E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(2): 412-415

www.fisheriesjournal.com
Received: 24-01-2017
Accepted: 25-02-2017

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Histopathological changes due to induced biopesticide Kethrin in the liver of freshwater fish, *Labeo rohita* (Hamilton)

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Abstract
The liver of *Labeo rohita* was histopathologically examined after acute exposure (4 days) and chronic exposure (30 days) of two sublethal concentrations (1/15th and 1/10th of 96h LC_{50} values) of Kethrin. After the acute exposure of 1/15th (1.445ppm) and 1/10th (=2.168ppm) of 96hr LC_{50} value of Kethrin to *L. rohita* for 4 days, histopathological changes observed includes severe necrosis, cloudy swelling of hepatocytes and disintegration of liver lobules. After the chronic exposure of 1/15th of LC_{50} (1.445ppm) and 1/10th (=2.168ppm) of LC_{50} value of Kethrin to *L. rohita* for 30 days, histopathological changes observed includes necrosis, cloudy swelling, hyperplasia, coagulation of blood vessel, lifting up of epithelium and infiltration of lymphocytes. The histopathological damage in the liver was found to be severe in case of chronic exposure as compared to the acute exposure of Kethrin. The results indicate that even the sublethal concentrations of Kethrin were enough to elicit significant changes in the liver histology of *L. rohita* and further suggest that even smaller concentrations of any toxicant in the environment can induce major histological changes and more care and vigil is needed before using such biopesticides into agricultural fields or near fish farms.

Keywords: *L. rohita*, Kethrin, Liver, histopathological changes

1. Introduction
In view of the environmental problems caused by the use of synthetic chemicals and the growing need for alternative methods of pest control that minimize this damage, there has been extensive research on pest control by substances from plants. One of the most promising natural compounds is matrine, an alkaloid extracted from the roots of *Sophora flavescens*. *S.flavescens* is an evergreen shrub growing upto 1-1.5m. Its roots have been found to contain many alkaloids including matrine and its oxide, oxymatrine. Characterization of alkaloids in *S. flavescens* Ait was reported by (Liu, 2011) [7]. Antifeedent activity and acute and residues toxicity of alkaloids from *S. flavescens* against formosan subterranean termites was reported by (Henderson, 2007) [5]. Recently plant based pesticides are popularised due to their high efficiency, broad spectrum, low toxicity, non-residue and green protectin to environment. However, many botanical pesticides have been found to be toxic to non-target organisms where they induce marked alterations in experimental animals (Mahboob *et al*., 1998; Anjaneyulu *et al*., 1999; Mondal *et al*., 2007) [8, 3, 9]. The toxic effects of such biopesticides are less documented. So there was a need to assess the potential effects of such biopesticides on some aquatic species. In the present study, the histopathological changes caused by biopesticide Kethrin in the liver of *L. rohita* were studied. Kethrin is a botanical insecticide obtained from the roots of *S. flavescens*. The formulation contains a minimum of 0.5% EC (500ppm) of matrine, the most bioactive compound of *Sophora*. Since fishes are the top consumers of aquatic ecosystem and thus chances of pesticide bioaccumulation are greater in them. Fishes act as bioindicators of aquatic pollution. In the present study, *L. rohita* has been selected as a test model animal because of its easy availability, high food value and wide distribution. Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Wester and Canton, 1991) [15] and field studies (Schwaiger *et al*., 1997) [13]. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible

for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al*., 2001) [4]. Histopathological alterations caused by synthetic as well as plant based pesticides in different fishes have been reported by different workers (Abraham *et al*., 2003; Sarkar *et al*., 2005; Mondal *et al*., 2007; Tilak *et al*., 2009; Saravanan *et al*., 2010; Prashanth, 2011 and Anita *et al*., 2012) [1, 12, 9, 14, 11, 10, 2]. The organ most associated with the detoxification and biomarker process is the liver due its function, position and blood supply. In the present study the histopathological changes produced in the liver of the test fish *L. rohita* after the induction of two sub lethal concentrations of Kethrin during acute (4 days) as well as chronic exposure (30 days) were studied.

2. Materials and Methods

2.1 Biopestide used in the Study

Biopesticide "Kethrin" (Manufactured by Ezzy Bio Sciences Pvt. Ltd., Khasra no. 90/1, Gram Meharja, Khargone, Madhya Pradesh) was used in this study. It contains a minimum of 0.5% EC (500ppm) of matrine. It is obtained from the roots of shrub *sophora flavescens*.

