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EFFECT OF THE PYRETHROID INSECTICIDE CYPERMETHRIN ON FERTILITY IN MALE RATS

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SUMMARY

The effect of cypermethrin (ectomin) at two dosage level on male reproductive organs were studied, the tested doses 1/40 and 1/30 LD_{50}, respectively were given for 65 successive days to male rats. Sex organ weight, semen picture, testosterone level were the criteria used to evaluate the productive efficiency of the treated male rats. Both doses decreased the weights of most genital organs, sperm cell concentration, percentage of live sperm, sperm motility and increased the percentage of total sperm, abnormalities. A decrease in the plasma testosterone level was observed in all treated groups. After 65 days on treatment and 30 days after last dose of drug administration as compared to control gorup. Histopathological examiantion was carried out on testes and accessory glands (prostate and seminal vesicle) there was incomplete spermatogenic process in the most of semineferous tubules, and activaiton of epithelial cell lining prostatic acini with polps formation protruded in the lumenae with cystic formation, while seminal vesicle showed cystic deilatation with polyps formation these changes were more potent in the large dose.

INTRODUCTION

Synthetic pyrethroids are widely used now for compating different external parasites on live stock (Griffith, 1977; appleyard et al., 1984; Hopecawdery, 1985 and Ron and Bakken, 1986).

The insecticidal activity, metabolism and toxicity of many pyrethroids were previously investigated (Elliott et al., 1976; Nolan et al., 1979; Guaguer et al., 1980; Arther and Young, 1985; Flanagane et al., 1989 and Legath et al., 1992). Most of these

pesticides are stable toxic compounds persist for several years and so constitute a great source of environmental pollution either for soil, water and hence for plants and anmals. Long exposute for these pollutants causes deteterious effects as neurologic effects, malignant tumour, teratogenic effects, abortions and reproductive failure (Cannon and kimbrough, 1979; Nafstad et al., 1983).

The study regarding the effect of repeated administration or application of synthetic pyrethroid on male fertility are rare, so the present work was designed to elucidate the effect of prolonged oral administration of the commonly and effectively used pyrethroid insecticide "Ectomin" on the fertility of male rats as well as to determine the level of testesterone in serum and histopathological changes.

MATERIAL AND METHODS

A) Cypermethrin highcis (Ectomin):

It was obtained from Ciba Geigy company, Basle, Switzerland, as an emulsifiable concentrate. Chemically, it is (R, S) a-cyano-3-phenoxy-benzyl (R]S)- Cis, trans - 3- (2-2- dichlorovinyl-2,2 dimethylcyclopropanecarboxylate).

B) Experimental animals:

30 Mature male albino rats weighing from 150-200 gm and of 4-6 months old each were divided into 3 equal groups of 10 animals each. Rats of the first group kept as control, while those of the second and third groups were given daily aqueous suspension of Cypermethrin in oral dose equal to 1/40th and 1/80th of LD50 respectively. According to Casida et al. (1983), Smith and Startoon, (1986) Cypermethrin has an oral LD50 to rats of 310-500 and has given for 65 succesive dyas to cover a complete spermatogenic cycle (Hershberger et al., 1969). The suggested parameters of 5 rats from each group were assessed after the end of administration. After 30 days of stopping administration another five rats were taken from the treated as well as from the control groups and used for evaluating the studied parameters to study the reversed effect of the insecticide. The rats were sacrified by decapitation and testes, epididymis, seminal vesicles and prostate glands were dissected out, examined macroscopically and weighed in relation to its body weight. The epididymal content was obtained and diluted 10 times with 2.9% sodium citrate solution for determining sperm cell concentration and percentages of progressive motility and sperm abnormalities as proceeded by Bearden and Fluquary (1980).

Blood smaples were obtained from each gorup, clear serum was separated by centrifugation at 3000 rpm for 15 minutes. The obtained sera was assayed for testesterone using radio-immuno-assay method.

Histopathological examination:

Specimens from the sexual organs of each rat "testes and accessory glands, prostate and seminal vesicle" were taken and fixed in 10% formalin, dehydrated in alcohol, cleared in xylol and embedded in paraffin. Prepared paraffin sections were stained by haematoxylin and eosin after (Carleton et al., 1967).

