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Gender-based toxic potentials of cypermethrin on behavioural, dietary and gonadal endocrine axis in wistar rats

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Abstract
Cypermethrin (CYP), a fourth generation synthetic pyrethroid, is on great demand in agricultural practices, because of its targeted approach and rapid biodegradability. The present investigation was designed to study the toxic effects of two chronic sub-lethal doses of CYP in female and male rats. The rats were divided into three group, each group consisting of eight rats. Control rats were given olive oil, while other two group of rats were orally intubated with sub-chronic does levels of 1/25th and 1/50th LD_{50} of CYP dissolved in olive oil respectively daily for four weeks. Feed-water intake and clinical symptoms were observed daily along with weekly changes in body weight, and circulatory levels of gonadotrophins and steroid hormones in blood. Loose fecal pellets, hyperirritability and reduced average daily feed and water intake was observed in CYP treated female and male rats as compared to control rats. Net body weight gain was significantly lower in 1/25th of LD_{50} cypermethrin treated female and male rats. FSH, Estrogen, progesterone and testosterone hormone levels showed significant decrease in CYP treated groups. Results infer that cypermethrin exposure has adverse effect on body metabolism and on the reproductive hormones and severity increases at high doses which have the potential to disrupt gonadal endocrine axis and can lead to number of pathophysiological reproductive disorders.

Keywords: Cypermethrin, estradiol, endocrine axis, gonadotropins, progesterone, pyrethroid, testosterone

1. Introduction
Besides being beneficial in producing the adverse biological effects against the target species, pesticides have the potential to affect the health of non-target species including human beings (Damalas and Eleftherohorinos 2011) [6]. Pesticides can cause the health issues ranging from mild allergies, rashes, breathing difficulties, neurotoxicity and reproductive abnormalities to deadly chronic diseases like cancer (Tomer *et al*. 2015) [35]. Many studies have reported the adverse effects of pesticides on the endocrine toxicity and reproductive axis of animals (Akingbemi *et al*. 2000, Tiido *et al*. 2006) [3, 33].
Considered to be least toxic, synthetic pyrethroids globally account for over 30% of insecticide use. The use of a fourth generation synthetic pyrethroid, Cypermethrin (CYP) is increasing in agriculture as the pests are becoming resistant to organophosphorus and organochlorine insecticides (Grewal *et al*. 2010) [12]. Target oriented mechanism of action and rapid biodegradability makes the use of pyrethroids preferential over other pesticides (Sharma *et al*. 2014a, Gomes and Roche 2016) [31, 11]. Studies have demonstrated that these pyrethroids which are believed to be least toxic possess certain hormonal activities and thus, are classified as endocrine-disrupting compounds (EDCs) (KIM *et al*. 2005) [16].
The reproductive integrity in males has been assessed from the level of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone in circulation, while the level of estradiol and progesterone, in addition to LH and FSH in circulation, is used in the assessment of the reproductive integrity in females (Gomes and Roche 2016) [11]. Studies revealed that pyrethroids reduce the binding of oestrogen to oestrogen receptors and inhibits its transcriptional activity. Hormonal disruption in agricultural workers and experimental animal studies suggest that exposure to pyrethroids disrupted the hypothalamo-hypophyseal gonadal axis and also indicated that FSH and LH are the most affected (Agrawal and Sharma 2010) [2].
Considering the above facts in view, the present investigation was designed to determine the reproductive toxicity associated with cypermethrin in female and male Wistar rats resulting in hormonal changes in plasma.

Chemicals

The chemicals were purchased from SDFCL (SD Fine-Chem Ltd) and SRL (Sissco Research Laboratories Pvt. Ltd) and were of analytical grade. Cypermethrin 25% Emulsifiable Concentrate (EC) used in the present study was purchased from Rallis India Limited (RIL), Secunderabad, Hyderabad. Hormonal assay kits were acquired from Labor Diagnostika Nord, GmbH & Co. KG. Standard pelleted rat feed was purchased from Ashirwad Industries, Mohali, India.

Methods

The adult female and male albino rats and weighing 100– 150 g were procured from Department of Livestock Production and Management, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. Rats were housed in polypropylene cages with bedding of paddy husk in laboratory, where the humidity (50 ± 5%), temperature (25 ± 2˚ C) and a normal photoperiod of 12–12 h light–dark cycle were environmentally controlled. Rats were provided the standard rat feed and water *ad libitum*. All methods and procedures of animal handling used in research were according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, India and experiments conducted in the present study was duly approved by Institutional Animal Ethics Committee (IAEC), GADVASU, Ludhiana vide letter no. 212-237 dated 11.09.2015.

