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***Research Article** (Open Access)*

HISTO- MORPHOLOGICAL ALTERATIONS IN TRUNK KIDNEY OF *CLARIAS BATRACHUS* (LINNAEUS, 1758) FOLLOWING ACUTE EXPOSURE WITH HYBRID PESTICIDE CONTAINING CHLORPYRIFOS (50 %) AND CYPERMETHRIN (5 % EC).

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ABSTRACT
The need of explosive increment in crop yield to meet the food demand of increasing human population lead to use of fertilizers and pesticides in the field. Now a days, hybrid pesticides containing the chemical molecules with differing modus operandi are being used to broaden their spectrum of action. This, in turn, may impose toxic effects on the non- target organisms. In the present study, a hybrid pesticide containing organophosphate, chlorpyrifos (50 %) and pyrethroid, cypermethrin (5 % EC) has been used to assess its toxic effects on the renal histo- morphology of freshwater catfish, *Clarias batrachus* (Linnaeus, 1758). Live and healthy specimens of *Clarias batrachus* of 20-25 cm and 75- 100 g were used for experimentation. They were kept for acclimatization to laboratory conditions for a period of two weeks. Fishes were fed with commercial fish feed once a day and water was changed after every 24 hour. They were divided into 3 groups with 5 individuals in each. Group 1 served as normal control (NC). In group II (LD) and III (HD), fish were exposed with sub- lethal concentration of $1/15^{th}$ and $1/5^{th}$ of 96 h LC_{50}, respectively, of hybrid pesticide. After 96 h, fishes from each group were sacrificed under anesthesia, trunk kidney was collected, fixed and processed for H & E double staining. Kidney sections of control group showed normal architecture of renal corpuscles, tubules and interstitial tissues. Whereas, acute treatment with hybrid pesticide showed a dose- dependent alterations in histo- morphology of renal tissue components expressed in terms of hyperplasia of renal corpuscles, nuclear and cytoplasmic degenerations in renal tubules, occlusion of tubular lumen, loss of haematopoietic tissues, etc. The toxicological changes in renal tissues advocate for the ill- effects of hybrid pesticide on the renal functioning of fish, thereby affecting their survival and growth.

Keywords: *Hybrid, Chlorpyrifos, Cypermethrin, Trunk kidney, Acute, Clarias*.

INTRODUCTION
Synthetic pesticides being used in agriculture are one of the major sources of contamination in aquatic ecosystem that possess high potential of bioaccumulation and biological alterations. Organophosphates and pyrethroids are the most commonly used pesticides in the agricultural fields. Although they have been useful in increasing the crop yield, but their persistence in the environment may cause harm to the biota present in surrounding aquatic as well as terrestrial ecosystem (Dorlikar and Mohite, 2019 & Mostakim *et al*., 2015). Recently, the use of combination of pesticides has become a trend to increase their spectrum of action. However, the cumulative efficacy may lead to cumulative toxicity also. Iyyadurai *et al*., 2014 reported that the combination of organophosphate and pyrethroid may pose greater toxicity to human as compared to either pesticide alone. However, it is not easy to predict the health hazards of hybrid pesticides on basis of effects caused by single pesticides (Falfushynska *et al*., 2022). So, it becomes a mandate to scientifically investigate the toxicological impacts of hybrid pesticides currently being used in agricultural practices on the aquatic health.

Being an inhabitant of aquatic ecosystem, fishes are continuously being exposed to these environmental contaminants. Fishes become a good bio- indicator for monitoring the ill- effects of these contaminants on environmental health due to their specific position in trophic chain and responsiveness to the contaminants even at low concentration (Mostakim *et al*., 2015; Sula *et al*., 2020). Apart from haematological, biochemical and physiological studies, histological examination of the concerned tissue provides an insight into health of the organism as well as its response towards the stressor (Akter *et al*., 2020). Kidney serves as an organ of excretion that helps in removal of the xenobiotics the organism is being exposed. It also participates in osmoregulation and haematopoietic activities (Raibeemol and Chitra, 2020). So, histopathological examination of kidney lesions can provide a better picture of effect of environmental pollution (Sula *et al*., 2020). There are plenty of research studies indicating the toxic effects of various organophosphates (Envoy 50 SC, Malathion, Profenofos, Quninalphos 25 EC, etc.) and pyrethroid on the renal tissues of the experimental fish (Akter *et al*., 2020; Bharti and Rasool., 2021; Moniruzzaman *et al*., 2017; Mostakim *et al*., 2015; Haque *et al*., 2017). However, the scientific study regarding effects of hybrid pesticides on the fish is scarce, so in the present investigation, an attempt has been made to explore the effects of hybrid pesticide containing chlorpyrifos and cypermethrin on histo- morphology of trunk kidney of *Clarias batrachus*.

