

# Systematic Toxicity of Cypermethrin and Alterations in Behavior of Albino Rats

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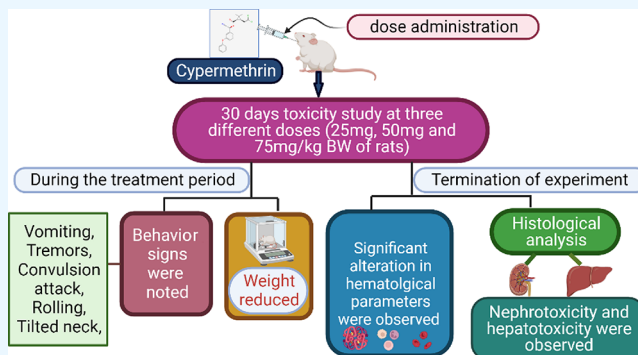
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**ABSTRACT:** The present study aimed to evaluate the effect of repeatedly orally administering cypermethrin (CYP) at different doses on the behavior, hematology, and histology of adult male and female albino rats for 1 month. For this, animals have divided into four different groups and each group contained 10 animals (5 males and 5 females). Group I served as a control group and groups II, III, and IV were represented as experimental groups and treated with CYP at doses of 25, 50, and 75 mg/kg body weight/day/rat, respectively. Through the behavioral results of this study, it was observed that cypermethrin intoxication causes dose-dependent moderate to high toxicity symptoms like vomiting, decreased food consumption, thick eye discharge, rolling, tremors, loss of coordination, tilted neck, and convulsion attacks. A significant reduction in body weight of high-dose (75 mg)-treated animals, especially in females, was noticed. Similarly, hematological data also revealed that CYP exposure caused a reduction in the level of Hb, RBC, WBC, neutrophil, and other blood indices such as PCV and MCV and an increase in the lymphocyte percentage in both male and female experimental groups. Microscopic observation stated that CYP produced infiltration of cells near the central vein, hepatocyte degeneration, congestion of the central and portal veins, hemorrhage, and necrosis in liver tissue. Shrinkage of the glomerulus, necrosis in the glomerulus and renal tubules, congestion of blood cells, and hemorrhage were seen in kidney tissue. The current study suggests that hepatotoxicity and nephrotoxicity due to cypermethrin exposure were more prominent in female rats.



## INTRODUCTION

Pesticides have been used worldwide for the last many decades to support the growth of the ever-increasing human population by increasing food production and also for their protection from vector-borne diseases.<sup>1,2</sup> The amount of insecticides used on the land is harmful to the environment as well as human and animal health. Due to this, public concern rises over health and the environment. Man is continuously advancing in the field of pesticides and is stuck in his own trap. Long-term pesticide exposure can be hazardous to human health and disrupt the normal function of various organs in the body.<sup>3</sup> Problems that are associated with the low residual level of pesticides are blood disorders like anemia, defects in blood coagulation, nervous system damage, paralysis, hepatic fibrosis, jaundice, allergic reactions, excretory problems, birth defects, infertility, cancer, and genetic disorders.<sup>4,5</sup> To find new insecticides that are less toxic to mammals and less insect-resistant, a large number of insecticides have been synthesized, and synthetic pyrethroids are the result of one of these attempts. Synthetic pyrethroids are a diverse class of more than a thousand powerful broad-spectrum organic insecticides used in domestic, veterinary, and agricultural applications. Cypermethrin belongs to the Type II class of synthetic pyrethroids

and it is used in agricultural and household applications. Despite its wide applications, cypermethrin is not free from toxic side effects. Cypermethrin's lipophilic nature allows it to easily cross the cellular membrane, causing changes in the internal structure of the cell membrane and cellular enzyme leakage.<sup>6,7</sup> The hydrophobic nature of pesticides also helps in their storage in the liver, kidney, body fat, skin, lungs, brain, adrenal glands, and reproductive organs of an organism.<sup>8</sup> They accumulate in biological membranes and stimulate reactive oxygen species in mammals, causing oxidative damage.<sup>9</sup> The tissue residue level of cypermethrin was found high in fat at a concentration of 4–5 mg/kg. Cypermethrin concentrations in the liver, kidneys, adrenals, gut, ovaries, and skin were ranging from 0.4–0.9 mg/kg. Small amounts of cypermethrin (0.04–0.07 mg/kg) were detected in the muscle, spleen, and bone.

