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Testicular Toxicity of Lambda Cyhalothrin Insecticide in Male Rabbits (*Oryctolagus cuniculus*)

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Abstract: An attempt was made to investigate the toxic effects of Pyrethroid (Lambda Cyhalothrin) on testicular tissues in rabbits. In this context animals randomly divided in to one control group and two test groups. The test group animals were orally administrated $^{1}/_{10}{}^{th}$ of LD_{50}lamda cyhalothrin at 8 mg/kg. body weight/ day for 10 and 15 consecutive days. During experimental period clinical observations were recorded in the treated animals. Highly significant decline ($P < 0.001$) in the body weight and testicular weight was observed in both test groups as compared to control group. Histopathological observations revealed that the seminiferous tubules become elongated and picnosis was noted throughout the tubular space in the 10 days treated animals. Whereas 15 days exposed animals showed tumor/hypertrophy formation in the seminiferous tubules and interstitial spaces. Leydig's cells eliminated and sheaths of the seminiferous tubules were broken down. Present study revealed that, lambda cyhalothrin insecticide showed drastic effects on the testicular tissues which can cause male reproductive disorders.

Keywords: Rabbits, Lambda Cyhalothrin, Toxic effects, Testicular tissues

1. INTRODUCTION

The various pesticides are used in the agriculture fields to kill the noxious insects for the protection of the crops. Controlled and proper use of agrochemicals to enhance the crop yield is not bad but random and indiscriminate usage is causing severe health and environmental problems. This harms the non target organisms confronting to the applications of pesticides/agrochemicals (Mc Kinlay *et al.,* 2008).These agrochemicals exert adverse effects on physiological systems and cause disruption in reproductive organs of animals (Rhind, 2002). Many pesticides have been documented as endocrine disrupting chemicals and cause disturbances in the mechanism of steroidogenesis at testicular tissues, exerts many defects in reproductive organs (Abro *et al*., 2005). Reproductive toxicity of pesticides in rats reveals histopathological alteration on testicular tissues (Zidan, 2009). The toxicity of synthetic pyrethroids reveals reproductive disorders including effects on testicular tissues, spermatogenesis, and sperm motility which ultimately cause infertility (Pike *et al*., 1993) and exposure of fish to Lambda Cyhalothrin exhibit significant decline in the level of testosterone (Saravanan *et al.,* 2010). Antiandrogenic activity of pesticides suggests that exposure to such chemicals may induce atrophy of the male reproductive organs inducing hyperplasia of the leydig's cells (Ostby *et al*., 1999).Definite effects of agrochemicals on reproduction and sex organs has been reported in different in *vivo* & in *vitro* studies (Ahmed, 2000).Lambda cyhalothrin is a synthetic Pyrethroid insecticide widely used in agriculture sector throughout world. Keeping in view the health hazards and disrupting characteristics of agrochemicals present study was aimed to assess the toxic effects of Lambda Cyhalothrin on testicular tissues in rabbits. This is an animal model to evaluate the endocrine disrupting effects with a perspective of reproductive toxicity.

2. MATERIALS AND METHODS

Experimental Animals and Design:

For evaluation of the toxic effects on testicular tissues, 30 male Rabbits *Oryctolagus cuniculus*, were used. Animals randomly divided into three groups -10 each and assigned as one control and two test groups (10 & 15 days exposure). These rabbits were kept for few days at animal house at Department of Zoology, University of Sinh, Jamshoro under biological observation for experimental studies. Animals were acclimatized for 15 days before starting of the experimental studies and maintained on drinking water and grass Alfalfa (*Medicago sativa*).

Test chemical and Experimental procedure:

In our experimental studies synthetic pyrethroid Lambda Cyhalothrin formulation 2.5% emulsifiable Concentrate (EC) was used. The solution of referred insecticide was prepared by adding in 10ml distilled water. The oral doses one tenth of LD_{50} of Lambda Cyhalothrin (08 mg./kg. body weight/day) administrated to each test group animals by disposable syringe for consecutive 10 and 15 days. Whereas each animal of control group received 10ml distilled water doses. During study control and treated animals were feeding on same quantity (Lucerne) and water. After doses clinical observations demonstrated on daily basis

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for 3 to 4 hours. At the termination of experiments on day 10 and 15, body weight of treated and control group animals were recorded. Subsequent to that animals sacrificed through anesthetized procedure and dissected. Weight of removed testes was recorded in the control and test groups.

