

## **DOSE-RESPONSIVE SYSTEMIC TOXICITY OF CYPERMETHRIN IN WISTAR RATS**

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### **ABSTRACT**

Pesticides are used frequently and have various adverse effects on human health in different ways. Cypermethrin (CYP), a synthetic pyrethroid, is used extensively to control a wide variety of pests in agriculture, forestry, horticulture, and public health. This study designed to investigate the dose-dependent gonadal, immune and systemic toxicity of CYP in mature male and female Wistar rats. Rats were randomly divided into nine groups, different doses (1/11<sup>th</sup>, 1/10<sup>th</sup>, 1/9<sup>th</sup>, 1/7<sup>th</sup>, 1/6<sup>th</sup>, 1/4.5<sup>th</sup>, 1/4<sup>th</sup>, 1/3<sup>rd</sup> incase of male rat; 1/11<sup>th</sup>, 1/10<sup>th</sup>, 1/9<sup>th</sup>, 1/7<sup>th</sup>, 1/6<sup>th</sup>, 1/5<sup>th</sup>, 1/4<sup>th</sup> incase of female rat) of CYP were administered for 14 consecutive days and different gonadal, immune and systemic parameters were assessed. Sperm viability, testicular acid phosphatase, WBC count, lymphocyte count, cerebellar and

cerebellum reduced glutathione (GSH) content were significantly diminished. Serum urea, creatinine, cerebellar and cerebellum malondialdehyde (MDA) content were increased following CYP treatment in male rats at 40 and 80 mg/kg body weight (1/9<sup>th</sup> and 1/4.5<sup>th</sup> LD<sub>50</sub>). Decreased SOD and GST levels were also observed in CYP-exposed female rats at a dose level of 34.33 and 51.5 mg/kg body wt. (1/9<sup>th</sup> and 1/6<sup>th</sup> LD<sub>50</sub>). In conclusion, cypermethrin induced gonadal, immune and systemic toxicity in mature male rats with a body weight of 40 mg/kg (1/9<sup>th</sup>LD<sub>50</sub>) and gonadal toxicity in mature female rats with a body weight of 34.33 mg/kg (1/9<sup>th</sup>LD<sub>50</sub>) and above.

**KEYWORDS:** Cypermethrin; Gonadal; Immune; systemic; Testicular ACP; Cerebellar MDA.

## 1. INTRODUCTION

Pesticides are the chemical formulation increasingly used in agriculture, animal husbandry and public health operation to kill the insects, weeds and fungus and to get rid of insect transmitted diseases. These pesticides are toxic not only to insects and pests but at different levels to animals and human beings (Aktar *et al.*, 2009). Improper use of these agrochemicals may pose serious hazards to human and animal health. The use of pyrethroids has been increasing during the past decade with the declining use of organophosphates, which are more acutely toxic to birds and mammals than pyrethroids (Shafer and Meyer, 2004).

However, although pyrethroids are less acutely toxic but some studies have demonstrated that synthetic pyrethroids possess hormone-mimicking action and have been classified as endocrine-disrupting compounds (EDCs), which potentially pose a threat to human and wildlife. Synthetic pyrethroids have been considered potentially toxic to male reproductive system (Kilian *et al.*, 2007).

Cypermethrin is a synthetic pyrethroid commonly used in agriculture, veterinary, and household-insect management (Solati *et al.*, 2010). Cypermethrin behaves as a fast-acting neurotoxin in insects. It causes neurotoxicity insects by causing a long-lasting prolongation of the normally transient increase in sodium permeability of nerve membrane channels during excitation. These long-lasting trains can cause hundreds to thousands of repetitive nerve impulses in the sense organs. This repetitive activity is induced by pyrethroid damage to the voltage-dependent sodium channel, causing sodium channels to stay open much longer than normal (Vijverberg and van den Bercken, 1990). Although considered to be safe for household applications, some studies reported the adverse effects of cypermethrin on nervous system of laboratory animals (Sayim *et al.*, 2005).

Kidney plays an essential role in health, disease and overall development and growth. The main function of kidney is to maintain total body fluid volume, its composition and acid base balance. A number of environmental variables including certain xenobiotics (e.g. pesticides) influence these functions (Rasoul *et al.*, 2012). In fact, oxygen free radicals are involved in toxicity of numerous chemicals including pesticides and in pathogenesis of many diseases (Kalender *et al.*, 2012; Mossa *et al.*, 2012).

