

## **Toxic Effects of Cypermethrin on Male Fertility and Some Hepatic Biochemical Parameters in Male Albino Rats**

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### **Abstract**

This study is aimed to evaluate the effect of Cypermethrin on some serum biochemical parameters , body weight and fertility in male rats. A total of thirty albino rats weighing (180 -200g) were used for this study. Rats were divided into 3 groups each of 10 rats. First group was given Cypermethrin 12.5 mg/kg bw. Second group was given Cypermethrin 25 mg/kg bw. and third group was left as control group. All doses were orally administered twice weekly for 65 successive days. At the end of the experimental period, blood samples were collected from each rat for biochemical analysis. Rats were humanely euthanized and testes and epididymis were taken for determination of weight of sexual organs and epididymal sperm characters in male rats. Oral administration of Cypermethrin twice weekly for 63 days in a dose of 12.5 mg/kg b.w. and 25 mg/kg bw. significantly increased the leakage enzymes AST , ALT and ALP. Also increased levels of cholesterol and triglyceride. The effect on the liver is one of the main toxic effects of this product as there was also a significant decrease in the total protein and albumin synthesized by the liver. Cypermethrin exposure resulted in a significant decrease in weight of testis and epididymis, epididymal sperm counts, sperm motility and increase in sperm abnormalities. In conclusion, all these changes were dose dependent, being less with low Cypermethrin dose and more even extensive with high Cypermethrin dose . Also, Cypermethrin has negative effects on hepatorenal cell function. In addition , Cypermethrin has dangerous effect on male fertility due to its effect on spermatogenesis (sperm number, motility and sperm abnormalities).

**Key word:** Cypermethrin ,serum biochemistry , male fertility

### **Introduction**

Cypermethrin is common synthetic pyrethroid used in agriculture, forestry as well as in public and animal health programmes. Although considered nontoxic to mammals, recent studies have shown the adverse effect of Cypermethrin on the nervous system (**Blindauer et al., 1999**) hepatic and renal system (**Mansour et al ., 2008**) .

Due to the lipophilic nature of pyrethroid insecticides (**Michelangeli et al., 1990**), they accumulate in biological membranes, leading to stimulation of the production of reactive oxygen species (ROS) and resulting in oxidative damage in mammals (**Mossa et al., 2013**). It is absorbed through the gastrointestinal and respiratory tracts and confers preferential distribution into lipid-rich internal tissues, including body fat, skin, liver, kidney, ovaries, the central and peripheral nervous systems (**Soderlund et al., 2002**)

Exposure to environmental toxicants including pesticides is a proven factor in impairment of male reproductive system and infertility. Cypermethrin initially thought to be safe for household application, a number of recent reports showed its reproductive toxicity in mammalian and nonmammalian laboratory and wildlife animal species (**WHO., 1993**). The reproductive toxicity of Cypermethrin is a major concern because spermatogenesis may be vulnerable to chronic exposure to chemicals at very low exposure. Other studies have shown that synthetic pyrethroids such as d-phenothrin esfenvalerate, fenvalerate and permethrin exhibited no potential to cause adverse effects on the male reproductive system (**Kunimatsu et al., 2002; Yamada et al., 2003**).

Therefore, it is necessary to clarify the male reproductive toxicity of the synthetic pyrethroid Cypermethrin in male Wistar rats and determination of liver function in male rats.

## Materials and Methods

### Chemicals

Cypermethrin (95%) was obtained from Jiangsu Yangnong Chemical Co., Ltd., China. Cypermethrin administered 2 times per week for 65 day for covering all the spermatogenesis period (**Hershberger et al., 1969**). Dose of Cypermethrin was chosen according to **Ray (1991)** as 1/10 of LD50 equal 25 mg/kg bw and 1/20 of LD50 equal 12.5mg/kg bw.

### Animals and groups:

30 male rats of the Wistar strain (*Rattus norvegicus*) weighing 180-200 g were obtained from a private Animal Breeding House for lab animal. Animals were kept in clean plastic cages with free access to food (standard pellet diet) and tap water *ad-libitum*, under standardized housing conditions in the laboratory animal room. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to three groups, each consisting of 10 rats, as follows: First group treated by oral intubation of Cypermethrin (12.5mg/kg bw.) . Second group treated by oral

intubation of Cypermethrin (25 mg/kg bw.) and third group left as control received food and water only.