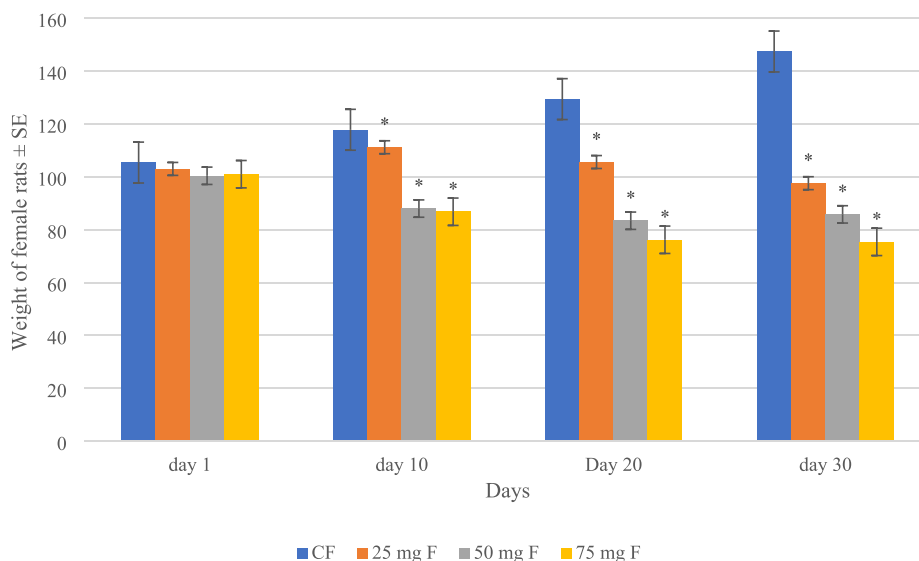
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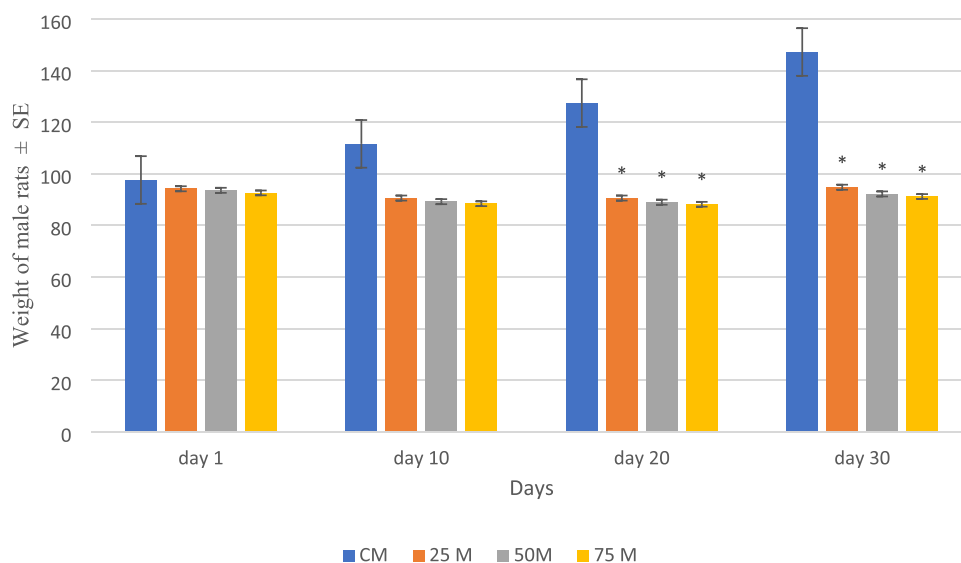


## Female's Weight



**Figure 1.** Mean weight of female rats  $\pm$  SE of control and CYP-treated experimental groups from day 1 to day 30. \*Values are statistically significant at  $p < 0.05$  from the control group determined by one-way ANOVA. Values are not significantly different ( $p > 0.05$ ).

## Male's Weight



**Figure 2.** Mean weight of male rats  $\pm$  SE of control and CYP-treated experimental groups from day 1 to day 30. \*Values are statistically significant at  $p < 0.05$  from the control group determined by one-way ANOVA. Values are not significantly different ( $p > 0.05$ ).

Less than 0.01 mg/kg of concentration was detected in the brain.<sup>10,11</sup> The residue level of  $\alpha$ -CP (14.5 mg/kg) following daily oral administration for 30 days was 0.07–1.02 ppm in the increasing order liver < brain < testis < heart < kidney < lungs.<sup>6</sup> The extent of accumulation of cypermethrin in tissue was: fat, 3.91 mg/kg; liver, 0.97 mg/kg; kidneys, 0.69 mg/kg; ovaries, 0.03 mg/kg; skin, 1.89 mg/kg; whole blood, 0.35 mg/kg; and plasma, 0.64 mg/kg.<sup>10–12</sup> However, the detected levels of pesticides were dose-dependent.<sup>13</sup> Both male and female rats excreted 50–65% of cypermethrin in their urine within 48 h and 30% of the cypermethrin in their feces within 3 days.<sup>11</sup> Several studies have found that CYP exposure in humans occurs primarily through their application or pyrethroid residue found in bread, vegetables, fruits, and cow's milk.