Histological Procedure:

Removed testes were fixed in Bouin's fluid for 24-48 hours and tissues passed through alcohol series for dehydration procedure and embedded in paraffin wax. Ribbon of 6µm thick sections were cut on Rotary microtome machine. Harris's hematoxylin (Gurr, 1956) and Eosin (Putt, 1948) staining procedure was used and selected slides taken for histological examinations and microphotography through Leica DM-2500 with digital camera 4LAS software.

Statistical Analysis:

Values described as Mean ± SD. One way ANOVA (IBM spss, 19) was used for statistical analysis. P value (< 0.05) considered as statistical significant.

3. RESULTS

Effects on Food intake, Health and Mortality:

After oral doses of insecticide, it was observed that treated animals temporarily stopped eating food provided to them whereas control group animals were comparatively normal in food intake. During treatment period clinical symptoms tremors, convulsion, tiredness, dizziness, occasionally diarrheas, fatigue and trembling were observed in both test group rabbits. During present study, three animals died due to the toxicity of insecticide one in 10 days test group and two in 15 days test group whereas no mortality recorded in control group.

Effect on Body and Testicular weight:

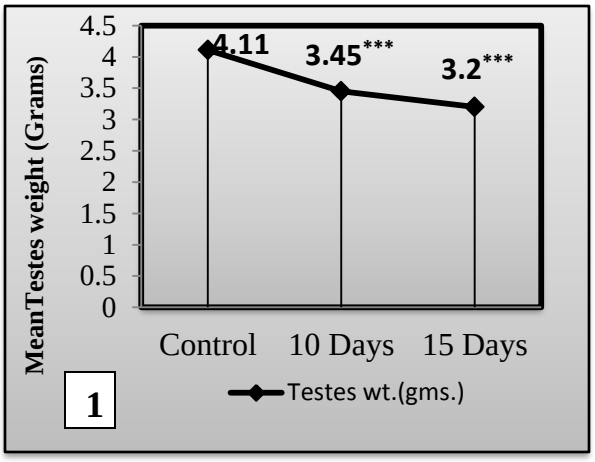
Administration of lambda cyhalothrin exerted toxic effects on body and testicular weight in the treated animals. Highly significant decline ($P < 0.001$) in the body weight was recorded when animals exposed to lambda cyhalothrin for 10 and 15 days. (Table. 1) The results of ANOVA revealed a highly significant ($P < 0.001$) reduction in the testicular weight of both exposed groups as compared to control group animals **(Table. 1).** This showed drastic toxic effects on male reproductive organs **(Fig.1-2).**

Table 01: Showing Parameters Examined in the Control & Test Group Male Rabbits

Animals Rabbits	No. of Rabbits n=30	Body weight (Mean±S.D) grams	Testes weight (Mean±S.D) grams
Control	10	1330±40.64	4.11±0.06
Test Group1 (10 Days)	09[1]	1245±23.7***	3.45±0.13***
Test Group2 (15 days)	08[1]	1170±22.3***	3.20±0.08***

Table Showing (Mean ± SD) body & testes weight in test groups with highly significant decrease ($P < 0.001$)***

As compared to control group.[1]Mortality recorded in three male rabbits during treatment period (01) in test group one & (02) in test group two.



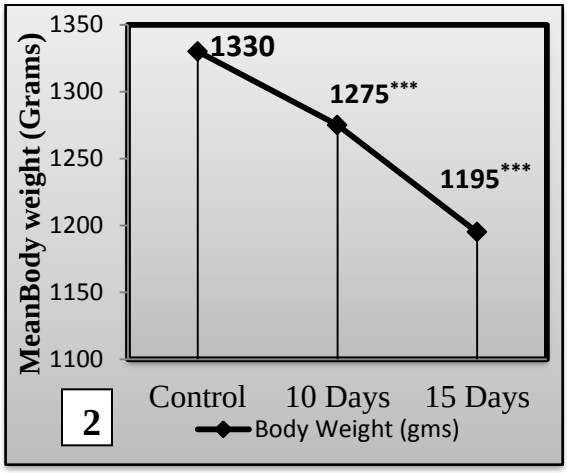


Fig.1-2. Showing decline in testes and body weight of treated rabbits for 10 & 15 days as compared to control rabbits as (Mean± SD) values with highly significant decrease ($P < 0.001$)***

