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Talhat Munir
Department of Zoology,
University of Gujrat, Punjab,
Pakistan

Razia Iqbal
Department of Zoology,
University of Gujrat, Punjab,
Pakistan

Sana Safdar
Department of Zoology,
University of Gujrat, Punjab,
Pakistan

Sana Naseem
Department of Zoology,
University of Gujrat, Punjab,
Pakistan

Ammara Zareen
Department of Zoology,
University of Gujrat, Punjab,
Pakistan

Correspondence
Talhat Munir
Department of Zoology,
University of Gujrat, Punjab,
Pakistan
munir.talat@yahoo.com

Histopathological changes in stomach and heart of frog, *Rana tigrina*, upon exposure to lambda-cyhalothrin

Talhat Munir, Razia Iqbal, Sana Safdar, Sana Naseem and Ammara Zareen

Abstract

This study was carried out in ICBS laboratory of Zoology researchers, Department of Zoology, University of Gujrat, Pakistan from April 2015 to August 2015. Analysis has been made to evaluate the effect of different concentration of lambda cyhalothrin on histopathology of stomach and heart of frog, *Rana tigrina*. In this study 24 frogs divided into 4 groups, 6 for each group. One group was control being untreated group while remaining received low, medium, and high doses respectively. A morphological and histopathological study reveals, it adversely affects the stomach and heart of frog that leads to limited ages of frog species and ultimate cause reduction in population size. Results suggested that these animals are sensitive to lambda cyhalothrin; therefore measures must be taken to secure these animals otherwise pernicious effects of agrochemicals will lead to extinction of this group.

Keywords: Amphibians, Pakistan, lambda-cyhalothrin, frog, stomach, heart

1. Introduction

Pesticides are used to eliminate undesired living forms, to prevent, destroy, repel, and mitigate any pest i.e. insect, rodent, nematode, fungus, weeds etc. and also used intentionally to cause damage to living forms but they also may affect other animals including man by their common mechanism ^[1]. Pyrethroids are powerful synthetic insecticides and have replaced older insecticides ^[2] because of their high efficiency, easy biodegradability, and low toxicity to birds and mammals. Pyrethroid insecticides disturb the normal functions of brain of insects and non-target organisms such as amphibians because when these bind to voltage-sensitive sodium channels they alter their gating Kinetics ^[3].

Lambda cyhalothrin is U.S. Environmental Protection Agency (EPA) registered insecticide belongs to pyrethroids and has the potential to affect terrestrial and aquatic life ^[4]. Amphibian's population is declining due to frequent use of pesticides and show adverse effects when exposed to lambda cyhalothrin because it causes severe damage in gonads, liver, and kidneys of frogs ^[5]. Cholinesterase activity in liver, kidney and brain of *Rana cyanophlyctis* is decreased when exposed to cyhalothrin (pyrethroid) and monocrotophos (organophosphate) pesticides ^[6]. When tadpoles of *X. laevis* are exposed to lambda cyhalothrin and deltamethrin for 168h, then tadpoles showed high toxicity symptoms toward lambda cyhalothrin as compared to deltamethrin. Both of these insecticides block the ACP and AST in *X.laevis* tadpoles. Lambda cyhalothrin also stop the LDH and AChE activity in these tadpoles. Due to inhibition of these enzymes, metabolism of tadpoles destroyed ^[3]. Amphibians population is declined when exposed to different pesticides especially λ -cyhalothrin because of the permeability of the frog skin and due to effect on male and female fecundity. They are exposed to low, median and high dose for several weeks and its affects observed on gonads i.e. testes and ovaries of *Rana tigrina*. Testis express breaking of cyst walls, scattering of sperm bundles, bunching of secondary spermatocytes, necrosis of sertoli cells and sprinkled masses of decayed sperm bundles. Similarly, ovaries express the increase of atretic follicles, contraction in follicles, reduction in the number of oocytes, vacuolation in the stroma, reduced maturity, less or no growth of previtellogenic oocytes, increase in atretic follicles, edema in stroma and formation of denatured yolk and necrosis ^[5].

Further study is needed to check the effects of lambda cyhalothrin on amphibians for their conservation. In this study frog's stomach and heart is under observation when exposed to lambda cyhalothrin.

