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Cypermethrin induced pathological and biochemical changes in reproductive organs of female rats

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Abstract

Cypermethrin, a synthetic pyrethroid, has broad spectrum use in domestic agriculture, and veterinary applications due to its high bioefficacy, enhanced stability and low mammalian toxicity. The present investigation was performed to investigate the sub acute effects of cypermethrin (25EC) in female rats. Cypermethrin (25EC) at a dose of 50mg kg^{-1} body weight (1/5th LD_{50}) was orally given to female rats for 4 weeks. Control rats received similar amount of ground nut oil. Significant decrease in ovarian weight (15.4%) was observed after four weeks of cypermethrin administration while the uterine weight (68.2%) and thickness of myometrium increased at 2 and 4 weeks. Cypermethrin caused degenerative changes in ovary as evidenced by increased follicular atresia and decreased concentration of proteins (38%), lipids (20%), phospholipids (18%) and cholesterol (37%). Acid (49.2%) and alkaline phosphatase (41%) activities were increased while lactate dehydrogenase (37.9%) and 3β–HSDH (31.3%) decreased in treated rat ovary.

Key words

Cypermethrin, Biomoleclules, Uterus, Albino rats.

Introduction

The use of pesticides has increased with the increasing awareness of their utility in agriculture, animal husbandry, post harvest technology and in public health (Milne, 2000). Pesticides may cause reproductive toxicity through several mechanisms; direct damage to structure of cells, interference with biochemical processes necessary for normal cell function and biotransformations resulting in toxic metabolites (Colborn, 1998; McLachlan, 2001; Agarwal and Sharma, 2010). Among the various classes of pesticides used, organophosphates and carbamates were extensively used in past for pest control to increase agricultural produce (Milne, 2000).

Cypermethrin, a synthetic pyrethroid, is a broad spectrum insecticide, used widely in domestic agriculture, and veterinary applications due to its rapid rate of degradation and low mammalian toxicity (Vijverberg and Vanden, 1990; Srivastava *et al.,* 2006). It acts by quickly affecting the insect's central nervous system (Tomlin 1994, Bradberry *et al.,* 2005). Earlier studies of Wolansky *et al.* (2006) have investigated the toxic effects of cypermethrin in mammals revealing increase in salivation, lack of coordination, muscle tremor and tonic-clonic convulsions.

Several pesticides are potential endocrine disruptors and female reproductive system may be regarded as sensitive target for these disruptors (Bretveld *et al*., 2006). They can alter development of reproductive system as well as ovulation and implantation (Stamati *et al*., 2007). Although Rustamov and Abbasov (1994) observed no adverse effect of cypermethrin on estrous cycle or its duration in rats however Saleem *et al.* (1996) revealed significantly decreased plasma progesterone levels in pyrethroid treated female rats. Fertility has also been found to be reduced in male rats ingesting cypermethrin (Elbietha *et al*., 2001). Studies concerning the molecular mechanism of chemical toxicity of cypermethrin on female reproductive organs are not well defined (Lasley and Overtreet, 1998).

The present study has been planned to investigate the histopathological and biochemical effects of cypermethrin (EC 25%) on ovary and uterus of albino rats.

Materials and Methods

Twenty four female albino rats (3 months; weight 110-150 gm) were acclimatized for 10 days before using them for experimentation. The rats were maintained under controlled conditions of temperature (22±2°C) and provided with standard pellet diet (Godrej Agrovet Ltd. Khanna, Ludhiana) and water *ad libitum*. In addition, presoaked black grams supplementation was also done. This experimental protocol was approved by the Institutional Animal Ethics Committee (I.A.E.C.) of the University.

