

The Study of Histopathological Changes in the Gonads of Frog upon Exposure to Lambda Cyhalothrin

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ABSTRACT-Pesticides frequently premeditated a quick source for scheming weeds and insect pests but they cause noteworthy risk to nontargeted organisms. This present work includes susceptible species such as amphibians show venomous effect and death by extensive used pesticide lambda cyhalothrin in rice fields of Pakistan and this ecological alteration may initiate a reduced biodiversity, trophic cascades' damage and ultimate harm to public health. Analysis has been made to assess the influence of various concentrations of lambda cyhalothrin on several histopathological constraints of gonads of frog. In this work 40 frogs were divided into four groups A (control), B (low lethal), C (median lethal) and D (high lethal) at no, 0.008, 0.010 and 0.012 mg/litre doses of different weight retaining frogs, respectively. The doses were given in water of aquarium mg/litre at 20th June, 2014 and frogs in took them through cutaneous respiration and inhalation. Effect differs with frogs' weight and morphological and histological alterations were observed in control group compared to treated group. At 2nd and 3rd day, frogs were pithed and observations concerning to morphology and histology of gonads were documented in all treatments, which incorporated discoloration and disruption of gonads and total degeneration of testis in less weighty frogs in morphological changes. Histopathological changes were foremost and included breaking of cyst walls, bunching of spermatocytes, necrosis of sertoli cells and sprinkled masses of decayed sperm bundles etc in testis and contraction in follicles, reduction in the number of oocytes, edema in stroma and formation of denatured yolk and necrosis etc. in ovaries which concluded that it has adverse affect on fertility of amphibians ultimately reducing their population size at all doses.

INDEX TERMS: Amphibian, Lambda-Cyhalothrin, Frog, Gonads, Rice fields, Decline, Pakistan

1 INTRODUCTION

Amphibians are exposed to pesticides due to their occupations or through dietary and environmental exposure (water, soil, air). (Radhaiah, V *et al.* 2007). According to report of IUCN 41% amphibians are endangered and this gigantic decline is due to many reasons including Lambda Cyhalothrin, as skin of frogs is highly permeable and this chemical developed as a synthetic version of the naturally occurring pesticide pyrethrin. (Milburn, 2004; Cavas, T, 2003). Lambda cyhalothrin targets a broad range of insects which includes aphids, Colorado beetles and larvae of coleopteran (Tucker, 1983). As other pesticides, in insects lambda cyhalothrin disrupts the nervous system causing paralysis and eventually lead to death. It is very toxic to bees having oral LD50 is 38 mg/bee, a lower toxicity in birds as oral LD50, in duck is more than 3,950 mg/kg. In mammals (rats and dogs), it is rapidly

absorbed and excreted and residue accumulates in fat having half life of 23 days (Verschoyle *et al.*, 1980). In dogs, it is administered orally and intravenously and excreted after 7 days and rats have LD50 of 56-79 mg/kg (Sparling *et al.*, 2001). Lambda cyhalothrin is very toxic to aquatic organisms especially to amphibians. Binding of lambda-cyhalothrin with soil and sediment reduces exposure of amphibians to it. In field study, researchers find no significant adverse effects on fish, amphibians and reptiles. FAO in 1984 studied that the effect of pesticides on amphibians has been come to the main focus of research for short time as great decline in the population of amphibians and they had been associated with greater amounts of agriculture, where the use of pesticide is common. This investigation lead to determine the effect of extremely applied field grade lambda-cyhalothrin on the blood, serum biochemical changes and tissue pathological changes of toads (*Bufo perreti*) and change in their level of excretory products (Davidson *et al.*, 2002). Recent limited research in

amphibians have been done to study the effect of pesticides on liver, kidney and gonads (Khan *et al*, 2005). The present study revealed that there is further need of research on pesticides for more conservation of this group otherwise it will lead to gross drastic amphibian decline due to the pesticides (Ezemonye, 2010).

2 MATERIALS AND METHOD

The animals used in the experimentation were healthy, uninjured specimens of adult frogs. They were kept in the different glass aquariums of 500litre capacity having appropriate water conditions with accurate surroundings according to protocol in ICBS laboratory of Zoology Researchers at UOG, Pakistan and provided with standard feed and food(arthropods, insects) and were acclimatized to lab with scrupulous background within 5 to 6 days. Water and chemicals were rehabilitated daily with insignificant disturbance to the specimen .From this stockpile adult specimens weighing in grams were selected for experimentation.

