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Histopathological effect of the insecticide imidacloprid on the liver of *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae)

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Abstract

Neonicotinoids are widely used pesticides which interact with nicotinic acetylcholine receptors (nAChR) of the central nervous systems of the pest. Neonicotinoids became popular because of their high-water solubility, which makes their soil application travel through the entire plant. Imidacloprid belongs to the first generation of Neonicotinoids, widely use all over the world. Histopathology is a useful biomarker for environmental contamination. The presence of insecticide in the environment, due to extensive use in agriculture is of potential toxicological concern for fish. Monitoring histopathological changes can help for evaluation of pathological side effects of water-borne pollution and assessment of histopathological alteration of fish organs in response to organic trace pollution. In the present study, exposure of *Clarias gariepinus* to Imidacloprid in various sublethal concentrations resulted in structural alterations like irregular hepatocyte, hepatocellular cytoplasmic vacuolisation, distorted hepatocyte, infiltration of central cord, hypertrophied nucleus and disorganized tissue, extreme vacuolation, ruptured hepatocyte, nucleus hypertrophy, accumulation of pyknotic nuclei, cytoplasmic degeneration and increased cellular spaces, damaged liver tissue, necrotic areas, vacuolation of tissue, nuclear pleomorphism, cellular oedema and clumping of hepatocyte, cytoplasmic degeneration, lesions, sinusoid dilation, cellular atrophy leading to clumping, necrosis of hepatocyte. The effect was dose and exposure time dependent.

Keywords: *Clarias gariepinus*, imidacloprid, histopathological, liver, neonicotinoids

Introduction

The usage of synthetic organic pesticides started around 1940 and resulted in maximum production of quality crops. The World Health Organization (WHO) estimates that each year there are around one million acute poisoning by pesticide exposure, with a fatality rate of 0.4% and 1.9%. Pesticides cause serious health hazards to living systems because of their rapid fat solubility and bioaccumulation in non-target organisms. Even at low concentration, pesticides may exert several adverse effects, which could be monitored at biochemical, molecular or behavioural levels (Agrawal *et al*., 2010) [1].

The Neonicotinoids are widely used pesticides which interact with nicotinic acetylcholine receptors (nAChR) of the central nervous systems of insects. Neonicotinoids became popular because of their high-water solubility, which makes their soil application travel through the entire plant. The first generation of this pesticide class used was 1-(6-chloro-1, 3-thiazol- 5-ylmethyl)-1, 3, 5-oxadiazinan-4-ylidenene (nitro) amine, known as Imidacloprid (Natalia & Robert, 2016) [16]. Neonicotinoids can persist in the soil for years, so it may contaminate other plants and non-target species over the time. They contaminate water, soil, fish, and other living species (Huseth & Groves, 2014) [9]. Neonicotinoid insecticides comprise 27% of the global insecticide market and have been detected in wetlands and other aquatic habitats (Hrynyk *et al*., 2018) [8]. In 2013, the European union and a few non-EU countries restricted the use of certain neonicotinoids. In 2018, the EU banned the three main neonicotinoids (Coltianidin, Imidacloprid and Thiamethoxam) for all outdoor uses.

Monitoring histopathological changes can help for evaluation of pathological side effects of water-borne pollution and assessment of histopathological alteration of fish in response to organic trace pollution. It has provided information to bio monitoring plan designed for various aspects of environmental risk assessment (Kazempoor *et al.*, 2015) [11]. Both short term and long-term effects of these environmental stressors can be measured by histopathological studies. The liver carries out essential body functions including regulation of metabolism, synthesis of plasma proteins, energy storage, storage of certain vitamins and trace metals and transformation and excretion of steroids and detoxification of pollutants (Salamat & Zarie, 2012) [20]. Fish liver comes in close contact with toxic chemicals absorbed by the body from polluted water as it is the primary organ of metabolism and detoxification. This results in histopathological changes of liver which serves as a valuable tool for the detection of effect of these contaminants (Verma, *et al.*, 2022 b). The fish *Clarias gariepinus* (Burchell, 1822) has been regularly used to study the histopathological effect of different types of pesticides on

different types of tissue (Verma et al., 2022a, b). The present study records the histopathological changes in the liver of the catfish *Clarias gariepinus* (Burchell, 1822).

Material and Methods

Young *Clarias gariepinus* fishes (12-13 gm and 10-11 cm long) were purchased from the market and acclimatized under laboratory conditions for 15 days and later treated with Imidacloprid.