Cypermethrin was dissolved in distilled water and given via oral route. Dosages of each administered were daily freshly prepared and adjusted weekly for body weight changes. The control group received an equivalent volume of distilled water (0.5 mL/rat).

### **Samples**

In all groups, body weights, clinical signs and mortality rate were recorded weekly all over the experimental period. The blood samples were drawn from all rats under ether anesthesia by puncturing the retroorbital venous plexus of the animals with a fine sterilized glass capillary and collected in glass tubes to separate the sera. Within 20 min of blood collection, the sera samples were drawn from blood after centrifugation at 3500 rpm for 10 min at 4°C. The sera was kept in a deep freezer (-20°C) until analyzed.

After blood collection, the rats were scarified by cervical dislocation, testes and epididymis were removed and weighted.

Epididymal contents were obtained immediately after sacrificing each rat by cutting the tail of epididymis and squeezing it gently in a clean Petri dish to proceed spermatozoa examination (Progressive motility, sperm cell concentration and Epididymal sperm abnormalities.)

### **Methods-**

Serum biomarkers were determined using a commercial kit in accordance with manufacturers' instructions. The activity of cellular enzymes such as aspartate aminotransferase and alanine transaminase (**Reitman and Frankel, 1957**) alkaline phosphatase were determined in sera according to **Tietz (1996)**. While, the concentration of albumin and total protein were determined by the methods of **Westgard and Poquette (1972)** and **Henry (1964)**, respectively.

Serum total cholesterol and serum triglycerides were measured as described by **Fredrikson et al. (1967)** & **Knight et al. (1972)**, respectively. All parameters were measured spectrophotometrically by using standardized test-kits

### **Epididymal spermatozoa examination :**

Progressive motility of sperms was examined according to the method reported by **Bearden and Feuquary (1980)**. A small droplet of epididymal contents was added to one drop of sodium citrate solution 2.9 – 3 % on warm slide. Several fields

were examined under microscope and the incidence of progressively motile sperms was estimated and recorded. Sperm cell concentration was performed according to the technique adopted also by **Bearden and Feuquary (1980)**.

### **Statistical Analysis:**

The results were subsequently analyzed following the statistical method established by **Snedecor and Cochran (1973)**.

## **Results and Discussion**

### **Signs of Toxicity**

No mortality occurred during the study period. Also, no signs of toxicity were observed in Cypermethrin treated rats. In addition, there was no effect on food and water consumptions in Cypermethrin -treated male rats compared to control groups (un-tabulated data).

Our results regarding absence of toxic signs in rats treated with Cypermethrin resembling the findings reported by **WHO (1989)**. It stated that no toxic signs were appeared on using Cypermethrin at a concentration of 150-1000 mg/kg in a diet of rats for 5 months to 2 years. **Ruzo et al. (1979)** revealed that decamethrin must reach a critical brain concentration to induce signs of toxicity.

### **Enzymatic profile**

As show in **Table (1)**, our results revealed that there was a significant dose dependant increase in AST, ALT and ALP activity in male rats orally administered Cypermethrin 25 mg/kg bw and 12.5 mg/kg bw. In agreement with our results, a significant increase in liver serum enzymes was observed in male rats given Cypermethrin at doses 12 mg/kg bw. for 30 days (**Abdou et al., 2012**) and 25 mg/kg bw. for 28 days (**Sankar et al., 2012**). In fact, aminotransferases are intracellular enzymes, most frequently utilized, and specific indicators of hepatocellular necrosis. AST and ALT catalyze the transfer of the amino acids of aspartate and alanine, respectively, to the keto group of ketoglutaric acid. ALPs are a family of zinc metalloenzymes, with serine at the active center, and they release inorganic phosphate from various organic orthophosphates and are present in nearly all tissues (**Thapa and Walia., 2007**). The increase in these enzymes in Cypermethrin treated male rats may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of the liver membrane takes place (**Goel et al., 2005**).

### **Protein profile**

Our results (**Table, 2**) revealed that, oral administration of Cypermethrin to male rats caused dose dependant significant decrease in serum total protein and albumin levels while no significant changes observed in globulin and A/G ratio in the two groups treated by Cypermethrin compared to control group. **Lakkawar et al. (2004)** noted that Cypermethrin toxicity decreased level of total protein in serum of young rabbits. **Catinot et al. (1989)** reported no significant changes in globulin and A/G ratio in rats after intraperitoneal administration of deltamethrin at 7.2 mg/kg bw. for 28 day . The decrease in total protein and albumin in Cypermethrin treated male rats may be due to the liver dysfunctions and disturbance in the biosynthesis of protein.