Histopathological Parameters

Histological Architecture of Testis in the Control Rabbit:

The histological structure of testis in the control group rabbit consist of proper size and round/oval shaped seminiferous tubules (STs.) containing different types of spermatogenic cells (Sgs.) with proper and narrow interstitial space (Ist.).These spermatocytes scattered throughout the (STs.) Sertoli cells were also present inside the tubules. The leydig's cells (Ldg.) of normal size examined in the interstitial space **(Fig. 3-4)**

Histopathological Effects of Lambda Cyhalothrin on Testes of the rabbits:

Histopathological observations in 10 days test group revealed picnosis throughout tubular spaces and (STs.) became elongated. Visible toxic effects of lambda cyhalothrin showed tumor formation in leydig's cells *(Fig. 5)* Seminiferous tubules became large sized and hypertrophied. It was observed that interstitial spaces became widened and tumor occurred between (STs.) **(Fig.6).** After 15 days exposure to insecticide visible effects occurred on the testicular tissues and no leydig's cells were present in the interstitial space of (STs.). Erosion noted throughout the tubular spaces **(Fig.7).** Multinucleated hypertrophied spermatogenic cells were present and tumor/ hypertrophy examined in leydig's cells. Due to toxic effect of insecticide broken sheath of seminiferous tubules was observed **(Fig. 8).** The results of present study revealed that, lot of histological changes demonstrated in the testes due to the toxic effects of Lambda Cyhalothrin insecticide.