However, the literature has proven that cypermethrin and other pyrethroids have carcinogenic, hepatotoxic, immunosuppressive, and neurotoxic effects on mammals.<sup>14–17</sup> The toxicity of pyrethroid insecticides in mammals has received considerable attention in recent years, owing to the fact that exposure to these insecticides in animals altered their physiological activities, in addition to other pathological features.<sup>18</sup> Oxidative stress in cerebral and hepatic tissues was observed in rats after oral exposure of cypermethrin.<sup>19</sup> Synthetic pyrethroids may also disrupt hematological, reproductive, and biochemical parameters.<sup>20</sup>

Hematological parameters are the most rapid and easily detectable variables that help in evaluating any health condition and at the same time, their importance in population

**Table 1. Effect of Exposure of CYP on Hematological Parameters of Albino Female Rats<sup>a</sup>**

parameters	control (female)	25 mg/kg CYP (F)	50 mg/kg CYP (F)	75 mg/kg CYP (F)
hemoglobin (g/dL)	14.2 ± 0.16	13.86 ± 0.10	13.26 ± 0.16	12.12 ± 0.41*
total leucocyte count (/cumm)	7990 ± 136.38	6840 ± 297.66	4940 ± 163.1	4520 ± 330.76*
neutrophil (%)	33.4 ± 2.91	29.8 ± 4.18	28 ± 2.17	24.8 ± 3.28
lymphocyte (%)	62.4 ± 3.47	67 ± 4.43	71.6 ± 3.39	74.4 ± 3.94
eosinophil (%)	1.2 ± 0.37	1.4 ± 0.24	1.8 ± 0.37	1.4 ± 0.24
monocyte (%)	1	1	1	1
basophil (%)	0	0	0	0
RBC count (millions/cmm)	8.3 ± 0.18	7.47 ± 0.23	7.07 ± 0.2	6.41 ± 0.29*
P.C.V. (hematocrit) (%)	42.16 ± 1.24	39.6 ± 0.64	37.4 ± 1.08	35.44 ± 0.65*
M C V (fl)	54.46 ± 0.98	49.34 ± 0.9	45.02 ± 1.66	44.56 ± 2.5*
M C H (pg)	19.44 ± 0.63	18.24 ± 0.19	16.54 ± 0.24	15.68 ± 0.33*
M C H C (g/dL)	36.42 ± 0.75	35.24 ± 0.82	33.86 ± 0.82	33.52 ± 0.77
platelet count (lakh/cmm)	6.31 ± 0.48	4.23 ± 1.24	4.12 ± 0.87	4.44 ± 0.4

<sup>a</sup>All values are mean ± SEM. \*Values are significantly different at  $p < 0.05$  from the control group.

**Table 2. Effect of Exposure of CYP on Hematological Parameters of Albino Male Rats**

parameters	control (male)	25 mg/kg CYP (M)	50 mg/kg CYP (M)	75 mg/kg CYP (M)
hemoglobin (g/dL)	14.56 ± 0.22	14 ± 0.16	13.64 ± 0.19	12.74 ± 0.34*
total leucocyte count (/cumm)	8160 ± 709.65	6620 ± 428.25	6160 ± 396.99	4840 ± 508.53*
neutrophil (%)	36.4 ± 4.41	31 ± 4.3	28.6 ± 4.32	25.8 ± 4.79
lymphocyte (%)	62.2 ± 4.02	67.8 ± 1.66	71 ± 3.46	74.4 ± 5.68
eosinophil (%)	1.4 ± 0.24	1.2 ± 0.37	1 ± 0.32	1.2 ± 0.2
monocyte (%)	1	1	1	1
basophil (%)	0	0	0	0
RBC count (millions/cmm)	8.78 ± 0.34	8 ± 0.28	7.84 ± 0.21	7.36 ± 0.19*
P.C.V. (hematocrit) (%)	47.46 ± 0.72	40.02 ± 0.43	38.38 ± 0.43	36.28 ± 0.34*
M C V (fl)	55.84 ± 0.55	50.28 ± 1.19	47.94 ± 0.88	45.16 ± 0.96*
M C H (pg)	19.96 ± 0.3	17.58 ± 0.67	15.84 ± 0.19	14.68 ± 0.49*
M C H C (g/dL)	37.1 ± 0.58	35.06 ± 0.55	33.02 ± 0.58	32.06 ± 0.45*
platelet count (lakh/cmm)	4.84 ± 1	4.65 ± 0.95	3.83 ± 0.76	3.79 ± 0.23

All values are mean ± SEM. \*Values are significantly different at  $p < 0.05$  from control group.