The exposure concentration is important for any type of pesticide, in analyzing the variation of its toxicity in any system. So, in this present study, Wistar rats were exposed to different concentrations of CYP to study the impact of CYP on gonadal, immune and systemic parameters in rats.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and reagents

A commercial formulation of cypermethrin 10% emulsifiable concentrate (EC), named “Ustad” (United Phosphorus Limited) was used in the experiments. Zinc sulphate ( $\text{ZnSO}_4$ ), WBC dilution fluid, chloroform, ethylene-diamine-tetra-acetic acid, phosphate buffer (0.1 M, pH 7.4), histopacque-1077, and all other chemicals used in this study were of analytical grade and obtained from Merck Ltd., Himedia, Mumbai, India.

### 2.2. Animal maintenance

Healthy Wistar albino male rats (weighing 130-150 g) were selected for this experiment. Standard laboratory feed and water were provided throughout the period of experimentation. Experimental protocol and surgical methods were approved by the Institutional Animal Ethical Committee, registered under CPCSEA.

### 2.3. Treatment protocol

After 10 days of acclimatization, the animals were randomly assigned to both the experimental groups and the control group, each containing six rats. Mature male, female rats were divided into nine groups where each group contains six animals. Group I was considered as control and Group II to IX were cypermethrin treated groups. According to FAO specifications (FAO specifications and evaluations for agricultural pesticides, 2006) oral LD<sub>50</sub> dose of cypermethrin formale and female rats were 360 and 309 mg/kg body weight respectively (Dee An Jones, 1992). Commercial formulation of cypermethrin 10% emulsifiable concentrate (EC) was administered at  $1/11^{\text{th}}$ ,  $1/10^{\text{th}}$ ,  $1/9^{\text{th}}$ ,  $1/7^{\text{th}}$ ,  $1/5^{\text{th}}$ ,  $1/4.5^{\text{th}}$ ,  $1/4^{\text{th}}$  and  $1/3^{\text{rd}}$  of LD<sub>50</sub> concentration respectively. After treatments for 14 consecutive days, all animals were anesthetized with pentobarbital sodium and sacrificed by cervical dislocation on 15th day. Blood samples were drawn from animals from all the treatment groups and allowed to fall drop by drop into a graduated centrifuge tubes containing anticoagulant ethylene-diamine-tetra-acetic acid (EDTA) for the estimation of haematological parameters. Tissue samples were collected and stored at -80°C until analysis. Epididymis were collected and washed immediately for sperm collection.

#### 2.4. Sperm viability assay

The eosin-nigrosin staining was measured to determine the sperm viability (WHO, 1999). One drop of sperm suspensions was added to two drops of 1% eosin Y. Then, after 30 s, three drops of 10% nigrosin were added and mixed well. A drop of mixture was placed on a clean glass slide, then air dried and was examined under the light microscope.

#### 2.5. Assay of testicular acid phosphatase

The acid phosphatase activity was measured using *p*-nitrophenol phosphate (PNPP) as a substrate (Vanha-Perttula and Nikkanen, 1973). Amount of liberated PNP was spectrophotometrically measured at 420 nm.

#### 2.6. Determination of ovarian superoxide dismutase (SOD)

The ovarian superoxide dismutase (SOD) was determined from its ability to inhibit the auto-oxidation of pyrogallol according to Marklund and Marklund (1974).

#### 2.7. Estimation of ovarian glutathione-S-transferase (GST)

Ovarian glutathione-S-transferase (GST) activity was measured spectrophotometrically by the method of Habig *et al.* (1974) using 1-chloro-2, 4-dinitrobenzene and detected at 340 nm. The activity of GST in testis was expressed in terms of  $\mu\text{mol}/\text{min}/\text{mg}$  protein.

#### 2.8. Total leukocyte count (TLC)

Total leukocyte count (Wintrobe, 1967) was determined by diluting blood in 1:20 dilution with white blood corpuscle (WBC) dilution fluid and then total leukocytes were counted in Neubaur haemocytometer chamber.

#### 2.9. Differential leukocyte count (DLC)

Thin blood smear was made by anticoagulant-added whole blood in a clean glass slide and was stained with Leishman's stain and then was observed under oil immersion objective of the microscope. The percentage of granulocytes and agranulocytes were calculated (Wintrobe, 1967).

#### 2.10. Estimation of malondialdehyde (MDA)

Malondialdehyde was assessed by the mixing of 1 ml of sample with 0.2ml of 8.1% sodium dodecyl sulfate, 1.5ml of acetate buffer (20%, pH-3.5) and 1.5 ml of aqueous solution of thiobarbituric acid (0.8%) and the mixtures were boiled for 60 min at 95°C. After heating when the red pigment was produced, that was extracted with 5 ml of n-butanol-pyridine

(15:1) and centrifuged at 5000 rpm for 10 min at room temperature. The optical density of supernatants was measured at 535nm (Ohkawa *et al.*, 1979).

