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RESEARCH ARTICLE

HARNESSING HERBAL POTENTIAL: *Bacopa monnieri*AMELIORATES LIVER TOXICITY FROM IMIDACLOPRID 70% WG IN *Labeo rohita*

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Abstract

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Pesticide induced toxicity possesses significant risks to all organisms including aquatic organisms particularly in regions with extensive agriculture activities. This study offers valuable insights into the toxicological effects of Imidacloprid 70% WG a widely used neonicotinioid pesticide on the fresh water fish species *Labeo rohita*. The study also sheds light on the therapeutic potential of *Bacopa monnieri* a medicinal plant known for its antioxidant properties, hepatoprotective and anti-inflammatory properties against Imidacloprid induced toxicity in *Labeo rohita*. Histopathological analysis revealed severe hepatic damage in imidacloprid exposed fish including necrosis and structural degeneration of hepatic cells. However treatment with *Bacopa monnieri* extract significantly ameliorated these effects restoring normal hepatic architecture. The results obtained from this investigation provide compelling evidence of the duality of pesticide toxicity and natural plant based mitigation leading to several key conclusions about the risk associated with immediately and the potential of alternative therapeutic interventions.

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Introduction:-

Imidacloprid is one of the most common used neonicotinoid insecticide. It is a broad-spectrum neonicotinoid insecticide, with an excellent systemic and contact activity that supports its use on many food crops, turf, and ornamentals and for termite and flea control [1].Imidacloprid acts as a neurotoxin by binding to nicotinic acetylcholine receptors (nAChRs) in insects, leading to continuous stimulation of the nervous system, paralysis, and death [2]. Although designed for insect control, this mechanism also affects other non-target species, including aquatic organisms.

Imidacloprid possesses specific environmental fate characteristics with moderate water solubility, equal to 610mg/L at 20 °C, low octanol-water partition coefficient (log Kow 0.57) and low soil adsorption (log Koc 6719), it may sustain a high runoff potential from irrigation of crops and other fields, mainly reaching surface waters and ground waters [3].

The 70% WG formulation of Imidacloprid is a wettable granule that dissolves in water, releasing the active ingredient for uptake by plants or aquatic systems. This formulation enhances the efficacy of pesticide and allows for targeted application. However, its solubility and persistence in water bodies raise concerns about prolonged

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exposure for aquatic life [4]. Imidacloprid has a moderate to high persistence in the environment, with a half-life ranging from several days to several weeks, depending on environmental conditions such as temperature, pH, and microbial activity [5].

The toxicity of imidacloprid to fish varies among species, but common effects include behavioral abnormalities, physiological stress, and increased mortality rates. For instance, studies on ***Labeo rohita***, a commonly used model fish, have shown that exposure to imidacloprid leads to significant alterations in swimming behavior, reduced feeding activity, and increased vulnerability to predation [6].

The aim of the study was to identify the ameliorating potential of medicinal plant *Bacopa monnieri* over the hepatotoxicity caused in the freshwater teleost *Labeo rohita* as a result of impact of Imidacloprid 70% WG. ***Bacopa monnieri***, commonly known as Brahmi, is a medicinal plant used in traditional ayurvedic medicine for its cognitive-enhancing and neuroprotective properties. The plant contains a variety of bioactive compounds, including bacosides, which have been shown to exhibit antioxidant, anti-inflammatory, and neuroprotective effects [7]. Given its properties, ***Bacopa monnieri*** has potential applications in mitigating the toxic effects of environmental pollutants.

Bacopa monnieri contains a variety of bioactive compounds that contribute to its pharmacological effects. The primary constituents include:

- **Bacosides**: They are triterpenoidsaponins that have been shown to enhance cognitive function, reduce oxidative stress, and exhibit neuroprotective properties. Two main types of bacosides are Bacopasaponins A and B[8].
- **Alkaloids**: The plant contains alkaloids such as brahmine and herpestine, which are believed to contribute to its neuroprotective effects. Brahmine has been shown to have sedative and anxiolytic properties [9].
- **Flavonoids**: ***Bacopa monnieri*** also contains flavonoids like luteolin, apigenin, and quercetin. These compounds exhibit antioxidant and anti-inflammatory activities [10].
- **Saponins**: Besides bacosides, the plant contains other saponins which contribute to its medicinal properties, including potential antimicrobial and anti-inflammatory effects [11].
- **Phenylethanoids**: These include compounds like acteoside, which have shown potential neuroprotective and anti-inflammatory effects in preclinical studies [12].