2.2 Experimental Set-up

The experiments of the present study were performed at Department of Zoology Government Science and Commerce College Benazir Bhopal (M.P) during the year 2012-13. Glass aquaria of the size 24"×12"×18" were set up in the laboratory. All the aquaria were of the capacity of 60 liters. Aquaria were provided with all the necessary equipment such as aerators, artificial light, facial matter extraction tube and water removing pipes to maintain the natural possible conditions for the test organism.

2.3 Collection of Experimental animal

The experimental fish, *L. rohita* was obtained from Patra fish farm Barkhedi Bhopal (M.P). They weighed 50g±2g and their length was in the range of 12cm±2.

2.4 Maintenance of the Experimental animal

They were brought to laboratory carefully in oxygen filled polythene bags in card board boxes to avoid any injury. They were disinfected by giving a bath for five minutes in $KMnO_4$ solution. Thereafter, they were transferred to glass aquaria filled with dechlorinated water. The fishes were acclimated to the laboratory conditions for 20 days prior to the experiment. During acclimatization fishes were fed daily with commercial fish food which was given at morning hours. Water was replaced every 24h after feeding in order to maintain a healthy environment for the fish during acclimatization period. This ensures sufficient oxygen supply for the fish and the environment was devoid of any accumulated metabolic wastes. Dead fishes whenever located were removed immediately to avoid the fouling of the water.

2.5 LC_{50} determination

Prior to conducting the bioassay for histopathology, a toxicity bioassay was run in the same water to estimate the 96hr LC_{50} value of Kethrin for *L. rohita* and the same was found to be 21.68 ppm.

2.6 Preparation of Biopesticide Doses

In the present study, two sublethal concentrations (1/15th and 1/10th of LC_{50} values) of Kethrin were prepared and induced to *L. rohita* for 4 days (acute exposure) and 30 days (chronic exposure).

2.7 Selection of Groups

Three groups were selected during the toxicity tests in which the group first was taken as the control group (no biopesticides used) and the other two groups were given sub lethal concentrations of Kethrin. The group II was treated with $1/15^{th}$ concentration of 96hr LC_{50} value of Kethrin and the group III was treated with $1/10^{th}$ concentration of 96hr LC_{50} value of Kethrin. Ten fishes were used in control as well as in experimental batches.

2.8 Histological examination

The liver at the end of exposure were taken out and rinsed in physiological saline solution to remove the debris etc. The liver was then fixed in Bouin's solution for 24 hours followed by thorough washing in 70% alcohol and dehydrated through alcohol series. The liver was then transferred in methyl benzoate for 24 hours. Then after washing with benzene, it was embedded in paraffin wax at 58°C in incubator. Blocks were prepared and cut at 5 microns. The ribbons were stretched on cleaned slides using Mayer's albumin. After 24 hours of drying, the sections were stained with haematoxylin and counter stain with eosin. The mounting was done in DPX. The microphotographs of selected sections were taken with the help of Olympus micrographic equipment.

3. Results

The histopathological changes were more evident in specimens exposed to Kethrin and were not observed in the control fish. The histology of the normal liver structure (Control group) showing hepatocytes with prominent nuclei was shown in Photo No.1. After the acute exposure of $1/15^{th}$ (1.445ppm) and $1/10^{th}$ (=2.168ppm) of 96hr LC_{50} value of Kethrin to *L. rohita* for 4 days, histopathological changes observed in the liver of the fish includes severe necrosis, cloudy swelling of hepatocytes and disintegration of liver lobules (Photo No. 2 & 3). After the exposure of $1/15^{th}$ of LC_{50} (1.445ppm) value of Kethrin to *L. rohita* for 30 days, histopathological changes observed includes necrosis, cloudy swelling, hyperplasia of hepatocytes, blood clotting and lifting up of epithelium (Photo No. 4, 5 & 6). After the exposure of $1/10^{th}$ (2.168ppm) of LC_{50} value of Kethrin to *L. rohita* for 30 days, histopathological changes observed were severe necrosis, hyperplasia, coagulation of blood vessel, pyknotic nuclei and infiltration of lymphocytes (Photo No. 7, 8 & 9).

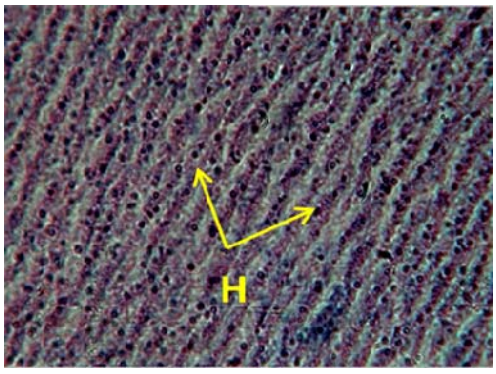

Photo1: Showing normal histoarchitecture of hepatocytes (H) with prominent nuclei cytoplasm in the liver of control fish *L. rohita* (x400).