### 2.11. Determination of reduced glutathione (GSH) content

At first 100µl of sulfosalicylic acid was mixed with 200µl of sample and the mixture was centrifuged at 3000 rpm for 10 min. With the supernatant, 1.8 ml of DTNB was mixed (Griffith, 1981) and the final reading of the supernatant was noted at 412 nm.

### 2.12. Estimation of urea

Urea was determined by the modified method of Natelson (Natelson *et al.*, 1951). To 0.1 ml of serum, 3.3 ml of water, 0.3 ml of 10% sodium tungstate and 0.67 N sulphuric acid were added. The suspensions were centrifuged at 2000 rpm and 1.0 ml of water, 0.4 ml of diacetylmonoxime and 2.6 ml of 0.67 N sulphuric acid-phosphoric acid reagents were added to the supernatant. Standard were prepared in a similar way and all the tubes were heated in a boiling water bath for 30 min and cooled. The developed color was measured at 480 nm in spectrophotometer (UV-Shimadzu-245, Japan).

### 2.13. Estimation of creatinine

Creatinine was measured according to the modified method of Brod and Sirota (Brod and Sirota, 1948). At first protein free filtrate was prepared by mixing 1ml of serum with 8.0 ml of water, 0.5 ml of 2/3 N sulphuric acid and 0.5 ml of 40% sodium tungstate. After that, 5.0 ml of filtrate was taken and 1.5 ml of saturated picric acid and 1.5 ml of 0.75 N sodium hydroxide were added to it. Standard and blank were also prepared similarly. The color intensity was measured at 530 nm in spectrophotometer.

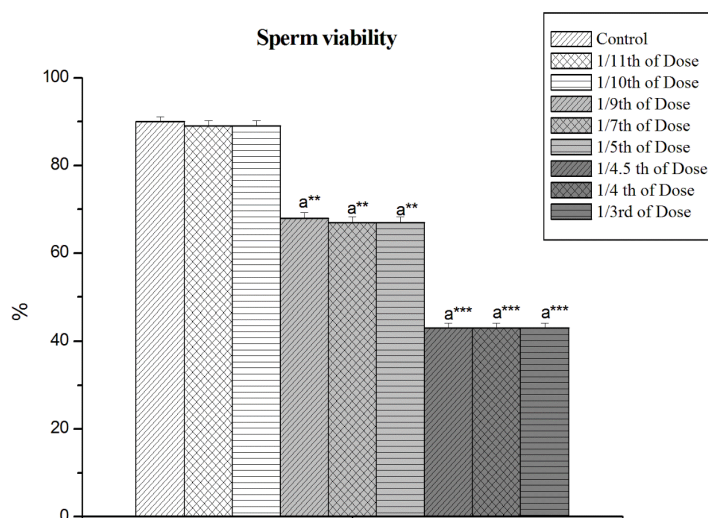
### Statistical analysis

The data was expressed as Mean±SEM, The differences between the means of each group were tested using a one-way ANOVA test (using a statistical package, Origin 6.1, Northampton, MA). P<0.05 was considered to indicate a statistically significant difference.

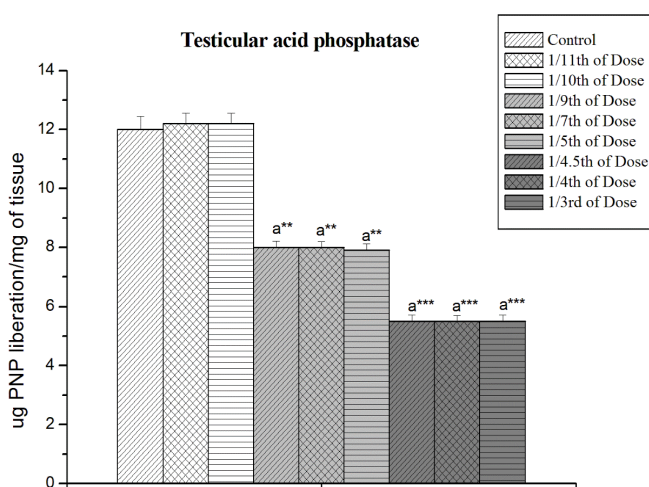
## 3. RESULTS

### 3.1. Effect of cypermethrin on sperm viability and testicular acid phosphatase

As shown in figure 1 the cypermethrin produced reproductive toxicity as reflected by the reduced sperm viability ( $p<0.001$ ). Acid phosphatase activities were lowered in the testis after cypermethrin treatment, in comparison to the controls (Figure 2).



