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

S Kurikose[1], P Verma[2*], D B Sawarkar[2]
[1] Centre for Higher Learning and Research in Zoology, N.H. College, Bramhapuri, Chandrapur, Maharashtra, India
[2] Centre of Higher Learning and Research in Zoology, Hislop College, Nagpur, Maharashtra, India
***Corresponding Author:** payalrverma@gmail.com

Abstract
The nicotinic acetylcholine receptors (nAChR) in pests' central nervous systems are the target of neonicotinoids, a class of extensively used pesticides. Due to their high-water solubility, neonicotinoids have gained popularity as a result of their ability to penetrate the entire plant when applied to the soil. The first generation of neonicotinoids, of which imidacloprid is a member, are widely used in agriculture around the globe. For environmental contamination, histopathology is a helpful biomarker. A potential toxicological hazard for fish is the presence of pesticide in the environment as a result of its widespread usage in agriculture. Assessing histopathological changes in fish organs in response to organic trace contamination can help in evaluating the pathological impacts of water-borne pollution. In the present study, exposure of *Clarias gariepinus* to Imidacloprid in various sublethal concentrations resulted in structural alterations like damaged renal tubule and hematopoietic tissue, tubular shrinkage, severe necrosis and swelling of lymphocytes, vacuolation of glandular epithelium, swelling of renal tubule, renal tubule dilation, glomerulus distortion, enlargement of sinusoids, disorganization of tissues, exudate in tubules, reduction of renal cells, desquamation of renal epithelium, balloon necrosis, distorted renal tubule. The effect was time- and dose-dependent.

Keywords: *Clarias gariepinus*, Imidacloprid, histopathology, kidney, neonicotinoids

Introduction

The use of synthetic organic pesticides led to the maximum production of high-quality crops in the year 1940. According to the World Health Organization (WHO), there are an estimated one million cases of acute poisoning from pesticide exposure each year, with a death rate between 0.4% and 1.9%. Pesticides pose substantial health risks to biological systems due to their quick lipid solubility and bioaccumulation in creatures other than their intended targets. Pesticides can have a number of negative consequences, even in low concentrations. These effects can be observed at the biochemical, molecular, or behavioural levels (Agrawal *et al.*, 2010) [1].

Neonicotinoids are commonly used systemic insecticides that interact with the nicotinic acetylcholine receptors (nAChR) found in insects central nervous system. Due to their high water solubility, neonicotinoids have gained popularity as a result of their ability to penetrate the entire plant when applied to the soil on which the pest feeds. The first version of this class of pesticides to be used was 1-(6-chloro-1, 3-thiazol- 5-ylmethyl)-1, 3, 5-oxadiazinan-4-ylidenene (nitro) amine, also known as imidacloprid (Natalia & Robert, 2016) [16]. Given that neonicotinoids can linger in the soil for several years, they have the potential to infect unintended species such as non-target flora and fauna (Huseth & Groves, 2014) [6]. These insecticides contaminate the soil, the water supply, fish, and other living beings. According to Hrynyk *et al.*, (2018) [5], wetlands and other aquatic ecosystems have been shown to contain neonicotinoid pesticides, which account for 27% of the insecticide market worldwide. The use of several neonicotinoids was limited in 2013 by the European Union and a few non-EU nations. All outdoor usage of the three main neonicotinoids (Coltianidin, Imidacloprid, and Thiamethoxam) were prohibited by the EU in 2018.