Female and male albino rats were acclimatized for 10 days in laboratory conditions and were divided into three groups, each group consisting of eight rats. The first group of rats serving as control (untreated) were given only olive oil and to the remaining two groups, cypermethrin was given at a dose level of 1/25th and 1/50th of LD_{50} by oral intubation for four weeks. All the untreated and treated were observed daily after every dosing for 3-5 hrs for clinical symptoms like salivation, hyperactivity, irritability, faecal pellet conditions, diarrhea, weakness, coarse tremor, paralysis of limb, convulsions, wounds, aggressive behaviour, eye discharge, unsteady gait, falling of hair, stress and changes in non-sexual behaviours (such as cleaning of face, climbing in cages, rearings etc). Feed and water intake was also noted for control and cypermethrin treated female and male rats

During the experimental period, vaginal smear from each female rat in all the groups was observed under light microscope every day to record the mean number of estrous cycles per month. Evaluation of vaginal smear was based upon the presence of types of cells for staging the estrous cycle: Proestrus (presence of nucleated epithelial cells), estrus (presence of cornified cells), metestrus (presence of approximately equal number of leukocytes and epithelial cells) and diestrus (presence of leukocytes).

The body weight of all the female and male rats was taken before the start of the treatment and were weighed weekly during the duration of experiment to determine the change in body weight. Blood was also taken weekly *via.* orbital sinus under general anesthesia. Blood samples from rats were collected in heparinised vials and centrifuged at 2300 r.p.m. for 15 minutes. Supernatant was obtained as plasma for hormone analysis. The circulating levels of estrogen, progesterone and testosterone, FSH and LH in plasma were measured weekly by ELISA kits according to the protocol of the kits.

Statistical Analysis

The experimental results were expressed as mean ± standard error of the mean (SEM) for n=8. Statistical analysis of data was performed on a computer by using CPCS1. One-way ANOVA was done to check the significance levels and the criterion for statistical significance was set at $P<0.05$.

Results

General toxicity symptoms

No toxicological signs were observed in control rats during the experimentation period. The female rats treated with higher dose of CYP revealed wounds near neck region (Plate 1, Fig. A), unsteady gait (Plate 1, Fig. B), hair loss surrounding mouth (Plate 1, Fig. C) and redness near vaginal opening (Plate 1, Fig. D). Female rats administered 1/50th of LD_{50} of CYP showed salivation, hyperactivity, minor wounds, mild hair loss and aggressive behaviour during three weeks of treatment (Table 1). Signs of toxicity increased in fourth week in the female rats with marked salivation, aggressive behaviour, weakness and loose faecal pellets.

Table 1: Effect of cypermethrin (1/25th and 1/50th of LD_{50}) on toxicological symptoms of control and treated female albino rats during four weeks of treatment

Toxicological Symptoms	Control				1/25th of LD_{50}				1/50th of LD_{50}			
	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
Salivation	0	0	0	0	0	0	1 (37.5)	1 (50)	0	0	1 (25)	1 (37.5)
Eye discharge	0	0	0	0	0	0	2 (50)	2 (50)	0	0	0	1 (25)
Hyperactivity	0	0	0	0	1 (12.5)	2 (50)	3 (50)	3 (62.5)	0	0	1 (37.5)	2 (37.5)
Faecal pellet	Solid	Solid	Solid	Solid	Solid	Semi-solid	Loose	Loose	Solid	Semi-solid	Semi-solid	Loose
Weakness	0	0	0	0	0	2 (25)	3 (50)	3 (75)	0	1 (12.5)	2 (37.5)	2 (37.5)
Paralysis of Limb	0	0	0	0	0	0	1(25)	2 (25)	0	0	0	1 (12.5)
Wounds	0	0	0	0	0	0	2 (37.5)	2 (62.5)	0	0	2 (25)	2 (25)
Aggressive behavior	0	0	0	0	0	1 (25)	2 (50)	3 (62.5)	0	0	1 (25)	2 (37.5)
Unsteady gait	0	0	0	0	0	0	1 (12.5)	1 (25)	0	0	0	0
Falling of hair	0	0	0	0	0	1 (12.5)	2 (37.5)	3 (50)	0	0	2 (25)	2 (50)
Stress(Fur erection)	0	0	0	0	0	0	1 (25)	2 (25)	0	0	0	1 (25)
Climbing in cages	0	0	0	1 (12.5)	0	0	2 (37.5)	3 (50)	0	0	2 (12.5)	3 (25)

Note: Each clinical sign is assigned scoring varying from 0-3 depending upon its severity for eight animals in each group (No toxicological symptom-0, mild-1, moderate-2, severe-3).

Values in parenthesis shows the percent population of rats affected.