MATERIALS AND METHODS

2.1. Experimental Animal

Live and healthy specimen of freshwater walking catfish, *Clarias batrachus* (Linnaeus, 1758) of either sex were purchased from local fish market in Ranchi, Jharkhand. The fish were carried to University Department of Zoology, Ranchi University, Ranchi, Jharkhand and were maintained in glass aquaria with 20 L of tap water. The fish were disinfected with 0.1 % $KMNO_4$ solution. The average body weight of selected fish was 75- 100 g and average length of the body was 20- 25 cm. The fish were kept for acclimatization in laboratory conditions for two weeks before the experimentation. The fish were fed with commercially available fish feed once a day. The water of the aquaria was changed after every 24 hour to get rid of excreta and left over feed. The physicochemical condition of the water, such as, temperature and pH was maintained constant throughout the study. They were subjected to a photoperiod of 12 h light/ 12 h dark condition.

2.2. Experimental Chemical

In this study, a hybrid pesticide containing chlorpyrifos (50 %) and cypermethrin (5 % EC) was used for experimentation. Anth- 505, a commercially available composition of the hybrid pesticide, was purchased from local supplier in Ranchi, Jharkhand.

2.3. Experimental Design

2.3.1. Determination of dose

The dose of the experimental chemical was determined on the basis of a previous study made by Kumar *et al*., 2020. According to their study, the 96 h LC_{50} value of hybrid pesticide containing chlorpyrifos (50 %) and cypermethrin (5 % EC) for adult *Clarias batrachus* of average body weight 150- 220 g and average body size 15- 25 cm was 6 µL/L of water. Based on their report, two sub- lethal concentration of the experimental chemical, that is, $1/15^{th}$ and $1/5^{th}$ of 96 h LC_{50} value was used for experimentation in the present study.

2.3.2. Grouping of experimental animal

For toxicological study, fish were divided into three groups and maintained in separate aquaria with five individuals in each. Group I served as normal control (NC) and maintained with tap water only. In group II, fish were exposed to low dose (LD) of hybrid pesticide, that is, $1/15^{th}$ of 96 h LC_{50} (0.4 µL/L of water). In group III, fish were exposed to high dose (HD) of hybrid pesticide, that is, $1/5^{th}$ of 96 h LC_{50} (1.2 µL/L of water). The experiment was conducted for a period of 96 h. A fresh dose of experimental chemical was administered after every 24 h. During the experimentation, animals were maintained as per the guidelines of institutional ethical committee of Ranchi University.

The grouping of experimental animals and dosing schedule followed in the present study is as per our previously published report, Kumar *et al*., 2023.

2.3.3. Collection of tissue sample

After 96 h of exposure with hybrid pesticide, fish from each group were sacrificed under anesthesia. Intact trunk kidney was removed carefully and fixed in Bouin's fluid.

2.3.4. Processing of tissues for histological study

After 24 h of fixation, tissues were transferred to 70 % alcohol. Further they were subjected to the process of dehydration, clearing, infiltration and embedding to form paraffin blocks. The kidney sections of 5 µm thickness were cut using rotary microtome and processed for H & E double staining. The stained kidney sections from each group were observed and photographed using image analyzer Olympus CH20i for histological analysis.

RESULTS

3.1. Anatomical localization of trunk kidney

In *Clarias batrachus*, a distinct head and trunk regions of kidney can be seen. The trunk region of kidney can be localized in retroperitoneal region in abdominal cavity, lying just ventral to the vertebral column. It was light brown and tubular in appearance, extending to the posterior region of abdominal cavity (Fig 1).

