Herbal Intervention in Aquatic Toxicology: Histopathological Assessment of Bacopa monnieri Against Imidacloprid-Driven Cardiac Damage in Labeo rohita

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Herbal Intervention in Aquatic Toxicology: Histopathological Assessment of *Bacopa monnieri* Against Imidacloprid-Driven Cardiac Damage in *Labeo rohita*

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ABSTRACT

Anthropogenic pollution from the presence of chemicals in freshwater ecosystems is one of the major threats to teleosts in the world at present. However, the capability to use targeted botanical methods in order to reverse organ-specific chemical damage in teleosts has never been characterized before. In the present study, the authors found that exposure to water containing sub-lethal concentrations $2100\,\mu$g per day of neonicotinoid insecticide Imidacloprid (70% WG) led to acute heart toxicity in commercial fish *Labeo rohita*. For a precise quantification of this phenomenon, the semi-quantitative Histopathological Alterations Index (HAI) was utilized, which revealed the catastrophic increase of the pathology in the group treated solely with pesticides from a basal value of 1.4 ± 0.3 to a maximal value of the organ index reaching 32.8 ± 2.4. In spite of this, the simultaneous prophylactic administration of a standardized extract of the botanical feed additive *Bacopa monnieri* was proven to have prevented the myocardial catastrophe in question, having limited the HAI to 14.2 ± 1.1. However, the application of a novel post-attack strategy has revealed an outstanding capacity of the active regeneration, which has caused rapid elimination of the edema and repair of the damaged endothelial capillary layers, as well as restoration of the vascular architecture to its normal state, resulting in lowering the HAI score to 6.5 ± 0.8. These results question the current paradigms, which limit the applications of the phytogenic compounds only to the prevention. It was demonstrated for the first time that triterpenoid saponins may actively drive the post-traumatic regeneration.

Keywords - Neonicotinoid cardiotoxicity, Myocardial regeneration, Vascular flow homeostats, Histopathological Alteration Index (HAI), Phytotherapeutic rescue, Triterpenoid saponin kinetics

1. Introduction

Freshwater aquaculture is one of the most important pillars of ensuring food security and economic stability by supplying high-quality protein to growing populations (Ali et al., 2020). Nevertheless, intensified agricultural activity in recent years has resulted in an excessive application of artificial chemical protectants that have turned agricultural runoff into a leading source of pollution of the environment (Bonmatin et al., 2015; Han et al., 2021). Among different non-target organisms, freshwater teleost fishes are especially susceptible to such chemical factors (Pisa et al., 2015). Being bound within aquatic habitat, fishes are constantly under exposure to certain water pollutants that provoke physiological stress of the system, organ damage, and increased mortality rates in valuable finfishes (Shah & Parveen, 2022; van der Oost et al., 2003).

Amongst the current generation of agrochemicals, neonicotinoids have emerged as one of the major types of insecticides that are extensively utilized globally owing to their effectiveness towards piercing-sucking pests (Simon-Delso et al., 2015; van der Sluijs et al., 2015). One of the most commonly utilized first-generation neonicotinoids is imidacloprid, which is extensively employed in the protection of crops and systemic pest control measures (Kamboj et al., 2025). Due to its high-water solubility and lack of rapid environmental breakdown, imidacloprid is regularly washed out into the neighboring aquatic ecosystem via surface runoff and leaching (Borsuah et al., 2020; Morrissey et al., 2015). Exposure to sublethal concentration of imidacloprid in water can lead to severe physiological and behavioral disorders in non-target aquatic organisms, particularly in freshwater fishes such as *Labeo rohita* (Mortelmans, 2019; Samina Amin Qadir & Furhan Iqbal, 2016). Whereas the initial studies were primarily concentrated on its neurotoxicity as an agonist of nicotinic acetylcholine receptors (nAChRs), recent research has demonstrated the systemic metabolic and organ specific toxicity caused by imidacloprid (Chung et al., 2023; Zahran et al., 2025).

