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Fenpropathrin Induced Histopathologic Changes in Tissues (Kidney, Liver, and Spleen) of Freshwater Fish, *Pethia conchonius* (Hamilton 1822)

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ABSTRACT

The most common threat to fish health is from agricultural runoffs. Among the runoffs are the insecticides that are applied regularly for crop protection. Fenpropathrin type II synthetic pyrethroid, was exposed to assess its histopathological effects on rosy barb, *Pethia conchonius*. The fish were exposed to two sublethal concentrations, 0.486 μg fenpropathrin/L and 0.243 μg fenpropathrin/L and a control for 30 days. At the end of study period, fish were euthnized humanely and three tissues kidney, liver and spleen, were dissected out and were fixed for histopathological evaluation. Tissues of fish in the control group showed normal histomorphology, however, in both treatment groups, kidney tissue showed dilation of renal tubules, degeneration of renal tubules, glomerular degeneration, while liver tissue of treatment groups showed dilated sinusoids, pyknotic nuclei and necrotic cell. Histological changes in spleen tissue in both treatment groups exhibited melanomacrophage centers and splenic congestion. All of these histological changes were clear indicative of fenpropathrin induced changes.

Keywords: Histopatholgy; *Pethia conchonius*; Kidney; Spleen; Liver; Fenpropathrin

1 INTRODUCTION

Introduction of pesticides has contributed to immense growth of production in agriculture. A pesticide is any agent that is intended to control the crop harming or damaging organisms; hence the usage of pesticide is meant to target specific class of organisms. An increased usage of pesticides has its own downfall, as many pesticides are often persistent and become biomagnified in trophic systems. There have been reports of pesticide poisoning; it is observed that, globally 3 million poisoning cases occur every year and more than 250,000 deaths occur due to pesticide poisoning [1]. Pesticides enter the water bodies through surface runoff, leaching or erosion [2]. Exposure and uptake of pesticides by the fish occurs through dermal or gills [3], but gills appear to be the most common way, as they are main organs for respiration and osmoregulation [4]. Pesticides are known to bioaccumulate in fish tissues and result in disruption in fish biology. The effect of pesticides in water affects fish directly or indirectly. When pesticides are present in sufficient concentration, it may result in fish deaths, but if present at low levels, invertebrates are affected thus impacting the availability of food to fish [5]. When present at lethal levels, pesticides may cause mortality, but at sublethal levels the effects may include impaired growth, impaired reproduction, reduced immunity and altered behaviour; these effects may vary between species and the life stage at which it is exposed to a particular type of pesticide [5].

Synthetic pyrethroids are the most recent insecticides that were produced. The first synthetic pyrethroid that was introduced was allethrin [6]. Synthetic pyrethroids were chemically derived from pyrethrin, that are naturally known toxins occurring in the plant, *Chrysanthemum cinerariaefolium* [7]. As their toxicity is low for mammals, their usage was envisaged to increase in the years to come [6]. Synthetic pyrethroids and carbamates are the next most utilised pesticides globally after organophosphates [8].

Synthetic pyrethroids are extremely toxic to fishes. The acute toxicity of synthetic pyrethroids differs among its representatives and fish species. Deltamethrin has a median lethal concentration of 2.3 μg/L (96 h) for common carp, while for *Tilapia nilotica* the acute toxicity is 14.5 μg/L (96 h) [9]. The median lethal concentration for cypermethrin, in

Tor putitora, was found to be 63 μg/L (96 h) [10], however, for *Labeo rohita*, median lethal concentration was reported to be 4 μg/L (96 h) [11]. At sublethal levels, pesticides are not fatal to fish, but can cause indistinct alteration in behaviour and physiology that hinders both survival and reproduction [12]. These subtle physiological changes that occur can be studied by histopathology. Histopathology studies are uncertain and are nonquantifiable, yet these studies are well known biomarkers for environmental contamination [2].

Histopathological effects of synthetic pyrethroids have been briefly reviewed [9]. However, till date, histopathological investigation of effects of fenpropathrin on fish are not reported. Fenpropathrin is a broad-spectrum insecticide, and bioconcentrates in tissues of organisms [13]. Fenpropathrin is widely used to control oriental fruit moth, thrips, leaf rollers and mites. Fish is an ideal model to study the effect of toxins in aquatic environment [14] because their behaviours can be observed easily and can be quantified in controlled settings. Accumulation of pesticides in vital organs of fish like liver, kidney and muscles results in dysfunction and mortality of fishes. *Pethia conchonius* has been used as ecotoxicological model for histopathological studies [15–17]. Hence in the present histopathological study, sublethal effects of fenpropathrin was evaluated on *Pethia conchonius*.