Assessing histopathological changes in fish as a result of organic trace pollution and evaluating the pathological impacts of waterborne pollution can both benefit from monitoring histopathological alterations. According to Kazempoor *et al.*, (2015) [11], histopathology has given information to bio-monitoring strategies created for several parts of environmental risk assessments. Histopathological studies can assess the short- and long-term impact of certain environmental stresses. Kidney is not only an excretory organ but also performs vital bodily tasks such as controlling metabolism, producing plasma proteins, storing energy, preserving some vitamins and trace minerals, converting and eliminating steroids, and detoxifying pollutants (Salamat & Zarie, 2012) [18]. In fishes, kidney is one of the main organ not only for metabolism but also for detoxification, since it comes into contact with hazardous compounds that the organism absorbs from polluted water. This results of histopathological changes of kidney serves as a valuable tool for the detection of influence of such insecticides (Verma, *et al.*, 2022 b) [25]. 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 (Kuriakose *et al.*, 2022; Verma *et al.*, 2022a, b; Verma, *et al.*, 2023) [24, 26]. The present study records the histopathological changes in the kidney of the catfish *Clarias gariepinus* caused by sublethal doses of Imidacloprid.

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 for *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. Chi-square test showed that the calculated values were less than the table values and is significant ($p<0.05$). Kidney tissue from each group of fishes was dissected post- treatment, fixed in Bouin's and stained with Delafield's Haematoxylin – Eosin (Humason, 1962).

Results and Discussions

The present results indicated that the kidney of *Clarias gariepinus* was affected by sub lethal concentrations of Imidacloprid. The kidney of fish exposed to 4.75mg/l concentration of Imidacloprid for 5 days exhibited reduced number of renal cells, disorganisation of tissue, balloon necrosis, damaged renal tubule, distorted glomeruli, and disorganized renal tubule. The changes observed in the kidney of fish exposed to 9.5mg/l Imidacloprid concentration for 5 days included enlargement of sinusoids, disorganization of tissues, cytoplasmic vacuolation, hydropic degeneration, narrowing and shrinkage of renal tubule, accumulation of haematopoietic tissue and disintegration of glomeruli. Fishes exposed to 19mg/l Imidacloprid concentration exhibited histological changes like vacuolisation and damaged renal tubule and hematopoietic tissue, necrosis, tubular shrinkage and vacuolation, desquamation of epithelial layer, focal vacuolization and occlusion of tubular lumen.

After 10 days at 4.75mg/l Imidacloprid concentration changes like renal tubular separation, glomerular oedema, vacuoles in the epithelial cells, loss of central canal, desquamation of renal tubule, increased cellular spaces and tubule distortion are observed. Fishes exposed to 9.5mg/l Imidacloprid concentration resulted in shrinkage and degeneration of the glomeruli, renal tubule damage, shrunken tubular lumen, reduction of glomeruli tissue, severe vacuolation, extensive cytoplasmic vacuolation, extreme haematopoietic and renal tissue damage are observed. Exposure of 19mg/l Imidacloprid concentration resulted in necrosis and exudates in tubules, haemorrhage, disorganized renal tubule, extreme vacuolation, single cell necrosis, vacuolation, damaged renal tissue, vacuolation, and extremely distorted renal tubule.

Exposure of 4.75mg/l Imidacloprid for 15 days treated fish showed damaged renal tubules, degenerated tubules, extreme vacuolation, damaged haematopoietic tissue, severe necrosis leading to tissue damage, desquamation of renal epithelium, increased inter cellular spaces and extreme vacuolation of tissue. At 9.5mg/l Imidacloprid concentration severe structural damages, shrinkages, degeneration of glomeruli, necrosis, loss of renal tissue, vacuolation and distortion of renal tubule are noticed. Exposure of 19mg/l Imidacloprid concentration treatment the kidney of treated fish along with the above effect also showed glomerular oedema, accumulation of hematopoietic tissue, pyknotic nuclei, vacuolization and atrophic glomeruli. The results of these studies clearly indicate that sublethal concentration of Imidacloprid has diverse effects on fish kidney (Figs. 1-12).

Kidney is the first organ of a fish what gets affected by water quality (Thophon *et al.,* 2003) [23] which disrupts the homeostasis by disrupting the process of selective reabsorption which maintains the volume and pH of blood, body fluid and erythropoiesis (Iqbal *et al.,* 2004) [8].