Cypermethrin (EC 25%) was obtained from Rallis India Limited. Adequate dilutions were made with ground nut oil to achieve the test concentration of 50 mg kg^{-1} b.wt. ($1/5^{th}$ LD_{50}). The test concentration of cypermethrin was calculated from the percentage of active ingredient of commercial formulation of cypermethrin. Twenty four rats were divided into two groups. 12 rats were orally given cypermethrin at a dose of 50 mg kg^{-1} b.wt., while 12 rats were given 0.2ml ground nut oil and served as control. The treatment continued for 4 weeks. Vaginal smears of rats were checked daily for cyclicity during the experimentation. For this vaginal secretion was collected with a plastic dropper containing 0.9% saline by gently inserting the tip into rat vagina. Vaginal fluid was placed on glass slides and observed under the light microscope with 10x objective lens to analyze the proportion among the three cellular types (leucocytes, epithelial and cornified cells) present in the vaginal smear. The proportion among them was used for the determination of the estrous cycle phases as described by Mandl (1951). The body weight of the rats was taken before the start of the treatment in control as well as in cypermethrin treated groups. During the experiment, the rats were weighed weekly to determine the change in body weight.

Half of the rats were sacrificed after 2 weeks remaining after 4 weeks. The reproductive organs viz; ovaries and uteri were excised, cleaned off the adhering tissue and weighed separately. One of the ovary (right) and uterus was processed for histopathological studies and the other ovary (left) of each animal was used for biochemical estimations.

For histopathological studies, ovary and uterus of treated and control rats were fixed in alcoholic bouin's solution for 24 hours. After routine processing and dehydration of each tissue, paraffin sections were cut at 5μm and stained with hematoxylin-eosin for microscopic examination. Serial sections of ovaries were studied for various observations like total number of follicles, number of normal and atretic follicles, diameter of follicle, oocyte and nucleus as described by Kaur and Guraya (1983). Sections of uterine tissue were also studied for observations like number of endometrial glands, height of uterine epithelium and thickness of myometrium using ocular micrometer.

For biochemical studies, ovaries were homogenized in 2 ml of phosphate buffered saline (PBS, pH 7.4) and ovarian homogenate was centrifuged at 5000 rpm for 10 min. The supernatant was used for estimation of total soluble proteins and various enzymes. Proteins were estimated by the method of Lowry *et al.* (1951). Total lipids from the ovarian tissue were extracted by the method of Folch *et al*. (1957). Total phospholipids were estimated by Ames (1966) method and cholesterol was estimated by Chiamori and Henry (1959) method from the above extracted lipids. Acid phosphatase (ACP) and alkaline phosphatase (ALP) activity was measured in citrate buffer (0.05M pH 4.8) and glycine buffer (0.05M pH 10.5), respectively using p-nitrophenol phosphate as substrate following the method of Bessey *et al.* (1946). Estimation of enzyme lactate dehydrogenase (LDH) was done by King's (1965) method using sodium pyruvate as substrate. Enzyme 3-β hydroxysteroid dehydrogenase (3- β HSDH) was estimated by the method Angular *et al.* (1992).

The data was analyzed on computer using analysis of variance (ANOVA) as a statgraphics statistical package, to evaluate the significance levels between the parameters studied. A "p" value of 0.05 was selected as a criterion for statistically significant differences.

Results and Discussion

During initial period of investigation all the rats were cycling normally. However, the estrous cycle was slightly disturbed in all the treated rats at 4^{th} week of treatment. The state of permanent estrous and consequently, the loss of cyclic characters of sexual activity indicated the disturbed endocrinology of reproduction (Bretveld *et al.,* 2006, Liu *et al*., 2006). Net body weight gain was significantly ($P < 0.05$) less in case of treated rats as compared to control rats (Table 1). In accordance with the present observations, Aldana *et al.* (2001), Elbietha *et al.* (2001) and Hussain *et al.* (2009) have also observed significantly lower body weight gain in cypermethrin treated rats as compared to control rats. Ovarian weight of rats decreased significantly after 2 weeks of treatment which further decreased at 4 weeks of treatment as compared to control rats (Table 1). Loss of weight in ovaries accompanied by anestrous was probably due to alteration in reproductive hormones (Liu *et al.,* 2006). Organophosphates like methyl parathion, dimethoate and monocrotophos given to female albino rats have also resulted in significant decrease

in the ovarian weights (Kaur and Dhanju, 2005). Uterus showed significant (P<0.05) increase in weight at 2 weeks and 4 weeks of treatment as compared to control groups. Length of uterus also increased in cypermethrin treated rats as compared to control rats (Table 1).

