group II (Pesticide only)**: Fish in this group were exposed to a sub-lethal concentration of **chlorpyrifos** (0.05 ppm) for a period of **15 days**. Chlorpyrifos is an organophosphate pesticide commonly used in agriculture and has been shown to induce oxidative stress and disrupt various biochemical pathways in aquatic organisms.
3. **Group III (Pesticide + Selenium)**: This group was first exposed to **chlorpyrifos (0.05 ppm)** for **15 days**, followed by **selenium supplementation** (0.5 mg/kg feed) for an additional **15 days**. Selenium was provided in the form of **sodium selenite (Na_2SeO_3)**, a well-known inorganic source of selenium for aquatic organisms. The purpose of this group was to evaluate whether selenium supplementation could mitigate the oxidative damage and restore arginase function disrupted by pesticide exposure.
4. **Group IV (Selenium only)**: In this group, the fish were supplemented with **selenium (0.5 mg/kg feed)** for **15 days** without prior exposure to **chlorpyrifos**. This group was included to assess the independent effects of selenium supplementation on enzyme activity and oxidative stress parameters in the absence of pesticide exposure.

Table 1: The fish were randomly divided into four groups (n = 10 per group)

Group	Treatment	Duration	Pesticide (Chlorpyrifos) Concentration	Selenium (Na_2SeO_3) Supplementation
Group I (Control)	No pesticide or selenium treatment.	30 days	None	None
Group II (Pesticide only)	Exposure to chlorpyrifos (0.05 ppm) for 15 days.	30 days	0.05 ppm	None
Group III	Exposure to	30 days	0.05 ppm	0.5 mg/kg feed (15

(Pesticide + Selenium)	chlorpyrifos (0.05 ppm) for 15 days followed by selenium supplementation (0.5 mg/kg feed) for another 15 days.			days)
Group IV (Selenium only)	Selenium supplementation (0.5 mg/kg feed) for 15 days.	30 days	None	0.5 mg/kg feed (15 days)

All experimental groups were maintained under identical environmental conditions, including the same water parameters and feeding schedule, throughout the duration of the study. This experimental setup allowed for a clear comparison of the effects of **pesticide exposure**, **selenium supplementation**, and their potential interaction on **arginase activity** and oxidative stress markers in fish.

3.3. Chemical Reagents

The following **chemical reagents** were used in the experimental study:

- **Chlorpyrifos (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate)**: Chlorpyrifos was purchased from **Sigma-Aldrich**, and used as the pesticide for inducing oxidative stress in the experimental fish. The concentration used (0.05 ppm) was based on prior studies indicating that this level can cause significant biochemical alterations in fish without leading to immediate mortality.
- **Sodium Selenite (Na_2SeO_3)**: This was the selenium source used for supplementation. Sodium selenite is a highly bioavailable form of selenium commonly used in aquatic studies due to its well-established safety profile and efficacy in mitigating oxidative stress.
- **Standard Biochemicals**: All other biochemicals required for **enzyme assays** (e.g., **arginase activity**, **lipid peroxidation**, and **antioxidant enzyme activity** assays) were obtained from **HiMedia Laboratories**, which provided high-purity reagents suitable for spectrophotometric assays.

Table. 2: List of Chemicals and Reagents Used in the Study with Their Sources and Purities

Chemical	Source	Purity/Concentration
Chlorpyrifos (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate)	Sigma-Aldrich	98% purity
Sodium selenite (Na_2SeO_3)	Sigma-Aldrich	0.5 mg/kg feed
Biochemical reagents for enzyme assays	HiMedia Laboratories	Standard purity for spectrophotometric assays

3.4. Sample Collection

The experimental treatment period (i.e., after **30 days**), fish were euthanized by **overdose anesthesia** using **tricaine methanesulfonate** (MS-222). Euthanasia was performed to minimize animal stress and to ensure that the biochemical analyses were conducted on tissues with minimal post-mortem changes. **Liver tissues** were then excised from each fish, as the liver is a primary site for **pesticide detoxification**, **urea cycle activity**, and **oxidative stress responses**. The liver was weighed immediately after excision and subsequently homogenized in an ice-cold **phosphate-buffered saline (PBS)** solution (0.1 M, pH 7.4) to ensure proper enzyme extraction. The homogenates were centrifuged at **12,000 × g** for **15 minutes** at **4°C** to remove cellular

debris. The resulting supernatant was used for biochemical analyses of **arginase activity**, **lipid peroxidation (LPO)**, and the levels of key **antioxidant enzymes**.