2. Material and Methods

This study was conducted in ICBS laboratory of Zoology researchers, Department of Zoology, University of Gujrat, Punjab, Pakistan from April to August 2015. In this case study healthy specimens of adult frogs were used. Specimens were kept in glass aquariums. These aquariums have the 40litre water capacity and proper conditions with right environment, according to protocol in ICBS laboratory of Zoology Researchers and having appropriate food and feed (arthropods, insects, worms etc.) supply. Specimens were adapted to lab with diligent background within 6 to 7 days. Water and chemicals (pesticide) were reintegrated daily with no disturbance to specimens. 24 frogs were taken randomly and divided into 4 groups i.e. A (Low Toxicity group), B (Medium Toxicity group), C (High Toxicity group), and control (with no Toxicity) hold 6 frogs in each group. Solution of chemicals (pesticide) was prepared and given to each group by defined toxicity category (Chemical brought from E. Merk Company, D-6100 Darmstadt, and F.R Germany). Experiment was conducted for 6 weeks to check the pathological changes in stomach and heart. Lambda Cyhalothrin is a chemical used as a pesticide in rice field in Pakistan and has side effect on amphibians. Group A was low toxicity group, 0.008mg/L of lambda cyhalothrin (25% formulation of lambda cyhalothrin i.e. 0.032mg/L) was mixed in aquarium water, and frogs got it through cutaneous respiration. Group B was medium toxicity group, 0.010mg/L of lambda cyhalothrin 25% formulation of lambda cyhalothrin i.e. 0.040mg/L) was mixed in aquarium water, and frogs obtained it through cutaneous respiration. Group C was high toxicity group, 0.012mg/L of lambda cyhalothrin 25% formulation of lambda cyhalothrin i.e. 0.048mg/L) was mixed in aquarium water, and frogs got it through cutaneous respiration. Control Group was without any toxicity because it was reference group without any pesticide treatment. Pithing of experimental frogs occurred at specific time after 3 days respectively. Frogs were dissected, organs of interest were removed and small pieces of require organ were taken for histopathological analysis. Frogs were pithed; abdominal skin and muscles from the body cavity removed by using scissors, then removed stomach and heart of frog. All organs washed with water and soaked with 0.085% saline solution to remove grim, dust and any type of impurity. Then organs were converted into 3-4mm thick pieces with the help of sterile dissection box. Then different morphological as well as histopathological changes were observed. Slides containing tissues were prepared by passing the tissue through step by step process of microtome. Staining of the slides was done following a series of steps using haematoxylin, eosin, and water, ascending and descending alcohol series in coplin jar. Histopathological changes were observed under microscope (LABOMED FLX 400; Jenoptik, Germany) and photomicrographs were taken under low (4x/0.10/0.17) and medium (10x/0.25/0.17) magnification.

3. Results and Discussion

Physical, morphological and histological studies on *Rana tigrina's* stomach and heart, upon exposure to different concentrations of lambda-cyhalothrin, revealed many defects.

It adversely affects the stomach causing rupture of stomach wall, tumors on stomach surface, thinning of muscles of muscularis externa, loosening of cells of muscularis externa, necrosis, and presence of macrophages, invagination of outer wall, lesions in smooth muscles, destruction of muscularis

externa, and severe destruction of gastric pits. Similarly, it severely damages the heart of frog, causing necrosis, rupturing of cell wall. But there was no single death of organism occurs due to pesticide exposure but low immunity occurred.

Pesticides (Carbaryl) affect stomach of Frog, *Pelophylax bedriagae*, by causing separation of epithelial layer, wide spaces among gastric glands, cellular swelling in gastric glandular epithelium, hemorrhage in submucosa layer, necrosis in glandular epithelium and epithelial cells lining the stomach and eroded epithelial layer, edema in tunica muscularis, congested vessel in submucosa layer, venous congestion in submucosa layer, vacuolization in gastric glands and necrosis and necrotic cells within the interstitial spaces [7]. Pesticides affect heart by causing necrosis, infiltration and reduced activity of heart [8].

3.1 Effect of 0.008mg/L Lambda Cyhalothrin on Histopathology of Stomach and Heart (Group A)

Group A frogs were exposed to 0.008mg/L of 25% formulation of lambda cyhalothrin. Gradual increases in morphological and histopathological defects in stomach and heart were observed. No mortality occurred.

Histopathological, necrosis, separation of epithelial layer, and rupture of cell wall in stomach observed. Morphologically, stomach had tumors on its surface. Both morphological and histopathological, heart showed necrosis.