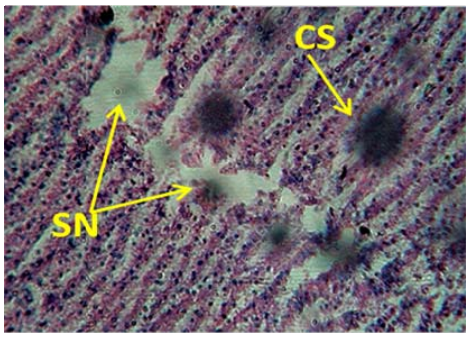

Photo 2: Showing severe necrosis (SN) and cloudy swelling (CS) of hepatocytes and, in 1/15th of 96h LC_{50} value of Kethrin (1.445ppm) treated *L. rohita* after acute exposure of 4 days (x400).

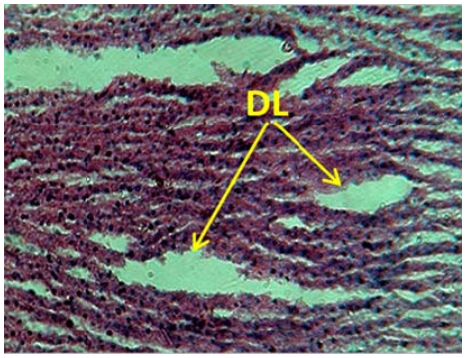

Photo 3: Showing disintegration of liver lobules swelling (CS) in 1/15th of 96h LC_{50} value of (2.168ppm) treated liver of *Labeo rohita* after acute exposure of 4days (x400).

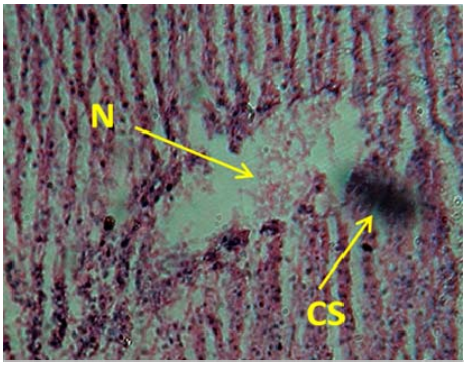

Photo 4: Showing necrosis (N) and cloudy (DL) in 1/10th of 96h LC_{50} value of Kethrin Kethrin (1.445ppm) treated liver of *Labeo rohita* after chronic exposure of 30 days (x400)

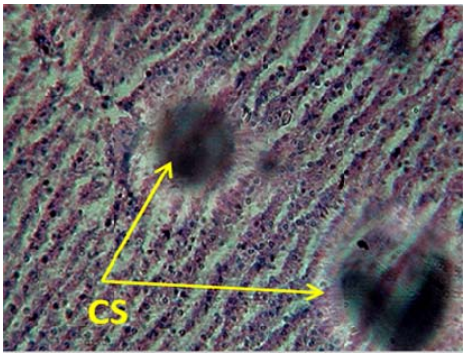

Photo 5: Showing cloudy swelling (CS) of hepatocytes in 1/15th of 96h LC_{50} value of Kethrin (1.445ppm) treated liver of *L. rohita* after chronic exposure of 30 days (x400).

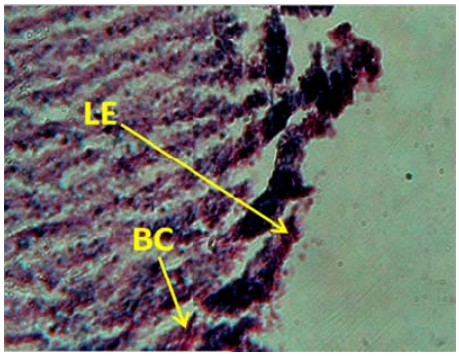

Photo 6: Showing lifting up of membrane (LE) and blood clotting (BC) in 1/15th of 96h LC_{50} value of Kethrin (1.445ppm) treated liver of *L. rohita* after chronic exposure of 30 days (x400).

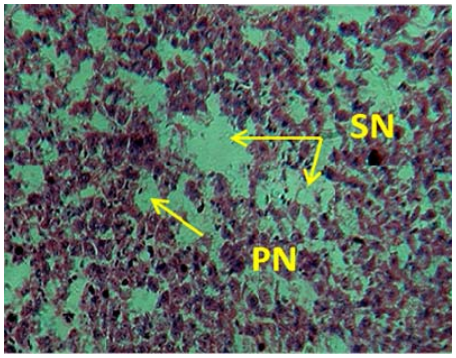

Photo 7: Showing severe necrosis (SN) and pyknotic nuclei (PN) in 1/10th of 96h LC_{50} value of Kethrin (2.168ppm) treated liver of day's days (x400).