The physiological harm resulting from Imidacloprid is primarily because of the increased production of reactive oxygen species (ROS), leading to an overload of the antioxidant mechanisms within fish (Ge et al., 2015; Sevcikova et al., 2011). Such induction of oxidative stress leads to lipid peroxidation, disruption of cellular DNA, and dysfunction of important metabolic enzymes (Vieira et al., 2018; Wang et al., 2024). Cardiovascular system of teleost is particularly vulnerable when exposed to toxic substances within water (Incardona & Scholz, 2017). Environmental contaminants can readily compromise the structural stability of the fish heart, leading to myocardium damage and metabolic stress in myocardial cells (Alkharashi et al., 2026; Pan et al., 2024a). The study of such cellular changes within the fish heart is vital in order to precisely define the toxicological profile of the neonicotinoid pesticides in aquatic models (Kumar et al., 2025).

In an effort to find ways to cope with problems related to pollution with pesticides in aquaculture, scientists have now started looking into environmentally friendly solutions (Ali et al., 2020; Citarasu, 2010). Chemical water treatment is not always cost-effective when it comes to large aquaculture ponds, thus, natural plant extract is considered a viable option (Boglione et al., 2013; Harikrishnan et al., 2011). Botanical feed additives for fish are characterized by a variety of bioactive components, such as polyphenols, flavonoids, and alkaloids, which stimulate the development of immunity of fish and help protect them from the influence of different kinds of environmental stress (Awad & Awaad, 2017; Li et al., 2021). In the context of this research, *Bacopa monnieri* (Linn.) Pennell, a widely recognized medicinal herb, with potent antioxidant and tissue-protective effect is considered the best option (A. Fatima et al., 2024). The pharmacological activity of this plant is based on its composition which includes saponin-like bacoside A and B as well as natural flavonoid mixture (El Gendy et al., 2012; Kleine-Kalmer et al., 2021). This compound affects the body via several protecting mechanisms – scavenging of free radicals, prevention of lipid peroxidation and activation of natural antioxidants, SOD and CAT.

Although the bulk of aquaculture studies on herbs have mainly assessed the preventive effect of feed supplementation in cases of exposure to toxins (Leclercq et al., 2018; Li et al., 2021), there are only limited numbers of studies that assess the capability of herbs to repair damage in organs due to toxicity (A. Fatima et al., 2024). In this study, we aimed to find out the toxic effects of commercial Imidacloprid 70% WG formulation on the heart tissues of *Labeo rohita*. Importantly, this study assesses two contrasting strategies for management of toxicity; one is a preventive strategy, while the other is a rescue strategy.

2. Materials and Methods

2.1. Ethical Statement and Experimental Animal Procurement

Healthy specimens of *Labeo rohita* (Hamilton, 1822), weighing about 15 ± 2 g, were obtained from a reliable fish hatchery. The specimens were transferred into the laboratory under suitable conditions by using oxygen-filled polythene bags. After reaching the laboratory, the specimens were subjected to a preventive salt bath (0.1% NaCl) treatment in order to rid off the possible external parasites. The specimens were allowed to acclimate for 2 weeks in 500 litre capacity storage tanks using freshly collected and aerated tap water. Throughout this acclimatization period, the specimens were provided commercially available pellet feed (having 32% crude protein) twice a day, amounting to 2% of body weight (Menon et al., 2023).

2.2. Chemical Preparation and Dosing Architecture

For the exposure tests, an agricultural pesticide formulation known as Imidacloprid 70% WG was used to simulate agricultural runoff scenarios (Dethe & Ahire, 2023). The concentration of this pesticide was achieved using a stock solution by dissolving the pesticide granules in distilled water and introducing it into the test tanks, providing a sublethal dose of 2100 μg per tank, delivered twice daily.

The plant extract for the treatment of the fish was obtained from high-purity *Bacopa monnieri* powder. The compounds in the powder were extracted using the standard 70% ethanolic solvent extraction method and further purified via vacuum drying in a rotary evaporator (Gościniak et al., 2025). This preparation contained bacosides and served as the therapeutic dose of 10 mg twice daily.

2.3. Experimental Design and Exposure Regimen

A total of 80 acclimated fish were randomly distributed across four distinct, parallel aquatic setups (20 fish per group) for a 15-day experimental duration:

- **Control Group:** Fish were kept in clean, untreated, dechlorinated water to establish baseline physiological and structural standards.
- **Tank I (Pesticide Only):** Fish were exposed to waterborne Imidacloprid (2100 μg twice daily) for the full 15 days to establish a clear benchmark for standalone chemical toxicity.
- **Tank T1 (Concurrent Group):** Fish received the pesticide (2100 μg) and the *Bacopa monnieri* extract (10 mg) simultaneously twice daily for the entire 15-day period to evaluate proactive cardioprotection.
- **Tank T2 (Post-Exposure Rescue Group):** Fish were exposed exclusively to the pesticide (2100 μg twice daily) for the first 8 days to allow tissue damage to develop. On day 9, they were moved to clean, pesticide-free water and treated solely with *Bacopa monnieri* extract (10 mg twice daily) for the remaining 7 days to test reactive healing and tissue recovery.