RESULTS

The effect of Cypermethrin on male fertility in rats is recorded in tables (1 & 2). It is evident from these tables that oral administration of cypermethrin in a dose of 7.7 and 3.8mg/kg b.w. equivalent to 1/40 and 1/80 LD50 respectively for 65 successive days to male rats decreased significantly the weight of testes, seminal vesicles and prostate glands at 65 days and 30 days after last dose of drug administration as compared to control gorup. Also it is clear that it decreased the sperm cell concentration, percentage of live sperm, sperm motility and increased the percentage of total sperm abnormalities. these changes in characteristics of epididymal spermatozoa were more potent in the large dose.

Table (3) showed plasma tetesterone concentration was significantly decreased with two dose levels of Cypermethrin and 30 days after stopping of its adminstration.

Table 1: Effect of prolonged administration of Cypermethrin in a dose of 7.7 & 3.8 mg/kg b.wt. for 65 successive days on the characters of epididymal spermatozoa of male rats (X ± S.E.) n=10.

Dose in mg/kg b.wt.	Weight of Sexual organs (g/100 g b.wt).			Epididymal sperm characters		
	Testes	Seminal vesicle	Prostate	Concentrration (10^6/ml)	Motility %	Abnormality %
Control	1.70±0.04	0.94±0.03	0.57±0.01	641.8±5.62	79.5±1.44	2.75±0.07
7.7 mg/kg	***1.24±0.03	***0.32±0.01	***0.11±0.005	***390.7±5.21	***46.7±2.19	***13.42±0.09
3.8 mg/kg	***0.79±0.01	***0.38±0.01	***0.09±0.004	***415.8±7.16	***67.4±2.6	***4.22±0.17

*** Significant at $P < 0.001$.

Table 2: Effect of Cypermethrin 30 days after stopping of administration in a dose of 7.7 & 3.8 mg/kg b.wt. for 65 successive days on the characters of epididymal spermatozoa of male rats (X ± S.E.) n=10.

Dose in mg/kg b.wt.	Weight of Sexual organs (g/100 g b.wt).			Epididymal sperm characters		
	Testes	Seminal vesicle	Prostate	Concentrration (10^6/ml)	Motility %	Abnormality %
Control	1.81±0.05	0.98±0.06	0.60±0.02	630.3±7.53	81.6±1.77	2.53±0.13
7.7 mg/kg	***0.76±0.02	***0.57±0.01	***0.08±0.002	***520.8±6.81	***50.1±2.19	***11.52±0.71
3.8 mg/kg	***0.75±0.02	***0.51±0.01	***0.10±0.002	*605.7±6.65	**68.2±3.03	**3.41±0.21

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

Tabel (3): Plasma testesterone (ng/ml) levles of male rats administered Cypermethrin in a dose of 7.7 & 3.8 mg/kg b.w. after 65 days continous administration and 30 days after stopping treatment (X ± S.E.) n=10.

Dose in mg/kg b.wt.	Plasma testesterone concentration (ng/ml)
Control	19.589 ±2.15
7.7 mg/kg 65 days after treatment	***3.138 ±0.265
7.7 mg/kg 30 days after stopping administration.	***2.191±0.25
3.8 mg/kg 65 days after treatment	**11.98±1.24
3.8 mg/kg 30 days after stopping administratio.	***7.663±0.55

** Significant at P< 0.01
*** Significant at P 0.001

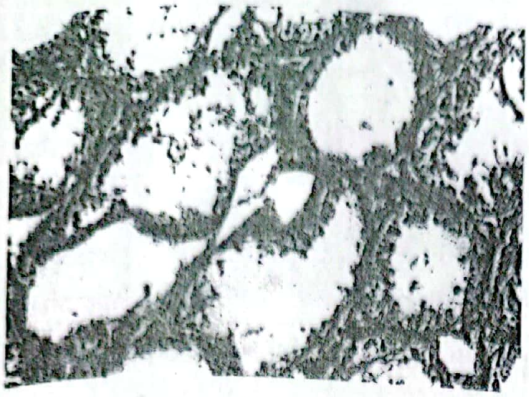

Fig (1): Testes of rats treated by 1/40 LD50 of Cypermethrin compound for 65 days showing inactivation of the semineferous tubules with incomplete spermatogenesis in most of them (H & E x 40).