### **Lipid profile**

The results in **Table (3)** shows that the administration of Cypermethrin induced significant ( $p < 0.05$ ) dose dependant increases in the serum total cholesterol and triglycerides in both groups treated by Cypermethrin compared with control group. These results are in agreement with **Yousef et al. (2003)**. The phospholipid levels dropped, while cholesterol content increased as a consequence of Cypermethrin toxicity . These results were due to lipogenesis and lipolysis occurring during Cypermethrin stress. (**Reddy et al., 1991**)

### **Effect on body weight**

**Table (4)** shows gradual decreases in body weight of male rats treated by Cypermethrin , this decrease was non significant till the 7<sup>th</sup> week in group received 12.5 mg/kg bw and till 5<sup>th</sup> week in group received 25 mg /kg bw . However this decrease was significant till the end of experimental period compared to control group . Previous studies show that Cypermethrin caused significant decrease in body weight gain in rats (**Hussain et al., 2009**) and rabbits (**Lakkawar et al., 2004**). Decrease in body weight gain in Cypermethrin treated male rats may be due to the combined action of neurotoxic effect and oxidative stress. (**Hincal et al., 2001**) reported that administration of Cypermethrin resulted in free radical formation; generation of ROS and production of oxidative stress.

### **Effect on testicular and epididymal weight**

As show in **Table (5)** Our results revealed that there was a significant dose dependant decrease in testes and epididymis weight in male rats orally administered Cypermethrin 25 mg/kg bw and 12.5 mg/kg bw. Testicular and epididymis weight is a valuable index of reproductive health. Accumulation of Cypermethrin in testis and

other reproductive organs may have accelerated oxidative stress leading to accelerated death of spermatogenic cells associated with sperm abnormalities (**Sharma and Singh ., 2010**). The decrease in weight of testis weight on xenobiotics exposure may be due to reduced tubule size, decrease number of germ cells and elongated spermatids (**Choudhary et al., 2008**).

### **Effect on Epididymal spermatozoa**

The results in **Table (5)** shows that the administration of Cypermethrin induced significant dose dependant decrease in sperm count and motility. While dose dependant increase in sperm abnormalities were observed in both groups treated by Cypermethrin compared with control group. The decrease in sperm count is in agreement with the findings of others, where Cypermethrin treatment was associated with the inhibition of T, LH and FSH level (**Liu et al., 2010 & Joshi et al., 2011**). Decrease in sperm motility, live sperm and increase in the number of the abnormal sperm may be due to enhanced ROS production by cypermethrin in the testis and epididymis as observed in this study. Pesticide induced ROS production is known to adversely affect sperm motility, live sperm, and increased sperm abnormality (**Joshi et al., 2011 & Kumar et al., 2004**). A significant elevation in the number of abnormal shape of sperm head was noticed in Cypermethrin exposed rats (**Li et al., 2013**). It is well known that testosterone plays a key role in the development of male reproductive tissues. Testosterone is needed for the continued production of different generation of germ cells in the seminiferous tubules. Therefore, reduction of testosterone level may lead to the separation of germ cells from the epithelium of the seminiferous tubules (**Elbetieha et al., 2001**). Reduction in sperm count on Cypermethrin exposure may be due to decrease in serum testosterone level observed in this study. Decreased testosterone might have suppressed the male spermatogenesis. The inhibition in spermatogenesis may also be due to low level of LH and FSH which are required for normal spermatogenesis in pubertal rats.

### **Conclusion**

Our study concluded adverse effect of Cypermethrin on serum marker enzymes such ALT, AST, ALP and total protein, albumin, cholesterol and triglyceride. These results suggested that Cypermethrin has negative effects on hepatorenal cell function. In addition, Cypermethrin has dangerous effect on male fertility due to its effect on spermatogenesis (sperm number, motility and abnormalities).