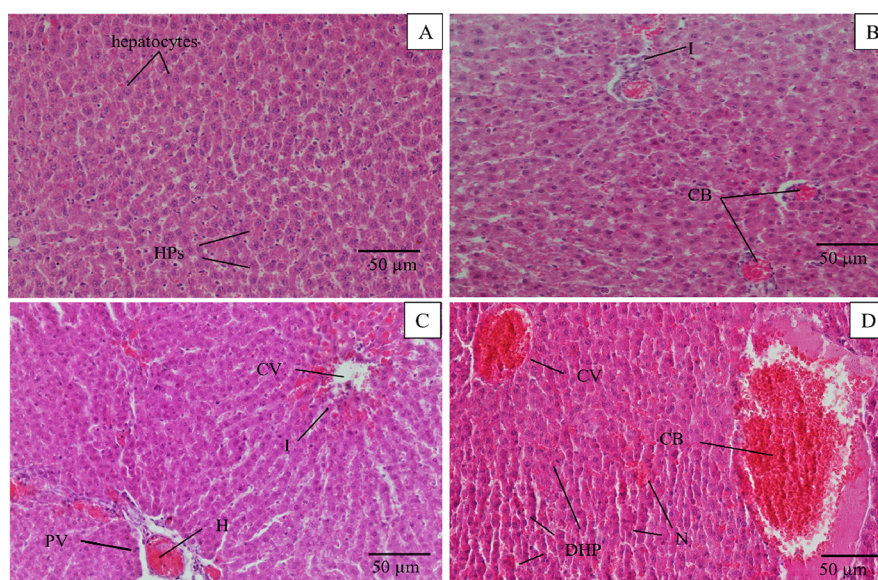
genetics and clinical biochemistry is well-known.<sup>21</sup> Most studies have shown that hematological parameters can be used to detect disease or stress in animals.<sup>22</sup> From the literature, it was found that many authors studied reproductive toxicity with selected cypermethrin doses, but data is much scarcer regarding hepatotoxicity, nephrotoxicity, hematological alteration, and behavioral toxicity with the selected dose of the current study. Furthermore, very few studies have compared the effects of cypermethrin on male and female albino rats. As a result, the current study was designed to assess the comparative systematic toxicity of cypermethrin and behavioral changes in albino rats.

## RESULTS

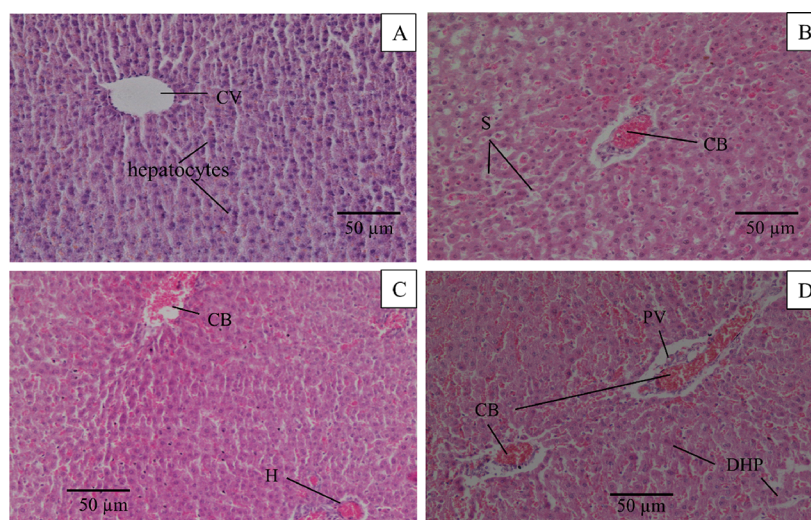
**Body Weight and Behavioral Changes.** Results showed that oral administration of cypermethrin (CYP) at doses of 25, 50, and 75 mg/kg of body weight of rats produced moderate-to-high levels of toxicity signs. In the 25 mg-treated group, animals showed vomiting, distress, and salivation. The 50 mg-treated group animals showed vomiting, less consumption of food, thick eye discharge, rolling, and tremors, but the 75 mg-treated group animals showed the highest level of toxicity symptoms such as aggression, convulsion attack, vomiting, irritation on their skin, distress, rolling, loss of coordination, tilted neck, and less consumption of food. At the beginning of the experiment, animals showed more symptoms of toxicity, but during the experiment or at the end of the experiment,

toxicity signs were minimized. Toxic symptoms were observed more in the female treated group than in the male treated group. Cypermethrin-treated groups showed a significant reduction in body weight compared to control groups. The body weight of the higher dose-treated group animal was significantly lower than that of the lower dose-treated group animal. The body weight of control group animals was increased during the experimental period. From this study, it was also concluded that cypermethrin-treated females (Figure 1) showed severe toxicity symptoms and a higher reduction in their body weight as compared to male groups (Figure 2). One female mortality was observed in the 75 mg CYP-treated group, showing signs of incoordination, a tilted neck, and tremors. According to the study, it was observed that the intensity of toxic levels of cypermethrin is dose- and time-dependent.