It has been demonstrated that***Bacopa monnieri***enhances endogenous antioxidant defences and reduce oxidative damage in various models [13]. By neutralizing free radicals and reducing oxidative stress, ***Bacopa monnieri*** can potentially counteract the deleterious effects of imidacloprid on aquatic organisms.

Material & Method:-

Materials:-

Chemical chosen for the study is Imidacloprid 70% WG. To prepare a stock solution of Imidacloprid 70% WG (Wettable Granule), a systematic and precise approach is followed, beginning with the procurement of the insecticide from a reputable supplier. In this case, the Imidacloprid 70% WG was sourced from "KrishiRasayan Exports Pvt. Ltd.", New Delhi, India, a well-known supplier of agricultural chemicals.

The preparation process starts by accurately weighing 615 milligrams of the Imidacloprid 70% WG granules and mixing it in 1000 ml of distilled water. This specific amount is selected based on the desired final concentration for the stock solution. The granules, which are designed to dissolve in water, are carefully measured using a high-precision analytical balance to ensure accuracy [14,15].

Plant *Bacopa monnieri* was ordered online from the reputable website Gachwala.in, a supplier known for providing high-quality botanical products. The initial procurement involved obtaining 96 grams of live plant then air dying it for 48 hours, during which the moisture content of the plant was gradually reduced, minimizing the risk of degradation of its active compounds [16].

Then plant was oven dried at 60 degree Celsius for 30 hours ensuring the complete removal of residual moisture while preventing thermal degradation of the plant's bioactive compounds [17]. This temperature was selected based on previous studies that have demonstrated its effectiveness in preserving the pharmacological properties of *Bacopa monnieri* while achieving the desired dry weight [18]. The dried plant was then crushed using mortar-pestle to get it in powdered form of 12 grams for the treatment of infected fish.

The experimental animal *Labeo rohita* fingerlings used in this study were sourced from the Subarnarekha River, located in Jugsalai, Jamshedpur, Jharkhand, India. The selection of this particular river is significant due to its relatively pristine environment, which is crucial for ensuring that the fish were free from prior exposure to pollutants, which could otherwise confound experimental results [19].

Method:-

Experimental Design

Tanks C, I, and T were set after a 15-day acclimatization period, 3 Labeo rohita fingerlings were placed in each tank, where Imidacloprid (2100 µg) sub lethal dose and *Bacopa monnieri* extract (10 mg) remedial dose were used based on past studies. Tank C was not exposed. Fish in Tank I were fed Imidacloprid twice daily for 15 days. Tank T was administered with Imidacloprid for 15 days and *Bacopa monnieri* for 15 days. Replacement of fresh de-chlorinated water took place every 48 hours so that all tanks were kept under optimum water quality.

Histological Examination

Liver tissues were collected from each tank after dissection of the fish. Tissues were then fixed in Bouin's solution further following the dehydration through graded alcohols, clearing in xylene, and then embedded in paraffin. Microtome was used to get thin 5 µm sections which was then stained using H&E. The slides further studied under the microscope for histopathological evaluation and imaging.

This brief design was intended to assess the toxicological effects of imidacloprid under controlled conditions and the remedial effect of *Bacopa monnieri*.

Result:-

In the present investigation, *Labeo rohita*exhibited several histopathological alterations when exposed to imidacloprid compared to the controlled group. The alterations observed in the tissues of imidacloprid 70% WG treated fish were indicative of severe toxicity, including cell apoptosis, vacuolization, necrosis, and degeneration of hepatic tissues. These findings align with the known toxic effects of neonicotinoids, which disrupt cellular structures and metabolic functions, primarily through oxidative stress mechanisms. The toxicological impact of imidacloprid on *Labeo rohita* was profound affecting the liver, which plays a crucial role in detoxification.