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**Table ( 1):** ALT, AST and ALP activities levels in the sera of male rats after administration of Cypermethrin (25 ,12.5 mg/kg bw) twice weekly for 65 day (n=10)

Treatment	AST (UL)	ALT(UL)	ALP(UL)
Cypermethrin 12.5mg/kgbw.	43.4 ± 0.56*	52.07 ± 1.72*	46.25 ± 2.10*
Cypermethrin 25 mg/kg bw.	52.4 ± 0.72**	61.2 ± 1.47**	58.4 ± 0.98*
Control	35.1 ± 1.03	40.8 ± 1.36	34.2 ± 2.13

\*Significant at (  $P \leq 0.05$  )      \*\* Significant at (  $P \leq 0.01$  )

Results are represented as mean ± standard error

AST: Aspartate transaminase, ALT: Alanine treansaminase, ALP\_: Alkaline phosphatase

**Table( 2):** Serum total protein, albumin, globulin concentration and A/G ratio in of male rats after administration of Cypermethrin (12.5 ,25 mg/kg bw) twice weekly for 65 day in male rats ( n=10)

Treatment	Total protein (g/ dL)	Albumin (A) (g /dL)	Globulin (G) (g/ dL)	A/G ratio
Cypermethrin 12.5mg/kg.bw	6.17 ± 1.2**	3.01 ± 0.58*	3.16 ± 0.41	0.952 ± 0.07
Cypermethrin 25 mg/kg.bw	5.55 ± 1.03**	2.984 ± 0.87**	3.60 ± 0.68	0.82 ± 0.09
Control	7.46 ± 0.98	3.86 ± 0.74	4.06 ± 0.35	0.95 ± 0.22

\*Significant at (  $P \leq 0.05$  )      \*\* Significant at (  $P \leq 0.01$  )

Results are represented as mean ± standard error

**Table ( 3):** Total cholesterol and triglyceride levels in the sera of male rats after administration of Cypermethrin (12.5 ,25 mg/kg bw) twice weekly for 65 day in male rats. ( n=10)

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Cypermethrin 12.5mg/kg.bw	159 ± 8.4*	140 ± 6.3*
Cypermethrin 25 mg/kg.bw	229 ± 7.6**	200 ± 6.1**
Control	96 ± 5.9	75 ± 3.8

\*Significant difference at (  $P \leq 0.05$  ) \*\*Significant at (  $P \leq 0.01$  )

Results are represented as mean ± standard error

**Table ( 4):** shows the effect of oral administration of Cypermethrin (12.5 ,25 mg/kgbw) twice weekly for 65 day in male rats. ( n=10)

Treatment	Cypermethrin 12.5mg/kg bw	Cypermethrin 25mg/kg bw	Control
<b>0 day</b>	190 ± 5.64	189.00 ± 4.89	191 ± 4.15
<b>1 week</b>	195.2± 4.2	195.8± 3.78	196 ± 5.25
<b>2 week</b>	194.98 ± 3.54	193.64 ± 4.22	200.0± 3.87
<b>3 week</b>	205.47 ± 4.77	202.12 ± 5.35	208 ± 6.41
<b>4 week</b>	216.3± 5.47	214.86 ± 5.22	219 .2± 3.76
<b>5 week</b>	224.5 ± 3.89	220.3 ± 4.21	230.45± 5.89
<b>6 week</b>	232 ± 5.24	226.3 ± 4.35*	239.24 ± 6.41
<b>7 week</b>	240.1 ± 5.8*	235. 42± 4.05*	250.01 ± 5.94
<b>8 week</b>	242.54 ± 4.8*	239.5 ± 5.43*	261.5± 6.23
<b>9 week</b>	247.44 ± 4.23*	241.65 ± 6.67*	269.43 ± 4.23

\*Significant difference at (  $P \leq 0.05$  )

Results are represented as mean ± standard error

**Table ( 5):** shows the effect of oral administration of Cypermethrin (12.5 ,25 mg/kg bw) twice weekly for 65 day on weight of sexual organs and epididymal sperm characters in male rats. ( n=10)

parameter	Cypermethrin 12.5mg/kg.bw	Cypermethrin 25mg/kg.bw	Control
testes weight (g)	4.21 ± 0.23*	3.88±0.07*	4.95 ± 0.054
weight of epididymis (g)	0.42 ± 0.32*	0.361 ±0.063*	0.54 ± 0.065
Sperm concentration (10 <sup>6</sup> mm <sup>3</sup> )	17.89 ± 0.75*	16.8 ± 0.82*	22.25± 1.04
Sperm motility%	56.0± 2.89*	43.0 ± 4.12**	76.0± 3.25
Life sperm%	61.0 ± 3.22*	48.0± 2.74**	82.0 ± 2.54
Sperm abnormality%	15.01 ± 0.28*	20.43 ±0.54**	9.11 ± 0.23

\*Significant difference at (  $P \leq 0.05$  ) \*\*Significant at (  $P \leq 0.01$  )  
 Results are represented as mean ± standard error

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