**Hematological Analysis.** Exposure of cypermethrin to male and female albino rats with different doses caused a significant alteration in hematological parameters. The hemoglobin level was significantly ( $p < 0.05$ ) decreased in both the treated male (Table 1) and female groups (Table 2) as compared to the control group. Total red blood cell count and RBC indices such as packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were reduced significantly ( $p < 0.05$ ) in both low- and high-dose-treated male and female groups. Further, all of



**Figure 3.** Micrograph of the liver section of female rats stained with H&E, 200×. (A) Control group showing a well-organized structure hepatocytes and hepatic plates; (B) 25 mg/kg CYP group, congestion of blood cells and distorted hepatic plates were seen; (C) 50 mg/kg CYP group demonstrating infiltration, hemorrhage, and distorted hepatic plates; (D) 75 mg/kg CYP group, congestion of blood cells, infiltration, hemorrhage, necrosis, and distorted hepatic plates were observed. CV, central vein; HPs, hepatic plates; I, infiltration; CB, congestion of blood vessels; H, hemorrhage; N, necrosis; PV, portal vein; DHP, distorted hepatic plates.

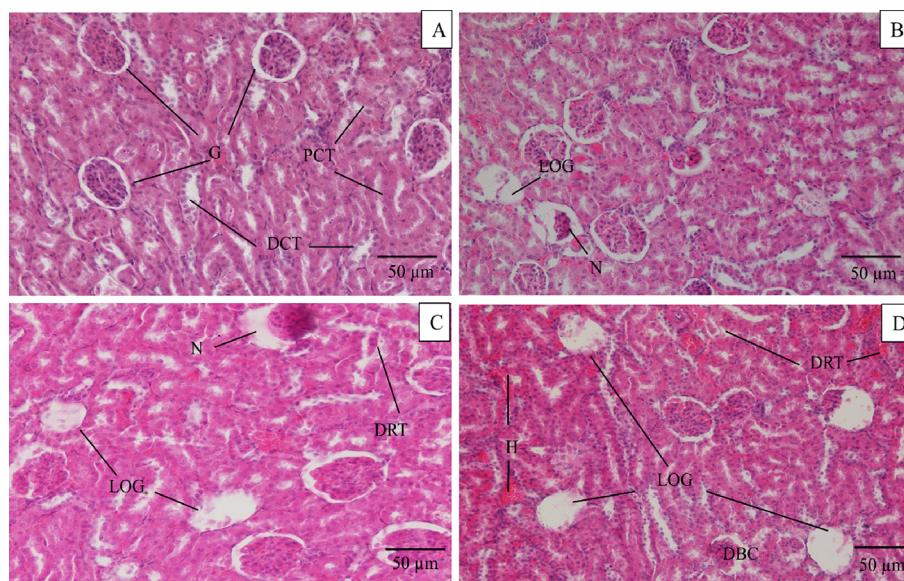


**Figure 4.** Micrograph of the liver section of male rats stained with H&E, 200×. (A) Control group showing a well-organized structure of central vein and hepatocytes; (B) 25 mg/kg CYP group, dilation of sinusoids, and congestion of blood cells were observed; (C) 50 mg/kg CYP group showing congestion of blood vessels with hemorrhage and distorted hepatic plates; (D) 75 mg/kg CYP group, congestion of blood cells, infiltration, congestion of blood vessels, and distorted hepatic plates were observed. CV, central vein; S, sinusoids; CB, congestion of blood vessels; H, hemorrhage; PV, portal vein; DHP, distorted hepatic plates.

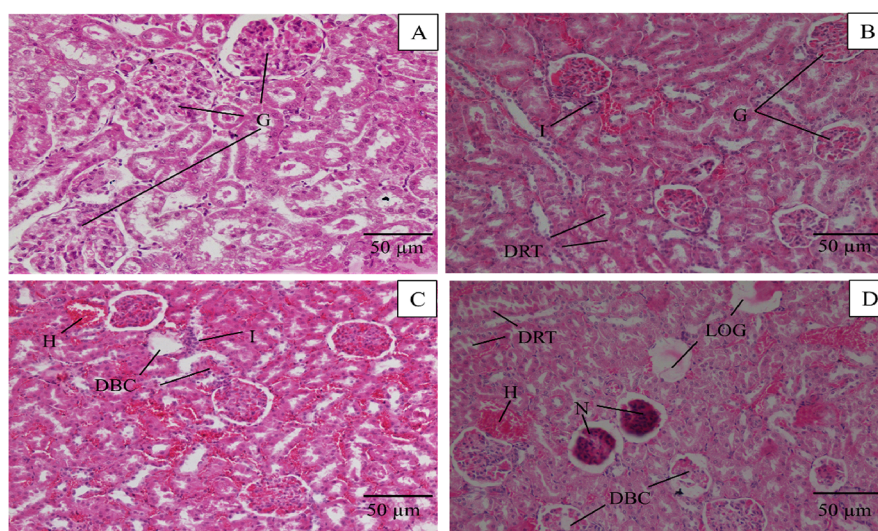
these parameters were reduced in a dose-dependent manner, with higher-dose animals being more affected than lower-dose animals. Both high- and low-dose treated male and female albino rats had significantly lower total leucocyte counts ( $p < 0.05$ ). The differential leucocyte count revealed a reduction in the neutrophil count but an increase in the count of lymphocytes compared to the control group. The count of other leucocytes such as eosinophils, basophils, and monocytes was comparable to the control groups. The platelet count was also decreased in treated groups as compared to control groups.