All the stages of follicular development *viz* primary, secondary, tertiary, early antral and antral were observed in control and cypermethrin treated rats. The total number of normal follicles did not differ significantly (P < 0.05) in control and cypermethrin treated ovaries (Table 2). However, percentage atresia of follicles was found to be higher in treated rat ovaries at 2 weeks and 4 weeks as compared to their respective control rats (Table 2) (Fig. 1 A-C). The diameter of follicles at various stages of development was significantly reduced in treated rats as compared to control rats where the early antral stage of the follicles was the most effected stage. However, the diameter of oocyte and oocyte nucleus of treated rats was comparable with control rats. Repeated administration of cypermethrin in rats also resulted in loss of follicular cells and oocytes in ovaries (Grewal *et al.,* 2010). Cypermethrin might have exerted some deleterious effects on ovaries in terms of increased atresia at early antral and antral stages of ovarian follicles either by directly targeting the tissues or indirectly via alterations of endogenous hormones (Borgest *et al.,* 2002; Bretveld *et al.,* 2006). Reduction in number of ovarian follicles have also been observed in mice with organochlorine administration (Rafia and Garieb, 2001)

In all the rats uterine luminal epithelium was of columnar type with nuclei located at the base (Fig. 1 D-F). Height of uterine luminal epithelium decreased significantly at two weeks (P < 0.05) in cypermethrin treated rats which further decreased at 4 weeks (Table 3) (Fig.1 E-F) as compared to respective control rats. In the uterus of cypermethrin treated rats, there was less number of endometrial glands as compared to their abundance in control groups (Fig.1 G-I). Myometrium was normal and intact in control groups (Fig.1 J) while in cypermethrin treated rats, myometrium was sometimes vacuolated. There was significant (P < 0.05) increase in thickness of myometrium at 2 weeks of treatment and it increased further in rats at 4 weeks of treatment (Fig.1 K-L). The distribution of leukocytes in myometrium was much less in rats treated with cypermethrin as compared to control groups (Table 3). Increase in uterine weights of cypermethrin treated rats may be attributed to increased thickness of myometrium and hypertrophy of endometrium (Raifa and Garieb, 2001). Hypertrophy of myometrium and endometrium in uterus has also been observed with the intraperitoneal treatment of insecticide DDT, an organochlorine compound (Raifa and Garieb, 2001). Significant reduction in the number of endometrial glands in cypermethrin treated rat uterus can be considered as one of pesticide-induced changes in the reproductive tract of female rats. Similar changes have also been observed in mice treated with ovarian steroid hormones and organochlorine compounds (Iguchi, 1976; Raifa and Garieb, 2001).

The treatment with cypermethrin resulted in significant (P<0.05) decrease in the level of total soluble proteins. Levels of total lipids were also decreased

Table 1 : Effect of cypermethrin (EC 25%) on weights of reproductive organs (g 100^{-1} b.wt.) and length of uterus (cm) in control and cypermethrin treated rats after 2 and 4 weeks

Name of organ	2 weeks		4 weeks	
	Control	Treated	Control	Treated
Net body weight gain	20.00±0.90	16.00±0.20 [a]	34.00±0.74	24.40±0.32 [ab]
Ovary	0.026±0.002	0.024±0.008 [a]	0.024±0.003	0.022±0.005[a]
Uterus	0.088±0.001	0.133±0.002 [a]	0.090±0.001	0.140±0.001[a]
Length of uterus	3.10±0.18	3.60±0.16 [a]	3.30±0.18	3.40±0.16 [a]

All the values are mean ± SE values of 6 animals in each groups; [a]Significantly different (P<0.05) between a group, [b]between two different time periods

Table 2 : Effect of cypermethrin (EC 25%) treatment on number of atresia in ovaries of treated rats as compared to control after 2 and 4 weeks of treatment