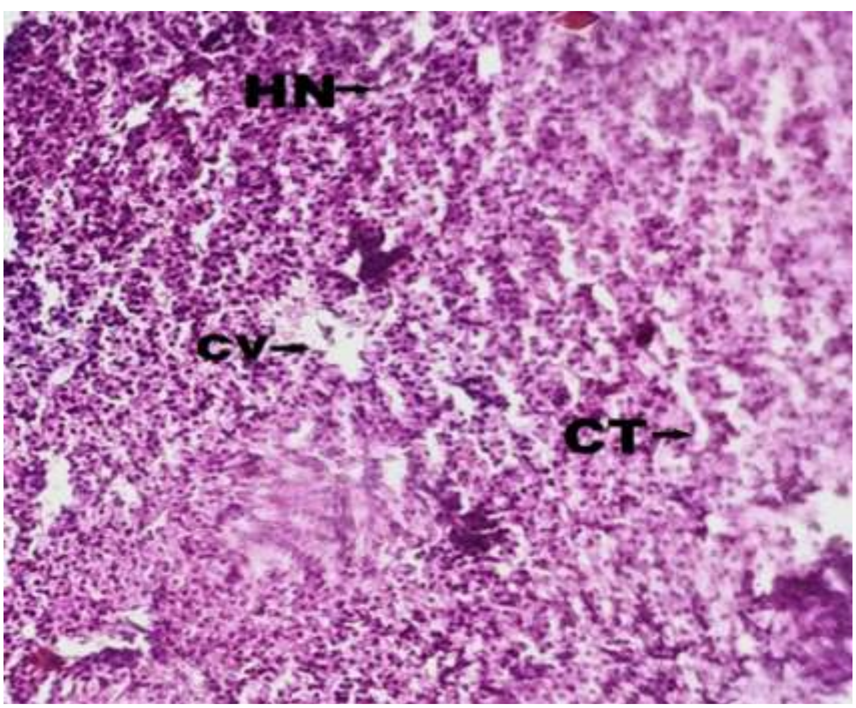

Fig I:- Photomicrograph Of fish (of Tank C) liver *Labeo rohita* (Hamilton) Showing Numerous Hepatocytes with Hepatic Nuclei, Central Vein & Connective Tissue (haematoxylin & eosin stain) **10x**

The liver tissue of the control group fish (Tank C) stained with Haematoxylin and Eosin (H&E) showed a well-preserved and healthy cellular architecture. The hepatocytes, the main cells of the liver, appeared normal, with round and distinct hepatic nuclei, indicating that the cells were functioning properly. These nuclei were centrally located and stained dark, a characteristic sign of their intact genetic material. The surrounding cytoplasm displayed a granular texture, reflecting the normal metabolic activity within the cells, with no signs of damage or disruption.