**Histological Analysis. Hepatotoxicity. In Female.** Histological results of the livers of control female albino rats showed

normal and compact architecture with a well-organized structure of sheets of hepatocytic cells and hepatocytes (Figure 3A). However, abnormalities were observed in the cypermethrin-treated groups. The 25 mg CYP-treated females showed infiltration of cells near the central vein, dilation of the sinusoids, congestion of blood vessels (Figure 3B), and degeneration of some hepatocytes. The portal vein in the 50 mg CYP-treated group was distorted, with infiltration of cells around the central vein, dilation of sinusoids, pyknotic nuclei, congestion, and hemorrhage (Figure 3C). Females given 75 mg of CYP experienced severe changes such as congestion and thickening of central and portal veins, pyknotic nuclei,



**Figure 5.** Micrograph of the kidney section of female rats stained with H&E, 200 $\times$ . (A) Control group showing a normal and well-organized structure of glomerulus, DCT, and PCT; (B) 25 mg/kg CYP group exhibiting necrosis and loss of glomerulus in kidney tissue; (C) 50 mg/kg CYP group, necrosis, loss of glomerulus, and distorted renal tubules were observed; (D) 75 mg/kg CYP group, necrosis, hemorrhage, damaged bowman's capsule, loss of glomerulus, and distorted renal tubules were observed in the CYP-treated group. G, glomerulus; PCT, proximal convoluted tubule; DCT, distal convoluted tubule; DRT, distorted renal tubules; H, hemorrhage; DBC, damaged bowman's capsule; N, necrosis; LOG, loss of glomerulus.



**Figure 6.** Micrograph of kidney section of male rats stained with H&E, 200 $\times$ . (A) Control group showing a normal and well-organized structure of glomerulus and renal tubules; (B) 25 mg/kg CYP group, infiltration and distorted renal tubules were seen; (C) 50 mg/kg CYP group showing hemorrhage and damaged bowman's capsule; (D) 75 mg/kg CYP group, necrosis, hemorrhage, loss of glomerulus, and distorted renal tubules were observed in the CYP-treated group. G, glomerulus; I, infiltration; DRT, distorted renal tubules; H, hemorrhage; DBC, damaged bowman's capsule; N, necrosis; LOG, loss of glomerulus.

distorted hepatic plates, hemorrhage, and necrosis (Figure 3D).

*In Male.* Microscopically analyzed liver tissue of control male albino rats showed a well-organized structure of the central vein, hepatic cords, and hepatocytes (Figure 4A). The 25 mg CYP-treated male animals showed dilation of the sinusoids (Figure 4B), vacuolization, and congestion of blood vessels. In 50 mg CYP-treated animals, distorted hepatic plates, and congestion of blood vessels with hemorrhage were observed (Figure 4C). In a higher dose (75 mg)-treated male group, congestion of blood vessels in the portal vein,

infiltration of cells, dilation of sinusoids, and degenerating hepatic plates were noticed (Figure 4D).

*Nephrotoxicity. In Female.* Control female albino rats showed normal renal architecture with the organized structure of the glomerulus, proximal convoluted tubules (PCT), distal convoluted tubules (DCT), and Bowman's capsules (Figure 5A). Mild to toxic histological changes were observed in low- to high-dose CYP-treated groups. Low-dose-treated female groups (25 mg) showed shrinkage of the glomerulus, infiltration of distorted renal tubules, and absence of the glomerulus at some places (Figure 5B). The 50 mg-treated

females showed more toxic signs than 25 mg dose-treated female animals. Necrosis and shrinkage of the glomerulus (Figure 5C), inflammation in renal tubules, damaged renal tubules, and absence of the glomerulus at many sites were noted in 50 mg dose-treated groups. The 75 mg dose-treated group showed necrosis in the glomerulus and renal tubules, congestion of blood cells, hemorrhage in the renal tubules, eroded Bowman's capsules, distorted tubules, shrinkage, and absence of the glomerulus (Figure 5D).

**In Males.** A histological study of the kidney tissue of male albino rats showed the well-organized structure of renal tubules and glomerulus (Figure 6A). The 25 mg-treated male animals showed infiltration of cells and deformed renal tubules (Figure 6B). In the 50 mg CYP-treated male group, congestion of blood vessels, infiltration of cells, damaged Bowman's capsules (Figure 6C), and distorted renal tubules were observed. A higher dose (75 mg) treated males showed necrosis, damaged Bowman's capsules, loss of glomerulus, congestion of blood vessels, and deformed renal tubules (Figure 6D).