**Figure 1: Effect of cypermethrin on sperm viability.** Results are expressed as Mean  $\pm$  SEM. Analysis is done by one way ANOVA. Superscript a Control group versus all other groups (\*\* indicates  $p < 0.01$ ; \*\*\* indicates  $p < 0.001$ ).

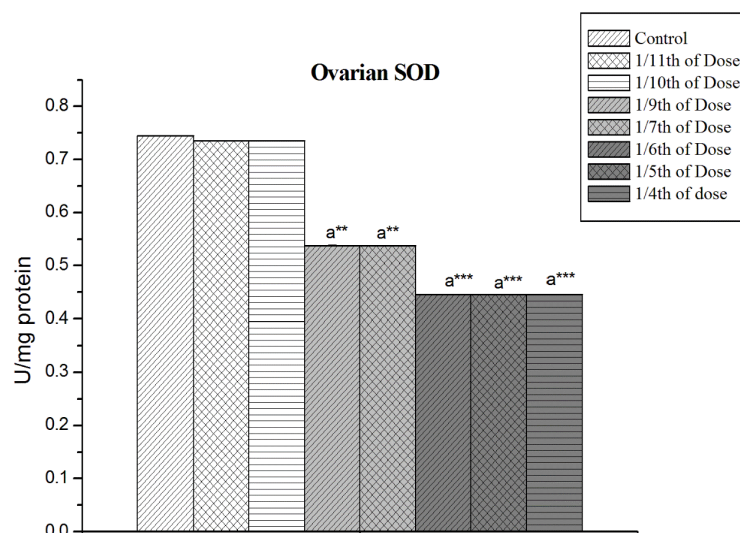


**Figure 2: Effect of cypermethrin on testicular acid phosphatase.** Results are expressed as Mean  $\pm$  SEM. Analysis is done by one way ANOVA. Superscript a Control group versus all other groups (\*\* indicates  $p < 0.01$ ; \*\*\* indicates  $p < 0.001$ ).

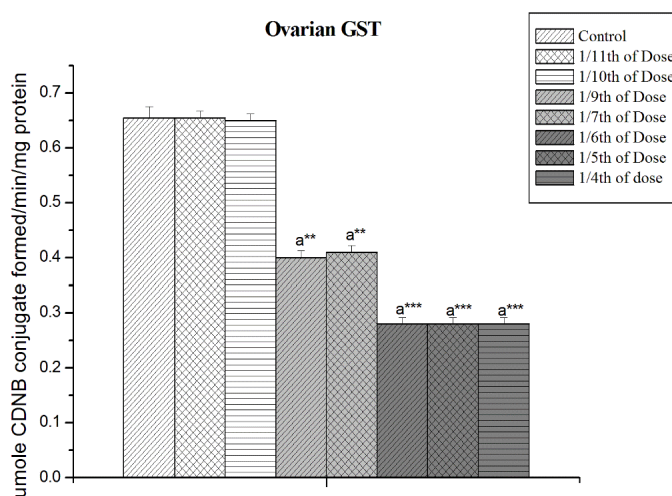
### 3.2. Effect on ovarian SOD and GST

Significant decrease in ovarian SOD and GST activity were observed at  $1/9^{\text{th}}$ LD<sub>50</sub> to  $1/6^{\text{th}}$ LD<sub>50</sub> dose level which indicates cypermethrin induced ovarian toxicity (Figure 3,4).





**Figure 3: Effect of cypermethrin on ovarian SOD.** Results are expressed as Mean  $\pm$  SEM. Analysis is done by one way ANOVA. Superscript a Control group versus all other groups (\*\* indicates  $p < 0.01$ ; \*\*\* indicates  $p < 0.001$ ).



**Figure 4: Effect of cypermethrin on ovarian GST.** Results are expressed as Mean  $\pm$  SEM. Analysis is done by one way ANOVA. Superscript a Control group versus all other groups (\*\* indicates  $p < 0.01$ ; \*\*\* indicates  $p < 0.001$ ).

