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Effect of Cypermethrin on Hematological and Histological Parameters in Male Albino Mice

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Abstract

The study investigated the behavioral, hematological, and histological changes of sub-lethal toxicity of Cypermethrin, a synthetic parathyroid insecticide, in male albino mice. The mice were divided into three groups, with the control group remaining untreated and the other groups treated with different doses. The behavioral effects produced mild to moderate toxic symptoms. The lower dose produced very mild toxicities characterized by intermittent diarrhea and decreased feed intake, whereas the higher dose displayed mild to moderate toxicities with diarrhea, decreased feed intake, loss of body weight, dyspnea, ataxia, eye discharge, and salivation. Hematological parameters such as packed cell volume (PCV), hemoglobin concentration (HB), white blood count (WBC), and red blood count (RBC) were evaluated. Hematological parameters such as PCV, HB, RBC, and WBC showed a dose-dependent reduction. At the same time, microscopically, cypermethrin produced neuronal degeneration and depletion of glycoprotein with accumulation of cholesterol in which the hepto-cells appear as a plant cell, and disorganization of hepatic laminae, dilation of sinusoids, and necrosis of hepatocytes in the liver. Section of kidney displayed hemorrhage and sloughing of renal epithelial cells in the convoluted tubules, shrinkage of glomeruli, and necrosis of renal tubules. The above observations clearly demonstrate that hematological parameters such as packed cell volume, hemoglobin concentration, total erythrocyte count, and total leukocyte count showed a dose-dependent reduction. There were relative variations in results in CYP-intoxicated groups. The changes in the blood picture indicate the toxic actions of CYP on the hematopoietic system. Nevertheless, we believe that the studies addressing the comparison of histopathological and behavioral effects would be very helpful in the evaluation of pyrethroid insecticides. In addition to the overall results of this study, which clearly demonstrate that oral administration of the (CYP) leads to histopathological changes in the liver, as well as the kidneys, in mammals and environmental systems.

Keywords: Albino rats, cypermethrin, histopathology, hematology parameters.

1. Introduction

A vast purpose of usage of many pesticides, like Cypermethrin (CYP), which is synthetic pyrethroids that are derived from pyrethrin, has as many applications for the control of Lepidoptera, cockroaches, and termites (1). The reverse effects due to its extensive use produce repeated exposure of (CYP). belongings of (CYP) ranged from slight local observations following from dermal contamination to neurological signs like seizures, tremors, coma, and gastrointestinal symptoms like nausea, vomiting, and gastrointestinal irritation (2, 3). Considerable influence that are markers for blood disturbance (anemia, defective blood coagulation), nerve damage, paralysis, jaundice and hepatic fibrosis, and infertility are commonly related with mild residual grades of this insecticide (4, 5). In mammals, decreased weight gain, decreased appetite, or reproductive problems could be associated with the persistence of low-level exposure of CYP (6). The issue of mutagenicity and carcinogenicity with pyrethroids is uncommon, which makes CPY not show serious residue problems (7). Because blood serves as a pathophysiological reflector of the entire body, blood markers play a crucial role in diagnosing the health status of fish exposed to toxicants (8). Alterations in the biochemical and blood profile reflect changes in the organism's metabolism and biochemical actions, resulting from the impacts of these pollutants (9). Several researchers (10-12) have reported a decrease in hematocrit, hemoglobin, and red blood cells in some fish following their exposure to insecticides. Researchers are exploring the possibility of using hematological markers as potential indicators of pesticides. The potential effects of pyrethroids on certain aspects of behavior, both in humans and in animal models, remain inadequately understood. The goal of the present paper was to investigate the impacts of selected pyrethroids on several biological tests of albino mice.

2. Materials and Methods

2.1. Animal housing

In this experiment, 40 healthy mature albino mice were used in this study. The Biotechnology Research Center/Al-Nahreen University (Baghdad, Iraq) housed the animals for breeding. Plastic cages measuring 25x12x11 cm housed the mice. Thirty-five mature mice (30g) were divided serially into 3 groups, each with 10 mice.

2.2. Experimental Animals' Treatment

Three groups, each containing ten animals, underwent experimental protocols after a week of acclimatization. Group 2 received 1.75 mg/kg of body weight from CYP orally for 2 and 4 weeks, while group 3 received 3.5 mg/kg of body weight from CYP orally for 2 and 4 weeks. All groups received these doses orally for 2 and 4 weeks.