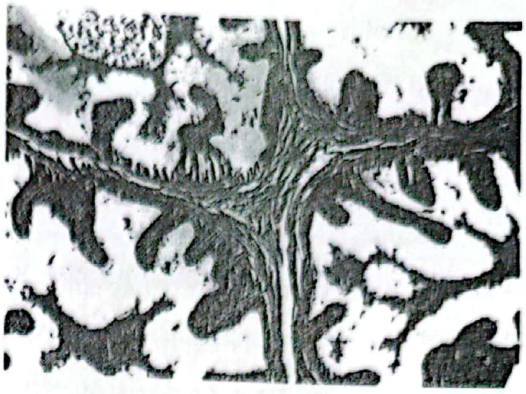

Fig. (2): Prostate of rat treated by 1/40 LD50 of Cypermethrin compound for 65 successive days showing polypoid formation in the acinar lumenae (H & E x 40).

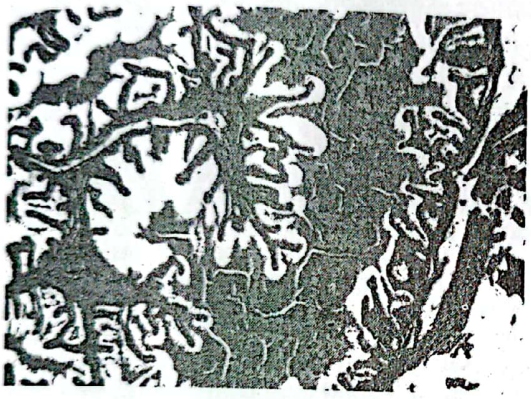

Fig. (3): Prostate of rat showing cystic dilatation and giant acinar formation (H & E x 160).

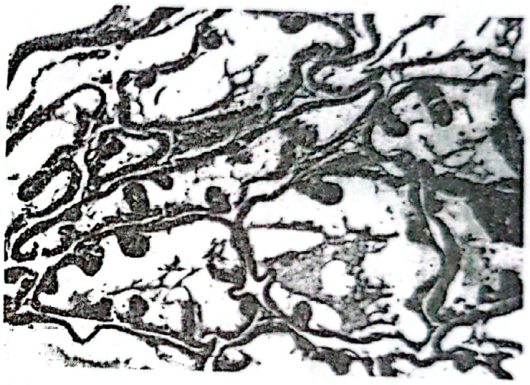

Fig. (4): Seminal vesicle showing polyps formation (H & E x 40).

Clinical symptoms

Rats treated with Cypermethrin in a dose of 7.7 and 3.8 mg/kg.b.wt. developed clincial symptoms which were progressing by time.

All treated rats 100% became irritable with marked distention of the abdomen; 60% and 20% of the treated gorups respectivley showed nervous manifestation appeared as tremors and convulsions.

At the terminal stage of experiment, rats showed loss of appetite and lost their vitality, weakness and emaciation.

2- Postmortem lesions

There were congestion and petecheal hemorrhages of different body organs.

Histopathological Findings:

Histopathological examination was carried out on testes and accessory glands (prostate and seminal vesicle).

1- Rats received a dose of 7.7 mg/kg Cypermethrin for 65 days showed pathological changes in sexual organ as follow.

Testes:

There was incomplete spermatogenic process in the most of semineferous tubules in association with absence of sperms and spermatids in many of them (Fig. 1).

Prostate gland:

There was activation of epithelial cells lining the prostatic acini with polyps formation protruded in the lumenae (Fig. 2). Cystic formation was observed in the prostatic acini with rupture of some acini forming giant one impacted by homogenous eosinophilic material (Fig. 3).

Seminal vesicle:

The acini showed cystic dilatation with flattening of lining epithelium, while others showed polyps formation (Fig.4).