Chronic Toxicity measures long-term effects of exposure (typically 21-28 days). Sub lethal or safe level concentrations were derived from 96h LC 50 (APHA, 1992). The sub lethal concentrations of Imidacloprid to *Clarias gariepinus* were calculated from the LC 50 value 95.09 mg/l are 9.5 mg/l (10%), 14.25 mg /l (15%) and 19 mg /l (20%). Ten fishes were exposed to each concentration for a period of 5, 10 and 15 days. A control batch was maintained simultaneously.

In the present study the 96 h LC_{50} value of Imidacloprid in *Clarias gariepinus,* was found to be 95.09mg/l with a 95% confidence limit ranging from 92.42mg/l (lower confidence limit) to 98.60mg/l (upper confidence limit) in the present study. LC_{50} values of 24, 48 and 72 h of Imidacloprid in *Clarias gariepinus* are 105.44, 102.64 and 99.41, respectively (Table 43). Chi-square test showed that the calculated values were less than the table values and is significant ($p<0.05$). Liver tissue from each group of fishes was dissected post- treatment, fixed in Bouin's and stained with Delafield's Haematoxylin – Eosin (Humason, 1962) [8].

Results and Discussions

The present results indicated that the liver of *Clarias gariepinus* was affected by sub lethal concentrations of Imidacloprid. Exposure of 4.75mg/l Imidacloprid for 5 days in *Clarias gariepinus* caused histological changes of liver such as irregular hepatocyte, hepatocellular cytoplasmic vacuolisation, necrosis, distorted hepatocyte, infiltration of central cord and hypertrophied nucleus. The liver of fish exposed to 9.5mg/l Imidacloprid for 5 days exhibited extreme vacuolation, ruptured hepatocyte, nucleus hypertrophy, accumulation of pyknotic nuclei, cytoplasmic degeneration and increased cellular spaces. Exposure of 19mg/l Imidacloprid for 5 days in *Clarias gariepinus* caused histological changes of liver such as lesions, hepatocellular cytoplasmic vacuolisation, increased vacuolation, hydropic degeneration, necrosis in hepatocyte leading to rupture of hepatocyte, clumping of hepatocyte leading to swelling, vacuolization, accumulation of pyknotic nuclei and damaged hepatic cords (Figs. 1-4).

Exposure of 4.75mg/l Imidacloprid for 10 days resulted in cytoplasmic degeneration, nuclear vacuolization and lesions, necrotic areas, vacuolation of tissue, ruptured hepatocyte, nuclear pleomorphism, cellular oedema and clumping of hepatocyte. Exposure of 9.5mg/l Imidacloprid for 10 days caused cytoplasmic degeneration, lesions, hypertrophy of nuclei, leukocyte infiltration, cytoplasmic vacuolation, sinusoid dilation, cellular atrophy, oedema of hepatocyte leading to clumping, necrosis of hepatocyte and accumulation of pyknotic nuclei. Exposure of 19mg/l Imidacloprid for 10 days resulted in necrosis, fibrosis, cytoplasmic degeneration, degenerated nuclei, vacuolated cytoplasm, clumping of hepatic cells, nucleus degeneration, necrotic areas, tissue disintegration, oedema of hepatocyte, hypertrophied nucleus and nuclear pleomorphism (Figs. 5-8).

Exposure of 4.75mg/l Imidacloprid for 15 days treatment showed lesions, fibrosis, clumping of hepatic cells, hypertrophy of nucleus, cellular degradation, leukocyte infiltration, necrotic areas, necrosis of hepatocyte, accumulation of pyknotic nuclei, infiltration of leukocyte and cytoplasmic degeneration. Exposure of 9.5mg/l Imidacloprid for 5 days treatment showed atrophy, total destruction of hepatic cords, vacuole degeneration with congestion of sinusoids, necrosis of hepatic cells, ruptured hepatocyte, infiltration of leukocyte, degeneration of cytoplasm, damaged hepatic cords, hypertrophied nucleus, vacuolation of cell cytoplasm, and damaged hepatocyte. Exposure of 19mg/l Imidacloprid for 15 days treated fish caused pronounced changes like nuclear vacuolisation, irregular shaped hepatocyte ruptured nucleus, dissociated hepatocyte, vascular congestion, leukocyte infiltration, dilation of extra cellular spaces, necrosis of the hepatocyte, diffuse vacuolation of hepatocyte, congestion destructed hepatic cords, damaged sinusoids (Figs. 9-12).