	2 weeks				4 weeks			
	Total	Atretic	Normal	% Atresia	Total	Atretic	Normal	% Atresia
Control	55.80±3.40	17.60±1.16	38.20±5.20	31.50	64.00±2.42	21.00±1.20	43.00±1.25	32.80
Treated	71.60±2.02 [a]	30.00±2.20 [a]	41.60±1.45	41.30 [a]	69.20±2.21 [a]	27.20±3.20 [a]	42.00±2.40	39.10 [a]

[a]Statistically significantly different (P<0.05) between a group; All the values are mean ± S.E. values of 6 animals in each group

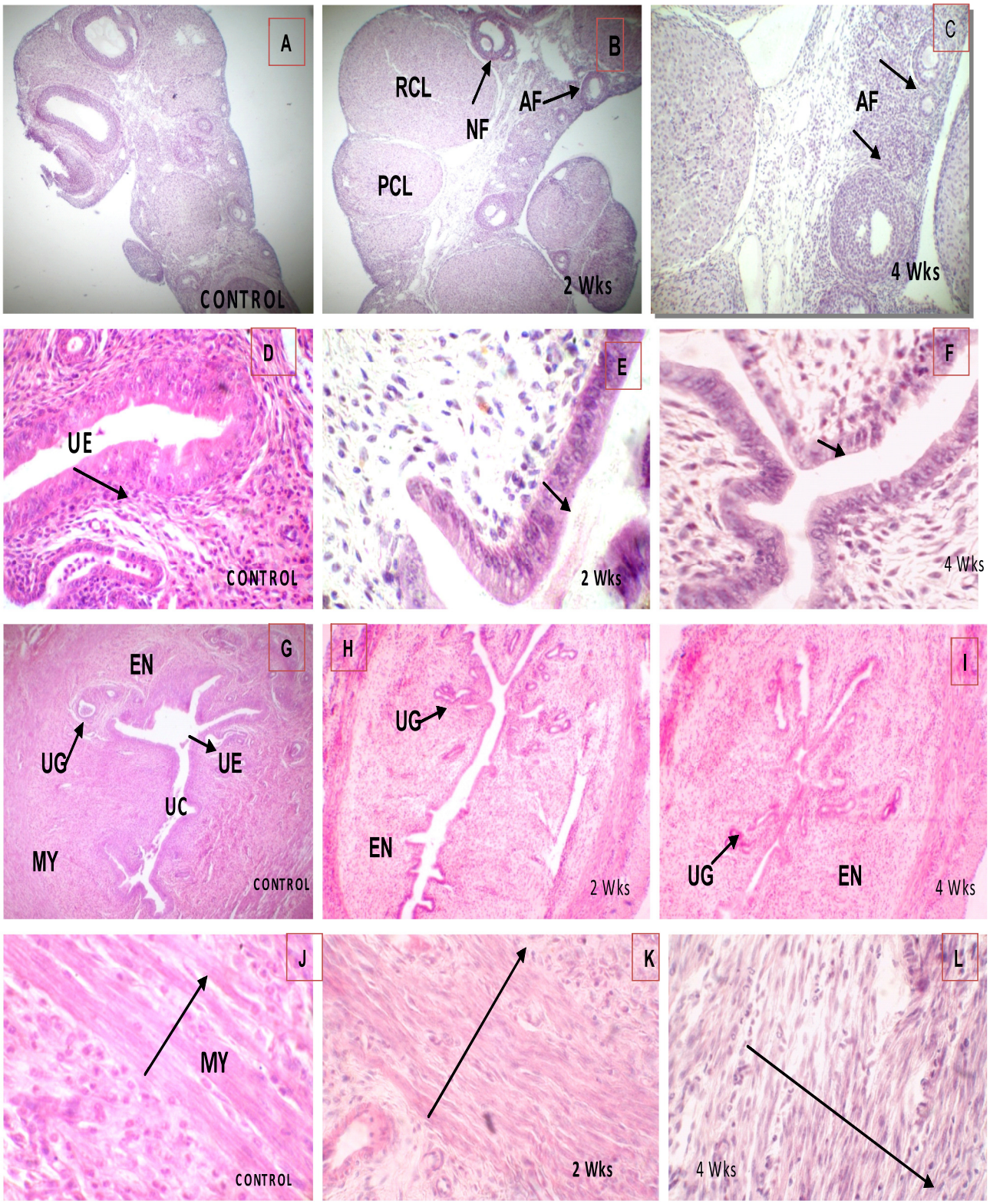

Fig. 1 : Histopathological observations : (A) Ovary of control rat and treated with cypermethrin (50mg kg^{-1} day^{-1}) after (B) 2 weeks and (C) 4 weeks of treatment. H&Ex100. Uterine wall with normal luminal epithelium (UE) of (D) control rat and reduced epithelium in cypermethrin (50mg kg^{-1} day^{-1}) treated rat after (E) 2 week and (F) 4 weeks of treatment. H&Ex160. Uterine wall showing endometrium (EN) and endocrine glands (UG) in (G) control rat and cypermethrin treated rats after (H) 2 weeks and (I) 4 weeks of treatment. H&Ex100. Myometrium (MY) in uterine wall of (J) control rat and cypermethrin treated rats after (K) 2 weeks and (L) 4 weeks of treatment. H&Ex400

significantly in treated rat ovaries as compared to control rats. The decrease was statistically significant at both 2 and 4 weeks of time period (P<0.05) (Table 4). The amount of phospholipids and cholesterol was also less in treated rats at 2 weeks which further decreased at 4 weeks in treated rats as compared to control rats (Table 4).

Biochemical parameters are sensitive index of the changes due to pesticide toxicity and can constitute important diagnostic tool in toxicological studies (Singh and Saxena, 2001). The decrease in the level of proteins in cypermethrin treated rats may indicate the induced degenerative changes in the ovaries of rats or general disturbance of the protein anabolism which may be due to androgen/estrogen deficiency leading to impaired gametogenesis. Little information of pyrethroids effect on ovarian protein is available so far, however, rats exposed to liquid mosquito repellent containing pyrethroid allethrin showed no change in levels of proteins in testes and epididymis (Srivastava *et al.,* 2006). Low levels of lipids, phospholipids and cholesterol in ovaries of cypermethrin treated rats indicated