2.3. Biological Parameters

At the end of the experiments, we anesthetized 10 mice from each group with ether and then collected two blood samples from each animal's heart using EDTA. We used an EDTA tube for hematological analysis, testing blood samples for blood parameters such as RBC (red blood cell), WBC (white blood cell), platelets (plt), hemoglobin (HB), packed cell volume (PCV), and mean capsular volume. MCV stands for mean corpuscular hemoglobin (MCH). The Biotechnology Research Center at Al-Nahreen University in Baghdad, Iraq, used the CELL-DYN 1700 counter (Abbott Hematology Analyzer Cell-DYN 1700, Abbott Laboratories, Abbott Park, Illinois, U.S.A.) to measure the average amount of hemoglobin in the blood.

2.3.2. Histological assay

After the end of the experiment, samples of the liver and kidney from all animals within different treatment groups were excised and mixed in 10% formal saline, followed by dehydration in ascending grades of alcohol, then clearing in xylene and embedding in paraffin wax. Paraffin sections (5 µm thickness) were stained with hematoxylin and eosin (H and E) for the histological examination (13) and the Feulgen method for histochemical investigation (14).

2.4. Statistical analysis

Results are expressed as mean±S.E. Data collected from various parameters were analyzed using two-way analysis of variance (ANOVA) and followed by Duncan's multiple range tests for grouping means having significance.

3. Results

3.1. Effect on behavioral and hematological parameters

There were no significant differences in weight gain between control and treated animals with 1.75 mg/kg for 2 weeks during the study, thus eliminating considerable alteration in general health, which may confound results. There were 2 out of 10 mice treated with CYP at 4 weeks in 3.5 mg/kg that died within 8 days of commencing the experiment, making a significant decrease in the life span of this group compared to the control animals. **Table (1**) summarizes the results of the hematological assays on albino mice in the control and in-exposed sets.

Table 1. Hemogram of male albino mice intoxicated with cypermethrin that treated with 1.75 mg/kg.

Parameter	Duration	Treatment 1.75mg/kg	
		CYP	SD
RBC (10^{12}/ l)	Control	7.967Aa± 0.757	
	2-Weeks	6.867Ba	0.751
	4-Weeks	6.000Ca	0.346
WBC (10^{9}/ l)	Control	7.293Aa	0.900
	2-Weeks	6.867Ba	1.464
	4-Weeks	4.367Ba	0.702
HB (g/dl)	Control	15.233Aa	0.850
	2-Weeks	13.300Ba	2.402
	4-Weeks	12.767Ba	1.193
PCV (%)	Control	51.000Aa	7.000
	2-Weeks	47.300Ba	6.855
	4-Weeks	45.367Ba	3.001
MCV (fl)	Control	47.333Aa	6.658
	2-Weeks	48.000Aa	6.000
	4-Weeks	42.433Aa	1.650
MCH (pg)	Control	16.367Aa	2.654
	2-Weeks	12.400Ba	1.453
	4-Weeks	15.067Ba	2.996
MCHC (g/dl)	Control	31.333Aa	1.528
	2-Weeks	26.333Ba	4.726
	4-Weeks	28.600Ba	1.114
PLT (10^{9}/ l)	Control	1231.333Aa	70.890
	2-Weeks	1141.333Ba	128.726
	4-Weeks	1081.667Ba	80.649

Different lower-case letters in the same row indicated significant differences. Different upper-case letters in the same column indicated significant differences. SD means (standard deviation)

When mice were given 1.75 mg/kg of CYP for two weeks, their WBC counts, HB, PCV, PLT, MCHC, and MCH levels all dropped significantly after two and four weeks compared to the control group. This was due to a dose-dependent effect.

Table 2. Hemogram of male albino mice intoxicated with cypermethrin that treated with 3.5mg/kg.

Parameter	Duration	Treatment 3.5mg/kg	
		CYP	SD
RBC	Control	7.966Aa	0.757188
(10^{12}/ l)	2-Weeks	6.866Ba	0.750555
	4-Weeks	5.733Ba	0.986577
WBC	Control	7.293Aa	0.900074
(10^{9}/ l)	2-Weeks	6.21Ba	0.958175
	4-Weeks	4.2667Ca	0.550757
	Control	15.233Aa	0.85049
HB (g/dl)	2-Weeks	13.3Ba	2.402082
	4-Weeks	12.766Ba	1.193035
	Control	51Aa	7
PCV (%)	2-Weeks	47.3Ba	6.854
	4-Weeks	45.366Ba	3.000
	Control	47.333Aa	6.658328
MCV (fl)	2-Weeks	48Aa	6
	4-Weeks	42.433Aa	1.650253
	Control	16.366Aa	2.653928
MCH (pg)	2-Weeks	12.4Ba	1.452584
	4-Weeks	11.733Ba	2.318045
	Control	35Aa	2.645751
MCHC (g/dl)	2-Weeks	30.7Ba	2.523886
	4-Weeks	24.6Ca	1.907878
PLT	Control	1264.667Aa	107.8208
(10^{9}/ l)	2-Weeks	1141.333Aa	128.7258
	4-Weeks	925.666Ba	138.3378

