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Assessing renal and hepatic alterations in schizothorax plagiostomus exposed to chlorpyrifos

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Chlorpyrifos (CPF) stands out as one of the most prevalent insecticides present in freshwater ecosystems, having been identified in agricultural and fish products on a global scale. The impact of pesticides on aquatic organisms is manifested through both direct and indirect pathways. While agricultural runoff serves as a direct entry point, the consumption within the food chain represents an indirect route. This study aimed to evaluate histopathological alterations in the kidney and liver of Schizothorax plagiostomus exposed to varying concentrations of chlorpyrifos. The fish were categorized into four groups for the experiment, with Group I serving as the control. Groups II, III, and IV were subjected to solutions of chlorpyrifos at concentrations of 4.0 mg/L, 4.5 mg/L, and 5.0 mg/L, respectively, during a short-term experiment lasting 96 hours. Histopathological changes in the kidney and liver were monitored at intervals of 24, 48, 72, and 96 hours following chlorpyrifos exposure.

Keywords: Fishes,*Schizothorax plagiostomus*,Kidney,Liver,Chlorpyrifos,Histopathology

INTRODUCTION

Tilapias are the second most significant farmed fish after carps; they are native to Africa and are produced in over 100 nations (Merrifield, Bradley et al. 2010). Tilapia adds 4.5 million metric tons to global food security each year by offering a low-cost protein source (Senapin, Shyam et al. 2018). The Nile tilapia, *Oreochromisniloticus,* sometimes known as tilapia, is one of the world's most significant aquaculture fish. (Garg 2015). The capacity to eat on a wide variety of food items, tolerance to a wide range of environmental conditions (pH, temperature, nitrogenous wastes), quick development, and high economic value make tilapia a good aquaculture option (Abarike, Obodai et al. 2013, Wang and Lu 2016)

According to recent accounts, an enhanced cultivation of O. niloticus over the years has resulted in a quadrupling of its productivity (Wang and Lu 2016, Chen, Fan et al. 2018), and its output will rise more during the coming decade. Many endemic and developing illnesses, such as hepatopancreatic necrosis disease and tilapia lake virus, are threatening the aquaculture sector's rapid expansion (Bacharach, Mishra et al. 2016, Senapin, Shyam et al. 2018). The use of veterinary drugs, such as antibiotics and vaccinations, has evolved as a conventional method of increasing biosecurity on farms. However, a paradigm change is required when dealing with aquaculture biosecurity hazards, because the inappropriate use of veterinary drugs has resulted in antimicrobial residues and antibiotic resistance (Magnadottir 2010, Kumar, Roy et al. 2016, Kavitha, Raja et al. 2018).

The use of probiotics (live microorganisms included in feed to affect the host positively) has been attributed to the successful combat of diseases and improved production in the aquaculture sector. Probiotics stimulate growth, improve food digestibility, increase stress tolerance, improve immune response, and increase disease resistance (Ghazalah, Ali et al. 2010, Nayak 2010, Singh, Kallali et al. 2011, C De, Meena et al. 2014,

Akhter, Wu et al. 2015, Van Hai 2015, Abarike, Cai et al. 2018, de Araújo, Barbas et al. 2018, Kuebutornye, Abarike et al. 2019).

MATERIALS AND METHODS

For this experiment 48 fingerlings of *Shizothorax plagiostmus were procured from Nagohafish hatchery* Swat.Thefishweightandlengthrangesfrom25to70gand13to19cm, respectively.Thefishwas initially acclimatized under laboratory conditions for one week in glass aquaria. During acclimatization period, carbohydrate-based diet was given to fish twice a day.

Preparation for stock solution of chlorpyrifos

A stock solution of chlorpyrifos was made by dissolving 1 mg of chlorpyrifos in 100 mL of acetone. Thefish were divided into four different groups and placed in different glass aquariums. Twelve fish were introduced in each aquarium filled with 35 litter of water. Group I was maintain in pesticide-free water to serve as control.GroupII, III and IV were exposed to different concentration of chlorpyrifos stock solution, 4.0 mgL^{-1}, 4.5 mgL^{-1}and5.0 mgL^{-1}respectively.Thefishwereexposedto these concentration for96h(Bhatnagar, Cheema, & Yadav, 2017).