decreased steroidogenic activity in atretic follicles and degenerating corpora lutea due to loss of intracellular membranes in degenerating tissues (Wellington *et al.,* 2004). The peroxidation of membrane phospholipids not only alters the lipid milieu and structural and functional integrity of cell membrane, but also affects the activities of various membrane bound enzymes including ATPases (Rhodes *et al.,* 1984). Shakoori *et al.* (1992) also reported a significant reduction in cholesterol levels in rabbits and rats treated with permethrin. Organophosphates like methyl parathion, dimethoate and monocrotophos when given to female albino rats at a dose level of $1/5^{th}$ of LD_{50} had also resulted in significant decrease in concentration of proteins, lipids, phospholipids and cholesterol in ovaries of treated rats (Kaur and Dhanju, 2005).

The activity of acid phosphatase increased at 2 weeks in treated rats which decreased non-significantly at 4 week as compared to control rats (Table 4). Alkaline phosphatase significantly increased in the ovaries of cypermethrin treated rats at 2 and 4 weeks (P<0.05) as compared to its levels in control rats (Table 4). Phosphatases are involved in many different processes that require mobilization of phosphate ions or dephosphorylation as part of anabolic, catabolic or transfer processes (Kaur and Dhanju, 2004). A change in enzyme activity is generally related to intensity of cellular damage (Muthuviveganandavel *et al.,* 2008). Increased acid and alkaline phosphatase activity in cypermethrin treated rats

Table 3 : Effect of cypermethrin treatment on histology of uterine components of control and treated female albino rats after 2 and 4 weeks

S.No.	Uterine component	2 weeks		4 weeks	
		Control	Treated	Control	Treated
1.	Epithelial height (µm)	32.60±2.42 (22.20-43.20)	16.00±1.47 [a] (13.30-19.00)	29.70±1.93 (18.30-32.70)	13.30±1.27 [a] (11.50-15.50)
2.	Myometrium thickness (µm)	92.02±0.10 (70.50-112.50)	109.37±0.93 [a] (92.50-128.10)	80.85±0.80 (75.00-112.50)	112.50±0.97[a] (98.50-128.00)

[a] Statistically Significantly different (P<0.05) between a group; All the values are mean ± S.E values of 6 animals in each group; Figures in parenthesis indicate the range of height of uterine epithelium and myometrium thickness.