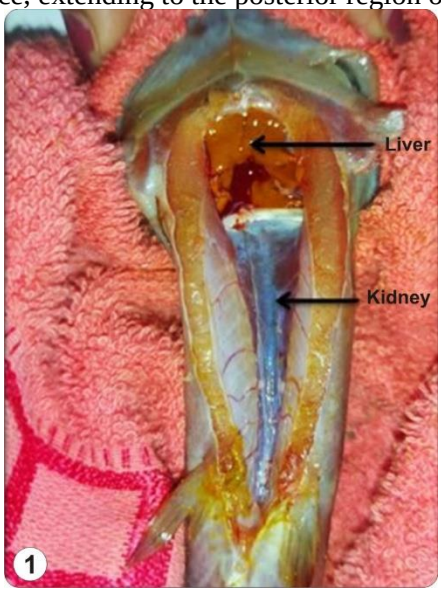

Figure 1: Anatomical localization of trunk kidney in *Clarias batrachus*

3.2. Histo- morphological study of trunk kidney

Fig 2 to 6 showed the histological structure of trunk kidney in control and treated group fish. In normal control (NC) group I, the T. S. of trunk kidney showed normal appearance of renal parenchyma with intact renal corpuscles/ malpighian body and renal tubules. Renal corpuscles consisted of vascular glomerulus surrounded by Bowman's capsule. Intact outer parietal and inner visceral epithelial layer of Bowman's capsule can be seen. The space between these two epithelial layers, termed as, Bowman's space was also distinguishable. Proximal convoluted tubules (PCT) composed of tall columnar epithelial cells with basal nuclei and brush-border lumen can be marked prominently. Few Distal convoluted tubules (DCT) made up of relatively lesser stained columnar cells with central nuclei and clear lumen can also be observed in kidney sections of control group fish. Group of haematopoietic tissues can be seen in interstitial space (Fig 2).

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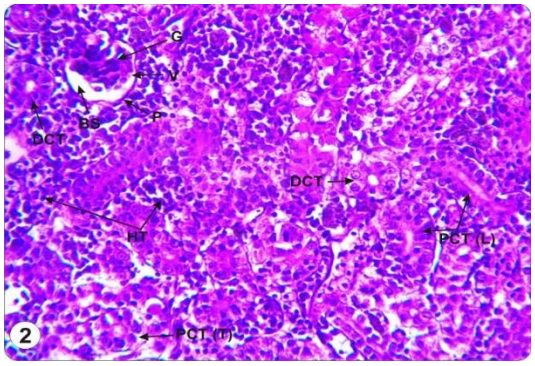

Figure 2: Photomicrograph of T. S. of trunk kidney of *Clarias batrachus* of normal control (NC) group (I) showing normal architecture of glomerulus, Bowman's capsule and renal tubules. Group of haematopoietic cells can also be seen in interstitial space (H&E, X400). Abbreviations: BS- Bowman's Space, DCT- Distal Convoluted Tubule, G- Glomerulus, HT- Haematopoietic Tissues, P- Parietal epithelium of Bowman's capsule, PCT (L)- Longitudinal section of Proximal Convoluted Tubule, PCT (T)- Transverse section of PCT, V- Visceral epithelium of Bowman's capsule.