Chemicals

Chlorpyrifosis abroad

spectrum,chlorinatedorganophosphateinsecticides.CommonName:Chlorpyrifos,Chemical Name: O,O-diethyl-O-(3,5,6-trichlor-2-pyridyl)-phosphorothioate, Empirical Formula: C9H11Cl3NO3PS

Acutetoxicity evaluation

Clean water was added to glass aquarium up to 70% of their water holding capacity and fish was introduced to gradually increasing test concentrations of chlorpyrifos with three replications of each doses. During the whole acute toxicity trial duration(96hrs).Test aquaria was examined at periodic intervals of24,48, 72and96hours.

Histopathologicalevaluation

Fish were collected at different time intervals 24, 48, 72 and 96 hours and dissected to collect organs i.e.kidneyandliver.Histopathologicalexaminationofkidneyandliverwasdonebypreservingitin70%formalin.Thesetissueswere dehydratedbypassingthroughvariousalcoholicgrades,washedwithclearingsolutionandembeddedin paraffin wax (molten). Fine sectioning of embedded tissue (by using a microtome), hemotoxylin and eosin stain were used and observe under microscope for histopathological findings(Kozawa, Hino, Minamino, Kangawa, & Matsuo, 1991). The histopathological observations wereclassified into 4 categories ranging from no changes (-), mild changes (+, < 10%), moderate changes (++, 10-50%)and severe changes (+++, >50%).

RESULTS

The experiment was conducted for a short duration of 96 hours to find out theacute exposure ofchlorpyrifos (CPF)onhistopathologyofkidneyand liverof*Shizothorax plagiostomus*

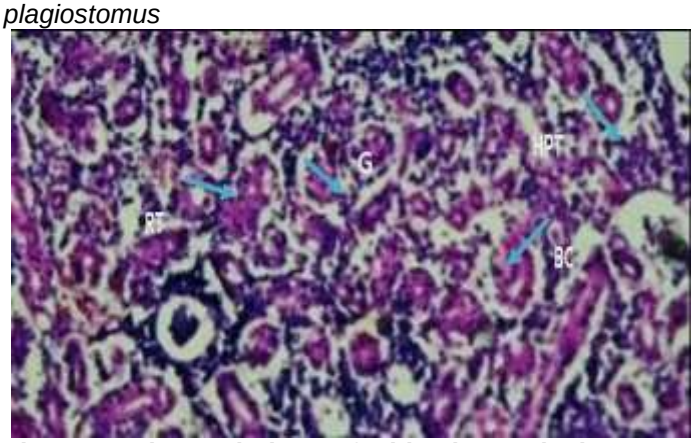

Figure 1: Histopathology of *Shizothoraxplagiostomus* kidney group I(control)

Kidney structure of control group showing normal: renal tubules(RT),glomerulus(G),bowmancapsule(BC),hematopoietic tissues (HPT).

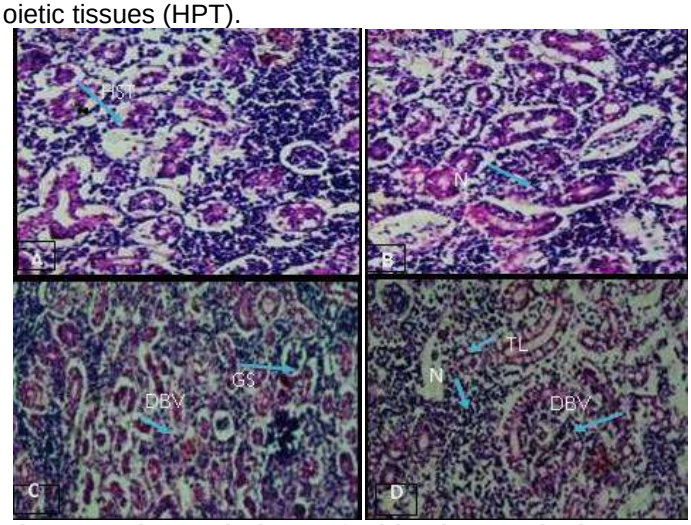

Figure2: Histopathology of *Shizothorax plagiostomus* kidney after exposure to chlorpyrifos 4.0 mg/L (GroupII)

After 24 hrs (A): exposure showing hydropic swelling of tubules (HST). After 48 hrs(B): necrosis (N). After 72 hrs(C): glomerular shrinkage (GS), damaged blood vessel (DBV). After 96 hrs (D): necrosis (N), increased tubular lumen (TL),damaged blood vessel (DBV).