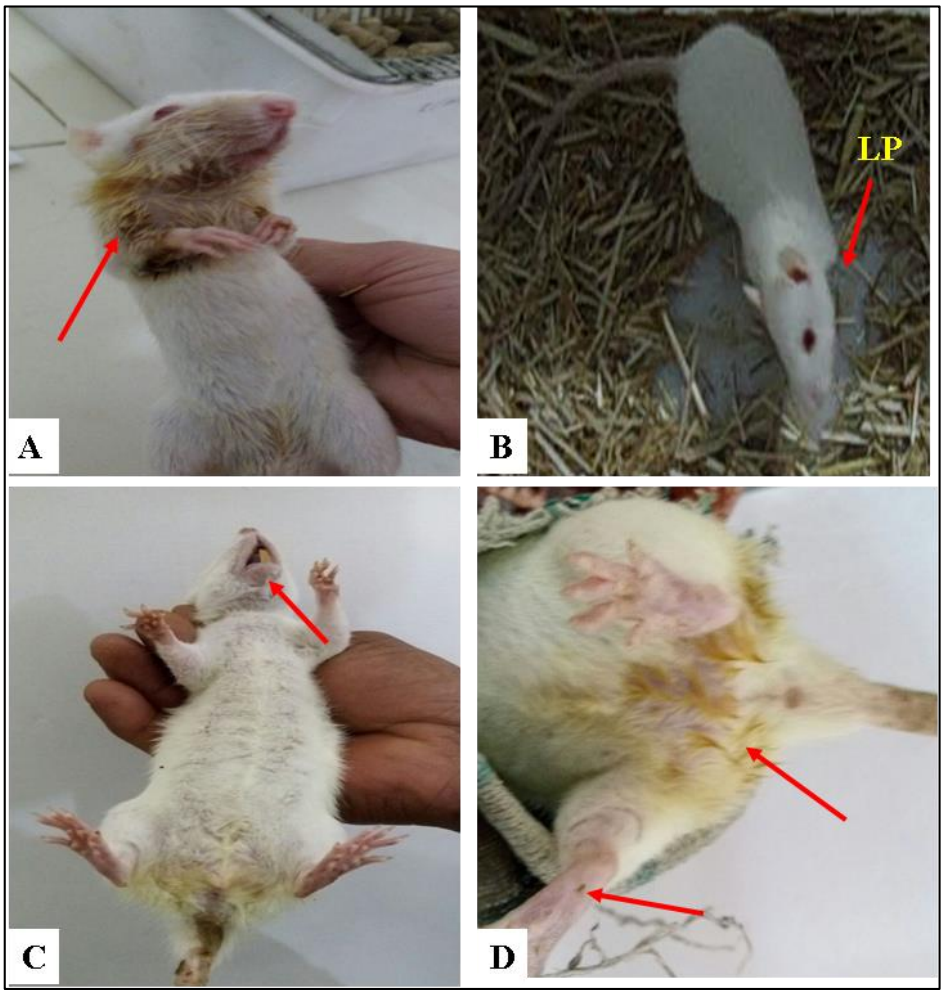

Plate 1: Fig (A): wounds in the neck region (arrow) (B): limb paralysis (LP); (C) Hair loss (arrow); (D): wounds in the vaginal region in 1/25th LD_{50} cypermethrin female rats.

In male rats, cypermethrin treatment at higher doses showed moderate signs of toxicity during first and second week. Salivation, thick discharge from the eye, wounds and unsteady gait was observed during the third week of treatment. Signs of toxicity in fourth week in the rats revealed severe hair loss (Plate 2, Fig. A, B), marked salivation, wounds (Plate 2, Fig. C), limb paralysis (Plate 2, Fig. D), aggressive behaviour, weakness and loose faecal pellets (Table 2). In 1/50th of LD_{50} cypermethrin treated rats, toxicity symptoms appeared in the third week of treatment with salivation, eye discharge, wounds and hair loss which further increased during the fourth week.

Table 2: Effect of cypermethrin (1/25th and 1/50th of LD_{50}) on toxicological symptoms of control and treated male albino rats during four weeks of treatment

Toxicological Symptoms	Control				1/25th of LD_{50}				1/50th of LD_{50}			
	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
Salivation	0	0	0	0	0	0	2 (50)	2 (62.5)	0	0	1 (12.5)	1 (37.5)
Eye discharge	0	0	0	0	0	0	1 (50)	1 (37.5)	0	0	1 (12.5)	1 (25)
Hyperactivity	0	0	0	0	0	0	2 (37.5)	3 (62.5)	0	1 (25)	1 (25)	2 (37.5)
Faecal pellet	Solid	Solid	Solid	Solid	Solid	Semi-solid	Loose	Loose	Solid	Solid	Semi-solid	Loose
Weakness	0	0	0	0	0	1 (25)	2 (37.5)	3 (62.5)	0	0	1 (37.5)	2 (37.5)
Paralysis of Limb	0	0	0	0	0	0	0	1 (25)	0	0	0	1 (12.5)
Wounds	0	0	0	0	0	0	1 (25)	2 (50)	0	0	1 (12.5)	2 (37.5)
Aggressive behavior	0	0	0	0	0	1 (25)	2 (50)	3 (50)	0	0	1 (25)	2 (37.5)
Unsteady gait	0	0	0	0	0	0	0	1 (25)	0	0	0	0
Falling of hair	0	0	0	0	0	0	2 (37.5)	3 (62.5)	0	0	1 (12.5)	2 (37.5)
Stress(Fur erection)	0	0	0	0	0	0	1 (25)	2 (37.5)	0	0	1 (12.5)	2 (12.5)
Climbing in cages	0	0	0	0	0	0	2 (37.5)	3 (62.5)	0	0	2 (25)	3 (37.5)