40 tadpole and adult frogs were taken and arbitrarily separated into 4 groups i.e. A(Control Group),B(Low Toxicity Category),C (Moderate Toxicity Category),D(High Toxicity Category) containing 10 frogs in every group. Each group was auxiliary subdivided on the basis of analysis days into 2 subgroups, each subgroup has 5 frogs. Experiment was maintained for 3 days to check pathological changes in gonads and it was conducted by bearing in mind the subsequent changes.

Color (regular or discoloration), swelling and necrosis. Experimental frogs were pithed at fixed time after 2 and 3 days respectively. Small pieces from gonads were collected for histopathological assessment.

Lambda Cyhalothrin is a chemical used in rice fields most frequently in Pakistan distressing amphibian life, was procured from local market. Group A was control group i.e. it was without any chemical treatment.Group B was low toxicity

group and 0.008mg/L of lambda cyhalothrin was mixed in water of aquarium and frogs attained it through cutaneous respiration.Group C was moderate toxicity group and 0.010mg/L of lambda cyhalothrin was mixed in water of aquarium and frogs acquired it through cutaneous respiration.Group D was high toxicity group and 0.012mg/L of lambda cyhalothrin was mixed in water of aquarium and frogs acquired it through skin by respiration. Effect varied with divergent weight.

Different morphological and histopathological symptoms were observed.

3 RESULTS & DISCUSSION

Effect of 0.008mg/L Lambda Cyhalothrin on Histopathology of Testis and Ovaries of Frog (Group B)

Upon exposure to 0.008mg/L of lambda cyhalothrin for 2-3days showed mild and harsh degenerative changes in testis like mutilation, disruption in steroli cells, cyst wall rupturing, loosening of cells, necrosis in steroli cells ,thinning of smooth muscles' wall and deformation of cells of epididymis and the ovaries showed pathological symptoms like increase of atretic follicles, changes in granulosa cells, disruption in oocytes, deformation in granulosa cells, high damage in oocytes and mild damage to nucleus as shown in table 01.

Renuka M. R. 2007 studied that the testis showed pathological changes upon exposure to sub-lethal concentration of nuvacron and carbaryl but with passage of time there were mild degenerative changes like breaking of cyst walls, scattering of sperm bundles in some tubules and necrosis of sertoli cells but the ovaries showed pathological symptoms like increase of atretic follicles, contraction in follicles, reduction in the number of oocytes, vacuolation in the stroma and decreased maturity .There is limited study about pathological changes in liver and kidney upon exposure to lambda cyhalothrin has been done(Omonoa,A.O &Emipke,B.O,2011).

Frogs weight pithed after 2 days(grams)	Effect on Testis	Effect on Ovaries	Frogs weight pithed after 3 days(grams)	Effect on Testis	Effect on Ovaries

45		Increase in Atretic Follicles, Changes in Granulosa Cells and Disruption in Oocytes	55	Breaking of Cyst Wall, Loosening of Cells and Necrosis in Steroli Cells	
70	Necrosis in Steroli Cells		68		High damage in Oocytes and Mild Damage in Nucleus
168		Increase in Atretic follicles	155	Deformation of Epididymis' Cells	
203	Mutilation in Testis		159	Thinning of Smooth Muscles' Wall	
205		Changes in Granulosa cells	159.5		Deformation in Granulosa cells

Table 01: Effect of 0.008mg/L Lambda Cyhalothrin on histopathology of Testis and Ovaries of Frog

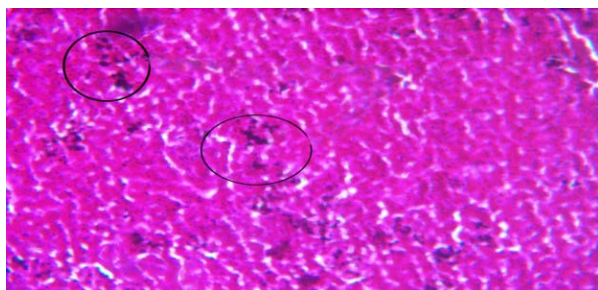


fig 01:Effect of 0.008mg/L Lambda Cyhalothrin on Histopathology of Testis showing Mutilation