II- Histopathological findings 30 days post stopage of dosing of Cypermethrin 7.7mg/kg b.w.):

Testes

There was complete absence of spermatids and sperms in the semineferous tubules (Fig. 5).

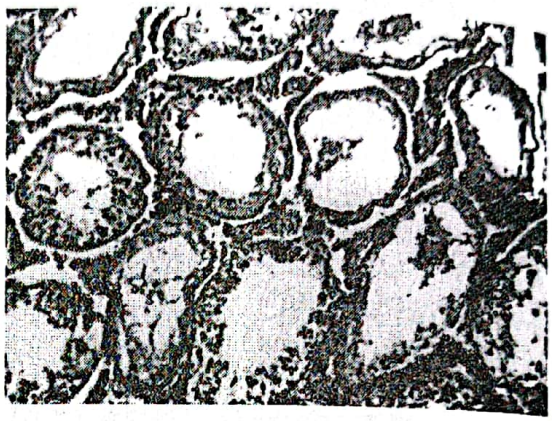

Fig. (5): Testes of rat post stopage of dosing by 30 days showing inactive semineferous tubules with stopage of the spermatogenesis (H & E x 40).

Seminal vesicle:

The acini showed severe dilatation with flattening lining epithelium in association with the appearance of homogenous esinophilic material in some lumenae. Polyps formation was observed in some other acini (Fig. 6).

Prostate gland:

Oedema with leucocytic inflammatory cells infiltration were observed in the connective tissue stroma (Fig. 7). Homogenous eosinophilic materi-

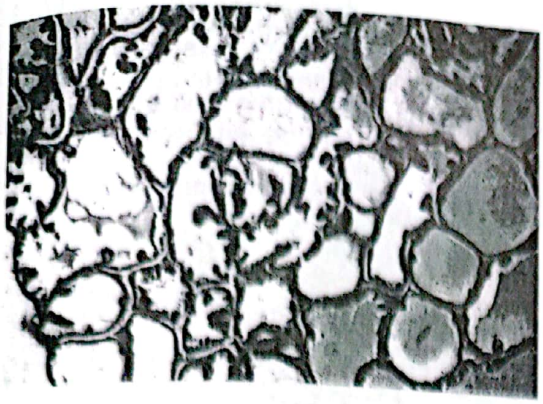

Fig. (6): Seminal vesicle showing polyps formation (H & E x 16).

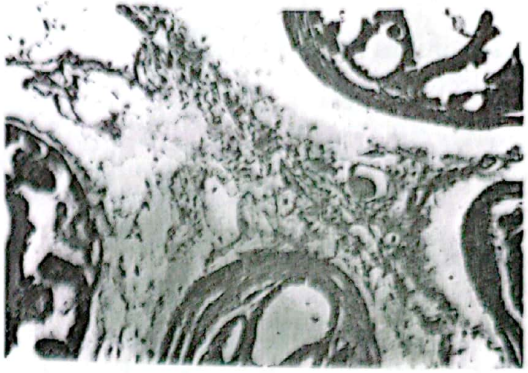

Fig. (7): Prostate showing oedema with leucocytic inflammatory cells infiltration in the connective tissue stroma (H&E x40).

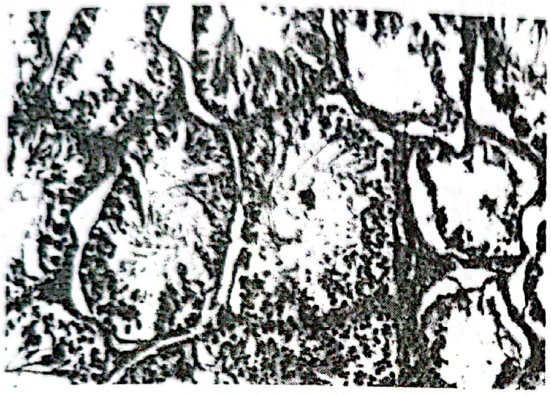

Fig. (8): Testes of rats treated by 1/80 LD50 of Cypermethrin compound for 65 days showing complete spermatogenic process in few of the semineferous tubules while other showing incomplete process (H & E x 40).