From the histopathological analysis, it was observed that the harmful effect of cypermethrin was more prominent in treated females than in the male groups.

## DISCUSSION

Results of the present study revealed that exposure to cypermethrin at different doses (25, 50, and 75 mg/kg body weight) causes an adverse effect on both male and female albino rats, but the level of toxicity was more pronounced in the females as compared to the males. The observed behavioral toxicity signs of the present study were found to be somewhat similar to the finding of Grewal et al.<sup>23</sup> where exposure to cypermethrin at a dose of 20 mg/kg/day causes moderate toxic signs such as loss of body weight, decreased food consumption, vomiting, excessive salivation, etc.

In the present hematological study, dose-dependent reductions in the levels of Hb, RBC, WBC, and other blood indices such as PCV, MCV, MCH, and MCHC were observed in all experimental groups. The lymphocyte count increased after cypermethrin treatment. The decreased levels of Hb and RBC in the current study showed similarities with the results of Hussien et al.<sup>7</sup> This decrease in blood parameter levels after cypermethrin treatment could be attributed to decreased bone marrow synthesis of red blood cells and hemoglobin.<sup>24</sup> Pesticide exposure adversely affects erythrocytes, which reduces erythropoiesis and then ultimately reduces the total erythrocyte count.<sup>25</sup> Moosa<sup>26</sup> also reported a reduction in the Hb and RBC count. In the current study, a significant reduction was observed in the hematocrit and MCV count because these values were directly correlated with the values of Hb and RBC. These results were also found to be consistent with the findings of Ambali et al.<sup>27</sup> and Sayim et al.<sup>41</sup> that exposure to cypermethrin and chlorpyrifos caused a significant reduction in Hb, RBC, MCH, and PCV count in treated rats. White blood cells act as the body's natural defender against foreign particles through antibody production and leucocytosis. In the current study, WBC and neutrophil count were also decreased because cypermethrin intoxication decreased the immunity of treated rats. Abbassy and Mossa<sup>28</sup> supported the current findings by exposing rats to formulated and technical-grade cypermethrin and deltamethrin for 90 days and observing a decrease in WBC count after treatment. Nair et al.<sup>29</sup> reported that cypermethrin-treated animals showed a decrease in total leucocyte count and neutrophil count and an

increase in lymphocyte count as compared to control animals. In the current study, histopathological analysis of liver tissue showed dilation of sinusoids and degeneration of hepatocytes, congestion and thickening of central and portal veins, distorted hepatic plates, hemorrhage, and necrosis. A change in the structure of liver tissue at a higher dose of cypermethrin was found to be more prominent in female rats than male rats. The current findings were also consistent with the findings of Grewal et al.,<sup>23</sup> who discovered that cypermethrin treatment resulted in a degenerative effect on the structure of the liver tissues via necrosis of hepatic cells, the presence of pyknotic nuclei, deformation of hepatic laminae, and sinusoidal dilation in the hepatic structure. Mamun et al.<sup>30</sup> suggested that cypermethrin intoxication at a lower dose (10 mg/kg) through the subcutaneous route also caused similar effects with congestion and dilation of blood vessels, congestion in a central vein, dilation of sinusoids, hemorrhage, and necrosis. Soliman et al.<sup>31</sup> and Mossa et al.<sup>32</sup> studies also showed similarities with current findings by concluding that CYP toxicity caused liver damage in rats. Yao et al.<sup>33</sup> also demonstrated that cypermethrin toxicity caused enlargement of sinusoid space, leucocyte infiltration, and inflammation in hepatic tissue. These findings also agreed with those reported by Ullah et al.<sup>34</sup> in pregnant rabbits and by Assayed et al.<sup>35</sup> in pregnant rats.

Cypermethrin exposure causes histopathological alteration in kidney tissue, including shrinkage of the glomerulus, distorted renal tubules, absence of glomerulus, necrosis, congestion of blood cells, and hemorrhage in the renal tubules. Grewal et al.<sup>23</sup> suggested that cypermethrin toxicity in albino rats caused glomerulus shrinkage, necrosis of renal tubules, hemorrhage, and sloughing off renal epithelial cells in kidney tissues. The present study also observed the same toxicity in CYP-treated groups. According to Shivanoor and David,<sup>36</sup> deltamethrin intoxication caused tubular dilatation of proximal tubules, inflammation, infiltration, and tubular cell desquamation necrosis. Raj et al.<sup>37</sup> suggested that medullary congestion in the kidney was observed when mice were orally treated with a combination of cypermethrin and endosulfan. The current findings are consistent with those of Lemos et al.,<sup>38</sup> who discovered that combining deltamethrin and *B. thuringiensis* subsp. *Aizawai* caused necrosis, degeneration of the convoluted tubules and collector ducts, proliferative glomerulonephritis, and a significant decrease in Bowman's spaces.