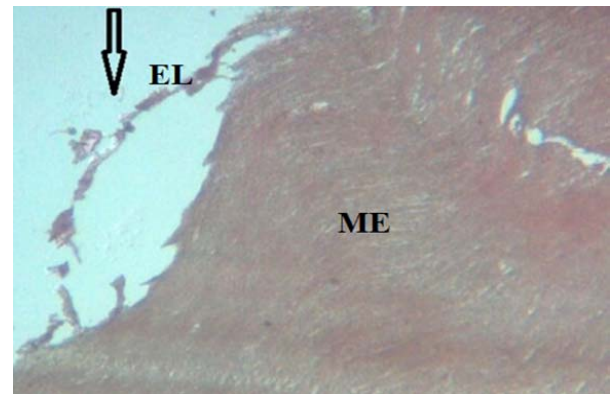


Fig 1: Effect of 0.008mg/L Lambda Cyhalothrin on Histopathology of Stomach showing Separation of Epithelial Layer (EL) of Muscularis Externa (ME) (LABOMED FLR 4X)

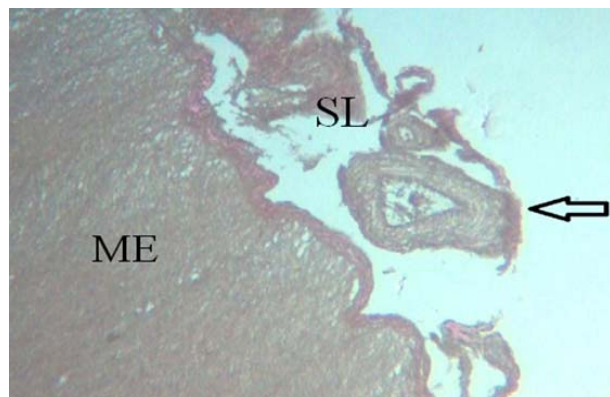


Fig 2: Effect of 0.008mg/L Lambda Cyhalothrin on Histopathology of Stomach showing Defect on Serosa Layer (SL) (LABOMED FLR 4X)

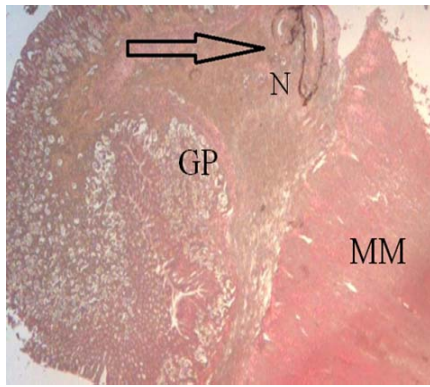


Fig 3: Effect of 0.008mg/L Lambda Cyhalothrin on Histopathology of Stomach showing Necrosis (N) (GP=Gastric Pits, MM=Muscularis Mucosa) (LABOMED FLR 10X)

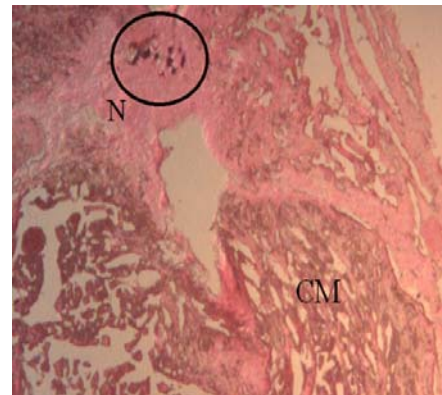


Fig 6: Effect of 0.010mg/L Lambda Cyhalothrin on Histopathology of Heart showing Necrosis (N=Necrosis, CM=Cardiac Muscle) (LABOMED FLR 4X)

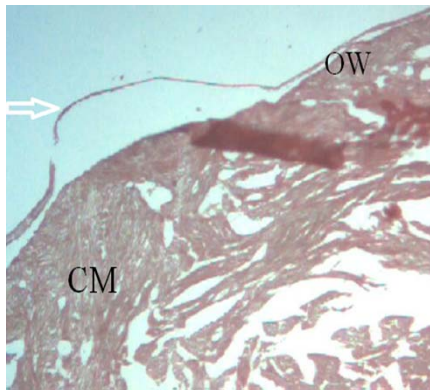


Fig 4: Effect of 0.008mg/L Lambda Cyhalothrin on Histopathology of Heart showing Disruption in cell wall boundary OW=Outer wall, CM= Cardiac Muscle (LABOMED FLR 4X)

3.2 Effect of 0.010mg/L Lambda Cyhalothrin on Histopathology of Stomach and Heart (Group B)

Group B frogs were exposed to 0.010mg/L of 25% formulation of lambda cyhalothrin. No mortality observed. Gradual increases in morphological and histopathological defects in stomach and heart were observed. Morphological, stomach had tumors and necrosis, Histopathological, stomach showed separation of epithelial layer, necrosis in muscularis externa, thinning of smooth muscles of muscularis externa. Morphological, heart showed necrosis. Histopathological, heart showed rupture of cell wall and necrosis.