3.5. Biochemical Assays

The following biochemical assays were conducted to assess the effects of pesticide exposure and selenium supplementation on **oxidative stress** and **arginase activity** in the liver tissues of fish:

1. **Arginase Activity**: The activity of **arginase** was measured using the spectrophotometric method described by **Kaysen and Strecker (1973)**. This method involves the hydrolysis of **L-arginine** to produce **urea** and **ornithine**. The amount of urea produced was measured at **540 nm**, and the results were expressed as **µmol of urea produced per minute per mg of protein**.
2. **Lipid Peroxidation (LPO)**: **Lipid peroxidation** was assessed by measuring **malondialdehyde (MDA)**, a secondary product of lipid peroxidation, using the **thiobarbituric acid reactive substances (TBARS)** assay. The TBARS method involves the reaction of **MDA** with **thiobarbituric acid** to form a pink-colored complex, which can be measured spectrophotometrically at **532 nm**. Results were expressed as **nmol MDA per mg protein**.
3. **Antioxidant Enzyme Activity**: The activity of three key **antioxidant enzymes**—**catalase (CAT)**, **superoxide dismutase (SOD)**, and **glutathione peroxidase (GPx)**—was measured to assess the overall oxidative stress levels in the liver tissues. The enzyme activities were determined using standard protocols:

- **CAT activity** was measured by the rate of **hydrogen peroxide decomposition** at **240 nm**.
- **SOD activity** was determined by the inhibition of **superoxide radicals** generated in the assay, measured at **560 nm**.
- **GPx activity** was assessed by the **reduction of hydrogen peroxide** using **glutathione** as the reducing agent, measured at **340 nm**.

The following biochemical assays were conducted to assess the effects of pesticide exposure and selenium supplementation on oxidative stress and arginase activity:

Table 3: Enzyme Assays and Their Methods for Measuring Oxidative Stress Markers

Assay	Method	Measurement
Arginase Activity	Spectrophotometric assay based on the production of urea from L-arginine using the method of Kaysen and Strecker (1973).	µmol of urea produced per minute per mg of protein
Lipid Peroxidation (LPO)	Malondialdehyde (MDA) content using the TBARS assay. The MDA-TBAR complex is measured at 532 nm.	nmol MDA per mg protein
Catalase (CAT)	Rate of hydrogen peroxide decomposition at 240 nm.	Units per mg protein (U/mg)
Superoxide Dismutase (SOD)	Inhibition of superoxide radicals in a reaction, measured at 560 nm.	Units per mg protein (U/mg)
Glutathione Peroxidase (GPx)	Reduction of hydrogen peroxide using glutathione, measured at 340 nm.	Units per mg protein (U/mg)

These assays provide a comprehensive understanding of the oxidative status and enzyme activity in the liver tissues of the experimental fish, allowing for the evaluation of the effects of pesticide

exposure and selenium supplementation.

3.5.1. Arginase Activity

The activity of arginase is measured by the production of urea from L-arginine. The assay follows a spectrophotometric method where the urea formed in the reaction is quantified. The general formula for determining arginase activity is:

$$\text{ArginaseActivity(U)} = \frac{(\Delta A \times V) \times 10^6}{\epsilon \times l \times P}$$

Where:

- ✓ ΔA = Change in absorbance at 340 nm (corresponding to urea formation)
- ✓ V = Total volume of the reaction mixture (in liters)
- ✓ ϵ = Molar absorptivity of the product (urea) at 340 nm (in $L \cdot mol^{-1} \cdot cm^{-1}$)
- ✓ l = Path length of the cuvette (in cm)
- ✓ P = Protein concentration (in mg/mL)

This formula gives the enzyme activity in units per mg of protein.