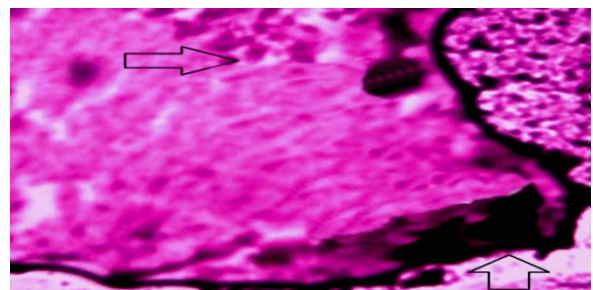


fig 03:Effect of 0.008mg/L Lambda Cyhalothrin on Testis Histology showing Disruption of Sertoli Cells and Thinning of Smooth Muscles' Wall

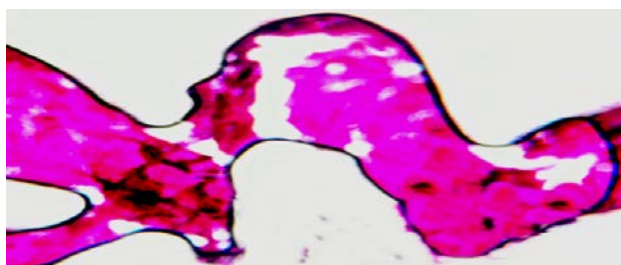
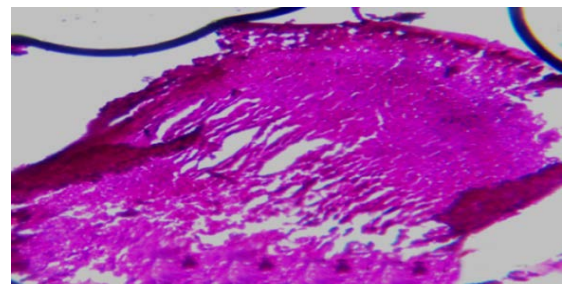


fig 02:Effect of 0.008mg/L Lambda Cyhalothrin on Histopathology of Testis showing Deformation in Epididymis



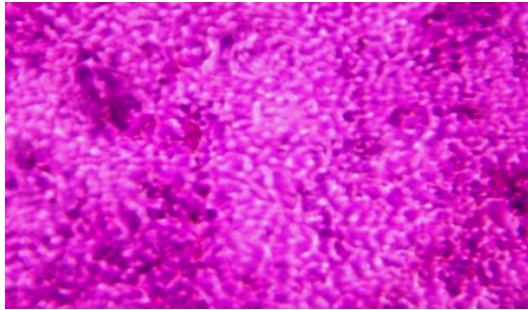


fig 05:Effect of 0.008mg/L Lambda Cyhalothrin on Ovaries showing Mild Changes

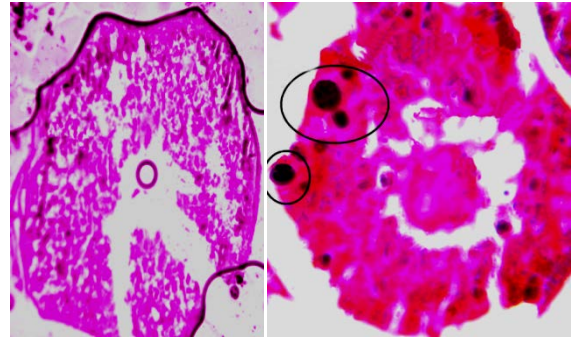


fig 04:Effect of 0.008mg/L Lambda Cyhalothrin on Cyst Walls' Breaking & Loosening of Cells

fig 06& 07:Effect of 0.008mg/L Lambda Cyhalothrin on Follicle of Ovaries at Different Weights showing disruption in Nucleus, Oocytes and Granulosa Cells

Effect of 0.010mg/L Lambda Cyhalothrin on histopathology of Testis and Ovaries of Frog (Group C)

Upon exposure to 0.010mg/L of lambda cyhalothrin for 2-3days showed that there were degenerative changes in testis and ovaries as mentioned in table 02. Renuka M. R. 2007 studied that upon exposure to median lethal concentration of nuvacron and carbaryl caused more serious

changes to the testis architecture showing scattering of sperm bundles and mild degenerative changes and the ovaries showed pathological symptoms like increase of atretic follicles, contraction in follicles, reduction in the number of oocytes, vacuolation in the stroma and decreased maturity. There is limited study of pathological changes in liver and kidney upon exposure to lambda cyhalothrin has been done (Omonoa, A.O &Emipke, B.O, 2011).