Note: Each clinical sign is assigned scoring varying from 0-3 depending upon its severity for eight animals in each group (No toxicological symptom-0, mild-1, moderate-2, severe-3).
Values in parenthesis shows the percent population of rats affected.

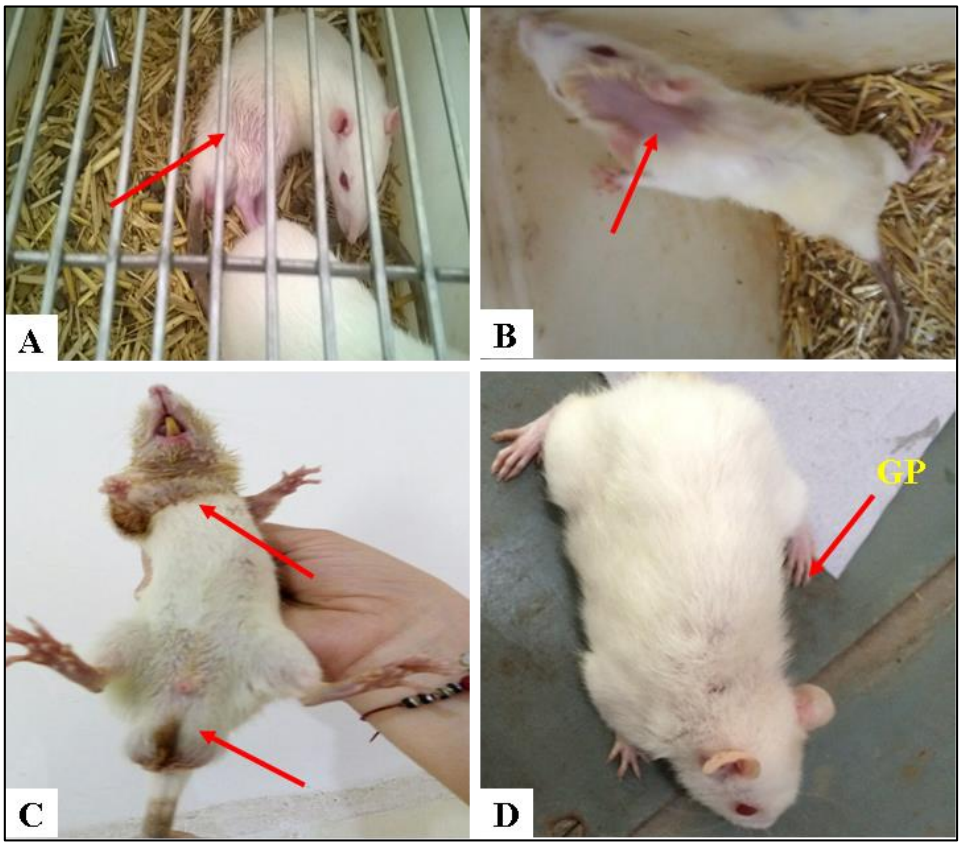

Plate 2: Fig (A,B) Hair loss (arrow) (C) wounds in the testicular region (D) limb paralysis (LP) in 1/25th LD_{50} cypermethrin male rats.

In the present study, cypermethrin treated rats at both the doses showed salivation, hyperirritability, aggressive behavior, lack of coordination, muscle tremor and convulsions. Similar toxicity symptoms were observed in animals administered with the combination of endosulfan and cypermethrin (1:1) at the dose of 172.95 mg/kg bw and 207.50 mg/kg bw which showed acute cholinergic symptoms like salivation, coarse whole body tremors, hyperactivity to sound and touch, abnormal gait, and limb paralysis (Raj *et al*. 2013) [25]. These signs of toxicity clearly indicates that the target for this chemical is the central nervous system (CNS) in mammals (Sangha *et al*. 2011) [29]. He also reported that deltamethrin at higher doses ranging from 125-225 mg/kg given to rats produced signs of CNS stimulation followed by prolonged depression. Pyrethroid use led to the generation of a variable sequence of motor symptoms developed that involved occasional pawing and burrowing, gradual development of hind limb extensor tone and coarse whole body tremor associated with movement. These motor symptoms following deltamethrin administration are strongly suggestive of central nervous system involvement (Nagarjuna and Doss 2009) [20]. This study is in agreement with the earlier studies of Manna *et al*. (2005) [19] who investigated the toxic effects of moderate toxicity in mammals, revealing neurotoxicity in terms of lack of coordination and aggression upon pesticides exposure.