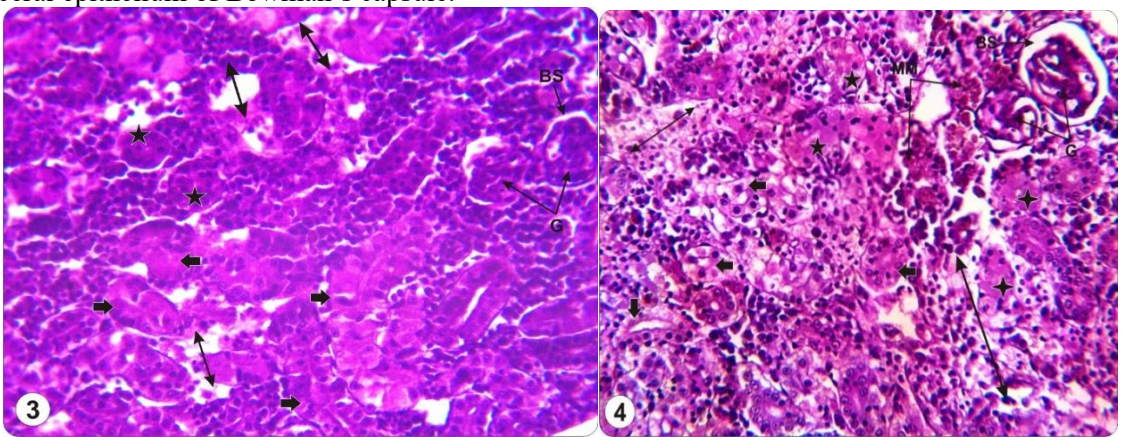

Figure 3: Photomicrograph of T. S. of trunk kidney of group II (LD) fish, *Clarias batrachus*, showing hyperplasia of renal corpuscles, expansion of glomerulus (G), shrinkage of Bowman's space (BS), degenerative and necrotic changes in epithelial cells of renal tubules (Arrow; ➞), loss of tubular lumen (Star; ★), vacuolization in interstitial tissues and infiltration of blood cells and tubular epithelial cells in the interstitial space (Double arrow; ↕) following exposure with low dose of hybrid pesticide ($1/15^{th}$ of 96 h LC50) for 96 h (H&E; X400).

Figure 4: Photomicrograph of T. S. of trunk kidney of group II (LD) fish, *Clarias batrachus*, showing hyperplasia of renal corpuscles, degeneration of glomerulus (G), shrinkage of renal tubules, necrosis and loss of structural integrity of renal tubules (★), appearance of cytoplasmic vacuolation and pyknotic nuclei in tubular epithelial cells (➞), occlusion of tubular lumen (✦), loss of haematopoietic tissues, vacuolar degeneration and aggregation of melanomacrophages (MM) and other blood components in interstitial space (↕) following acute exposure of 96 h with hybrid pesticide at low dose (0.4 µL/L of water) (H&E; X400).

In group II and III, fish were intoxicated with low (1/15th of 96 h LC_{50}) and high (1/5th of 96 h LC_{50}) dosage of hybrid pesticide containing chlorpyrifos (50 %) and cypermethrin (5 % EC), respectively, for a duration of 96 h. Acute exposure with hybrid pesticide in treated group (II and III) fish showed toxic changes in histological structure of trunk kidney. A distorted morphology of renal corpuscles and renal tubules can be seen. However, the severity of damage was directly proportional to the amount of dose administered.

In low- dose (LD) treated group (II), a hyperplasia of renal corpuscles can be seen. There was an expansion in glomerulus resulting in narrow Bowman's space. Degenerative changes can also be seen in glomerulus (Fig 4). Most of the renal tubules can be marked with degenerative and necrotic changes in columnar epithelial cells (Fig 3 & 4). Loss of structural integrity, cytoplasmic vacuolization, loss of nuclei and appearance of pyknotic nuclei can be observed in tubular epithelial cells. There was a loss of lumen in many renal tubules (Fig 3 & 4). Toxic changes can be observed in interstitial tissues also. Due to shrinkage of renal tubules and vacuolization of interstitial tissues, the kidney parenchyma appeared loosely arranged. Although there was a loss of haematopoietic tissues, a local aggregation of melanomacrophages and other blood components can be seen in interstitial space (Fig 4).