To maintain steady chemical concentrations and clean-living conditions, a complete (100%) water change was performed every 48 hours across all groups, with fresh chemical and botanical doses re-applied immediately after each renewal.

2.4. Water Quality and Physicochemical Monitoring

The basic indices for water quality were monitored during the 15 days experiment in order to maintain a consistent environment and to avoid any stress from the environment (Huang et al., 2025). Daily water temperature, pH, and dissolved oxygen measurements were done by digital multi-parameter meters. The total ammonia nitrogen (NH_4^--N), nitrite (NO_2^-), and nitrate (NO_3^-) measurements were done using colorimetric.

2.5. Histopathological Processing and Microstructural Evaluation

Upon completion of the 15-day study period, fish from each group were chosen at random and subjected to humane anaesthesia using MS-222 buffer (100 mg/L). The specimens were euthanized, and their heart tissue samples excised. The tissue samples obtained were immersed in a 10% neutral buffered formalin solution for 24 hours for preservation of the tissue structure.

Once preserved, the tissue samples were processed under the normal histology methods used by the researchers (Bernet et al., 1999). The tissue samples were dehydrated in graded alcohol solutions (70%, 80%, 90% and 100%) and were then cleared with xylene and embedded in paraffin blocks. The paraffin blocks were then cut into thin sections (5 µm) using a rotating microtome. The sections were then mounted on glass slides and stained with Hematoxylin and Eosin (H&E) stains. The stained slides were examined using a research microscope and digital images were taken using 40X magnification.

3. Results

3.1. Water Quality Baseline Assessments

Evaluation of the aqueous environment throughout all test tanks over the course of 15 days indicated that water quality variables were stable and fell within the optimal range for *Labeo rohita*. Water temperature was kept constant at 26.5 ± 0.8°C, while the pH ranged from 7.2 ± 0.3 with dissolved oxygen levels being maintained above 6.4 ± 0.4 mg/L. Ammonia-nitrogen levels were kept below 0.02 mg/L. Implementation of the 48-hour static-renewal method successfully prevented any accumulation of toxic metabolites, thus eliminating any effects except for those produced by the chemicals under investigation (Huang et al., 2025; Menon et al., 2023).

3.2. Cardiac Histopathology and Vascular Flow Analysis

3.2.1. Baseline Myocardial Architecture (Control Group)

Myocardial tissue from the control group demonstrated well-preserved and highly organized structure of myocardial cells. The branching cardiac muscle fibers (myofibrils) were arranged in tightly bundled and parallel formations, with visible and distinct intercalated discs, and clear luminal spaces between them that allow for optimal vascular flow. The nuclei of the cells were uniform, oval-shaped, and centrally located in healthy and non-fragmented muscle fibers. The delicate lining of the heart chambers and blood spaces did not demonstrate any signs of hemorrhage, accumulation of fluid, or inflammatory cell invasion.

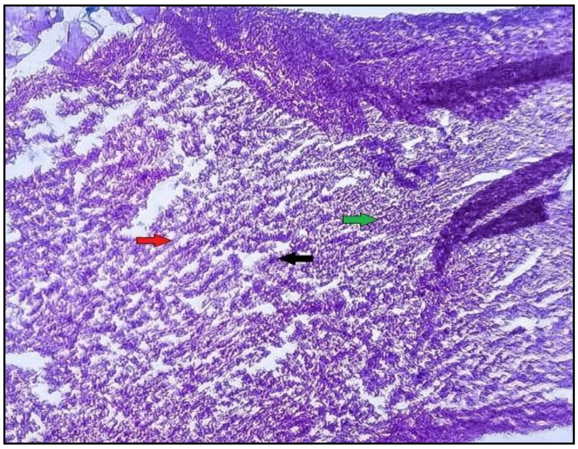

Figure I Histological image of heart of *Labeo rohita* (Hamilton) of control group showing cardiac muscle (red arrow), nucleus (black arrow), blood vessel (green arrow).