After 24 hrs (A): exposure showing hydropic swelling of tubules (HST). After 48 hrs(B): necrosis (N). After 72 hrs(C): glomerular shrinkage (GS), damaged blood vessel(DBV). After 96 hrs (D): necrosis (N), increased tubular lumen(TL), damaged blood vessel(DBV).

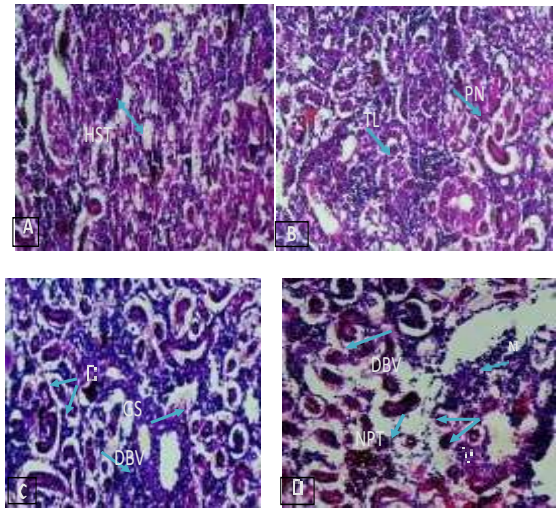

Figure3:Histopathology of *Shizothorax plagiostomus* kidney after exposure to chlorpyrifos4.5mg/L(GroupIII)

After 24 hrs (A): exposure showing hydropic swelling of tubules (HST). After 48 hrs (B): increased tubular lumen(TL),pycnotic nuclei (PN). After 72 hrs(C): degeneration of tubular epithelium (D), glomerular shrinkage (GS),damaged blood vessel (DBV). After 96 hrs (D): necrotic proximal tubules (NPT), necrosis (N), vacuolized(V),damagedblood vessel(DBV).

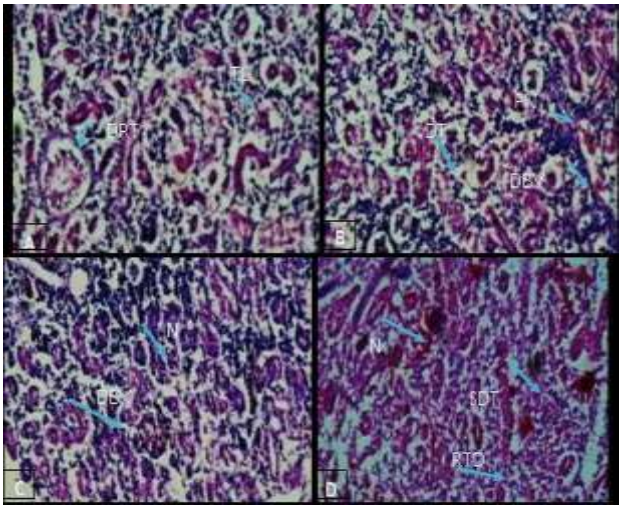

Figure 4: Histopathology of Shizothorax *plagiostomus* kidney after exposure to chlorpyrifos 5.0mg/L(Group IV)

(A) 24 hrs: degeneration of renel tubules (DRT), tubuler lumen (NL). (B) 48 hrs: degeneration of blood vessels(DBV),necroris(N),pyknoticnuclei(PC).(C)72hrs:de generationofbloodvessels(DBV),necroris(N).(D)96hrs: renel tubules damges (RTD), necrotic (N) and severe tubules damges (STD).

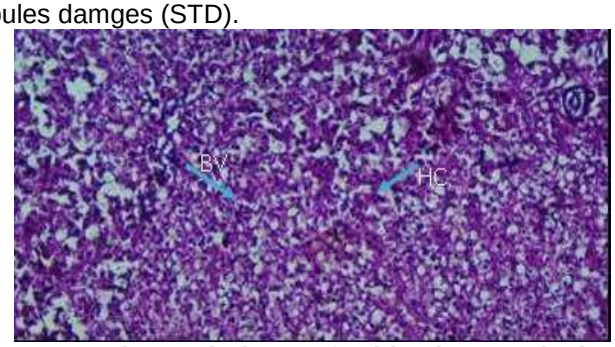

Figure 5: Histopathology of *Shizothorax plagios to mus*liver group I(control)

Norma hepatic cells (HC), blood vessels (BV),noinflammation and hydropicde generation