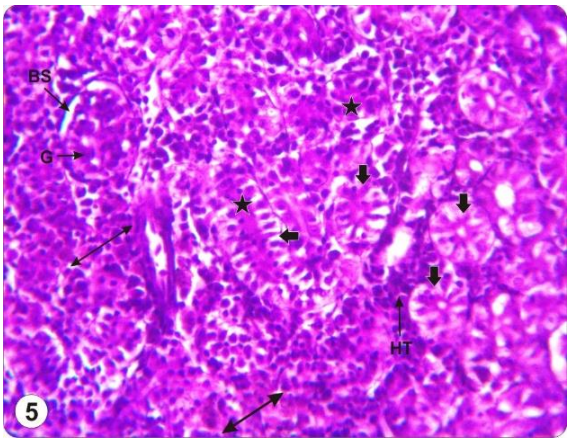

Figure 5: Photomicrograph of T. S. of trunk kidney of group III (HD) fish, *Clarias batrachus*, exposed to high dose of hybrid pesticide (1/5th of 96 h LC50) for 96 h showing expansion of glomerulus (G) leading to shrinkage of Bowman's space (BS), severe necrotic and degenerative changes in renal tubules (➞) such as, cytoplasmic vacuolation, loss of nuclear material, pyknotic nuclei along with occlusion of tubular lumen (★). There was infiltration with blood cells in the interstitial space (↕). Haematopoietic tissues (HT) were sparse (H & E; X400).

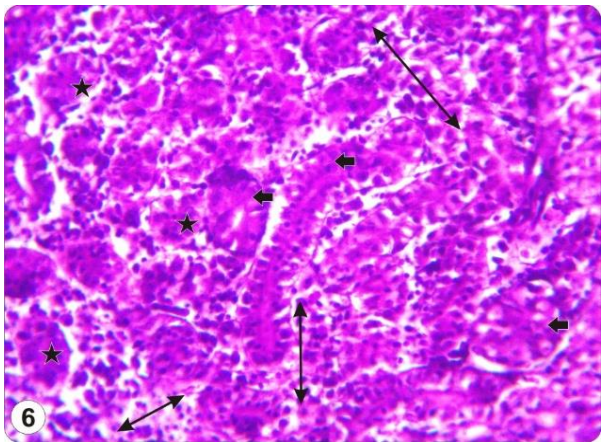

Figure 6: Photomicrograph of T. S. of trunk kidney of group III (HD) fish, *Clarias batrachus*, following acute exposure of 96 h with high dose of hybrid pesticide (1.2 µL/L of water) showing extensive degeneration of renal tubules indicated by loss of structural integrity, shrinkage of tubules, necrotic changes in cytoplasm and nuclear material of tubular epithelium (➞) and occlusion of lumen (★). Loosely packed interstitial space can be seen with blood components and detached tubular epithelial cells (↕) (H & E; X 400).

Exposure with high dose of hybrid pesticide resulted in severe degeneration of renal tubules in group III fish. Degenerative deformities in the tubules can be noticed in terms of loss of structural integrity and shrinkage of the tubules. The boundary of tubular epithelial cells was not intact. There was a loss of cytoplasm with extensive vacuolization. The nucleus showed variable degree of necrotic signs, such as, karyolysis (fading of nuclear material), pyknosis (Shrinkage of nuclear material) and anuclear condition with complete loss of nuclear material. (Fig 6). In some cells, the nucleus was present in periphery, towards the lumen. The epithelial cells were detached from tubular structure and scattered in the interstitium. An occlusion of tubular lumen was also evident (Fig 5 & 6). Although the hyperplasia of renal corpuscles was not visible, an expansion in glomerulus and narrowing of Bowman's space was visible in HD group fish also (Fig 5). Loosely packed interstitium consisted of sparse haematopoietic cells, remnants of tubular epithelial cells and infiltration of blood components (Fig 5 & 6). Altogether, acute exposure with the hybrid pesticide negatively affected the structural integrity of renal tubules, primarily of PCT, and interstitial tissues at both the dosage.