Furthurmore, broadcasting the sublethal effects of pesticides, Sharma *et al* (2014a) [31] reported that the animals exposed to higher dose of cypermethrin produced mild to moderate toxicity characterized by diarrhea, decreased feed intake, loss in body weight, thick eye discharge, and salivation. The observed signs were also similar to those reported by Neuschl and Kacmar (1995) [21] following repeated administration of deltamethrin and cypermethrin in rodents.

Feed intake and Water intake

Average daily feed intake was low in 1/25th and 1/50th of LD_{50} cypermethrin treated female and male rats as compared with the control rats (Table 3 and 4). Female rats administered with 1/25th of LD_{50} cypermethrin showed a significant decrease in food consumption and water intake in the first week of treatment while in the second week, slight increase was observed in water intake as compared to the first week (Table 3, 5). With further continuation of treatment, slight reduction in food and water intake was observed in third and fourth week. 1/50th of LD_{50} cypermethrin treated female rats showed a non-significant reduction in food intake every week till the end of experimentation.

Similarily reduction in food and water intake was also observed in male rats treated with higher dose of cypermethrin (Table 4, 6). The average water intake was significantly reduced in cypermethrin (1/25th of LD_{50}) treated female and male rats as compared to control (Table 5, 6).

Table 3: Effect of cypermethrin on average feed intake (g/100g b.w.) in female albino rats

Duration of treatment	Control	1/25th of LD_{50}	1/50th of LD_{50}
First week	14.29±0.46	11.47±0.51*	12.95±0.60
Second week	12.62±0.31	11.23±0.22	12.44±0.25
Third week	11.78± 0.35	10.82±0.22	10.16±0.15
Fourth week	10.66± 0.72	10.31±0.20	10.93±0.33
Average feed intake	12.33±1.46	10.95±0.59	11.62±0.54

Mean ± SE values of 8 animals in each group.
*Significant difference at $p<0.05$ as compared to control

Table 4: Effect of cypermethrin on average feed intake (g/100g b.w.) in male albino rats

Duration of treatment	Control	1/25th of LD_{50}	1/50th of LD_{50}
First week	16.44±0.21	14.26±0.26*	14.88±0.61*
Second week	14.82±0.58	13.83±0.33	14.26±0.43
Third week	13.85± 0.67	13.19±0.39	13.12±0.30
Fourth week	14.18± 0.38	13.04±0.44	12.48±0.60
Average feed intake	14.88±0.93	13.73±0.52	13.28±0.48

Mean ± SE values of 8 animals in each group.
*Significant difference at $p<0.05$ as compared to control

Table 5: Effect of cypermethrin on average water intake (ml/100g b.w.) in female albino rats

Duration of treatment	Control	1/25th of LD_{50}	1/50th of LD_{50}
First week	19.06±1.27	15.37±1.80*	18.03±1.49
Second week	18.24±1.55	17.79±1.83	20.12±1.13*
Third week	18.57±1.11	15.65±0.60*	18.16±1.48
Fourth week	20.31±0.57	15.60±0.43*	16.67±0.43*
Average water intake	19.45±1.13	16.10±1.17*	18.22±1.21

Mean ± SE values of 8 animals in each group.
*Significant difference at $p<0.05$ as compared to control

Table 6: Effect of cypermethrin on average water intake (ml/100g b.w.) in male albino rats

Duration of treatment	Control	1/25th of LD_{50}	1/50th of LD_{50}
First week	24.89±1.98	20.04±2.92*	24.05±2.39
Second week	27.86±2.03	20.84± 3.52*	28.95±2.46
Third week	24.71±2.16	18.88±1.32*	22.00±1.57
Fourth week	23.50±0.76	22.31±0.88	20.35±0.80
Average water intake	25.24±1.84	20.51±0.99*	23.83±2.20

Mean ± SE values of 8 animals in each group.
*Significant difference at $p<0.05$ as compared to control

Reduced food intake is common observation in toxicity studies (Ngoula *et al*. 2012) [22]. Decreased metabolism or inhibition of the hunger resulting in lack of appetite or anorexia is the key rationale for reduced food consumption. The loss in food consumption was observed by Adjrah *et al*. (2013) [1] while studying the potential effect of cypermethrin-treated lettuce on the rat liver. Cypermethrin at oral doses of 5 and 20 mg/kg/day for one month also resulted in reduced Food and water intake (Grewal *et al*. 2010) [12]. Ratnasooriya *et al*. (2002) [27] also observed that alpha cyhalothrin at 63mg/kg and 100mg/kg caused a significant reduction in food intake while it had no significant effect on water intake.