### 3.3. Effect of cypermethrin on immunological parameters

From the study it was seen that total WBC count was decreased significantly ( $p < 0.05$ ) from the dose level of  $1/9^{\text{th}}$  LD<sub>50</sub> to  $1/4^{\text{th}}$  LD<sub>50</sub> dose level in male rat. It was seen that total leukocyte count was enhanced significantly ( $p < 0.01$ ) at the dose level of  $1/9^{\text{th}}$  LD<sub>50</sub> but decreased

significantly ( $p < 0.01$ ) from the dose level of  $1/4.5^{\text{th}}$  LD<sub>50</sub> to  $1/3^{\text{rd}}$  LD<sub>50</sub> in male rat. No significant changes were observed below  $1/9^{\text{th}}$  LD<sub>50</sub> dose. Maximum toxic effect of cypermethrin was seen in the  $1/4.5^{\text{th}}$  LD<sub>50</sub> dose without showing any mortality. From the  $1/4^{\text{th}}$  LD<sub>50</sub> dose, the rate of mortality was increased.

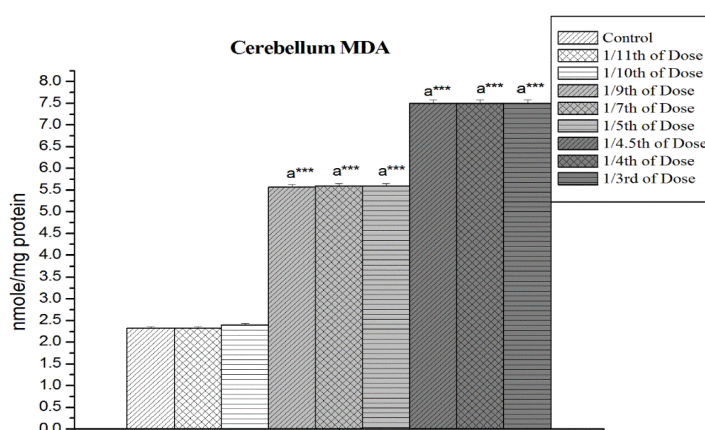
**Table 1: Effect of different doses of cypermethrin on WBC count, leukocyte count.**

	WBC Count / $\mu\text{l}$	Lymphocyte count (%)
Control	5591 $\pm$ 58	54.33 $\pm$ 0.557
1/11 <sup>th</sup> Dose	5591 $\pm$ 58	54.33 $\pm$ 0.57
1/10 <sup>th</sup> Dose	5591 $\pm$ 58	54.33 $\pm$ 0.881
1/9 <sup>th</sup> Dose	7275 $\pm$ 83 a***	63.66 $\pm$ 0.666 a***
1/7 <sup>th</sup> Dose	7275 $\pm$ 83a***	63.66 $\pm$ 0.666 a***
1/5 <sup>th</sup> Dose	7275 $\pm$ 83a***	63.66 $\pm$ 0.666 a***
1/4.5 <sup>th</sup> Dose	6866 $\pm$ 102 a***	64.833 $\pm$ 0.477 a***
1/4 <sup>th</sup> Dose	6866 $\pm$ 102 a***	64.833 $\pm$ 0.477 a***
1/3 <sup>th</sup> Dose	6866 $\pm$ 102 a***	64.833 $\pm$ 0.477 a***

Results are expressed as Mean $\pm$ SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript **a**, Control versus all other experimental groups; (\*\*\*) indicates  $p < 0.001$ ).

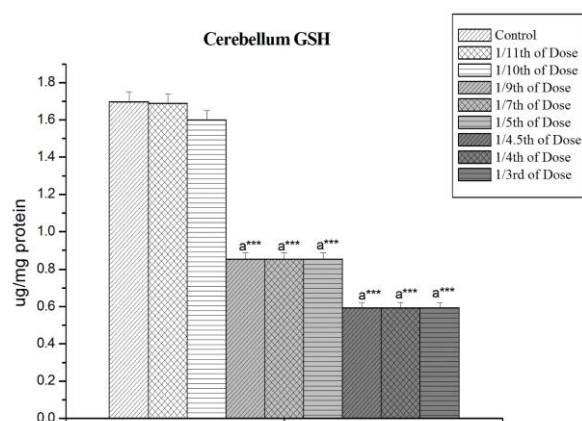
### 3.4. Effect of cypermethrin on cerebellar and cerebellum MDA and GSH content

A noticeable dose-dependent increase ( $p < 0.001$ ) in the MDA level and decline in GSH content were seen in cypermethrin induced rat from  $1/9^{\text{th}}$  LD<sub>50</sub> dose level onwards (Figure 5,6,7,8).