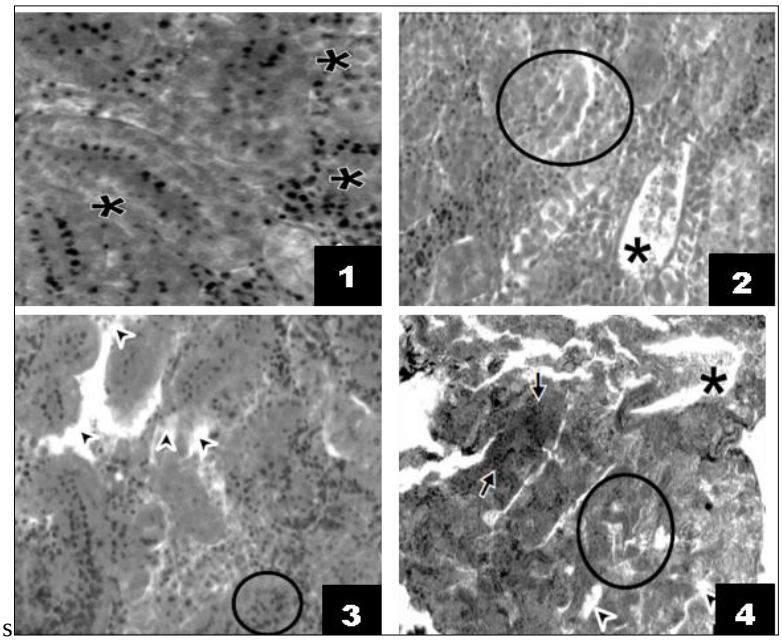

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Fig 1-4: Section of Kidney of control and treated fish, *Clarias gariepinus* (Haematoxyline- Eosine stain).

Fig 1: Section of kidney of control fish, *Clarias gariepinus* showing normal tissue vacuoles (arrowhead) and normal organization of tissue cells (asterix).
Fig 2: Fish exposed at 4.75mg/l Imidacloprid for 5 days showing balloon necrosis (asterix), damaged renal tubules (encircled) (HE x400).
Fig 3: Fish exposed at 9.5mg/l Imidacloprid for 5 days showing cytoplasmic vacuolation (arrowheads) and hydropic degeneration (encircled) (HE x400).
Fig 4: Fish exposed at 19mg/l Imidacloprid for 5 days showing necrosis (asterix), damaged hematopoietic tissue (arrows), tubular shrinkage (encircled) and vacuolation (arrowheads) (HE x100).