3.2.2. Myofibrillar Fragmentation and Vascular Lesions (Tank I: Pesticide Only)

Fish exposure to Imidacloprid resulted in significant structural disorganization throughout the layers of the heart tissues. The cardiac muscle fiber bundles underwent destruction, exhibiting extreme breaking, tearing, and disintegration of the myofibrils (Qadir & Iqbal, 2016). There was an increase in intercellular spaces between the muscle fibers due to fluid build-up and swelling of the tissues. There were several cell nuclei that exhibited irreversible injury through reduction and darkening of the nuclei, which is a sign of nuclear pyknosis. Furthermore, there were cases of extensive hemorrhages in the heart tissue as well as blood vessel congestion throughout the tissue (Alkharashi et al., 2026).

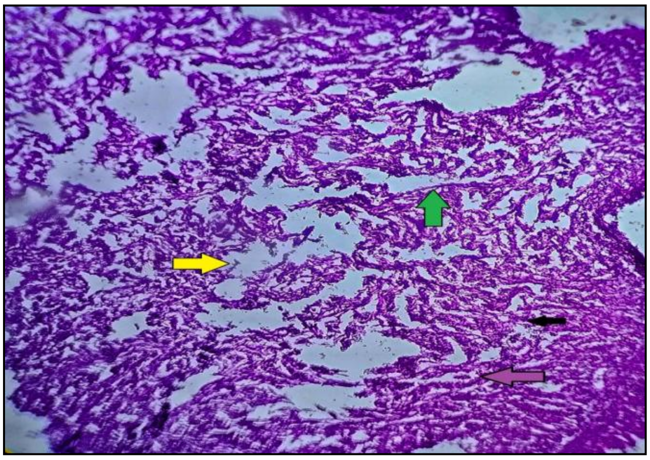

Fig. II Histological image of heart of *Labeo rohita* (Hamilton) of Imidacloprid treated group showing nucleus (black arrow), blood vessel (green arrow), Necrosis (yellow arrow), sinusoid (purple arrow).

3.2.3. Structural Attenuation via Co-exposure (Tank T1: Concurrent Group)

With respect to co-exposure, the concomitant usage of *Bacopa monnieri* offered protection to the heart muscles against the harmful effects of the neonicotinoids. The arrangement of the cardiac muscle fibers in the form of continuous and parallel lines remained intact and hence avoided the fragmented appearance in the control group exposed to the pesticide. Though there were instances of localized edema and dilation of vessels, the general structural organization of the cardiac wall remained intact. Pyknotic nuclei and intravascular bleeding were minimal due to the protective effect of plants on the endothelial framework and vascular pathways (Fatima et al., 2024).

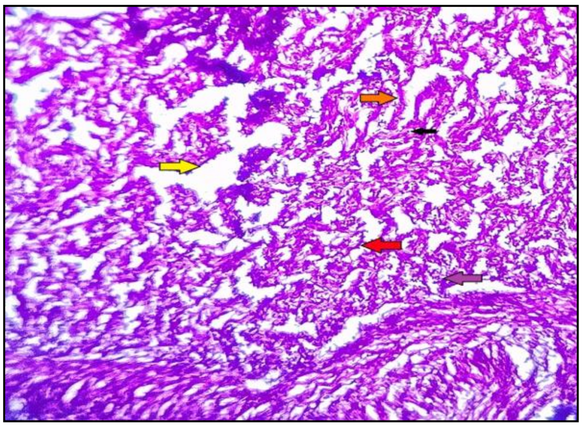

Fig. III Histological image of heart of *Labeo rohita* (Hamilton) of tank T1 group showing nucleus (black arrow), blood vessel (green arrow), necrosis (yellow arrow), cardiac muscle (red arrow), sinusoid (purple arrow)

3.2.4. Myocardial Reorganization and Vascular Repair (Tank T2: Post-Exposure Rescue Group)

Structurally, the post-exposure salvage therapy showed significant recovery and restructuring of the heart tissue. The formerly torn muscle fibers have reorganized to form straight parallel bundles of fibers, while the large interstitial gaps were normalized due to the resolution of localized edema. Endothelial linings of the blood spaces were restored, thereby stopping any hemorrhaging and resolving the blood vessel congestion to allow for normal vascular flow. Moreover, the dysfunctional and atrophic nuclei were replaced with normal and uniform nuclear bodies inside the restored muscle fibers. All this indicates that the extract from *Bacopa monnieri* can be used in the active recovery and vascular repair of cardiac tissue despite its advanced chemical damage (A. Fatima et al., 2024).