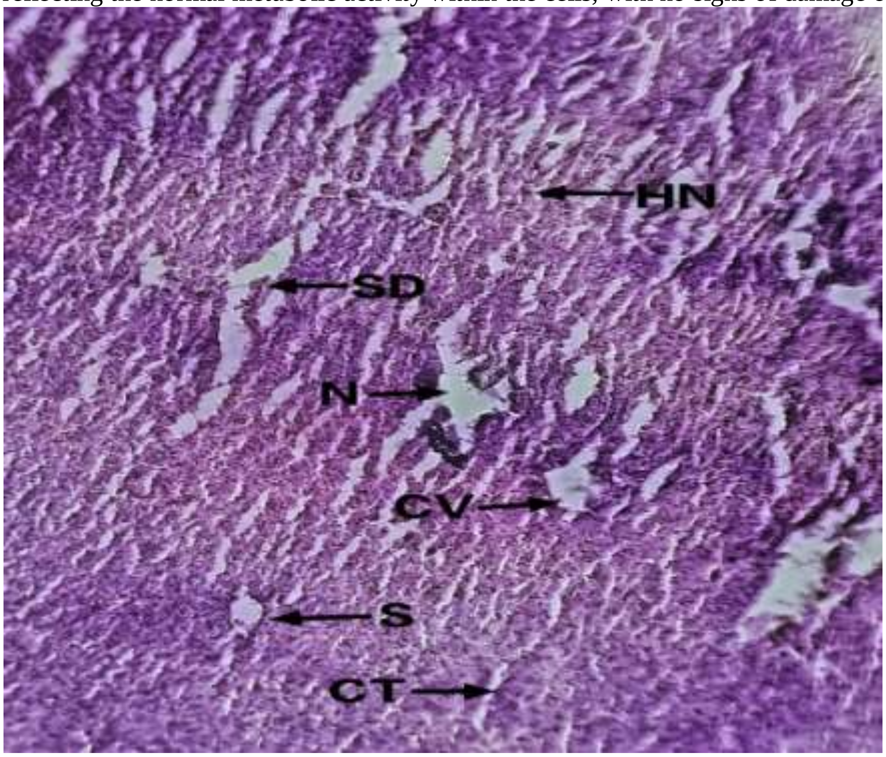

Fig II:-Photomicrograph Of imidacloprid treated Liver Of *Labeo rohita* (Hamilton) showing Loss of Hepatocytes, Vacuolation of Cytoplasm, Sinusoid Openings, Structural Degeneration, Necrosis, Lack Of Central Vein & Connective Tissue (haematoxylin & eosin stain) **10x**

The liver of the imidacloprid 70% WG treated fish showed vacuolation of the cytoplasm, which is often associated with metabolic disruptions caused by pesticide exposure. Apoptotic changes, characterized by condensed nuclei and fragmented cells, were prominent. Furthermore, necrosis was evident in various sections of the liver, with cells losing their integrity and undergoing premature death. These observations indicate that imidacloprid significantly impairs liver function, leading to metabolic imbalances and oxidative damage.

The hepatocytes in the *Bacopa monnieri*treated group displayed reduced vacuolization, with cells regaining much of their normal structure. The apoptotic and necrotic areas observed in the imidacloprid-exposed fish were significantly diminished, indicating that Bacopa monnieri has a protective effect on liver cells, facilitating tissue rejuvenation.

Discussion:-

The observation that we got in this project after the histopathological study of liver tissues of *Labeo rohita* with effects of pesticide imidacloprid 70% WG and protective effects of *Bacopamonnieri*, clearly shows that the pesticide has degenerative effects on the tissues. The tank where the treatment was done after infection clearly shows that the cells regenerate and the mitigative effects were clearly demonstrating that the plant's extract cures the harmful effects of Imidacloprid 70% WG.

Initially, pesticides enter through fish's skin, gills, and digestive systems, pass many biological membranes, and accumulate in tissues. Some pesticides are metabolized in the body and get eliminated with the help of body fluids such as urine. Pesticide accumulation causes neurophysiological and hematological damage, endocrine disruption, DNA damage, apoptosis, and lipid peroxidation, leading to oxidative stress [20].

Furthermore, cytochrome P450 enzymes, such as CYP3A4 and CYP2C19, can metabolize imidacloprid in hepatocytes. In the hepatocytes, hydroxylation of imidacloprid leads to the production of 5-hydroxy imidacloprid. Then, this metabolite may convert to 5-hydroxy imidacloprid-olefin, olefin-imidacloprid, and 5-hydroxyimidacloprid-urea. Finally, these metabolites may conjugate with glucuronic acid and sulfate and be excreted from the body [21].