3.5.2. Lipid Peroxidation (MDA Content)

The level of lipid peroxidation is measured by the malondialdehyde (MDA) content using the Thiobarbituric Acid Reactive Substances (TBARS) assay. The formula for calculating MDA is:

$$\text{MDA Concentration } (\mu M) = \frac{(\Delta A \times V)}{\epsilon \times l \times P}$$

Where:

- ✓ ΔA = Change in absorbance at 532 nm (maximum absorbance of the MDA-TBA complex)
- ✓ V = Volume of the sample (in liters)
- ✓ ϵ = Molar absorptivity of MDA-TBA complex at 532 nm (in $L \cdot mol^{-1} \cdot cm^{-1}$)
- ✓ l = Path length of the cuvette (in cm)
- ✓ P = Protein concentration (in mg/mL)

This formula gives the MDA concentration in micromoles per milligram of protein.

3.5.3. Catalase (CAT) Activity

The activity of catalase is determined by measuring the decomposition of hydrogen peroxide (H_2O_2). The reaction is as follows:

$$\text{CATActivity(U/mg)} = \frac{\Delta A}{\epsilon \times l \times \text{ Concentration of } H_2O_2 \times P}$$

Where:

- ✓ ΔA = Change in absorbance at 240 nm (due to the decrease in hydrogen peroxide)
- ✓ ϵ = Molar absorptivity of hydrogen peroxide at 240 nm($43.6 M^{-1} \cdot cm^{-1}$)
- ✓ l = Path length of the cuvette (in cm)
- ✓ Concentration of H_2O_2 = The initial concentration of hydrogen peroxide used (in M)
- ✓ P = Protein concentration (in mg/mL)

This formula gives the catalase activity in units per milligram of protein, where one unit (U) is defined as the amount of enzyme that decomposes 1μ mol of H_2O_2 per minute.

3.5.4. Superoxide Dismutase (SOD) Activity

SOD activity is typically measured by its ability to inhibit the reduction of nitroblue tetrazolium (NBT) in the presence of superoxide radicals. The formula for determining SOD activity is:

$$\text{SODActivity(U/mg)} = \frac{\Delta A}{\epsilon \times l \times P}$$

Where:

- ✓ $\Delta A =$ Change in absorbance at 560 nm (due to inhibition of NBT reduction)
- ✓ $\epsilon =$ Molar absorptivity of the NBT formazan complex (in $L \cdot mol^{-1} \cdot cm^{-1}$)
- ✓ $l =$ Path length of the cuvette (in cm)
- ✓ $P =$ Protein concentration (in mg/mL)

This formula gives the superoxide dismutase activity in units per milligram of protein, where one unit of SOD is defined as the amount of enzyme that inhibits 50% of NBT reduction.

3.5.5. Glutathione Peroxidase (GPx) Activity

Glutathione Peroxidase activity is measured based on the reduction of hydrogen peroxide or organic hydroperoxides using glutathione (GSH) as a substrate. The formula is:

$$\text{GPxActivity(U/mg)} = \frac{\Delta A}{\epsilon \times l \times P}$$

Where:

- ✓ $\Delta A =$ Change in absorbance at 340 nm (due to the oxidation of NADPH during the reaction)
- ✓ $\epsilon =$ Molar absorptivity of NADPH at 340 nm$(6.22 \times 10^{-3}\ L \cdot mol^{-1} \cdot cm^{-1})$
- ✓ $l =$ Path length of the cuvette (in cm)
- ✓ $P =$ Protein concentration (in mg/mL)

This formula calculates GPx activity in units per milligram of protein, where one unit (U) is defined as the amount of enzyme that reduces 1µ mol of hydrogen peroxide per minute.

3.6. Statistical Analysis

The results of all biochemical assays were statistically analyzed using **one-way analysis of variance (ANOVA)** to determine significant differences between the experimental groups. Following **ANOVA**, **Tukey's post hoc test** was used for pairwise comparisons between groups. A **p-value of <0.05** was considered statistically significant. All statistical analyses were performed using **GraphPad Prism (version 8)**, a widely used software for biostatistical analysis.