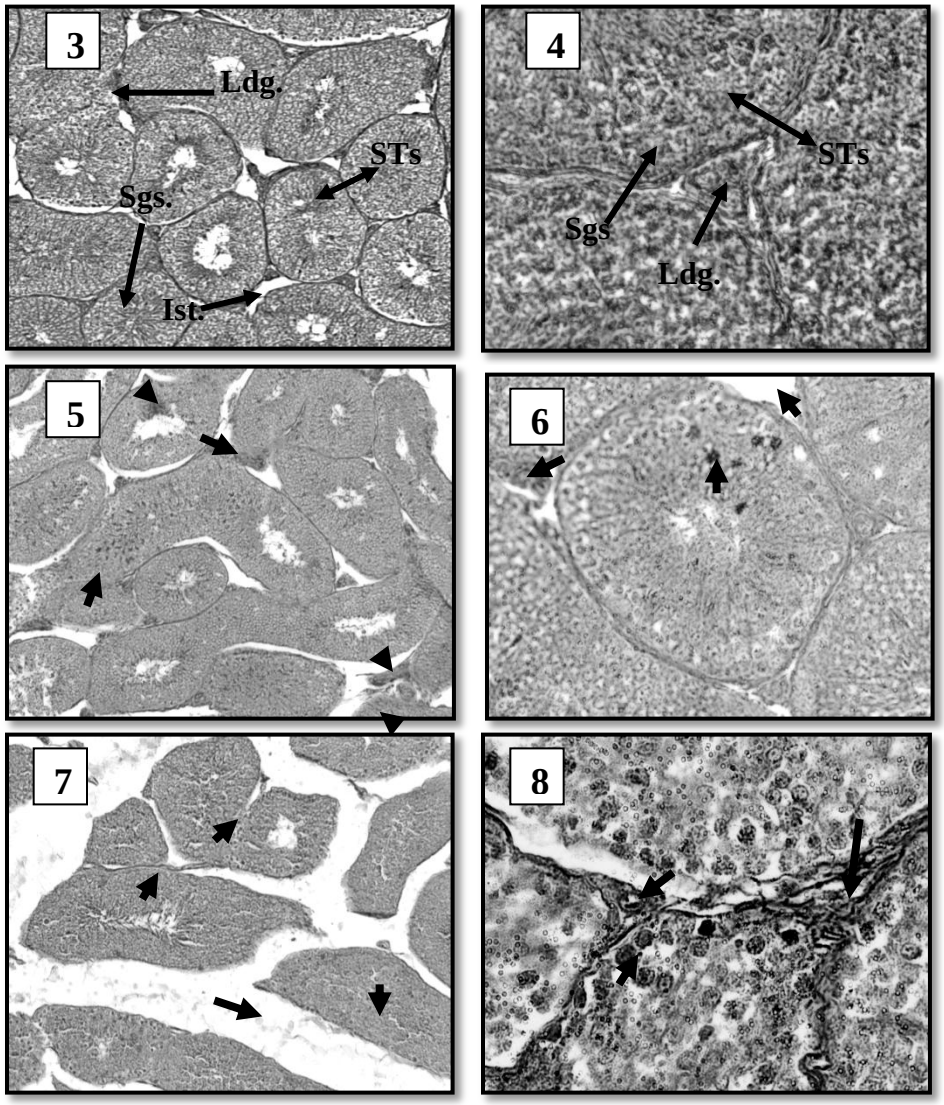


Fig. 3-4. Histological sections of testis of control group rabbits showing (2 &3), oval shaped seminiferious tubules (STs) spermatogenic cells (Sgs.) with normal Interstitial space (Ist.),interstitial space having numerous leydig's cells (Ldg.), (X 10, 63).

Fig.5-8. Histopathological sections of testis of test group (10 Days) showing (5 &6), STs. elongated shaped with widened Ist spaces small number of Ldg. having tumor formation and lumen of tubules containing Sgs. and test group (15 Days) showing (7), widened Ist spaces and degenerated STs. present (8), STs. Sheath broken down, severe tumor and hypertrophy formation, multinucleated number of Sgs. are present in the STs with tumor/hypertrophy (X 10, 63).

4. DISCUSSION

Lambda cyhalothrin administration exerted adverse effects on health in treated animals. Acute and sever health problems tremors, tiredness, dizziness, impaired movement, and low consumption of food have been reported by (Fukuyuama *et al.* 2009; Snipes *et al.,* 2009). In the present study similar type of symptoms were observed in the both test group animals. Toxicity of lambda cyhalothrin causing death in experimental animals, reduction in body, testicular weight and exert toxic effects on behavior in animals (Ratnasooriya *et al.,* 2002). Exposure to lambda cyhalothrin exerts significant changes in sperm motility and decline in the weight of male reproductive organs. Animals reduce food intake consequently decline in body growth takes place. Therefore lambda cyhalothrin induces major toxic effects on male genital organs and their performance (Yousef, 2010). Reduction in body weight reveals many other health problems in animals and these might be reproductive disorders (Aly *et al*., 2009).In accordance to earlier findings results of present study revealed that treated animals in the test groups showed highly significant decrease in body and testicular weight as compared to control group animals Mortality also found in 3 rabbits during treatment period. It is very well documented effect of pyrethroids as endocrine disrupting chemicals and this has been reported by (Wang *et al*., 2009). Histological alteration like elongated STs. and testicular atrophy, picnosis by lambda cyhalothrin exposure occurs in animals (Lebaili *et al.*, 2008). Results of present study with regard to the histopathological changes conformed reported data on the toxic effects of lambda cyhalothrin on male reproductive organs. The result of the reported studies reveals that an exposure of pyrethroids creates anti androgenic effects and disruption in reproductive organs and its secretions (Zhang *et al*., 2007; Melissa *et al*., 2007). Pyrethroids are rapidly replacing other insecticides due to relatively lower toxicity for mammals. However, they have now become an environmental issue due to excessive use in agriculture, livestock production, leather industry and shampoos etc. In addition to various clinical and neuro-toxicological effects, hazards of pyrethroids are suspected with respect to reproductive toxicity. The testicular and epididymal sperm counts and serum testosterone concentrations in pyrethroid treated animals decrease (Ahmed *et al*., 2011). In the present study drastic histological changes occurred in the testicular tissues of test group animals due to the lambda cyhalothrin exposure for 10 and 15 days. Hence, testicular damage demonstrated. The seminiferous tubules elongated and degenerated. Leydig's cells hypertrophied and leading to tumor formation noticed in the animals exposed for 15 days to lambda cyhalothrin. Significant decline in testicular weight observed, which means that reproductive toxicity induced by lambda cyhalothrin in male rabbits. In view of observed results this can be conclude that pyrethroids (lambda cyhalothrin) exhibited disorders in male gonads result from hazardous effects on seminiferous tubules, sertoli and leydig's cells. Hence, chronic exposure to this compound can cause male infertility problems that probably lead to decline in male sex hormones. Further molecular trails are recommended for better understanding of cytotoxicity and disturbance of the androgen.

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