Different lower-case letters in the same row indicated significant differences. Different upper-case letters in the same column indicated significant differences. SD means (standard deviation)

Additionally, RBC levels dropped significantly over 2 and 4 weeks at a dose of mg/kg, but MCV values did not change significantly over the same time periods (CYP caused a substantial dose-dependent drop in MCV values across all treatment groups). WBC counts did not rise statistically, although some hematological measurements had noticeable alterations that were neither dose-dependent nor statistically significant. The hematological investigation on albino mice in the control and treatment groups yielded the findings listed in Table 2. When mice were given 3.5 mg/kg of CYP for two weeks, their RBC counts, HB, PCV, and MCH levels all dropped significantly compared to the control group after two and four weeks. These drops were dose-dependent. The value of MCV exhibited an insignificant decrease for 2 and 4 weeks, but in PLT measurement there was a significant decrease for 2 weeks compared with control as well. Furthermore, despite the significant declines in WBC and MCHC values at both 2 and 4 weeks mg/kg, the addition of CYP led to a significant dose-dependent decrease in MCV values across all treated groups. The statistical result showed that WBC counts did not rise, although other hematological indicators had noticeable alterations that were neither dose-dependent nor statistically significant.

3.2. The histopathological results

The light microscope examination of the liver tissues of male albino mice in all treatment groups, compared to the control group (**Figure 1A**), revealed a normal histological structure composed of the vein and threads of hepatocyte cells with sinusoids. Compared to the histopathological examination of the liver tissue of the control subjects (**Figure 1A**), there was no significant difference. A section of the liver from group 2 underwent a 2-week treatment with (CYP) at a dose of 1.75 mg/kg, resulting in the depletion of glycoprotein and the deposition of cholesterol. This treatment also pushed organelles into the cell, as shown in **Figure (1.B)**. Furthermore, when (CYP) was exposed to a section of liver treated with (CYP) 3.5 mg/kg for 2 weeks, the hepatocyte cell with pyknotic nuclei, the disorientation of hepatic laminae, and the presence of plant cells in the liver tissue showed increased reduction of glycoprotein and more buildup of cholesterol (**Figure 1.C**). Additionally, the histological analysis in the liver tissue has demonstrated a dose-dependent increase in CYP. Parts of the liver that were exposed to CYP showed some tissue flaking, death of hepatocellular cells, disorganized hepatic laminae, and inflammatory cells entering the MNC along with bleeding cells (**Figure 1.D**). Furthermore, the histopathological modifications in the liver tissue, which were exposed to a section of liver treated with 3.5 mg/kg (CYP) for four weeks, revealed a dose-dependent increase in (CYP). These changes included increased tissue sloughing, necrosis of hepatocellular cells, and inflammatory cell infiltration of MNC with hemorrhaged cells (**Figure 1.E**).

Cypermethrin produced significant alterations in the histoarchitecture of the kidney, especially at higher dosages. The kidney tissues of male albino mice in all treatment groups exhibited a normal histological structure, consisting of glomeruli and renal tubules, when compared to the control group (**Figure 2A**). In contrast to the histological examination of the liver tissue of controls (**Figure 2A**), the section of the kidney in group 2 that was treated by CYP 1.75 mg/kg for 2 weeks showed mild degeneration of renal tubular epithelial cloudy swelling and vacuolar degeneration, signs of fibrosis and inflammatory cellular infiltration., thickening of the basement membrane, and a narrow lumen (**Figure 1B**). In addition, the histopathological changes in the liver tissue have shown a dose-dependent increase in CYP exposed to a section of liver treated by CYP 3.5 mg/kg for 2 weeks, which showed an increase of degeneration of cells and presence of necrosis because of condensed chromatid cells of few cells with inflammatory cells dilatation and of congestion of blood vessels. Bleeding in interstitial space; some glomeruli showed completely degenerated others showed vacuolar degeneration in some tubular epithelial cells (**Figure 2.C**). In addition, the histopathological changes in the liver tissue have shown a dose-dependent increase in CYP exposed to a section of liver treated by 1.75 mg/kg (CYP) for 4 weeks, which showed shrinkage of glomeruli, necrosis, and disruption of renal tubules. moderate degeneration of renal tubular epithelium with necrosis and heavy inflammatory cell infiltration with pyelonephritis, vacuolar degeneration, and cloudy degeneration, interstitial cellular infiltration. Some glomeruli showed lobulation, and others completely degenerated (**Figure 2D**). Also, the histopathological changes in the liver tissue showed that the amount of CYP increased with time. For example, a section of liver that was treated with 3.5 mg/kg (CYP) for 4 weeks had more degeneration of the renal tubular epithelium with necrosis and heavy inflammatory cell infiltration with pyelonephritis (**Figure 2E**).