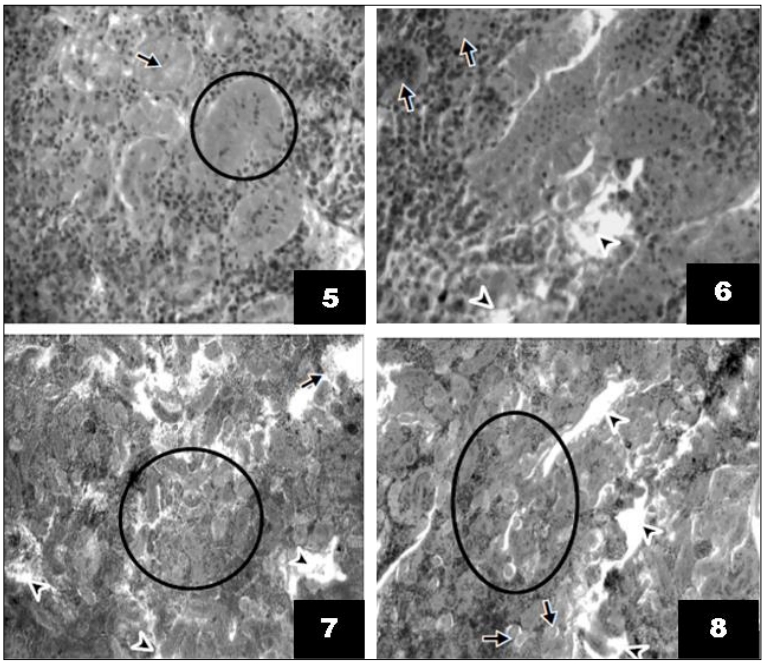

Fig 5-8: Section of Kidney of treated fish, *Clarias gariepinus* (Haematoxyline- Eosine stain).
Fig 5: Fish exposed to 4.75mg/l Imidacloprid for 10 days showing loss of central canal (arrow) and desquamation of renal tubules (encircled) (HE x400).
Fig 6: Fish exposed at 9.5mg/l Imidacloprid for 10 days showing shrunken glomerulus (arrows) and extensive cytoplasmic vacuolation (arrowheads) (HE x400).
Fig 7: Fish exposed at 19mg/l Imidacloprid for 10 days showing hemorrhage (arrow), disorganized renal tubules (encircled) and extreme vacuolation (arrowheads) (HE x100).
Fig 8: Fish exposed at 19mg/l Imidacloprid for 10 days showing single cell necrosis (arrows), vacuolation (arrowheads) and disorganized renal tissue (encircled) (HE x100).

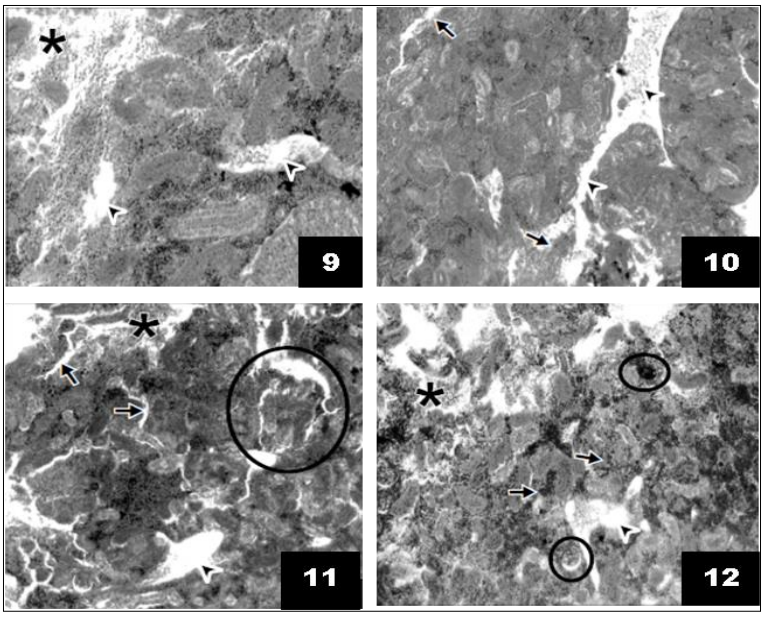

Fig 9-12: Section of Kidney of treated fish, *Clarias gariepinus* (Haematoxyline- Eosine stain).
Fig 9: Fish exposed at 4.75mg/l Imidacloprid for 15 days showing necrosis (asterix), increased inter-cellular spaces and extreme vacuolation (arrowheads) (HE x100).
Fig 10: Fish exposed at 9.5mg/l Imidacloprid for 15 days showing increased inter-cellular spaces (arrowheads) and desquamation of renal epithelium (arrows) (HE x100).
Fig 11: Fish exposed at 9.5mg/l Imidacloprid for 15 days showing loss of renal tissue (asterix), distortion of renal tubules (encircled), increased inter-cellular spaces (arrows) and vacuolation (arrowhead) (HE x80).

Fig 12: Fish exposed at 19mg/l Imidacloprid for 15 days showing single cell necrosis (encircled), distorted renal tubules (asterix), tubular shrinkage (arrows) and vacuolation (arrowheads) (HE x100).