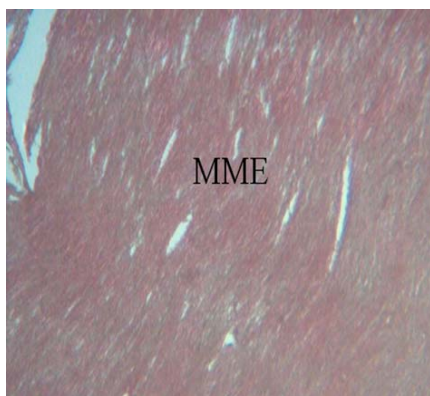


Fig 5: Effect of 0.010mg/L Lambda Cyhalothrin on Histopathology of Stomach showing Thinning of Smooth Muscles of Muscularis Externa (MME=Muscles of Muscularis Externa) (LABOMED FLR 4X)

3.3 Effect of 0.012mg/L Lambda Cyhalothrin on Histopathology of Stomach and Heart (Group C)

Group C frogs were exposed to 0.012mg/L of 25% formulation of lambda cyhalothrin. No mortality observed. Gradual increases in morphological and histopathological defects in stomach and heart were observed. Morphological, stomach showed tumors, inflammation and necrosis. Histopathological, stomach showed necrosis, lesions in muscularis externa, losing of cells of muscularis externa, and severe destruction of gastric pits. Heart showed infiltration, necrosis, and disruption of cell wall.

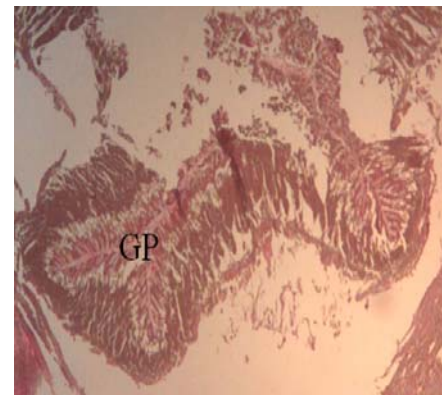


Fig 7 : Effect of 0.010mg/L Lambda Cyhalothrin on Histopathology of Stomach showing Destruction of Gastric pits (GP) (LABOMED FLR 4X)

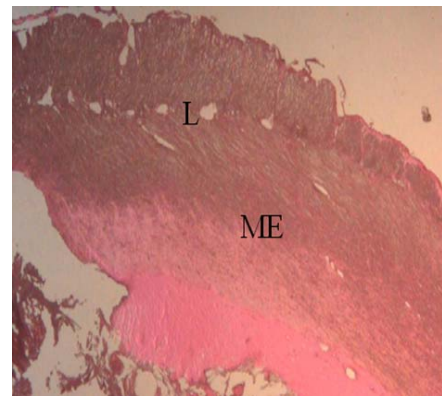


Fig 8: Effect of 0.012mg/L Lambda Cyhalothrin on Histopathology of Stomach showing Lesions (L) in Smooth Muscles of Muscularis Externa



Fig 9: Gross photograph of stomach of frog from Group C (0.012mg/L) showing tumors (T)



Fig 10: Gross photograph of Heart of frog from Group C (0.012mg/L) showing Necrosis (N)

4. Conclusion and Recommendations

Lambda-cyhalothrin treatment displayed that stomach and heart of frog adversely affected even in low concentrations. The absurd use of this pesticide for human benefits not only causing environmental pollution but also extinction of aquatic life especially amphibians. The morphological and histopathological studies on stomach and heart of frog (*Rana tigrina*) showed that lambda-cyhalothrin caused destruction to the organs and increased risk of parasitic infection. It is recommended that further study is needed to check the both direct and indirect effect of lambda-cyhalothrin on frogs to prevent future haphazard. By using this chemical, safety measures always should be in mind and must avoid excessive use.

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