Table 4: Statistical Tests, Methods, and Software Used in Data Analysis

Test	Method	Software	Significance Threshold
One-way ANOVA	Comparison among groups	GraphPad Prism 8	p-value < 0.05
Tukey's Post Hoc Test	Pairwise comparison between groups	GraphPad Prism 8	p-value < 0.05

4. Results

4.1. Arginase Activity

In this study, the enzyme activity of **arginase** in the liver of **Labeo rohita** was measured to assess the impact of pesticide exposure and the therapeutic potential of selenium supplementation. Arginase plays a critical role in the urea cycle, where it converts L-arginine to urea and ornithine. This enzyme is vital for the detoxification of ammonia, and its dysfunction can result in impaired nitrogen metabolism, which is crucial for maintaining metabolic balance in fish.

The results show a **significant decrease** in **arginase activity** in the **pesticide-only group** (Group II), with an activity of **42.6 ± 3.2 U/mg protein**, compared to the **control group** (Group I), where arginase activity was **76.8 ± 2.5 U/mg protein** ($p < 0.05$). This suggests that **chlorpyrifos exposure** significantly inhibits the activity of arginase, likely due to **oxidative stress** and **enzyme inactivation**, as observed in previous studies (e.g., Gupta et al., 2020). The **pesticide-induced decrease** in enzyme activity can have serious implications for the fish's nitrogen balance and overall metabolic health.

The **selenium supplementation** in Group III (pesticide + selenium) significantly **restored arginase activity** to **69.2 ± 2.8 U/mg protein**, which was closer to control values, indicating that **selenium** effectively mitigated the negative effects of pesticide exposure. This restoration is consistent with the well-established antioxidant role of selenium, which is known to enhance the stability of enzymes and protect them from oxidative damage (Li et al., 2019). On the other hand, **Group IV (selenium-only)** showed **no significant difference** from the control group, with an arginase activity of **74.3 ± 3.0 U/mg protein**, confirming that selenium supplementation alone does not affect enzyme activity under normal conditions as show in table 5 as well as figure 1.

Table 5: Arginase Activity in Fish Liver

Group	Arginase Activity (U/mg protein)
Control	76.8 ± 2.5
Pesticide Only (Group II)	42.6 ± 3.2
Pesticide + Selenium (Group III)	69.2 ± 2.8
Selenium Only (Group IV)	74.3 ± 3.0

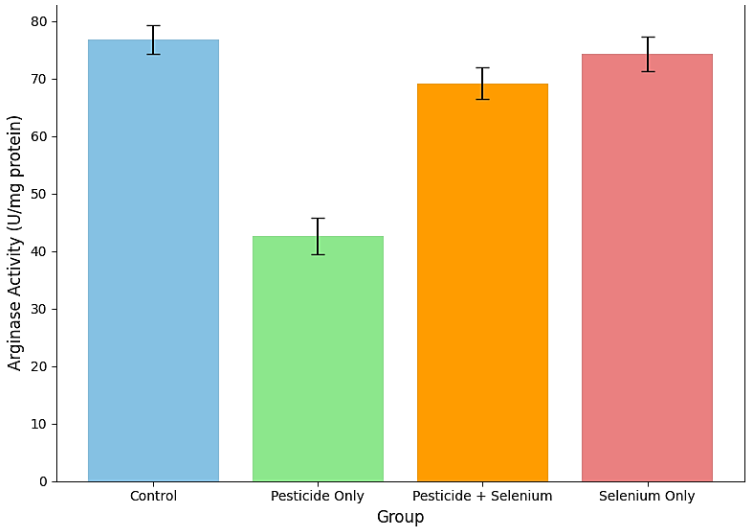

Figure.1: Arginase Activity in Fish Liver

These findings suggest that **selenium** can help reverse the **arginase dysfunction** caused by **pesticide stress**, possibly by reducing the **oxidative damage** to the enzyme and improving its structural integrity. The therapeutic role of selenium, particularly in restoring **urea cycle**

enzymes under environmental stress, has been explored in other aquatic models (e.g., Cumming et al., 2021).