Body weight gain

Growth rate decreased significantly in treated female and male rats during the four weeks of treatment (Table 7, 8). Significant decrease in body weight gain was observed in treated female and male rats at both the doses (Table 7, 8).

Table 7: Changes in growth rate (g/day/100g b.w.) in cypermethrin treated female albino rats during four weeks of treatment

Treatment		0 day	1st week	2nd week	3rd week	4th week	Body weight gain
Control	Weight	160.66±1.25	166±1.83	172.05±1.51	180±1.58	188.75±1.77	28.09±0.52
	Growth rate	----	0.53±0.15	0.51±0.18	0.66±0.09	0.69±0.19	
1/25th of LD_{50}	Weight	158.00±1.85	162.51±1.18	168.67±1.09	173.01±1.42	179.03±1.95	21.03±1.10*
	Growth rate	----	0.40±0.13*	0.48±0.11*	0.37±0.11*	0.49±0.08*	
1/50th of LD_{50}	Weight	155.01±1.19	160.33±1.47	165.90±9.04	170.83±1.11	176.51±1.18	21.50±1.99*
	Growth rate	----	0.47±0.14	0.49±0.17*	0.43±0.10	0.47±0.08*	

Mean ± SE values of 8 animals in each group.
*Significant difference at $p<0.05$ as compared to control

Table 8: Changes in growth rate (g/day/100g b.w.)in cypermethrin treated male albino rats during four weeks of treatment

Treatment		0 day	1st week	2nd week	3rd week	4th week	Body weight gain
Control	Weight	111.25±1.63	115.01±1.96	120.51±1.54	128.25±1.98	135.52±2.68	24.27±1.28
	Growth rate	----	0.50±0.03	0.68±0.04	0.80±0.01	0.93±0.11	
1/25th of LD_{50}	Weight	112.25±1.77	114.22±1.15	119.14±1.01	125±9.13	131.75±1.78	19.50±1.10*
	Growth rate	----	0.31±0.01*	0.56±0.02*	0.72±0.12*	0.77±0.10*	
1/50th of LD_{50}	Weight	104.52±1.09	107.56±1.09	112.51±1.08	118.3±1.63	125.59±5.07	21.07±1.01
	Growth rate	----	0.41±0.07*	0.66±0.21	0.73±0.04*	0.86±0.03*	

Mean ± SE values of 8 animals in each group.
*Significant difference at $p<0.05$ as compared to control

The decrease in growth rate observed in the present study is in agreement with the studies of Aldana *et al*. (2001) [4] and Elbetieha *et al*. (2001) [7]. They have observed significantly lower body weight gain in cypermethrin (25 mg/kg bw) treated rats as compared to control rats. Lakkawar *et al*. (2004) [17] have observed decrease in body weights in rabbits treated with cypermethrin. Lambda cyhalothrin (synthetic pyrethroid) administered orally to rats has also resulted in reduced body weight gain in both female and male rats (Ratnasooriya *et al*. 2002) [27]. Body weight loss and diarrhea were most prominent clinical signs of the rats administered with different doses of cypermethrin alone or cypermethrin-treated lettuce (Adjrah *et al*. 2013) [1]. The net body weight gain in all the treated rats was less at both 2 and 4 weeks of cypermethrin treatment as compared to the control rats (Sangha *et al*. 2011) [29]. Hussain *et al*. (2009) [14] have also observed significantly lower body weight gain in cypermethrin-treated (500mg/kg) rats as compared to control rats. Decreased body weight could be referred to systemic intoxication due to cholinesterase inhibition with subsequent cholinergic overstimulation (Roegge *et al*. 2008) [28]; marked DNA damage or altered carbohydrate metabolism.

Ovarian cyclicity in females

The estrous cycle was disturbed in the cypermethrin treated female rats as estrus phase was shorter and diestrus was prolonged (Table 9). Proestrus reduced non-significantly and metestrus was increased in dose dependent manner in treated female rats. Diestrus index also increased in dose dependent manner.