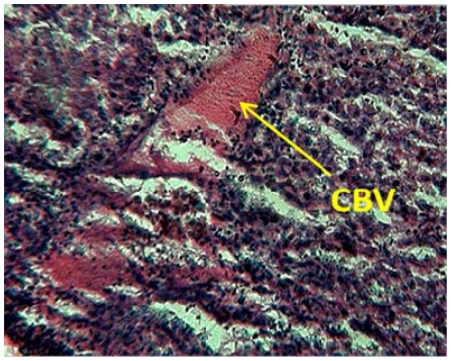

Photo 8: Showing coagulation of blood vessel (CBV) in 1/10th of 96h LC_{50} value of Kethrin (2.168ppm) treated liver of *Labeo rohita* after chronic exposure of (x400).

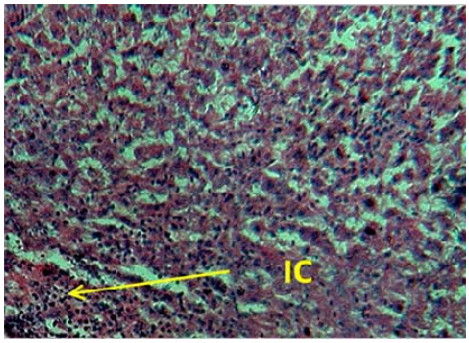

Photo 9: Showing infiltration of inflammatory cells (IC) in 1/10th of 96h LC_{50} value of Kethrin (2.168ppm) treated liver of *L. rohita* after chronic exposure of 30 days (x400).

4. Discussion

Many investigators have observed similar histopathological changes in the biopesticides and synthetic pesticide induced liver of fishes. Abraham *et al*., (2003) [1] studied the histopathological effect of Neemax on the liver of *Anabas testudineus*. The histopathological lesions elucidated by Neemax (Neem based biopesticide) were hypertrophy, hyperplasia, rupture and lifting of the epithelium, appearance of pyknotic nucleus, aggregation of hepatic cells, infiltration of lymphocytes and overall necrosis of the tissue. Sarkar *et al*., (2005) [12] investigated the carbofuran and cypermethrin induced histopathological alterations in the liver of *L. rohita* (Ham.). Major damage caused by carbofuran toxicity was diffuse necrosis, cordal disarrangement, individualization of hepatocytes. Significant changes induced by cypermethrin were hyperplasia and disintegration of hepatic mass. Joshi *et al*., (2007) [6] studied the histopathological changes in the liver of *Heteropneustes fossilis* exposed to Cypermethrin. The changes observed were cloudy swelling, necrosis, hypertrophy, vacuolization and pyknotic nucleus. Nagaraju *et al.,* (2014) [16] studied the histopathological changes in the liver of *L. rohita* (Ham.) exposed to Novaluron. The histopathological changes observed were hyperplasia of hepatocytes, necrosis, hypertrophy and epithelial lifting. Most of the histopathological lesions observed in the present study are similar to those reported in earlier studies. One of the important functions of liver is to eliminate toxicant through metabolism. Hence the liver becomes hyper-active to eliminate the intoxicants. Due to the hyper activity and accumulation of compounds, the cells may become larger in size and to meet the requirement, cells proliferate much faster, which may be the reasons for hypertrophy and hyperplasia. Similarly the liver tissue will try to avoid such intoxicant being absorbed for which the epithelial tissues will lift up to avoid the toxicants. Shrunken and pyknotic nuclei indicated that cells became hypo functional and at the end, necrosis was extensive. The results of the present study indicate that the biopesticide such as Kethrin also cause far reaching consequences in the aquatic system. Even the sublethal concentrations of Kethrin were enough to elicit significant changes in the liver histology of fishes like *L. rohita*. The results further suggest that even smaller concentrations of any toxicant in the environment can induce major histological changes and more care and vigil is needed before using such biopesticides into agricultural fields or near fish farms. It would be a big mistake to consider products of plant origin, and this includes botanical insecticides, harmless merely because they are natural. We must not forget that the toxic potential of a molecule is the nature of its chemical structure and not its origin.

Botanical pesticides are presently used against different pest species in the agricultural farms and gardens but their effect on non-target organisms cannot be ruled out. So, these botanical pesticides should also be used cautiously like synthetic pesticides.

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