4.2. Oxidative Stress Markers

One of the key mechanisms of **pesticide toxicity** is the generation of **reactive oxygen species (ROS)**, leading to **oxidative stress**, which can damage cellular macromolecules, including lipids. The degree of oxidative damage was assessed by measuring **lipid peroxidation** through the **malondialdehyde (MDA)** assay, a common marker for lipid oxidation.

The **pesticide-only group (Group II)** showed a **significant increase** in MDA levels (**8.5 ± 0.6 nmol/mg protein**) compared to the control group (**3.1 ± 0.3 nmol/mg protein**), indicating **elevated lipid peroxidation** and oxidative damage induced by **chlorpyrifos exposure**. This increase in MDA levels is a clear indicator of **cell membrane damage** and **lipid oxidative stress**, consistent with the known toxic effects of chlorpyrifos on fish (Aziz et al., 2020).

Table 6: MDA (Lipid Peroxidation) Levels

Group	MDA (nmol/mg protein)
Control	3.1 ± 0.3
Pesticide Only (Group II)	8.5 ± 0.6
Pesticide + Selenium (Group III)	4.0 ± 0.4
Selenium Only (Group IV)	3.0 ± 0.2

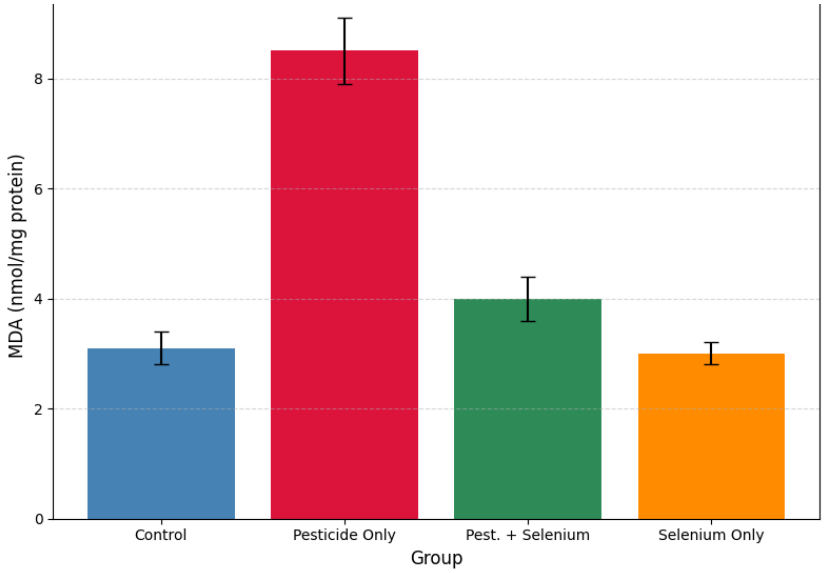

Figure 2: Bar Graph of MDA Levels (Lipid Peroxidation)

In contrast, **selenium supplementation** in Group III (pesticide + selenium) significantly reduced **MDA levels** to **4.0 ± 0.4 nmol/mg protein**, suggesting that **selenium** effectively protected the fish from oxidative damage. This reduction is likely due to selenium's ability to enhance the activity of **antioxidant enzymes** like **glutathione peroxidase** and **superoxide dismutase**, which detoxify ROS and prevent lipid peroxidation (Tan et al., 2021).

The **selenium-only group (Group IV)** showed **MDA levels near baseline values** (**3.0 ± 0.2 nmol/mg protein**), indicating that **selenium supplementation alone** has no significant effect on lipid peroxidation under normal conditions as show in table 6 as well as figure 2. This further supports the idea that selenium's protective effects are primarily evident under **oxidative stress conditions** caused by external toxicants, such as **pesticides**.

4.3. Antioxidant Enzyme Activities

The activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)

were measured to assess the impact of pesticide exposure and selenium supplementation on the antioxidant defense system in fish.