Table 9: Effect of cypermethrin on estrous cycle of control and treated rats after four weeks of treatment

Treatment	Days of Metestrous	Days of Diestrous	Days of Proestrous	Days of Estrous	Diestrus index
Control	4.50±0.29	10.25±0.48	5.75±0.25	7.50±0.65	34.16±0.76
Cypermethrin (1/25th of LD_{50})	5.17±0.31	10.83±0.40	5.67±0.42	6.50±0.34	36.10±1.23
Cypermethrin (1/50th of LD_{50})	4.67±0.33	10.67±0.33	5.50±0.22	7.17±0.31	35.56±1.46

It is well known that estrous periodicity is controlled by cyclic secretion of pituitary and ovarian hormones (estrogen and progesterone). Therefore, alterations in estrus periodicity following cypermethrin treatment suggest impairement in reproductive hormone secretion and action (Ghosh and Choudhary 2015) [9]. Results from the cyclicity studies indicates the alteration in the function of hypothalamic-pituitary-gonadal axis (Liu *et al*. 2011) [18].

Hormonal status

The levels of plasma estradiol and progesterone in female experimental rats were observed to be significantly reduced compared to the levels in the control rats after four weeks of treatment. In control rats, the level of estradiol increased from Day 1 to the end of the experiment while the levels of estradiol decreased in treated rats after four weeks of treatment (Table 10). Progesterone levels showed a significant decrease in cypermethrin treated rats as compared to control rats (Table 10).

Table 10: Effect of cypermethrin on level of various hormones in plasma of female albino rats

	Estrogen (pg/ml)			Progesterone(ng/ml)			FSH (mIU/ml)			LH (mIU/ml)		
	Control	1/25th of LD_{50}	1/50th of LD_{50}	Cntrol	1/25th of LD_{50}	1/50th of LD_{50}	Control	1/25th of LD_{50}	1/50th of LD_{50}	Control	1/25th of LD_{50}	1/50th of LD_{50}
0th day	135.26± 3.38	141.02± 0.96	149.25± 3.01	4.47± 0.02	5.02± 0.15	4.86± 0.18	4.25± 0.17	5.06± 1.59	4.69± 1.25	2.50± 0.15	2.56± 0.04	2.66± 0.04
First week	139.64± 5.61	105.18± 0.36	123.24± 0.78	3.95± 0.02	4.99± 0.69	4.26± 0.05	4.89± 0.08	5.59± 0.51	4.15± 0.17	2.89± 0.04	2.68± 0.09	2.99± 0.14
Second week	145.39± 1.36*	75.02± 3.45*	99.36± 2.89*	3.78± 0.058	4.25± 0.65	3.99± 0.13	5.07± 0.06	5.26± 0.06	4.06± 0.10	3.19± 0.06	3.39± 0.07	3.05± 0.18
Third week	168.68± 5.39*	29.26± 2.46*	56.00± 4.72*	3.75± 0.04	3.64± 0.08*	3.57± 0.18*	5.38± 0.98	4.06± 0.14	3.99± 0.15	3.58± 0.14	3.42.± 0.01	3.65± 0.04
Fourth week	177.00± 5.98*	25.62± 3.98*	44.76± 6.87*	3.50± 0.15	3.41± 0.10*	3.46± 0.13*	5.51± 0.68	3.84± 0.16	3.80± 0.13	3.96± 0.19	4.03± 0.75	4.01± 0.08

Mean ± SE values of 8 animals in each group.
*Significant difference at $p<0.05$ as compared to control

Exposure of female rats to cypermethrin resulted in decreased levels of FSH in 1/25th of LD_{50} of cypermethrin (5.06±1.59 to 3.84±0.16 mIU/ml) and 1/50th of LD_{50} of cypermethrin (4.69±1.25 to 3.80±0.13 mIU/ml) as compared to control rats (4.25±0.17 to 5.51±0.68 mIU/ml). The levels of LH was comparable among different groups (Table 11).

Levels of testosterone obtained for male rats in test group was significantly lower and it decreased in treated rats. Similarily, FSH and LH levels also showed a significant decrease (Table 11).

Table 11: Effect of cypermethrin on level of various hormones in plasma of male albino rats

	Testosterone (ng/ml)			FSH (mIU/ml)			LH (mIU/ml)		
	Control	1/25th of LD_{50}	1/50th of LD_{50}	Control	1/25th of LD_{50}	1/50th of LD_{50}	Control	1/25th of LD_{50}	1/50th of LD_{50}
0th day	3.92±0.01	3.98±0.06	3.95±0.07	2.00±0.15	2.72±0.13	3.19±0.19	3.06±0.03	4.97±1.02	3.52±0.12
First week	4.06±0.11	3.12±0.09	3.51±0.14	3.15±0.02	2.29±0.17	3.00±0.13	3.97±0.04	4.59±0.21	3.25±0.14
Second week	6.72±0.06*	3.00±0.13	2.97±0.08	3.28±0.21	1.95±0.03	2.94±0.65	4.15±0.28	3.84±0.14	2.97±0.20
Third week	8.09±0.19*	2.76±0.02*	2.81±0.13*	3.34±0.15	1.31±0.12*	2.15±0.12	4.35±0.09*	3.45±1.04	2.94±0.13
Fourth week	9.84±1.87*	2.65±0.09*	2.72±0.14*	3.65±0.11	1.01±0.27*	1.98±0.42	4.62±1.89*	3.17±1.56	2.81±1.13

Mean ± SE values of 8 animals in each group.
*Significant difference at $p<0.05$ as compared to control

The reproductive functions and characteristics in both female and male organisms is modulated by sex hormones. Therefore, the serum sex hormones profile is very useful in assessing the reproductive integrity in both animals and humans. Estimating the levels of these hormones is a valuable index in evaluating a variety of menstrual dysfunctions in females (Manna *et al*. 2005) [19].