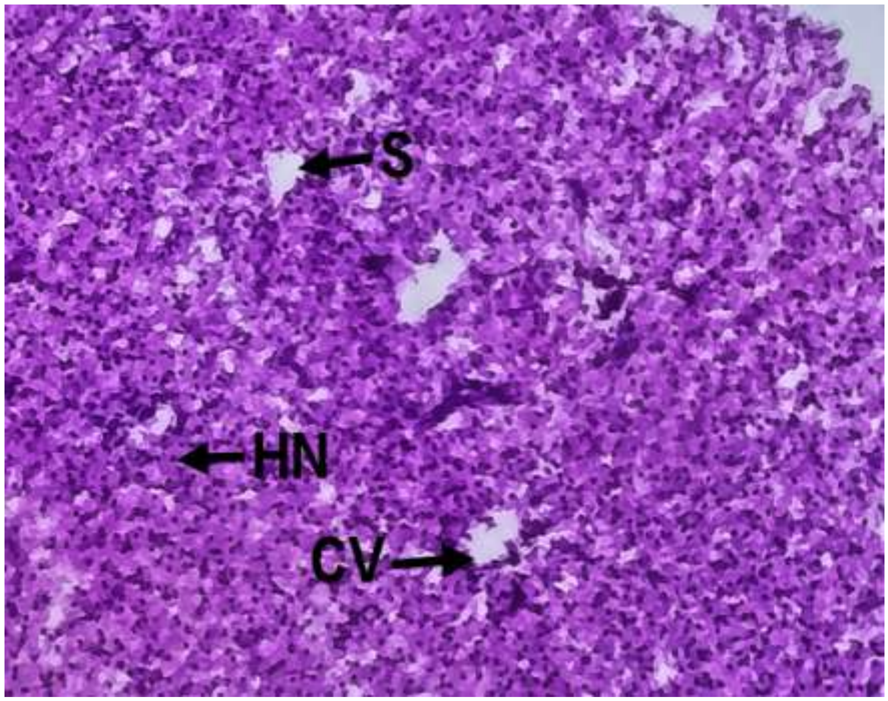

Fig III:- Photomicrograph Of Treatment with plant extraction after infection with insecticide Liver Of *Labeo rohita* (Hamilton) Showing Hepatic Nuclei, Hepatocytes, Sinusoid Openings, Necrosis, Central Vein & Connective Tissue (haematoxylin & eosin stain) **10x**

Imidacloprid-olefin, a major oxidative metabolite of imidacloprid, is highly toxic to aquatic organisms, particularly fish. In fish, it can disrupt physiological processes, leading to various toxicological effects. Imidacloprid-olefin increases the production of reactive oxygen species (ROS), which can overwhelm the antioxidant defenses in fish. This oxidative stress damages lipids, proteins, and DNA, leading to cell dysfunction or apoptosis.

The liver is the primary organ involved in detoxifying chemicals. In fish, hepatocytes metabolize imidacloprid-olefin, but excessive exposure can cause **hepatic injury**, disrupting liver function and causing elevated levels of liver enzymes such as **alanine aminotransferase (ALT)** and **aspartate aminotransferase (AST)**. These enzymes are markers of liver damage.

Several compounds in *Bacopa monnieri* contribute to its therapeutic effects on fish recovering from pesticide toxicity, including hepatic cell and cardiac tissue recovery. The active chemicals include bacosides, bacopasaponins, flavonoids, and alkaloid.

Bacosides (Bacoside A and B)

Bacosides are the primary active compounds in *Bacopa monnieri*. These compounds have been shown to improve antioxidant enzyme activity and reduce oxidative stress, which is critical for protecting and repairing hepatic cells after pesticide exposure.Bacosides protect liver cells by reducing lipid peroxidation, improving the levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). This can reverse damage caused by toxins such as imidacloprid [22].

Bacopasaponins

Bacopasaponins, a group of triterpenoidsaponins, have hepatoprotective and anti-inflammatory properties. These compounds help in cellular repair by promoting the regeneration of liver cells damaged by pesticide-induced oxidative stress.Bacopasaponins facilitate liver regeneration by improving detoxification processes and stabilizing cellular membranes, protecting hepatocytes from pesticide-induced toxicity [23].

Bacopa monnieri has been shown to reduce liver enzyme levels (AST, ALT, and ALP), indicating a protective effect on liver function after damage induced by toxicants like imidacloprid. The plant enhances detoxification by increasing glutathione levels and promoting the activity of detoxifying enzymes [24].

Conclusion:-

The comprehensive research presented in this study offers valuable insights into the toxicological effects of Imidacloprid 70% WG, a widely used neonicotinoid pesticide, on the freshwater fish species *Labeo rohita*. Additionally, the study sheds light on the therapeutic potential of *Bacopa monnieri*, a medicinal plant known for its antioxidant properties, in mitigating the damage caused by pesticide exposure. The findings underscore the profound impact of imidacloprid on aquatic organisms, illustrating the broader environmental and ecological risks posed by the widespread use of such chemical compounds.

In conclusion, the results of this study underscore the urgent need for more sustainable and environmentally friendly agricultural practices. The widespread use of imidacloprid and other neonicotinoid pesticides poses a significant threat to aquatic ecosystems and non-target organisms, and the findings presented here highlight the potential of natural alternatives, such as *Bacopa monnieri*, to mitigate these risks. By incorporating these alternatives into pest management strategies, it may be possible to reduce the environmental impact of pesticide use while still maintaining agricultural productivity.

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Conflict of Interest

There is no conflict of interest.

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