DISCUSSION

Chlorpyrifos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyrinidyl)-phosphorothioate) is a widely used insecticide. It is chlorinated organophosphate in nature and acts via inhibition of activity of Acetylcholinesterase, responsible for conduction of nerve impulses in cholinergic synapses. Despite of its wide applicability in agricultural, industrial as well as domestic purposes, it has been documented with severe toxic impacts on the health of fishes (Georgieva *et al*., 2021; Raibeemol and Chitra, 2020). On the other hand, cypermethrin is a synthetic pyrethroid used as an insecticide. It is moderately toxic in nature and acts via inhibition of voltage- gated sodium channel (Georgieva *et al*., 2021). Both the pesticides have been reported to cause renal toxicity to the fishes when used individually. Exposure with chlorpyrifos have been documented to cause vacuolization in epithelial lining of proximal and distal tubules, presence of aggregation of mononuclear cells, hyperactivated melanomacrophages, haemorrhage and an increase in interstitial space in the kidney of Nile tilapia (Zahran *et al*., 2018). Duration- dependent degenerative changes in the kidney of *Heteropneustes fossilis* exposed with chlorpyrifos at the dose of 75 % of 96 h LC_{50} was reported by Verma *et al*., 2020. The changes were evident in the form of enlargement of glomerulus and Bowman's capsule, loss of nuclear material, necrosis, shrinkage and dilation in renal tubules and vacuolization in interstitial space. Similarly, atrophic and degenerative changes in the renal tissues of *Cirrhinus mrigala* was observed by Prashanth MS, 2011 when the fish were exposed with cypermethrin at the dose of $1/5^{th}$ of 96 h LC_{50} value. Exposure with cypermethrin caused a reduction in cell number in proximal and distal tubules resulting in narrowing of tubular lumen, loss of tubular shape, vacuolization, infiltration of epithelial cell nuclei and leucocytes in the surrounding tissue and necrotic changes in the nucleus with presence of pyknotic nuclei. The extent of damage caused by the pesticide was dependent on the dose administered, duration of exposure, chemical nature of the pesticide and susceptibility of the experimental animal used (Prashanth MS, 2011). Renal toxicity induced by cypermethrin intoxication was also reported in *Oreochromis niloticus* and *Cyprinus carpio* (Majumder and Kaviraj, 2022; Neelima *et al*., 2015). Acute exposure with cypermethrin for 96 h caused glomerular shrinkage and necrotic changes in renal tubules as well as interstitial tissues in kidney sections of *Ophiocephalus striatus* (Dorlikar and Mohite, 2019). In the present study, a hybrid pesticide containing chlorpyrifos and cypermethrin was used to investigate the toxicological effects on trunk kidney of *Clarias batrachus*. Acute exposure with low ($1/15^{th}$ of 96 h LC_{50}) and high ($1/5^{th}$ of 96 h LC_{50}) dosage of the pesticide for a duration of 96 h resulted in a dose- dependent degenerative damages in the renal parenchyma. The kidney sections of fish intoxicated with the hybrid pesticide showed hyperplasia of renal corpuscles, expansion of glomerulus leading to shrinkage of Bowman's space, degenerative damage in renal tubular epithelial cells, shrinkage of renal tubules and occlusion of tubular lumen (Fig 3-6). Necrotic changes in the interstitial tissues can also be marked. The aberrant renal cells may lead to a disturbance in the normal metabolism of the individual, thereby inducing diseases and even death at long-

term exposure (Xing *et al*., 2012). The toxic changes were more evident in proximal tubules, as were also reported by Prashanth MS, 2011. They further explained that the presence of comparatively larger number of mitochondria make the PCT more susceptible to the damage. On the other hand, higher number of PCT in the renal sections also advocated for prevalence of damage. The degenerative damages in the renal tubules, occlusion of tubular lumen, degeneration of glomerulus, cellular rupture and necrosis following toxicant exposure might be due to hyperactivity of the renal cells to combat the toxicity induced by the chemical, increased cell volume and damage to cell membrane due to impaired ion flow and cation pump and subsequent accumulation of the toxicant in the tissues (Pal *et al*., 2012). The extent of severity and nature of renal tissue damage observed in the present study was in accordance with the previous studies reporting the toxic effects of chlorpyrifos and cypermethrin alone. This made it tough to conclude if the pesticides are synergistically acting to cause the damage or one of the pesticide is more prevalent. Georgieva *et al*., 2021 reported a higher toxicity of cypermethrin as compared to chlorpyrifos on various behavioural, biochemical and histological markers in common carp, even when cypermethrin was used at lower concentration than chlorpyrifos. On the other hand, a cumulative toxicity of the hybrid pesticide containing chlorpyrifos and cypermethrin was reported in Nile tilapia (Thanomsit *et al*., 2020).

CONCLUSION

Based on the findings of the present investigation, a dose- dependent toxic effect of hybrid pesticide on the renal tissues can be documented in *Clarias batrachus*. However, further study is needed to elaborate the long-term toxicity of the hybrid pesticide and mechanism of their action.

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