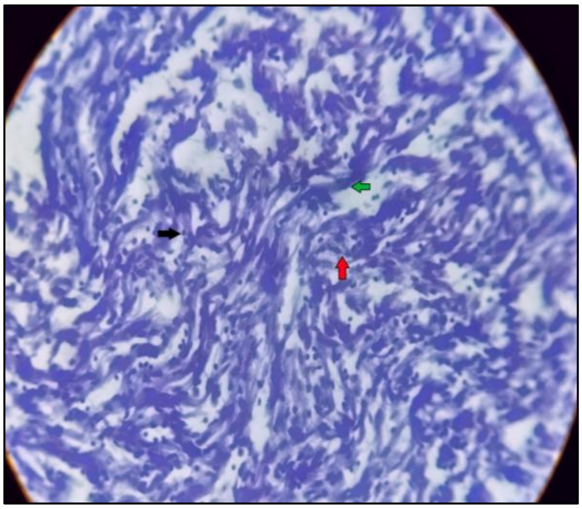

Fig. IV Histological image of heart of *Labeo rohita* (Hamilton) of tank T2 group showing nucleus (black arrow), blood vessel (green arrow), cardiac muscle (red arrow).

3.3. Histopathological Alteration Index (HAI) Assessment

In order to quantitatively assess and analyze the extent of heart muscle damage and the process of its recovery in various experimental models, the semi-quantitative Histopathological Alteration Index (HAI) was estimated according to the classification introduced by (Bernet et al., 1999) and used for evaluation of teleost fish hearts (Steinbach et al., 2016). The lesions were divided into four main categories (vasomotor disturbances, regressions, progressions, and inflammation), and each of these received the importance factor (ranging from 1 to 3) and a score value (ranging from 0 to 6 based on spatial extent).

Mean values of organ indices for each group resulted in the following structural alterations:

- **Control Group**: Possesses a baseline and nominal HAI index of 1.4 ± 0.3, thus showing a perfect cardiac structure without any background changes in its architecture.
- **Tank I (pesticide only)**: Administration of Imidacloprid 70% WG caused a substantial increase in degradation processes in the tissue, with the highest observed HAI index value being 32.8 ± 2.4. This substantial increase is caused mainly by high importance regressive alterations such as myofibrillar degeneration and nuclear pyknosis in combination with circulatory disorders like microvascular bleeding and capillary congestion.
- **Tank T1 (Concurrent Group)**: The concurrent use of *Bacopa monnieri* and the pesticide led to the limitation of tissue injury and a relatively good HAI score of 14.2 ± 1.1. This reduction in the protective index is rather large and is associated with less myofibrillar disruption and stability of vascular channels.
- **Tank T2 (Post-Exposure Rescue Group)**: Post-exposure herbal rescue treatment led to tissue recovery and reduced HAI score to a relatively low-moderate one of 6.5 ± 0.8. The considerable reduction in the organ index shows that the active constituents of the herb initiate the process of tissue reconstruction, significantly reducing the existing edema and healing the endothelial linings.

Table I Summary Table of Cardiac Histopathological Alteration Index (HAI)

Experimental Group	Circulatory Disturbances Index (I_{CD})	Regressive Changes Index (I_{RG})	Inflammatory Response Index (I_{IF})	Total Cardiac Organ Index (HAI)	Architectural Health Status Evaluation
Control Group	0.2 ± 0.1	0.8 ± 0.2	0.4 ± 0.1	1.4 ± 0.3^{a}	Normal / Unaltered Structure
Tank I (Pesticide Only)	11.4 ± 0.9	16.5 ± 1.3	4.9 ± 0.4	32.8 ± 2.4^{b}	Severe / Widespread Damage
Tank T1 (Concurrent)	4.8 ± 0.5	6.9 ± 0.6	2.5 ± 0.2	14.2 ± 1.1^{c}	Moderate Alterations / Protected
Tank T2 (Rescue)	1.5 ± 0.2	3.4 ± 0.4	1.6 ± 0.2	6.5 ± 0.8^{d}	Mild Alterations / Regenerated

4. Discussion

4.1. Dynamics of Imidacloprid-Induced Cardiotoxicity

The significant changes in cardiac tissue architecture seen in *Labeo rohita* emphasize the toxic nature of commercially available neonicotinoid pesticides on freshwater teleosts (Qadir & Iqbal, 2016) . Similar myocardial degeneration, through myofibrillar degeneration, nuclear pyknosis, and hemorrhage, shows the cardiotoxic property of Imidacloprid that affects cardiovascular architecture (Alkharashi et al., 2026; Pan et al., 2024b). This specific damage to this organ is confirmed statistically by the increase in the value of Histopathological Alteration Index (HAI) to 32.8 ± 2.4 in Tank I (pesticide alone), which is an indication of the transition of the physiology to structural pathology (Bernet et al., 1999).