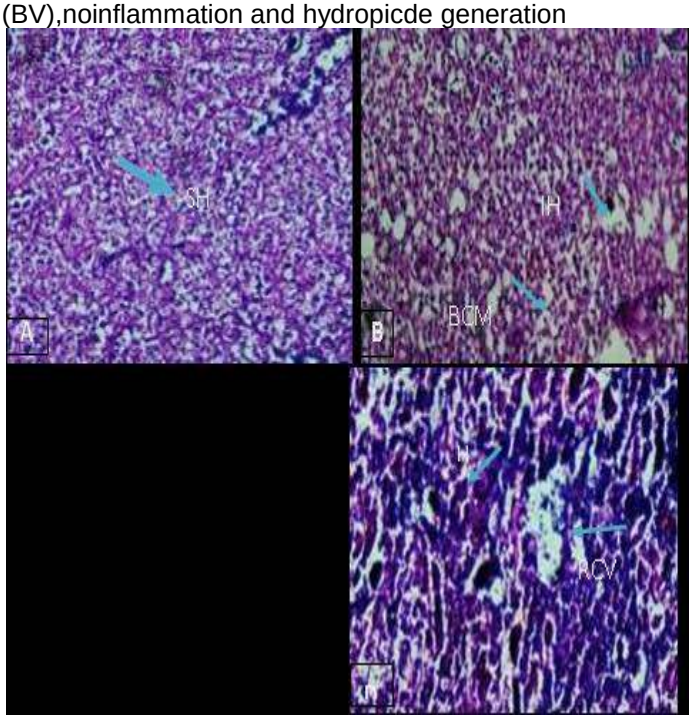

Figure 6: Histopathology of Shizothorax *plagiostomus*liver aftere xposuretochlorpyrifos4.0mg /L(GroupII)

(A):swellinginhepatocytes(SH)after24hrs.(B):brokence llmembrane(BCM),irregularstructureofhepatocytes(IH)after 48hrs.(C):showingaccumulationofvacuoles(V),rupturedcen tralveins(RCV)after72hrs.(D):showingabnormal hepatocytes(H), ruptured central veins.

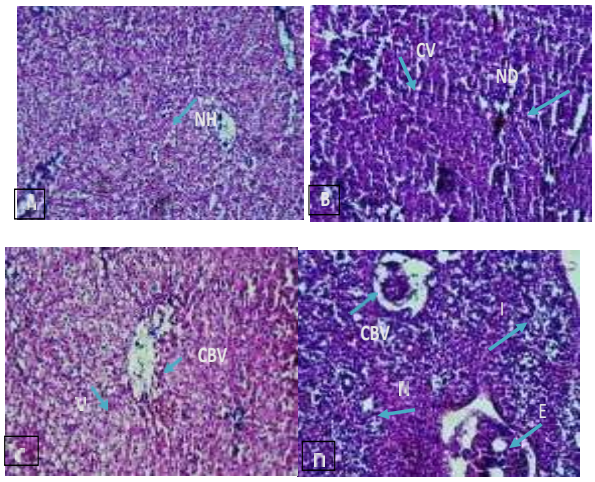

Figure 7:Histopathology of *Shizothorax plagiostomus*liver after exposure to chlorpyrifos 4.5mg/L (GroupIII)

The liver shows the nuclear hypertrophy (NH) after 24 h (A). Cytoplasmic vocuolation(CV), nuclear degeration (ND)after48h(B).Vaculationinhypatocytes(V),congestedblo odvessels(CBV)after72h(C).Necrosis(N),inflammation(I),e dema (E)after96h.

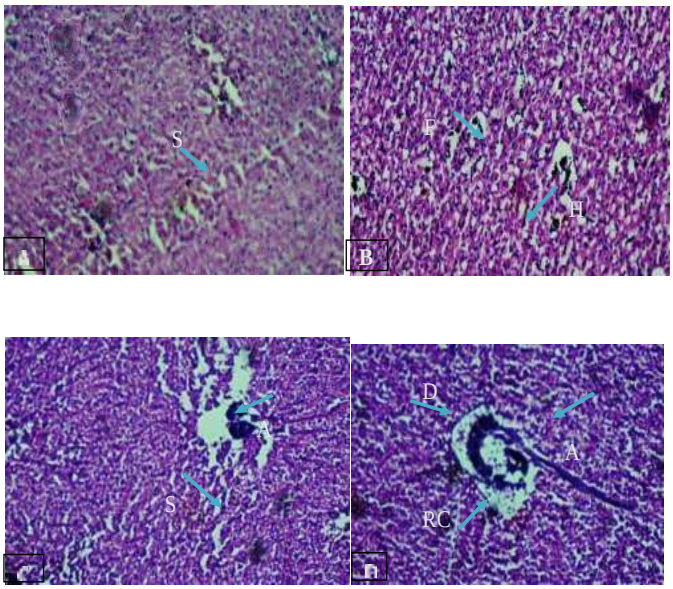

Figure 8:Histopathology of *Shizothorax plagiostomus*liver after exposure to chlorpyrifos 5.0mg/L (GroupIV)