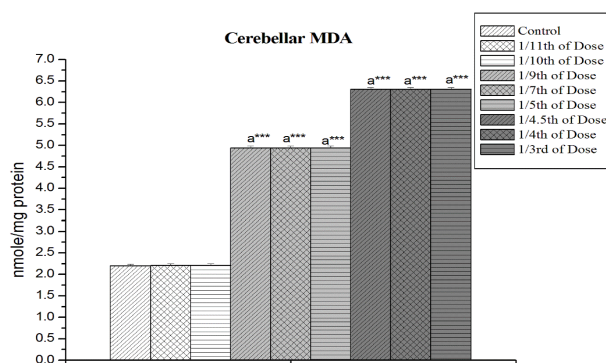


**Figure 5: Effect of cypermethrin on cerebellum MDA.** Results are expressed as Mean  $\pm$  SEM. Analysis is done by one way ANOVA. Superscript **a** Control group versus all other groups (\*\*\*) indicates  $p < 0.001$ ).

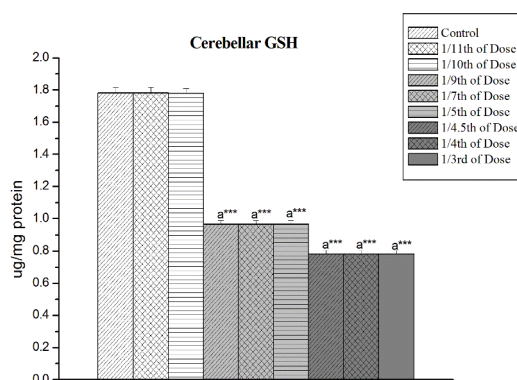




**Figure 6: Effect of cypermethrin on cerebellum GSH.** Results are expressed as Mean  $\pm$  SEM. Analysis is done by one way ANOVA. Superscript a Control group versus all other groups \*\*\* indicates  $p < 0.001$ ).



**Figure 7: Effect of cypermethrin on cerebellar MDA.** Results are expressed as Mean  $\pm$  SEM. Analysis is done by one way ANOVA. Superscript a Control group versus all other groups \*\*\* indicates  $p < 0.001$ ).



**Figure-8 Effect of cypermethrin on cerebellar GSH.** Results are expressed as Mean  $\pm$  SEM. Analysis is done by one way ANOVA. Superscript a Control group versus all other groups \*\*\* indicates  $p < 0.001$ ).

### 3.5. Effect of cypermethrin on serum urea and creatinine content

Significant decrease in serum urea and creatinine were observed at 1/9<sup>th</sup> LD<sub>50</sub> to 1/6<sup>th</sup> LD<sub>50</sub> dose level which indicate cypermethrin induced nephro-toxicity (Table-2).

**Table 2: Effect cypermethrin on serum creatinine and urea at different dose levels.**

	Creatinine(mg/dl)	Urea(mg/dl)
<b>Control</b>	2.8±0.002	25±0.15
<b>1/11<sup>th</sup> Dose</b>	2.82±0.003	26±0.21
<b>1/10<sup>th</sup> Dose</b>	2.82±0.003	26±0.21
<b>1/9<sup>th</sup> Dose</b>	8±0.001a***	55±0.85a***
<b>1/7<sup>th</sup> Dose</b>	8±0.001a***	55±0.85a***
<b>1/5<sup>th</sup> Dose</b>	8±0.001a***	55±0.85a***
<b>1/4.5<sup>th</sup> Dose</b>	10.5±0.001a***	65±0.92a***
<b>1/4<sup>th</sup> Dose</b>	10.5±0.001a***	65±0.92a***
<b>1/3<sup>rd</sup> Dose</b>	10.5±0.001a***	65±0.92a***

Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript **a**, Control versus all other experimental groups; (\*\*\*) indicates  $p < 0.001$ ).

## 4. DISCUSSION

The present study was conducted to explore the systemic toxic effect of cypermethrin in male and female Wistar rats.

In this study, reduced sperm viability was observed and this may be due to reduced spermatozoal mitochondrial enzyme activity, altered fructose synthesis and secretion by the accessory glands.

Acid phosphatases are enzymes capable of hydrolyzing orthophosphoric acid esters in an acid medium. The testicular acid phosphatase gene is up-regulated by androgens and is down-regulated by estrogens (Yousef *et al.*, 2001). Activities of phosphatase enzymes have been shown to rise when testicular steroidogenesis is increased (Mathur and Chattopandhyay, 1982). From our result it is confirmed that after cypermethrin exposure testicular steroidogenesis was decreased as testicular acid phosphatase was reduced.