Frogs weight pithed after 2 days(grams)	Effect on Testis	Effect on Ovaries	Frogs weight pithed after 3 days(grams)	Effect on Testis	Effect on Ovaries
45		Further increase of Atretic Follicles, Disruption in Oocytes and Deformation in Granulosa Cells	25	Testis total disintegration	
82	Leydig Cells disintegrate, Nourishing Cells rupture		45	Leydig cells disintegrate, Developing Cells and Sperms disintegration	

135	Developing Cells disintegrate		55		Further increase of Atretic Follicles, Disruption in Oocytes ,Deformation in Granulosa Cells and Necrosis in Oocytes
172		Further increase of Atretic Follicles, Deformation in Granulosa Cells	157	Leydig Cells disintegrate	
189	Leydig Cells darkening		220		Disruption in Oocytes

Table 02: Effect of 0.010mg/L Lambda Cyhalothrin on histopathology of Testis and Ovaries of Frog

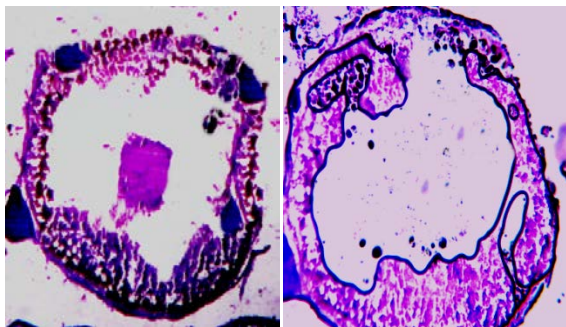


fig 08& 09: Effect of 0.010mg/L Lambda Cyhalothrin on histopathology of Testis of Frog showing disruption in Seminiferous Tubules(a)Mature and Developing Sperms disintegrate & Nourishing Cells ruptured(b)High Disruption in Seminiferous Tubules

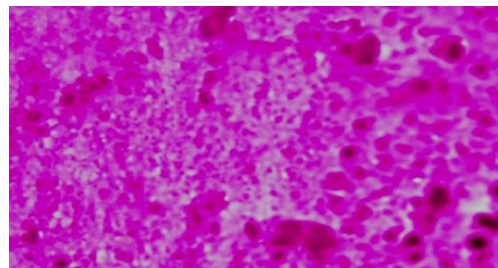


fig 10:Effect of 0.010mg/L Lambda Cyhalothri on Histopathology of Ovaries of Frog showing Deformation of Granulosa's Cells

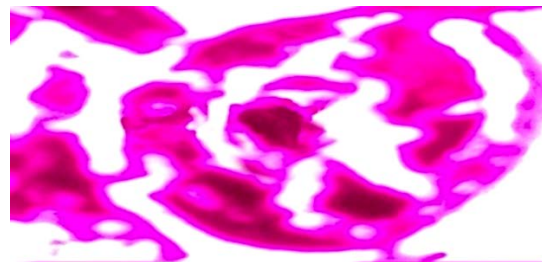


fig11:Effect of 0.010mg/L Lambda Cyhalothrin on Histopathology of Testis of Frog showing Degeneration of Cells and Oocytes

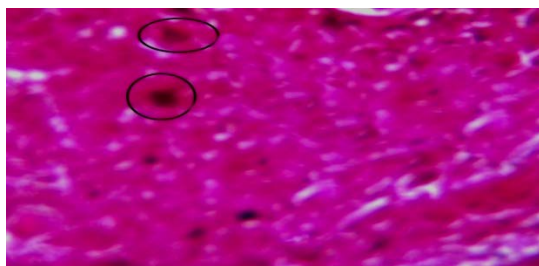


fig12:Effect of 0.010mg/L Lambda Cyhalothrin on Histopathology of Testis of Frog showing Discoloration in Stroma

Effect of 0.012mg/L Lambda Cyhalothrin on histopathology of Testis and Ovaries of Frog (Group D)

Upon exposure to 0.012mg/L of lambda cyhalothrin for 2-3days showed that there were extensive degenerative changes in testis and ovaries as shown in table 03.

Renuka M. R. 2007 studied that upon exposure to high dose of carbaryl and nuvacron there were high pathological alterations in testis like scattering

of sperm bundles, vacuolation of cytoplasm, shrunken tubules and reduction in sperm numbers and even there was exfoliation of sperms and the spermatids were degenerated. While the ovaries showed increase in atretic follicles, edema in stroma and formation of denatured yolk, reduction in the number of SGP oocytes and total degeneration and necrosis. There is limited study on the pathological changes in liver and kidney upon exposure to lambda cyhalothrin has been done (Omonoa, A.O &Emipke,B.O,2011).