(A): swelling in hepatocytes (SH) after 24 h. (B): fat vacuoles (FV), hepatocytesnecrosis (HN) after 48 h. (C):accumulationofvacuoles(AV),sinusoidbloodcongestion(SB)after72h.(D):abnormalhepatocytes(AH),dilationofsinus oid (DS),rupturedcentralveins(RCV)after 96h.

DISCUSSION

Effect of chlorpyrifoson Shizothorax plagiostomus

The principal haematological and osmoregulatory organs in fish are the kidneys. Because the majority of post-branchial blood flows to fish kidneys, alteredfish histology is a good indicator of environmental pollution. In the present study kidney tissues show no clear changes in control group showing normal renal tubules,glomerulus, bowman capsule, hematopoietic tissues. The kidney offish exposed to 4.0 mg/L chlorpyrifos stock solution, showing hydropic swelling of tubules ,shrink age of glomerulus and damaged blood vessels were observed. The kidney of fish exposed to 4.5 mg/L chlorpyrifos stock solution, showing hydropic swelling of tubules. The kidney of fish exposed to 5.0 mg/L chlorpyrifos stock solution, the histopathological changes in the kidney of fish such as degeneration of renel tubule and tubuler lumen were observed. Degeneration of blood vessels was also seen with necroris.

Many researchers have observed histological changes inthe kidney at the glomerulus and tubular epithelium level in fish following exposure to harmful substances like insecticides. In *Labeo rohita* treated tohexachloro-cyclohexane, dilationof proximal tubule and inflammatory alterations characterised by karyorrhexix and karyolysis. After exposure to fenvalerate, *Ctenopharyngodon idella* kidney tissues showed necrosis, cloudyedoemaintherenal tubules, glomerular shrinkage and vacuolization (Pal, Kokushi, Koyama, Uno, & Ghosh, 2012).

The liver is the principal organ for metabolism, xenobiotic detoxification, and hazardous chemical excretion. It has the potential to digest harmful chemicals, but high concentrations of these compounds can exceed its regulatory mechanisms, which can lead to structural damage(Paulino, Sakuragui, & Fernandes, 2012).

In the present study, liver tissue of control group fish revealed normal hepatic and sinusoids architecture. The histological examination of chlorpyrifos treated *Shizothorax plagiostomus*groups were showed that at 4.0 mg/L liver tissues showing abnormal hepatocytes, dilation of sinusoid as compared to control group, and 4.5 mg/Lliver shows the nuclear hypertrophy, cytoplasmic vacuolation and nuclear degeration and 5.0mg/L the histopathological changes observed in the liver tissues were swelling in hepatocytes. broken cell membrane,irregular structure of hepatocytes and necrosis. Hepatocyte degeneration and accumulation of vacuoles, hepatocytes necrosis were seen in Nile tilapia treated to delta methrin. In *O. niloticus*, histopathological alterations were observed in hepatocytes where diazinon-exposed liver revealed bleeding or hemorrhage, fatty deterioration,

and hepatocytes. Fish behavior changes are important indicators of pesticide exposure which cause possible harmful effects. In the present study, numerous abnormal behaviors such as loss of balance, motionlessness, erratic swimming and changes in the skin colouration were exposed to chlorpyrifos. They stated that due to the toxicity of diazinon, the fish equillibrium became paralyses in all cases, and eventually settled down to the bottom of the aquarium, wherethey remained until death(Ayoola et al. 2008)

CONCLUSION

Chlorpyrifos is a commonly used organophasphate insecticides that cause toxicological effects in aquatic organism especially in fish. In the present study,it was concluded that exposure of chlorpyrifos caused histopathological changes in *Shizothorax plagiostomus* after 96 hours of trial. After exposure,histopathological study of organ (kidney,liver) of*Shizothorax plagiostomus* showed prominent variations in these organs as compared to control group.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

AK presents the idea, OZ has supervised and wrote the manuscript, SR, NS and NK did the experiment. BU and EA reviewed and reform the manuscript. All authors read and approved the final version.

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