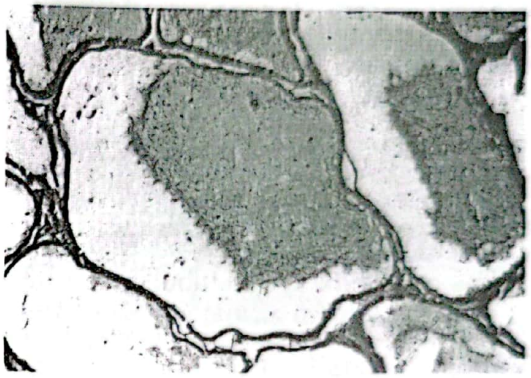

Fig. (9): Seminal vesicle showing cystic dilatation with flattening lining epithelium (H & E x 40).

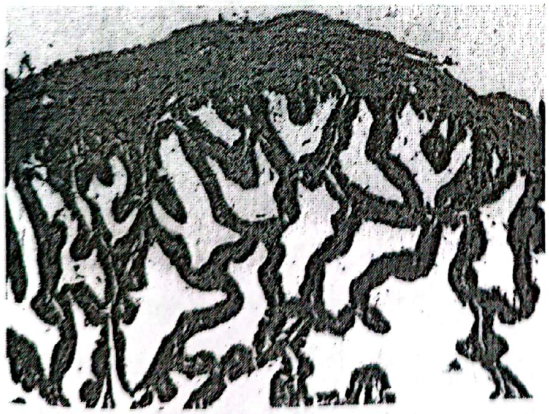

Fig. (10): Prostate showing intact empty acini (H & E x 40).

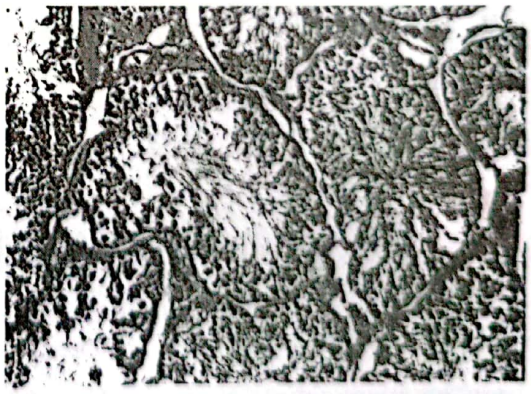

Fig. (11): Testes of rat 30 days post stopage of dosing 1/80 LD50 showing complete process of spermatogenesis in all semineferous tubules (H & E x 40).

al was noticed in the lumenae.

III- Histopathological findings in sexual organs of rats received Cypermethrin in a dose of 3.8 mg/kg b.w. for 65 days.

Testes:

There was incomplete spermatogenesis with absence of sperm and spermatids in some seminefer-ous tubules, while others showed complete spermatogenic process (Fig. 8).

Seminal vesicle:

There was cystic dilatation with flattening lining epithelium of the acini (Fig. 9).

Prostate gland:

The acini were intact and empty (Fig. 10).

IV- Histopathological findings 30 days post stopage of Cypermethrin in a dose of (3.8 mg/kg b.w.).

Testes:

Complete process of spermatogenic activity was observed in most of the semineferous tubules (Fig. 11).

Seminal vesicle:

There were severe cystic dilatation of the acini which were impacted by homogenous eosinophil-ic material and desquamated cells (Fig. 12).

Prostate gland:

Homogenous pink material was noticed in some acini (Fig. 13).

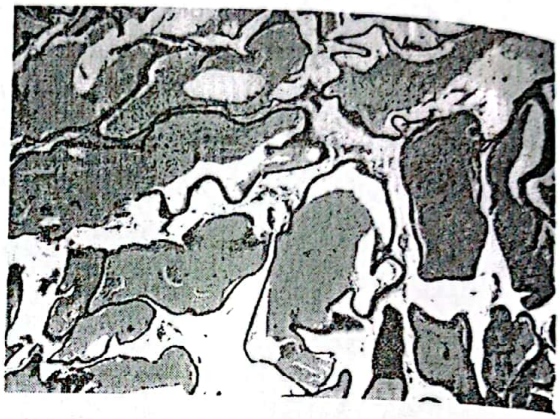

Fig. (12): Seminal vesicle showing cystic dilatation with flattening lining epithelium (H & E x 16).