Maintaining the balance between ROS and antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione-S-transferase (GST), is, therefore, crucial and could be an important mechanism for preventing damage by oxidative stress. Superoxide dismutase

(SOD) is involved in the clearance of superoxide. In the present investigation, decreased SOD level was observed in cypermethrin induced male Wistar rats.

The increased WBC counts were noted in CYP-treated groups. These findings were also supported by the findings of Yousef *et al.* in sheep (Yousef *et al.*, 1998) and in rabbits (Yousef *et al.*, 1999). This increase in leukocyte count may indicate an activation of the animal's defence mechanism and immune system of the body (Nasuti *et al.*, 2007). This may also result in an increase in the release of WBC from the bone marrow storage pool into the blood. The primary function of WBCs is to defend the body from foreign bodies, which are generated by leukocytosis and antibody production. Pathological leukocytosis may occur due to exposure to chemicals or acute hemorrhages and hemolysis. Leukocytosis may result due to a resistance developed by the animal towards the localization of the inflammatory response. Another possible cause of leukocytosis may be severe hemorrhages in the liver and lungs (Latimer *et al.*, 2004). This increase may be related to an increase in the lymphocyte percentage.

In the present study, serum urea levels exhibited a significant increase in cypermethrin-treated rats according to dose-dependent manner. Also, our results revealed that the concentration of creatinine was significantly elevated in cypermethrin-treated group compared to the control value. The creatinine excretion is dependent almost on the process of glomerular filtration, though tubular secretion contributes slightly (Kassirer, 1971). The slight and significant rise in the serum creatinine level of male rats may be due to the impairment of the glomerular function and tubular damage of the kidneys. The increased levels of these end products in blood especially serum creatinine and serum urea indicate poor clearance of these substances by the kidneys, rather than excessive production.

Oxidative stress defines an imbalance between the production of reactive oxygen species (ROS) and antioxidative defense mechanisms. During pyrethroid metabolism, ROS are generated and cause oxidative stress in intoxicated animals. In oxidative stress, lipid peroxidation occurs due to excessive free radical production and is considered a primary mechanism of cell membrane destruction and cell damage. MDA is the end product of lipid peroxidation. Increased MDA levels in brain suggested that CYP caused testicular damage (Gupta *et al.*, 1999). Reduction in GSH levels in brain following CYP treatment is indicative of oxidative stress, whereas GSH was utilized for the detoxification of ROS. As one of the most essential biological molecules, GSH played a key role in the detoxification of the ROS.

Normal cellular function was executed based on a balance between ROS production and antioxidant defense mechanisms existing in the cell (Ghosh and Maiti Choudhury, 2015).

## 5. CONCLUSION

The present study confirmed that cypermethrin exposure was responsible for gonadal, immune and systemic toxicity at 40 mg/ kg body weight ( $1/9^{\text{th}}$  LD<sub>50</sub>), and 80 mg/kg body weight ( $1/4.5^{\text{th}}$  LD<sub>50</sub>) in male rats, and at 34.33 mg/kg body weight ( $1/9^{\text{th}}$  LD<sub>50</sub>) and 51.5 mg/kg body weight ( $1/6^{\text{th}}$  LD<sub>50</sub>) in female rats.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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## REFERENCES

1. Aktar, M.W., Sengupta, D., Chowdhury, A. Impact of pesticides use in agriculture: their benefits and hazards. *2009*; 2(1): 1-12.
2. Shafer, T.J., Meyer, D.A. Effects of pyrethroids on voltage-sensitive calcium channels: a critical evaluation of strengths, weaknesses, data needs, and relationship to assessment of cumulative neurotoxicity. *Toxicol Appl Pharmacol*, 2004; 196: 303–318.
3. Kilian Delpont, E.R., Bornman, M.S., De Jager, C. Simultaneous exposure to low concentrations of dichlorodiphenyltrichloroethane, deltamethrin, nonylphenol and phytoestrogens has negative effects on the reproductive parameters in male Sprague Dawley rats. *Andrologia*, 2007; 39: 128-35.
4. Solati, J., Hajikhani, R., Zaeim, R.T. Effects of Cypermethrin on Sexual Behaviour and Plasma Concentrations of Pituitary-Gonadal Hormones. *International Journal of Fertility and Sterility*, 2010; 4(1): 23-28.
5. Vijverberg, H.P., van den Bercken, J. Neurotoxicological effects and the mode of action of pyrethroid insecticides. *Crit Rev Toxicol*, 1990; 21: 105-126.
6. Sayim, F., Yavasoglu, N.U., Uyanikgil, Y., Aktug, H., Yavasoglu, A., Turgut, M. Neurotoxic effect of cypermethrin in Wistar rats: A haematological, biochemical and histopathological study. *J Health Sci*, 2005; 51: 300-7.