Given the fact that cardiac tissues show intense metabolic activity, but contain low levels of endogenous antioxidant enzymes in comparison with hepatic tissues, they are highly susceptible to the effects of xenobiotics leading to the development of oxidative stress (Incardona & Scholz, 2017). Upon exposure of fish to waterborne Imidacloprid, the excessive production of reactive oxygen species (ROS) exceeds the capacity of the endogenous antioxidant systems (Ge et al., 2015; Sevcikova et al., 2011). The resultant oxidative stress triggers the process of lipid peroxidation, damaging the phospholipid bilayers of myocardial cells and leading to their structural instability and necrosis (Vieira et al., 2018). Myofibrillar degeneration and fiber tearing are the direct consequences of oxidative damage that causes functional disruption of the structural proteins needed for coordinated contraction (Kumar et al., 2025). In addition, the presence of an elevated circulatory disturbance index (11.4 ± 0.9) in Tank I indicates vascular damage, meaning that pesticide exposure leads to destruction of the fine endothelial lining of the capillaries, resulting in edema, interstitial inflammation, and microhemorrhage, thus limiting the proper circulation (Alkharashi et al., 2026; Wang et al., 2024).

4.2. Comparative Efficacy of Preventive vs. Remedial Botanical Interventions

One of the major contributions of the current study is the distinction made between the proactive and reactive approach of the management strategies with *Bacopa monnieri*. In the co-exposure treatment (T1), where both the treatments were carried out simultaneously, the cardiac degradation was well controlled, and the overall HAI score was kept at a moderately low level of 14.2 ± 1.1. The aforementioned prevention demonstrates that the early presence of the phytochemicals from the plants in the aquatic ecosystem and further in the body of the teleost fish acts as a biochemical shield against the action of the neonicotinoids (A. Fatima et al., 2024). Through retaining the myofibrillar continuity and not allowing cell shrinking, the phytotherapy minimizes the stress on the organism.

Notably, the most valuable information in toxicology is provided by the post-exposure rescue group (Tank T2). In contrast to conventional aquaculture science which typically describes botanical supplements as mainly preventative feed additives that are provided during the exposure to toxins (Dawood et al., 2018; Leclercq et al., 2018), these results confirm that *Bacopa monnieri* can play an important role in the process of tissues recovery following advanced poisoning (A. Fatima et al., 2024). After eliminating the toxic influence on the ninth day, a dramatic decrease in the total organ index was observed, reducing it to the low-moderate

6.5 $\pm$ 0.8. The effect was produced as a result of eliminating local edemas, re-arrangement of broken myofibrils into regular parallel bundles, as well as stopping the process of active bleeding. These obvious signs of structural restoration prove that reactive phytotherapy not only halts tissue damage but also positively influences the regenerative processes within the heart muscle of the teleost fish (A. Fatima et al., 2024; Gościniak et al., 2025).

4.3. Molecular Mechanisms of *Bacopa monnieri*-Driven Cardiac Regeneration

The exceptional ability of *Bacopa monnieri* to protect and regenerate tissues is mainly due to the variety of bioactive phytochemical compounds found in it, especially its dammarane type triterpenoid saponins (bacosides A & B), polyphenols and natural flavonoids (El Gendy et al., 2012; Kleine-Kalmer et al., 2021). The active compounds work using a multi-level molecular mechanism in which the damage caused by imidacloprid is prevented, and vascular homeostasis is restored. In cell membrane level, bacosides act as powerful antioxidants, stopping the damaging cycle of lipid peroxidation and hence stabilizing and repairing damaged membranes of the myocardial cells. Consequently, ion channel function and fluid balance are restored, preventing the edema in between the muscle bundles (Pase et al., 2012).