Frogs weight pithed after 2 days(grams)	Effect on Testis	Effect on Ovaries	Frogs weight pithed after 3 days(grams)	Effect on Testis	Effect on Ovaries
39	Total degeneration of Testis		49	Total degeneration of Testis	
50		Highly disrupted and degenerative Structures of Ovaries	89	Total degeneration of Testis	
170		Edema in Stroma, Contraction in Follicles, Decrease in number of Oocyte	199		Highly disrupted and degenerative Structures of Ovaries
190	Deformation of testis		201		Highly disrupted and degenerative Structures of Ovaries

233	High disruption in Epididymis		235		Highly disrupted and degenerative Structures of Ovaries
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Table 03: Effect of 0.012mg/L Lambda Cyhalothrin on Histopathology of Testis and Ovaries of Frog

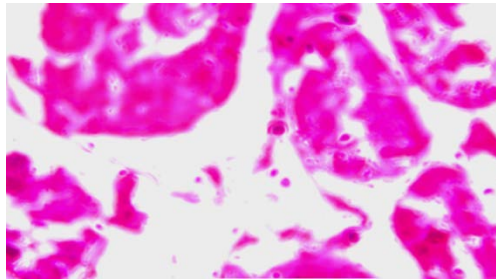


fig13:Effect of 0.012mg/L Lambda Cyhalothrin on Oocytes

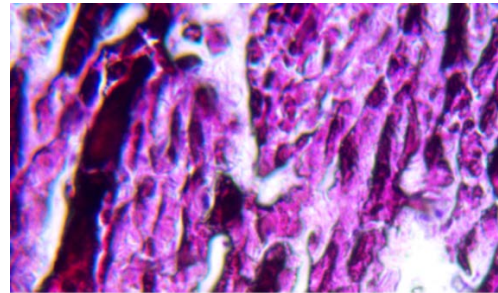


fig14:Effect of 0.012mg/L Lambda Cyhalothrin on Histopathology of Ovaries of Frogs showing Contraction in Follicles

4 CONCLUSION & RECOMMENDATION

It is concluded that studies done on the histopathological changes in the gonads of frogs upon exposure to lambda cyhalothrin uncovered that lambda cyhalothrin treatment shows that gonads are badly affected by the low, median and high lethal doses of concentrations of 0.008, 0.010 and 0.012mg/litre within a period of 2-3 days and cause weighty damages in the gonads of injected frogs as testis show breaking of cyst walls, scattering of sperm bundles, bunching of secondary spermatocytes, necrosis of sertoli cells and sprinkled masses of decayed sperm bundles etc and ovaries show the increase of atretic follicles, contraction in follicles, reduction in the number of oocytes, vacuolation in the stroma, decreased maturity, less or no growth of previtellogenic oocytes, increase in atretic follicles, edema in stroma and formation of denatured yolk and necrosis which adversely affect male and female fertility of amphibians ultimately reducing their population size. The conclusion of the present exploration recommend that more research is needed to investigate both direct and indirect impacts of lambda cyhalothrin on frogs.

5 APPENDICES

HEMATOXYLIN

Hematoxylin 2.00gm

Ammonium alum(NH₄Al. (SO₄)₂.12H₂O) 3.00gm

100% ethanol 100ml

Glycerin 100ml

Distilled water 100ml

ALCOHOLIC EOSIN

Eosin , saturated solution in 95% 10.0ml.

ETOH (approximately 0.5gm/100ml)

95% ethanol 45.0ml.

BOUIN'S FLUID

Picric acid(saturated aqueous) 75.0 ml.

Formalin 25.0 ml.

Glacial acetic acid 5.0 ml.

LAMBDA CYHALOTHRIN

Equal quantities of (S)- α -cyano-3-phenoxybenzyl

(Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

(R)- α -cyano-3-phenoxybenzyl

(Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

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Every honor is due to **Allah Almighty**. The supreme, the ubiquitous, the compassionate, the most merciful and the beneficent, Who knows the hidden facts of universe and we do not encompass anything of His knowledge except as He wills, His

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8 AUTHORS' DETAIL

throne extends over the heavens and the earth and Who blessed me with the ability to do this work and thanks to the **Holy Prophet Hazrat Muhammad (Peace Be Upon Him) and His AAL**, Who has enabled me to know my Creator and lead me to the right path and save me to astray from the faithful tract, without His teachings and perfect life I was nothing but he leads this dirt-made nonentity to the entity of success.

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