7. Rasoul, M.A., Marei, G.I., Abdelgalei, S.A. Evaluation of antibacterial properties and biochemical effects of monoterpenes on plant pathogenic bacteria. *Afr J Microbiol Res*, 2012; 6(15): 3667-3672.
8. Kalender, Y., Kaya, S., Durak, D., Demi, F. Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation, and testis-histoarchitecture induced by chloropyrifos in male rats. *Environ Toxicol Pharmacol*, 2012; 33: 141-148.
9. Mossa, A.T., Heikal, T.M., Omara, E.A. Physiological and histopathological changes in the liver of male rats exposed to paracetamol and diazinon. *Asian Pac J Trop Biomed*, 2012; 2: S1683-S1690.
10. Dee An Jones. Environmental fate of cypermethrin, Environmental Monitoring & Pest Management, Department of Pesticide Regulation, Sacramento, Data from EPA's Pesticide Fact Sheet Database. 1992; CA 95814-3510.
11. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Fourth edition. New York. Cambridge University Press; 1999.
12. Vanha-Perttula, T., Nikkanen, V. Acid phosphatases of the rat testis in experimental conditions. *Acta Endocrinology*, 1973; 72: 376-390.
13. Marklund, S., Marklund, G. Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 1974; 47: 469-474.
14. Habig, W.H., Pabst, M.J., Jakoby, W.B. Glutathione-S-Transferase: the first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry*, 1974; 249: 7130-7139.
15. Wintrobe, M.M. Clinical hematology. Lea and Febiger, Philadelphia, Library of Congress, Print USA; 1967.
16. Ohkawa, H., Onishi, N., Yagi, K. Assay for lipid per-oxidation in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry*, 1979; 95: 351-358.
17. Griffith OW. Glutathione turnover in human erythrocytes. *The Journal of Biochemistry*, 1981; 256: 4900-4904.
18. Natelson, S., Scott, M.L., Beffa, C. A rapid method for the estimation of urea in biological fluids. *American Journal of Clinical Pathology*, 1951; 21: 275-81.
19. Brod, J., Sirota, J.H. The renal clearance of endogenous "creatinine" in man. *Journal of Clinical Investigation*, 1948; 27: 645-654.

20. Yousef, G.M., Diamandis, M., Jung, K., Diamandis, E.P. Molecular cloning of a novel human acid phosphatase gene (ACPT) that is highly expressed in the testis. *Genomics*, 2001; 74: 385-395.
21. Mathur, P.P., Chattopadhyay, S. Involvement of lysosomal enzymes in flutamide-induced stimulation of rat testis. *Andrologia*, 1982; 14: 171-176.
22. Yousef, M.I., Ibrahim, H.Z., Yacuot, M.H.M., Hassan, A.A. Effects of cypermethrin and dimethoate on some physiological and biochemical parameters in Barki sheep. *Egyptian J. Nutr. Feeds*, 1998; 1: 41-52.
23. Yousef, M.I., Abbassy M.S., Yacout, MH.M. Assessment of cypermethrin and dimethoate toxicity in Barki sheep: Biochemical and histological changes and tissue residues. *Egyptian J. Anim. Prod*, 1999; 36: 25-41.
24. Nasuti, C., Rosita, G., Maria, L.F., Antonio, D.S., Franco, C. Dopaminergic system modulation, behavioral changes and oxidative stress after neonatal administration of pyrethroids. *Toxicol*, 2007; 229: 194-205.
25. Latimer, K.S., Mahaffey, E.A., Prasse, K.W. Clinical Pathology: Veterinary Laboratory Medicine. 4<sup>th</sup> ed. Iowa State University Press, Ames, Iowa, USA. 2004.
26. Kassirer, J.P. Clinical evaluation of kidney function—glomerular function. *N Engl J Med*, 1971; 285(7): 385-389.
27. Gupta, A., Agarwal, A.K., Shukla, G.S. Functional impairment of blood-brain barrier following pesticide exposure during early development in rats. *Human Exp Toxicol*, 1999; 18: 174-9.
28. Ghosh, R., Maiti Choudhury, S. Alteration in estrous cycle, lipid peroxidation and antioxidant status in female rat after exposure to lambda cyhalothrin and its attenuation by taurine. *International Journal of Bioassays*, 2015; 4: 4526-4532.