The kidney of *Clarias gariepinus* exposed to Imidacloprid undergo several structural changes like severe necrosis and swelling of lymphocytes, vacuolation of glandular epithelium, swelling of renal tubule, renal tubule dilation, glomerulus distortion, enlargement of sinusoids, disorganization of tissues, exudate in tubules, tubular necrosis, reduction of renal cells, desquamation of renal epithelium, balloon necrosis, distorted renal tubule, single cell necrosis and damaged renal tubules. The most frequent changes observed in fish exposed to water contamination, according to Takashima & Hibya (1995) [22], are tubular degeneration, glomerulus capillary dilatation, and a reduction in Bowman's capsule space. Histopathological changes were reported by Gupta & Srivastava (2006) [4] in the kidney of *Channa punctatus* when exposed to sub-lethal concentration of Zinc. The changes included enlargement of renal tubules, desquamation of epithelial lining, hypertrophied nuclei, oedema, dilation of renal tubules, severe necrosis, pyknotic nuclei, vacuolization, and disorganized blood capillaries in glomerulus. Altinok & Capkin (2007) [2] found highly degenerative alterations in *Onycorhynchus mykiss* exposed to the pesticide methiocarb, including severe necrosis, increased sinusoids, and exudates in tubules. In kidney tissue of *Channa punctatus* subjected to the pesticide Alachlor, Butchiram *et al.,* (2009) [3] reported structural alterations such as severe necrosis, hypertrophy, enlargement of renal tubule, and vacuole formation. *Tilapia zillii* and *Solea vulgaris* treated to contaminated drainage water showed vacuolization and necrotic alterations in their kidneys (Mohammed, 2009) [14]. Since kidney of fish receives most of the largest portion of postbranchial blood, renal lesions are expected to be a good indicator of environmental pollution. When the fish *Oreocromis mossambicus* was exposure to dimethoate, Parikh *et al.,* (2010) [17] noticed oedema, vacuolar degeneration, and necrosis. Jalaludeen *et al.,* (2012) [9] reported major damage and disorganization of tubules, glomerular oedema, and necrosis in *Tilapia mossambica* exposed to Cadmium Sulphate. In the kidney of a phenol-exposed *Clarias gariepinus*, Ibrahem (2012) [7] observed deformed and damaged renal tubules. The most frequent histological consequence of several pesticides, according to Salim & Majeed (2014) [19], was glomeruli congestion and vacuolization in the kidney of *Cyprinus carpio*. The kidney tubules of *Clarias gariepinus* and *Mugil capito* exhibited degeneration, focal-necrosis and fibrosis when exposed to heavy metals (Mahmoud & Abd El-Rahman, 2017) [13]. Kidney tissue of *Ctenopharyn godonidella* exposed to Deltamethrin exhibited structural changes like severe necrosis and vacuole formation (Srinivasrao *et al.,* 2018) [21]. Kaur *et al.,* (2018) [10] reported variations in histo-architecture of kidney like desquamation of renal epithelium, necrosis and disorganization of renal tubules when exposed to toxic heavy metals like Arsenic, Chromium, Cadmium, Manganese and Lead. Shah *et al.,* (2017) [20] studied cadmium-induced histological variations like necrosis, loss of lumen, disorganization of renal tubules probably due to bioaccumulation of cadmium in kidney of *Heteropneustes fossilis.* Murali *et al.,* (2018) [15] reported degeneration of renal tubules, presence of melanomacrophages and sinusoidal space along with necrotic cells in the hematopoietic tissue, aggregation of blood cells, membrane damage in the blood cells, vacuolation, increased space in between glomerulus and enlarged bowman's capsule when freshwater fish *Oreochromis mossambicus* was exposed to Aluminium oxide nanoparticles. These pathological changes are consistent with the histological findings of the kidney tissue in the current investigation, evidencing unequivocally that imidacloprid has a significant impact on the kidney of *Clarias gariepinus*.

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