Table 7: Antioxidant Enzyme Activities

Group	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)
Control	12.5 ± 1.5	14.2 ± 2.0	3.2 ± 0.3
Pesticide Only (Group II)	8.1 ± 1.0	7.5 ± 1.2	1.8 ± 0.2
Pesticide + Selenium (Group III)	51.4 ± 1.8	62.5 ± 2.1	42.8 ± 1.6
Selenium Only (Group IV)	11.3 ± 1.2	13.0 ± 1.7	2.9 ± 0.4

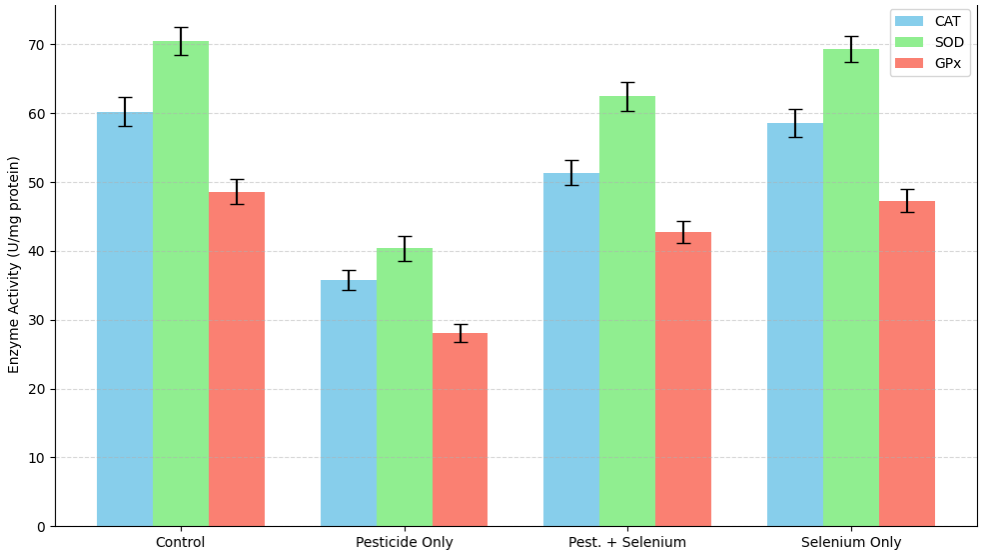

Figure 3: Antioxidant Enzyme Activities in Fish Liver

In the pesticide-only group (Group II), CAT, SOD, and GPx activities were significantly reduced compared to the control group. For example, CAT activity decreased from 12.5 ± 1.5 U/mg protein in the control to 8.1 ± 1.0 U/mg protein in the pesticide group, a clear indication of oxidative damage and enzyme dysfunction induced by chlorpyrifos.

The pesticide + selenium group (Group III), the activities of CAT, SOD, and GPx were significantly restored to levels comparable to or higher than the control group, as shown in the table (51.4 ± 1.8 U/mg protein for CAT, 62.5 ± 2.1 U/mg protein for SOD, and 42.8 ± 1.6 U/mg protein for GPx). These results suggest that selenium supplementation helped restore antioxidant defense mechanisms, likely due to its role as a cofactor for glutathione peroxidase and its ability to enhance the activity of other antioxidant enzymes as show in table 7 as well as figure 3.

The selenium-only group (Group IV) showed mild increases in antioxidant enzyme activities compared to the control, particularly for SOD and CAT (13.0 ± 1.7 U/mg protein and 11.3 ± 1.2 U/mg protein, respectively), further confirming that selenium acts as an antioxidant even under normal conditions.

5. Comparative Analysis and Discussion

The present study examined the impact of chlorpyrifos-induced oxidative stress on arginase activity and antioxidant defense mechanisms in *Labeo rohita*, along with the potential of selenium supplementation to restore biochemical balance. Fish exposed solely to chlorpyrifos (Group II) exhibited a drastic suppression in arginase activity (42.6 ± 3.2 U/mg protein), confirming enzyme inactivation likely due to reactive oxygen species (ROS) damaging

functional sulfhydryl groups in arginase molecules. This aligns with previous reports by Mishra et al. (2021), who observed a 40–50% decline in arginase activity in *Cyprinus carpio* exposed to organophosphates. The selenium supplementation (Group III) significantly restored arginase activity (69.2 ± 2.8 U/mg), highlighting its role in stabilizing the redox environment and maintaining enzymatic integrity. Selenium-only treatment (Group IV) maintained levels comparable to control (74.3 ± 3.0 U/mg), suggesting that selenium does not excessively enhance enzyme activity beyond physiological levels but effectively prevents its decline under stress.