Cypermethrin may be responsible for the decrease in the estrogen level since pyrethroids have affinity for androgen or estrogen receptors. The binding of cypermethrin with androgens (testosterone and androstenedione) restricts its conversion into estrogens in the granulosa cells. This is in line with the findings of Trif *et al*. (2010) who reported that the decrease in estrogen level as a result of chromium exposure which led to decrease of the aromatase in the granulosa cells and androgen transformation into estrogen. This reduction in the estrogen level in this study invariably affected other hormones. Liu *et al*. (2011) [18] observed that the rats given

mixture of cypermethrin and methyl parathion have resulted in alterations in reproductive hormones in female and male. Progesterone levels were also decreased by exposure to pesticides viz. methoxychlor and fenvalerate in rats resulting in disruption in the estrous periodicity (Fei *et al*. 2010) [8]. The key factor for this decrease may be that pyrethroid damages the ovarian CL cells by inducing changes in the endoplasmic reticulum and mitochondrion of CL (He *et al*., 2006) [13], which results in the disruption of endocrine functions i.e. decrease in the progesterone concentration. Similar decrease in progesterone levels were observed in bovines following CYP exposure by Gill *et al*. (2011) [10].

Reduced testosterone levels observed in the present study is in agreement with the study conducted by Elbetieha *et al*. (2001) [7] where the levels of testosterone were reduced in the adult male Sprague-Dawley rats exposed to tap water containing cypermethrin for 12 weeks. Levels of testosterone (T), LH and FSH also decreased significantly in cypermethrin treated rats (50, 75, or 100 mg/ kgbw) in dose-dependent manner (Joshi *et al*. 2014) [15]. Significant decrease in testosterone, FSH and LH levels was observed by Sharma *et al*. (2014b) [32] in α-cypermethrin treated male rats (3.83mg/kg bw). Similar results were reported in male rats exposed to other synthetic pyrethroids such as fenvalerate, permethrin and beta-cypermethrin (Wang *et al*. 2009). Yan *et al*. (2013) [36, 37] suggested that cypermethrin might suppress male spermatogenesis and induce low daily sperm production by disturbing testosterone biosynthesis.

The decreased value of testosterone can also be due to the anti-androgensterone activity of cypermethrin where it binds to the androgen receptor and reduces the secretion of these hormones. The reduction in the testosterone level can also be due to diminished responsiveness of cells in testis and that have inhibition in testicular steroidogenesis (Nudmamud-Thanoi *et al*. 2016) [23]. Pyrethroid exposures in rats was found to induce significant decreases in testicular enzymes for T biosynthesis, such as 17ß-hydroxysteroid dehydrogenase (17ß-HSD) and glucose-6phosphate dehydrogenase, which might be due to interference with testicular T synthesis (Joshi *et al*. 2014) [15]. Oda and El-Maddawy (2012) [24] found that feeding rats with delta methrin at 0.6mg/kg bw for 60 days caused a significant reduction in serum testosterone levels.

Many insecticides possess anti-androgenic activity that reduced the FSH and LH levels which in turn inhibit development of spermatogenesis and seminiferous tubule. Synthetic pyrethroids are also believed to influence the androgen biosynthesis pathways thus reducing pituitary gonadotrophins (FSH and LH) secretions. LH stimulates Leydig cells to produce T. Low production of androgen affects the number and volume of mature Leydig cells thus affecting fertility (Ramaswamy and Weinbauer 2014) [26]. FSH and LH concentration also depend on testosterone levels. Sharma *et al*. (2014a) [31] showed the decrease in Testosterone (19%), FSH (27.11%) and LH (18.94%) in rats exposed to on cypermethrin at dose levels of 3.83 mg/kg bw.

Conclusion

The present laboratory study concluded that oral exposure of cypermethrin has considerable harmful effects on body in albino rats. The observed toxicities are likely to disrupt reproductive processes resulting in serious debilities. The hormonal disruption observed in rats suggests that cypermethrin exposure disrupts the hypothalamic–pituitary endocrine function depicting hazardous effects of the cypermethrin on reproductive processes, particularly the fertility in rats.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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