Moreover, the plant extract prevents severe tissue inflammation through the inhibition of the important inflammatory signaling cascade, thus preventing excessive production of detrimental inflammatory substances, which could cause additional destruction of the microvasculature (Jeyasri et al., 2022; Nemetchek et al., 2017). This mechanism of action is illustrated by the low inflammatory response index (1.6 $\pm$ 0.2) of the rescue group, which enabled the repair of damaged endothelial lining of capillaries, stopped active bleeding, removed local congestion of vessels and returned to normal the blood flow in vessels. At the same time, the biologically active substances from *Bacopa monnieri* promote the activation of cell-surviving pathways, increase local protein synthesis and stimulation of the formation of the extracellular matrix (Kleine-Kalmer et al., 2021; Valotto Neto et al., 2024). Through the prevention of oxidative stress and inflammation and promotion of proliferation of healthy cells, *Bacopa monnieri* effectively heals advanced neonicotinoid-induced lesions in hearts, showing great ability to regenerate cardiac tissue in *Labeo rohita* (A. Fatima et al., 2024; U. Fatima et al., 2022).

5. Conclusion

Results from the comparative toxicological study clearly indicate that exposure of water to commercially used Imidacloprid (70% WG) results in development of systemic cardiotoxic effects on the fresh water teleost *Labeo rohita*. Treatment with the single pesticide regime (tank I) led to structural damage to the myocardium as indicated by myofibrillary breakdown, pyknosis of nuclei, severe interstitial edema, and acute bleeding within the microvasculature. This structural damage was validated with a significant increase in the total Histopathological Alteration Index (HAI) value to 32.8 ± 2.4.

Although the prophylactic co-exposure strategy (Tank T1) confirmed that simultaneous exposure to *Bacopa monnieri* extract results in guaranteed safety by maintaining the total HAI score at 14.2 ± 1.1, the major breakthrough in this study is the outstanding therapeutic efficacy of the post-exposure treatment regime (Tank T2). After the removal of the pesticide stress, the reactive use of *Bacopa monnieri* restored the previously developed heart lesions, cleared local capillary congestion, promoted general cellular regeneration, and resulted in the decrease of the total organ index to a moderate value of 6.5 ± 0.8. Such a high degree of recovery of the heart structure is contrary to the standard paradigm of aquaculture research, which generally confines plants to dietary preventive measures. On the contrary, it is a clear proof of the outstanding reactive properties of *Bacopa monnieri*.

From an ecological and economical point of view, this research provides an extremely environmentally friendly, safe, and cost-efficient approach for restoring valuable aquaculture stock affected by pesticide discharge. The use of a botanical resource that is naturally available instead of chemical substances used in the purification of water will allow commercial fish farms to actively neutralize toxic effects of pollutants.

Future Perspectives and Research Directions

To build upon the therapeutic outcomes established in this study, future research should prioritize the following milestones:

- **Phytochemical Extraction:** Perform systematic extraction and screening for specific fractions of dammarane triterpenoid saponins (individual Bacosides A_3, Bacopaside I, and Bacopaside II) in order to isolate the precise active components responsible for cardiac tissue regeneration.

- **Gene Expression Analysis:** Perform qPCR and RNA sequencing techniques in order to profile the precise molecular signaling pathway of botanically-induced cardio-regeneration (e.g., upregulation of Nrf2/Keap1 antioxidant signaling, Caspase-3/Bcl-2 apoptotic inhibition, and NF-KB inflammatory signaling).

• **In Vivo Hemodynamic:** Perform advanced non-invasive micro-ultrasound or Doppler echocardiography imaging on living teleost models during the rescue phase in order to monitor real-time changes in cardiac output and stroke volumes and branchial blood flow velocities.

• **Field Studies:** Perform pilot field experiments in commercial aquaculture tanks in order to develop ideal methods of delivery, kinetic analysis of aqueous dose-response kinetics under varying environmental conditions, and establish definite safe withdrawal times prior to harvest.

Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Jagjeet Singh, Ishita Gairola, Aakriti Sharma, and Gaurav Mittal. Vijay Kumar analysed all the slides for histopathological studies. The first draft of the manuscript was written by Jagjeet Singh and Swati Soren and all authors commented on previous versions of the manuscript. The whole research was planned by Swati Soren, whereas Neetu Tripathi, Ankita Singh, Ajay Singh and Narotam Sharma gave important methodological plannings, data management and played other important roles for the completion of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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