The liver plays a significant role in the metabolism and biochemical transformations of pollutants from the environment, which unavoidably reflects on its integrity by generating lesions and other histological abnormalities of the liver parenchyma (Doherty *et al.*, 2013) [6]. This organ is connected with the detoxification and strangely, due to its role, position and blood supply, it is also one of the primary organs which is mostly impacted by toxins in water (Camargo & Martinez, 2007; Verma et al., 2022) [5, 23, 24]. Sakr & Lail (2005) [19] described histological alterations in the liver including cytoplasmic vacuolization, inflammatory reactions, and necrosis of *Clarias gariepinus* subjected to Fenvalerate. According to Liu *et al.* (2006) [13], increased hepatocyte vacuolization is a marker of a degenerative process that may indicate metabolic impairment as a result of exposure to contaminated water. Altinok & Capkin (2007) [2] revealed degenerative effects such necrosis and hypertrophy in the fish *Onycorhynchus mykiss* exposed to Methiocarb. Bifenthrin exposure caused degeneration, vacuolar degeneration and necrosis in the liver cells of *Onycorhynchus mykiss, Tilapia zillii* and *Solea vulgaris* (Velisek *et al.*, 2009; Mohammed, 2009) [22, 15]. Butchiram *et al.* (2009) [4] described structural alterations in the liver tissue of *Channa punctatus* subjected to Alachlor, including atrophy, vacuole development, blood vessel rupture and necrosis. Hepatocellular necrosis and cytoplasmic vacuolization were observed in *Labeo rohita* after exposure to Imidacloprid (Indirabai *et al.*, 2010) [10]. *Oreocromis mossambicus* treated to Dimethoate showed vacuolar degeneration and lymphocyte enlargement (Parikh *et al.*, 2010) [17]. Salim & Majeed (2014) [21] documented vacuolization and expansion of hepatocytes caused by several pesticides in *Cyprinus carpio.* The

liver of the fish *Etroplus maculates* subjected to both low and high doses of Fluben Diamide exhibited vacuolar degeneration, swelling in the hepatocytes, and indistinct cellular shape (Reethamma, 2014) [18]. Since liver is the site of detoxification for all types of toxins and chemicals, these changes are attributed to the direct toxic effects of pollutants on hepatocytes. Additionally, there is a temporal sequence of the events that begins with vacuolization, swelling, necrosis, and other situations as reported in the present study.

When exposed to Herbex-D, young catfish *Clarias gariepinus* liver underwent histological alterations (Makinde *et al.*, 2015) [14]. According to Srinivasrao *et al.* (2018), *Ctenopharyngodon idella* treated to Deltamethrin experienced blood cell degradation, necrosis, atrophy, and rupture. Kumari *et al.* (2018) [12] discovered liver cell necrosis and vacuolization in *Channa gachua* subjected to Sedaxane. Elias *et al.* (2018) [7] reported histological alterations in *Clarias gariepinus* liver tissue treated to sublethal concentrations of the herbicide Thiobencarb, which included necrosis, changes in nuclear structure, formation of vacuoles, and atrophy of hepatocytes. In the current investigation, *Clarias gariepinus* treated to different sublethal concentrations for different period of time showed similar histological alterations of varying intensities depending upon the level of concentration and exposure period.