2 METHODOLOGY

2.1 Animals

Healthy adult rosy barbs were purchased from local pet vendor (fish fair, Hubli, India) with mean length of 3.78 $\pm$ 0.27 cm and mean weight 1.45 $\pm$ 0.3 g. Fish were transferred to 40 L capacity glass tanks (60 cm $\times$ 30 cm $\times$ 30 cm). Fish were allowed to acclimatize for 15 days. Water quality parameters are given in Table 1 . Permission to conduct experiments were obtained from the Animal Ethical Committee, Committee for the Purpose of Control and Supervision of Experiments on Animals under institutional registration (registration number: 639/GO/Re/S/02/CPCSEA).

Table 1: Water quality parameters during the exposure of *Pethia conchonius* to fenpropathrin

Parametrers	Values
Dissolved Oxygen	12.6 $\pm$ 0.536 mg/L
Total Hardness	225$\pm$7.9 mg/L
Temperature pH	26 $\pm$ 0.5° C
pH	7.3 – 7.4

2.2 Chemicals

Commercial formulation of fenpropathrin (Danitol® 10% EC; Sumitomo Chemical India Limited, India) was purchased from a local vendor.

2.3 Experimental design

Fish (n = 7) were randomly distributed into three groups, viz., a control group and two treatment groups (0.486 μg fenpropathrin/L and 0.243 μg fenpropathrin/L). The exposure was semi-static, wherein the test medium was refreshed for every 24 h. The duration of exposure period to fenpropathrin was for 30 days.

2.4 Histology

After the study period, fish from all the groups were euthanized in 2-phenoxyethanol. The fish from each group were dissected and target organs, kidney, liver and spleen, were collected and fixed in Bouin's fluid for a period of 24 to 36 h. The tissue were dehydrated with series of alcohol gradients and were embedded in paraffin. The blocked tissue was sectioned at 5 μm thickness by semi-automatic microtome (Leica rm2255, Leica microsystems, Germany). The sectioned tissue was stretched and spread on slides. The slides were cleared in xylene two times and hydrated in alcohol gradients and stained with hematoxylin and dehydrated with increasing alcohol gradients and counter-stained with eosin and cleared again in xylene. The slides were cover slipped and observed for histopathological evaluation using microscope (BX50, Olympus, Japan). Sections of tissue on slides were photographed using camera attached to the microscope (BX50) at magnification 400X (40X objective, 10X eye piece) using ProgRes 2.5 software.

3 RESULTS AND DISCUSSION

Histopathological studies are indispensable in the identification of tissue perturbances that result from xenobiotics. The structural changes in the tissues caused by the exogenous agents are important to understand their properties. Major and rapid changes in the tissues depend on the concentrations of insecticides and the time duration that fishes are exposed to the toxicants [18]. Kidney, liver, and spleen of control fish group appeared normal in the present study.

3.1 Histopathology of Kidney

The histomorphology of kidney of control fish group is shown in Figure 1 A. Kidney in teleost has prominent Bowman's capsule, glomerulus, distal tubules [19], and hematopoietic tissue. Kidney is a major target for histopathological alterations as it is also a site for detoxification and depending upon the concentration levels of a pollutant, kidney can show abnormal changes [20]. Figure 1 B, shows kidney of fish exposed to 0.243 μg fenpropathrin/L. The kidney tissue of the fish exposed to 0.243 μg fenpropathrin/L showed degenerated glomeruli, dilated renal tubules and disruption of hematopoietic tissue was observed. Kidney of fish exposed to 0.486 μg fenpropathrin/L hemorrhage, ruptured renal tubules, melanomacrophage center (MMC) aggregates (Figure 1 C). In the present study, at both tested concentrations of fenpropathrin, kidney showed degenerated glomeruli and renal tubule alterations. Degeneration and dilation of renal tubules, damage to glomeruli

in silver carp exposed to deltamethrin, a pyrethroid similar to fenpropathrin, is also reported [21]. In another study, *Cyprinus carpio* exposed to deltamethrin, showed degeneration of glomeruli and degeneration of renal tubule epithelial cells [4]. Another synthetic pyrethroid insecticide, lamda-cyhalothrin, exerted necrosis of tubular epithelium and shrinkage of glomerulus [22]. Hemorrhage and MMC were observed in kidney tissue of fish exposed to 0.486 μg fenpropathrin/L. Occurrence of MMC in the kidney tissue have been reported in fish exposed to stressors [19, 23].