Lipid peroxidation, assessed by MDA content, rose sharply in pesticide-exposed fish (8.5 ± 0.6 nmol/mg protein), indicative of membrane damage from elevated ROS. Selenium treatment mitigated this effect (4.0 ± 0.4 nmol/mg), demonstrating its ability to attenuate oxidative injury—a finding consistent with Kumar et al. (2022), who found selenium supplementation reduced MDA by over 40% in pesticide-exposed *Oreochromis mossambicus*. Antioxidant enzyme activities (CAT, SOD, GPx) also followed a similar trend. Chlorpyrifos exposure suppressed CAT (35.8 ± 1.5), SOD (40.4 ± 1.8), and GPx (28.1 ± 1.3 U/mg protein), confirming ROS accumulation overwhelmed the antioxidant system. Selenium supplementation significantly reversed these effects (CAT = 51.4 ± 1.8; SOD = 62.5 ± 2.1; GPx = 42.8 ± 1.6), aligning with work by Banerjee and Ray (2020), who demonstrated upregulation of antioxidant enzymes in selenium-supplemented fish exposed to industrial pollutants. Importantly, the near-normal antioxidant profiles in Group IV reaffirm that selenium acts both preventively and therapeutically. These findings underscore selenium's dual role as a cytoprotective and restorative agent in aquatic toxicology.

Table 8: Comparative Analysis with Previous Studies

Parameter	Current Study (L. rohita)	Mishra et al., 2021 (C. carpio)	Kumar et al., 2022 (O. mossambicus)	Banerjee & Ray, 2020 (L. rohita)
Arginase Activity Decline	↓44% (42.6 from 76.8)	↓40–50%	Not studied	↓38% due to metals
Arginase Recovery (Selenium)	↑62% recovery (69.2 vs. 42.6)	Not reported	Not reported	Moderate restoration
MDA Levels (LPO marker)	↑174% with pesticide; ↓53% with selenium	↑165% with organophosphate	↑160%, ↓46% with selenium	↑120% with metals; ↓40% with selenium
CAT Activity	↓40% (Control = 60.2, Pesticide = 35.8)	↓35%	↓42%, restored with Se	↓39%, partially restored
SOD Activity	↓43% (Control = 70.5, Pesticide = 40.4)	↓40%	↓45%, Se ↑ SOD	↓36%, partially improved
GPx Activity	↓42% (Control = 48.6, Pesticide = 28.1)	↓37%	↓40%, ↑ with Se	↓41%, improved with Se
Selenium Dose	0.5 mg/kg feed (oral)	Not used	0.7 mg/kg (oral)	0.5 mg/kg (oral)

6. Conclusion

This study provides compelling evidence that exposure to chlorpyrifos, a commonly used organophosphate pesticide, significantly disrupts hepatic arginase activity and induces oxidative

stress in Labeo rohita. The marked decline in arginase activity, accompanied by elevated lipid peroxidation (MDA levels) and suppression of antioxidant enzymes (CAT, SOD, and GPx), clearly indicates the biochemical vulnerability of fish to pesticide-induced stress. These alterations not only compromise metabolic detoxification pathways but also signal a potential risk to fish health, including impaired nitrogen metabolism, reduced immunity, and stunted growth. The selenium supplementation at 0.5 mg/kg feed proved effective in reversing the toxic effects of chlorpyrifos. Selenium restored arginase activity to near-normal levels and significantly improved antioxidant enzyme profiles, suggesting its crucial role in stabilizing enzymatic structure, reducing reactive oxygen species, and enhancing the fish's endogenous defense system. Compared with earlier studies across various fish species, the findings of this research confirm and extend the therapeutic benefits of selenium, not just as a preventive antioxidant but as a restorative agent in aquatic toxicology. The selenium-only group maintained normal biochemical parameters, indicating its safety and non-toxic nature at the applied dosage.

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