## CONCLUSIONS

This study concluded that repeated exposure to cypermethrin has an injurious side effect on the bodies of both male and female albino rats. The intensity and degree of damage caused by pesticides were time- and dose-dependent. The observed hematological, histological, and behavioral toxicity suggested that cypermethrin exposure disrupts the body's normal functioning, resulting in serious problems. This study also suggested that cypermethrin has a toxic effect on the liver and kidney, due to which biochemical, metabolic, and excretory processes were disrupted. These effects are more adverse in female albino rats than in male rats.

## MATERIALS AND METHODS

**Chemicals.** Cypermethrin (25% EC) was purchased from the pesticide store Rohtak and manufactured by Dhanuka Agritech Limited. To achieve the test concentration, adequate

dilutions were made with water. The test concentration of cypermethrin was calculated from the percentage of the active ingredient in the commercial formulation of cypermethrin. Doses for the current study were selected according to the previously published studies<sup>39,40</sup> with slight modifications. So, accordingly, 25 mg (1/10th of LD50) was a low dose, 50 mg (1/5th of LD50) was a medium dose, and 75 mg (approximately 1/3 of LD50) was the higher dose selected.

**Animals.** In this study, 40 male and female albino rats (3–4 months old) were purchased from the animal house of DFSAH (disease-free small animals house), LUVAS (Lala Lajpat Rai University of Veterinary and Animal Sciences), Hisar, Haryana. They were housed in plastic polypropylene cages (five animals per cage) under controlled conditions with an LD 12:12 h cycle and temperature 25 °C with proper ventilation and air conditioning. Animals were fed on a standard laboratory pellet diet with free access to drinking water. The protocols were approved by the Institutional animal ethics committee of M.D.U, Rohtak. They were acclimatized under laboratory conditions for 1 week before the commencement of experimental work. After the acclimatization period, animals were randomly divided into experimental and control groups.

**Experimental Design (OECD 407).** The rats were divided into four groups (one control and three experimental groups). Each group contained 10 animals (5 males and 5 females).

Group I animals served as a control group, and they remained unexposed to the pesticide.

Groups II, III, and IV animals were considered experimental groups.

Groups II, III, and IV animals were treated with cypermethrin at a dose of 25 mg/kg body weight (BW), 50 mg/kg BW, and 75 mg/kg BW of rats, respectively, per day through oral gavage for a period of 1 month.

The dose was freshly prepared daily after measuring the body weight of the rat. During the experimental period, body weight, water, food intake, and behavioral activities were closely observed. At the time of termination of the experiment, animals fasted overnight, and then the next day they were sacrificed. Blood was collected to study hematological parameters, and histology was performed to check hepatotoxicity and nephrotoxicity in male and female rats (Figure 7).

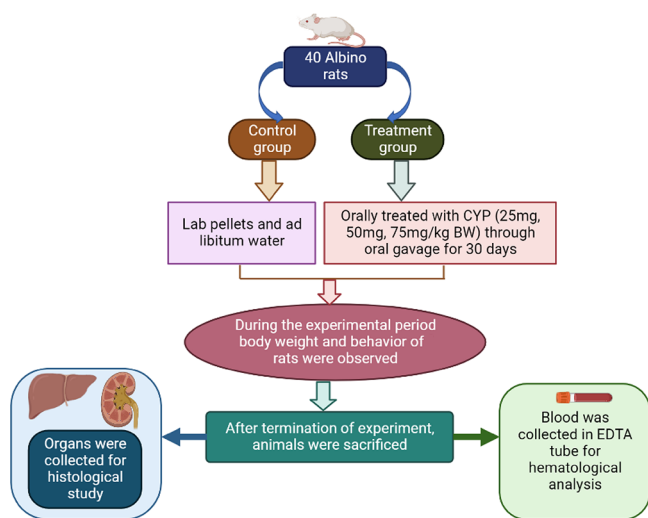


Figure 7. Experimental design.

**Hematological Study.** Blood samples were collected from each sacrificed animal into EDTA tubes for the study of blood parameters such as hemoglobin level, RBC count, WBC count, neutrophil, eosinophil, basophil, monocytes, lymphocytes, platelet count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).<sup>41</sup>

**Histology.** Organs such as the liver and kidney were taken from the dissected animals and placed in a 10% formalin solution. Tissue was washed under running tap water for slide preparation, then dehydrated with an ascending series of ethanol, and cleared in xylene; the block was prepared with paraffin wax; and 5 μm tissue sections were stained with hematoxylin and eosin for histopathological study.<sup>42</sup>

**Statistical Analysis.** The data were presented as mean standard error (SEM). Statistical significance was calculated by performing a one-way analysis of variance (ANOVA) followed by Tukey's post hoc comparisons test. At  $p \leq 0.05$ , the difference was considered statistically significant.

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### Notes

The authors declare no competing financial interest.

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