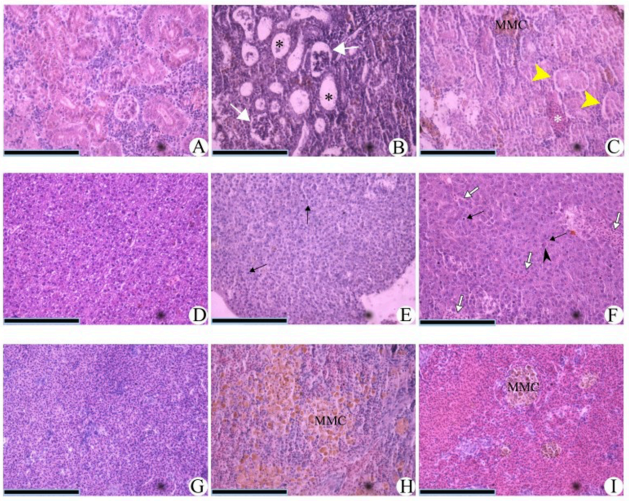

Figure 1: Histopathology of Kidney, Liver, and Spleen of *Pethia conchonius* exposed to sublethal levels of fenpropathrin for 30 days. A-kidney, D-liver and G-spleen of control fish.B-kidney (white arrow -degenerated glomeruli, black asterisk-dilated renal tubule), E-liver(Black arrow- pyknotic nuclei) and H-spleen of fish exposed to 0.248 μg fenpropathrin/L. C-kidney (yellow arrowhead -lysed renal tubules, white asterisk-haemorrhage, F-liver (white arrow-dilated sinusoids and congestion, black arrow-pyknotic nuclei, black arrowhead-necroticcell) and I-spleen (white asterisk-splenic congestion) of fish exposed to fenpropathrin/L. MMC-Melanomacrophage centers. Bar, A-I = 50μm, H&E-Haematoxylin and Eosin, 400X (40X objective, 10X eye piece)

3.2 Histopathology of liver

Liver tissue has normal appearance with hepatocytes in polygonal shape and central nuclei (Figure 1 D). Liver of fish exposed to 0.243 μg fenpropathrin/L, had no serious alterations, except with the low to moderate occurrence of pyknotic nuclei (Figure 1 E). Fish exposed to 0.486 μg fenpropathrin/L showed most alterations in liver (Figure 1 F). The occurrence of pyknotic nuclei, dilated sinusoids and congestion, and necrotic cell were observed in liver. Liver is an important organ for detoxification [24]. Histopathology of liver is an excellent way to gauge the effects of a chemical toxicant [25]. Liver of fish exposed to 0.243 μg fenpropathrin/L exhibited no serious signs of toxicity. The alterations observed were pyknotic nuclei. The occurrence of pyknotic nuclei suggests, fenpropathrin role in inducing cell death. In fish exposed to 0.486 μg fenpropathrin/L, liver tissue had more prominent lesions such as dilated sinusoids and congestion, necrotic cell, and pyknotic nuclei. Occurrence of necrotic cell in portions of liver is an indication of stress experienced by the liver cells to eliminate excessive toxicant from the body; further, inability to regenerate new hepatic cells leads to necrosis [26]. Similar reports on fish liver histopathology exposed to pesticides have been reported [10, 22, 25, 27–29].

3.3 Histopathology of Spleen

The teleost fish spleen is covered by a thin capsule that encapsulates the splenic parenchyma, which contains blood vessels, ellipsoids, red pulp, white pulp and macrophages [30]. Spleen is an immunological organ and functions in phagocytosis of erythrocytes and possess hematopoietic activity [31]. The spleen of control fish (Figure 1 G) exhibited no MMC, indicating healthy spleen. Fish exposed to 0.243 μg fenpropathrin/L (Figure 1 H) showed occurrence of MMC and disruption in the splenic cords. In spleen of fish exposed to 0.486 μg fenpropathrin/L, (Figure 1 I) showed occurrences of MMC and exhibited splenic congestions. A characteristic of teleost spleen is presence of phagocytic corpuscle MMC, which are made of phagocytosing macrophages that contain melanin granules, peroxisome and other phagosomes in their cytoplasm [31]. Formation of MMC is directly correlated to the concentration of pollutant in the environment [32]. Exposure of deltamethrin with increase in exposure duration caused larger MMC in spleen of silver carp [21].

In summary, fenpropathrin induces histopathological changes in the organs of *Pethia conchonius*. These changes reflected the severity caused by the chemical, wherein kidneys appeared to be most affected at both tested concentrations, while changes in liver was more prominent at 0.486 μg fenpropathrin/L. Spleen, at both tested concentrations, showed MMC prevalence.

4 CONCLUSION

Based on our results, fenpropathrin affected the vital organs, kidney, liver, and spleen, of *Pethia conchonius*. Thus, it can be concluded that, fenpropathrin affects vital organs, kidney and spleen, and can impair immune status of fish, which needs to be further investigated to correlate the histopathological changes observed in the study.

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