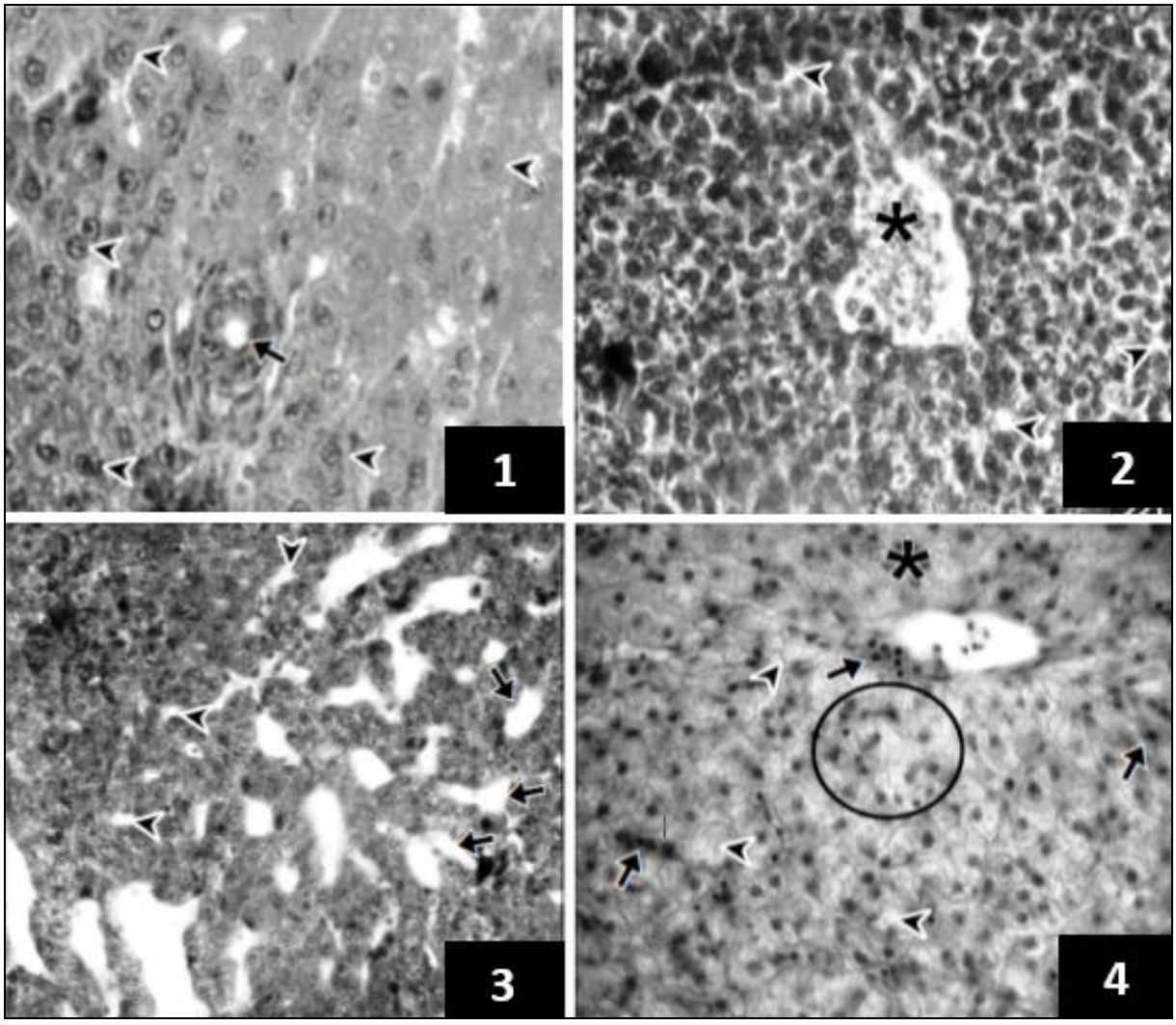

Fig 1-4: Section of Liver of control and treated fish, *Clarias gariepinus* (Haematoxyline- Eosine stain). Fig. 1 Section of control fish showing normal histological structure of the portal vein (arrow) with hepatocytes (arrowheads) (x400). Fig. 2. Fish exposed at 4.75mg/l Imidacloprid for 5 days showing focal area of necrosis (asterix) and vacuolization of cell cytoplasm (arrowheads) (HE x400). Fig. 3. Fish exposed at 9.5mg/l Imidacloprid for 5 days showing vacuolation of cell cytoplasm (arrowheads) and extreme vacuolation (arrows) (HE x400). Fig. 4. Fish exposed to 19mg/l Imidacloprid for 5 days showing clumping of hepatocyte leading to swelling (asterix), vacuolization (arrowheads), accumulation of pyknotic nuclei (arrows) and damaged hepatic cords (encircled) (HE x400).