Table 4 : Effect of cypermethrin treatment on biochemical constituents in ovary of female albino rats after 2 and 4 weeks

Biochemical constituents	2 weeks		4 weeks	
	Control	Treated	Control	Treated
Proteins (mg g^{-1} wet wt. of ovary)	3.63±0.12	2.45±0.09[a]	3.68±0.08	2.23±0.08 [a]
Total lipids (mg g^{-1} wet wt. of ovary)	23.15±1.70	18.05±0.74 [a]	23.80±1.85	18.50±1.06 [a]
Phospholipids (mg g^{-1} wet wt. of ovary)	6.23±0.02	5.19±0.03 [a]	6.10±0.02	5.11±0.04[a]
Cholesterol (mg g^{-1} wet wt. of ovary)	0.54±0.03	0.36±0.01 [a]	0.53±0.02	0.34±0.02 [a]
ACP (µmole mg^{-1} protein)	2.52±0.07	3.81±0.03 [a]	2.40±0.10	3.76±0.03 [a]
ALP (µmole mg^{-1} protein)	9.55± 0.14	10.98±0.56 [a]	10.33±0.02	12.47 ±0.18 [a]
3βHSDH (µmole mg^{-1} protein)	27.65±0.52	20.52±0.23 [a]	26.11±0.19	19.00 ±0.58 [a]
LDH (µmole mg^{-1} protein)	0.132±0.001	0.084±0.002 [a]	0.130±0.001	0.082±0.001 [a]

All the values are mean ± SE values of 6 animals in each groups; [a] Significantly different (Pd"0.05) between a group and between two different time periods

can be attributed to degenerative changes induced in tissues (Kaur and Dhanju, 2004). Alpha-cypermethrin administered intradermally to male albino wistar rats had also resulted in higher levels of ACP and ALP in testes of male rats (Muthuviveganandavel *et al.,* 2008). Insecticide DDT, an organochlorine compound injected intraperitoneally to newly born female mice also resulted in increased activity of ALP in ovaries (Raifa and Garieb, 2001). The increase in ALP activity represents the functional status of different vital organs like testes, ovary and liver.

The activity of 3 β-hydroxysteroid dehydrogenase decreased significantly in the ovaries of cypermethrin treated rats at 2 and 4 weeks (Table 4) as compared to their level in respective control. Lactate dehydrogenase (LDH) also decreased significantly at 2 and 4 week in treated rats as compared to control rats (Table 4). Decrease in 3 β-HSDH activity in treated rat ovary indicates altered levels of reproductive hormones in rats (Bretveld *et al.,* 2006; Liu *et al*., 2006). Oral administration of cypermethrin to female rats has resulted in significant decrease in plasma progesterone levels (Saleem *et al.,* 1996). Earlier studies of Mani *et al.* (2004) also observed significant reduction in testicular enzyme 17 β-hydroxysteroid dehydrogenase, responsible for testosterone biosynthesis in male rats exposed to fenevalerate which may ultimately be leading to net decrease in testosterone concentration in group of rats. A low activity of LDH in the ovarian tissue indicates a decreased metabolism and a poor oocyte growth in treated rat ovaries (Guraya, 2000). Srivastava *et al.* (2006) also observed mild decrease in activity of LDH in testes and epididymis of rats exposed to liquid mosquito repellent (LMR) containing pyrethroid allethrin (3.6% w/w).

In conclusion our results suggest that exposure to pesticide cypermethrin has adverse effects on female reproductive system.

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