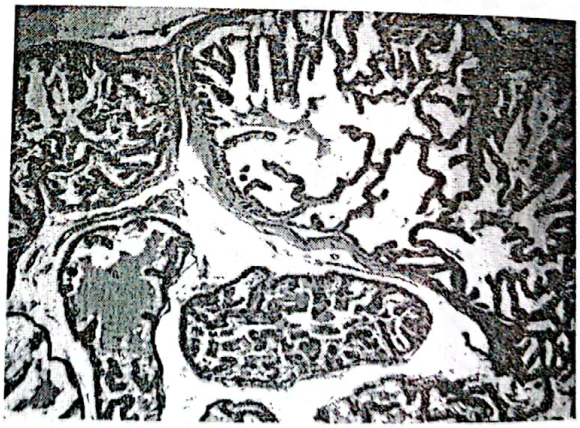

Fig. (13): Prostate showing homogenous pink material in the acinar lumenae (H & E x 16).

DISCUSSION

Oral administration of Cypermethrin in doses of 1/40 and 1/80 LD50 equivalent to 7.7 & 3.8mg/kg b.w. respectively daily for 65 consecutive days

produced significant changes in the weights of reproductive organs and semen picture. There was also an increase in the percentage of dead and morphologically abnormal spermatozoa. Moreover, the studied insecticide caused a decrease in the level of the teststerone in serum of rats during 30 days after the end of treatment. The inhibitory effect of the pyrethroid Cypermethrin in the reproductive organs and spermatozoal picture in male-treated rats may be attributed to the direct cytotoxic effect of the pyrethroid on the tissue of testes. This openion was proved by the marked histopathological alterations observed in testes, prostate and seminal vesicles which revealed severe pathological degenerative changes. These effects could impair or even stop the process of spermatogenesis resulting in infertility of male rats. Our explanation agreed with Paz (1978) who attributed the reduction in sperm motility to decrease in androgen production.

The available literature concerning the effect of pyrethroids on male reproductive system was scanty, but El-Ashmawy et al (1983) recorded similar results, showing that male rats exposed to the aerosols of the pyrethroid matox at a level of one tablet / $36m^3$ for 60 hours daily up to 2, 4 and 8 weeks caused a significant decrease in the weight of testes, epididymis, seminal vesicle and prostate glands after 4 and 8 weeks. Furthermore, the sperm mtility was significantly decrease after 2, 4 and 8 weeks of exposure to Matox aerosols while the sperm cell concentration was significantly decreased after 4 and 8 weeks of exposure to Matox aerosols. Sobhy (1991) also revealed that oral administration of sumicidin and S-3206 in oral doses of 20, 100 and 1 and 50 mg/kg b.w. for 60 successive days produced significant decrease in the weight of sexual organs, sperm count and percentage of progressive motility, also significant increase in the percentage of sperm abnormalities.

The obtained data revealed that Cypermethrin induced marked reduction in sperm concentration. This effect may be attributed to the reduction in meiotic index of the testicular cells which might be due to the passage of the pyrethroid across the blood testis barrier (BTB) and gain access to the germ cells in semineferous tubules. Similar findings have been recorded by Hassan et al. (1993) who recorded that pyrethroid cause severe degenerative changes in the semineferous tubules which cause severe hypoplasia and complete necrosis. This degeneration may be due to fluctuation in the level of fSH which has a dirct effect upon the germinal epithelium and Sertoli cells which are responsible for development of spermatozoa.

Our histopathological results are in agreement with Hess et al. (1988) and Blackburn et al. (1988) therefore, the histopathological changes obtained in the present study could be used as confirmatory diagnostic method in cases of chronic toxicity of pyrethroids.

Dixon and Lee (1973) reported that the BTB appeared to represent an important aspect in the consideration of reprocutive and mutagenic effects of environmental chemicals.

It is concluded that great attention should be taken during deltamethrin field application to avoid the possible adverse reproductive effects in farm animals and occupationally exposed human.

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