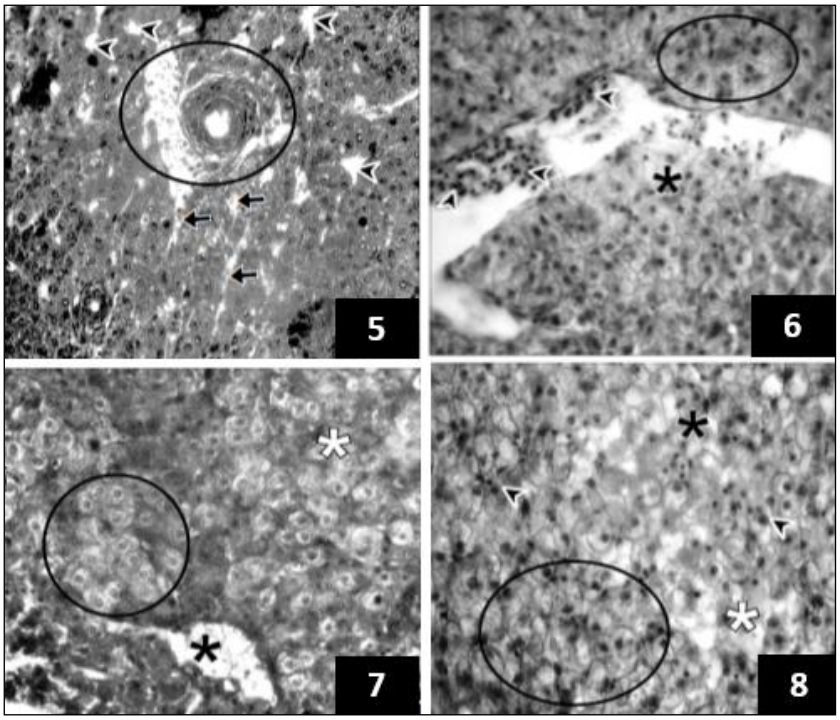

Fig 5-8: Section of Liver of treated fish, *Clarias gariepinus* (Haematoxyline- Eosine stain). Fig. 5. Fish exposed at 4.75mg/l Imidacloprid for 10 days damaged liver tissue (encircled), necrotic areas (arrows) and vacuolation (arrowheads) (HE x320). Fig. 6. Fish exposed at 9.5mg/l Imidacloprid for 10 days showing oedema of hepatocyte (encircled) leading to clumping, necrosis of hepatocyte (asterix) and accumulation of pyknotic nuclei (arrowheads) (HE x400). Fig. 7. Fish exposed at 19mg/l Imidacloprid for 10 days showing oedema of hepatocyte (encircled), nucleus degeneration (white asterix) and necrotic area (asterix) (HE x400). Fig. 8. Fish exposed at 19mg/l Imidacloprid for 10 days showing tissue disintegration (asterix), oedema of hepatocyte (encircled), hypertrophied nucleus (white asterix) and nuclear pleomorphism (arrowheads) (HE x400).

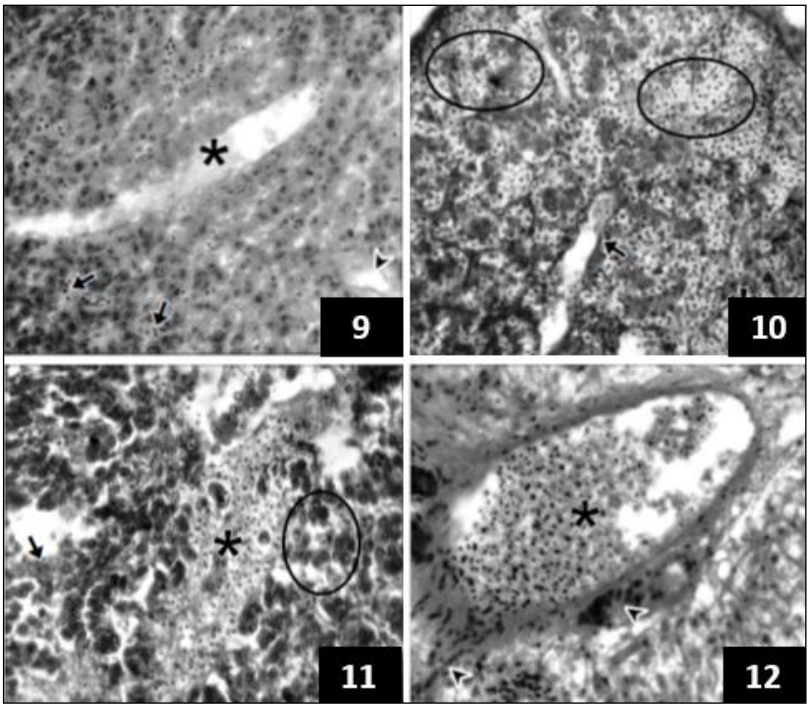

Fig 9-12: Section of Liver of treated fish, *Clarias gariepinus* (Haematoxyline- Eosine stain). Fig. 9. Fish exposed at 4.75mg/l Imidacloprid for 15 days Necrotic areas (asterix), vacuolation (arrowhead) and accumulation of pyknotic nuclei (arrows) (HE x160). Fig. 10. Fish exposed at 9.5mg/l Imidacloprid for 15 days showing total destruction of hepatic cords (encircled), vacuolar degeneration with congestion of sinusoids, necrosis of hepatic cells (HE x320). Fig. 11. Fish exposed at 19mg/l Imidacloprid for 15 days showing ruptured nucleus (arrow), dissociated hepatocyte (encircled), vascular congestion, leukocyte infiltration (asterix) (HE x320). Fig. 12. Fish exposed at 19mg/l Imidacloprid for 15 days showing damaged sinusoids (arrowheads) and infiltration of leukocyte (asterix) (HE x400).

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