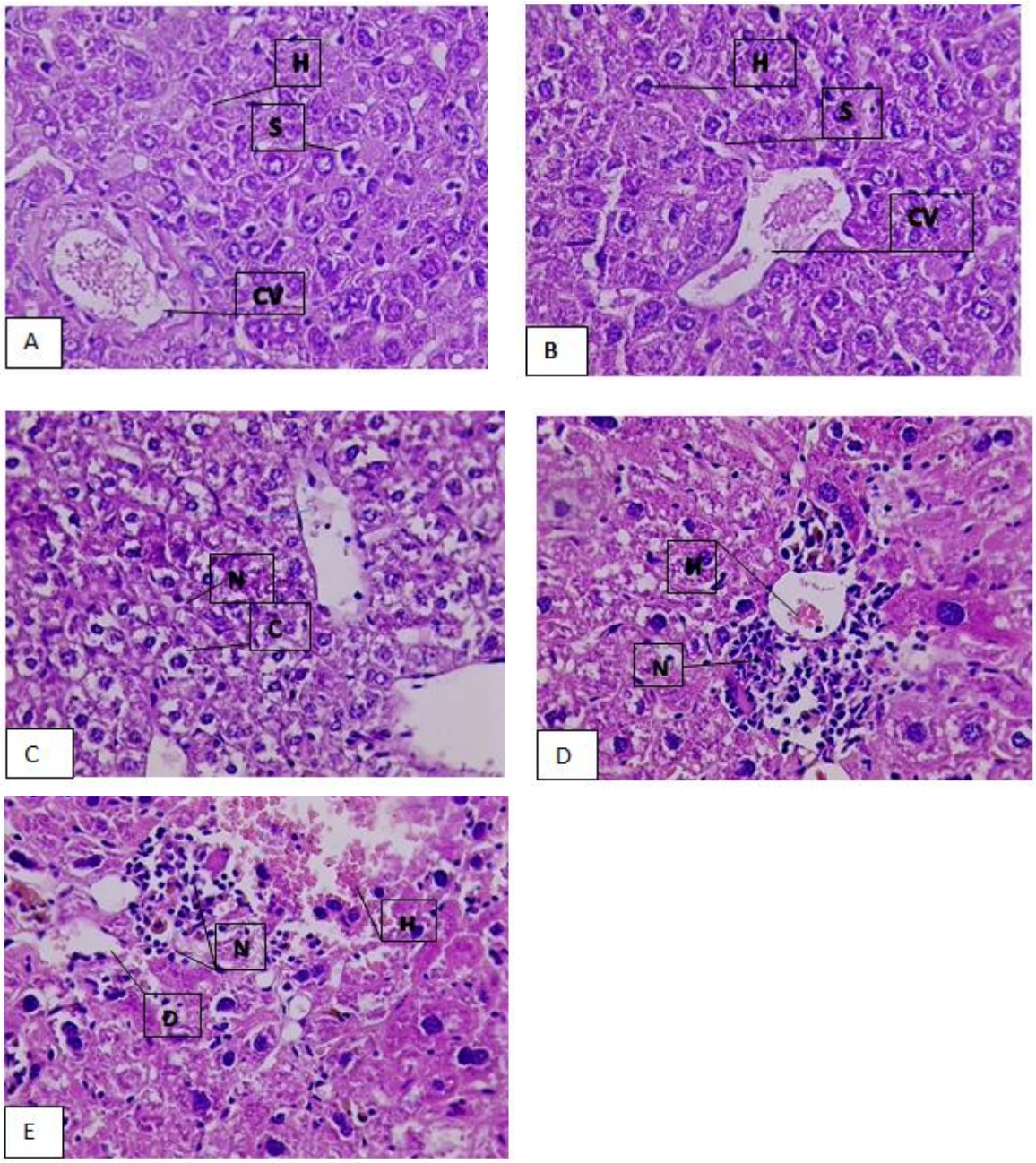

Figure 1. A section of the liver (A) displays the normal structure of the hepatocyte, complete with the central vein (cv) and sinusoid (s). Figure B: Animals treated with 1.75 mg/kg for 2 weeks displayed mild degeneration. C: Cypermethrin treatment at 3.5 mg/kg for 2 weeks resulted in an accumulation of cholesterol (C) and the presence of necrosis (N) in a section of the liver. D: A section of liver treated with 1.75 mg/kg cypermethrin for 4 weeks exhibited hemorrhagic cells (H) and necrosis (N). E: The liver section, treated with 3.5mg/kg cypermethrin for 4 weeks, exhibited sloughing of the tissue, necrosis (N) of hepatocellular cells, (O) edema, and hemorrhaged cells (H). (H&E)(x40). (H&E)(x40).

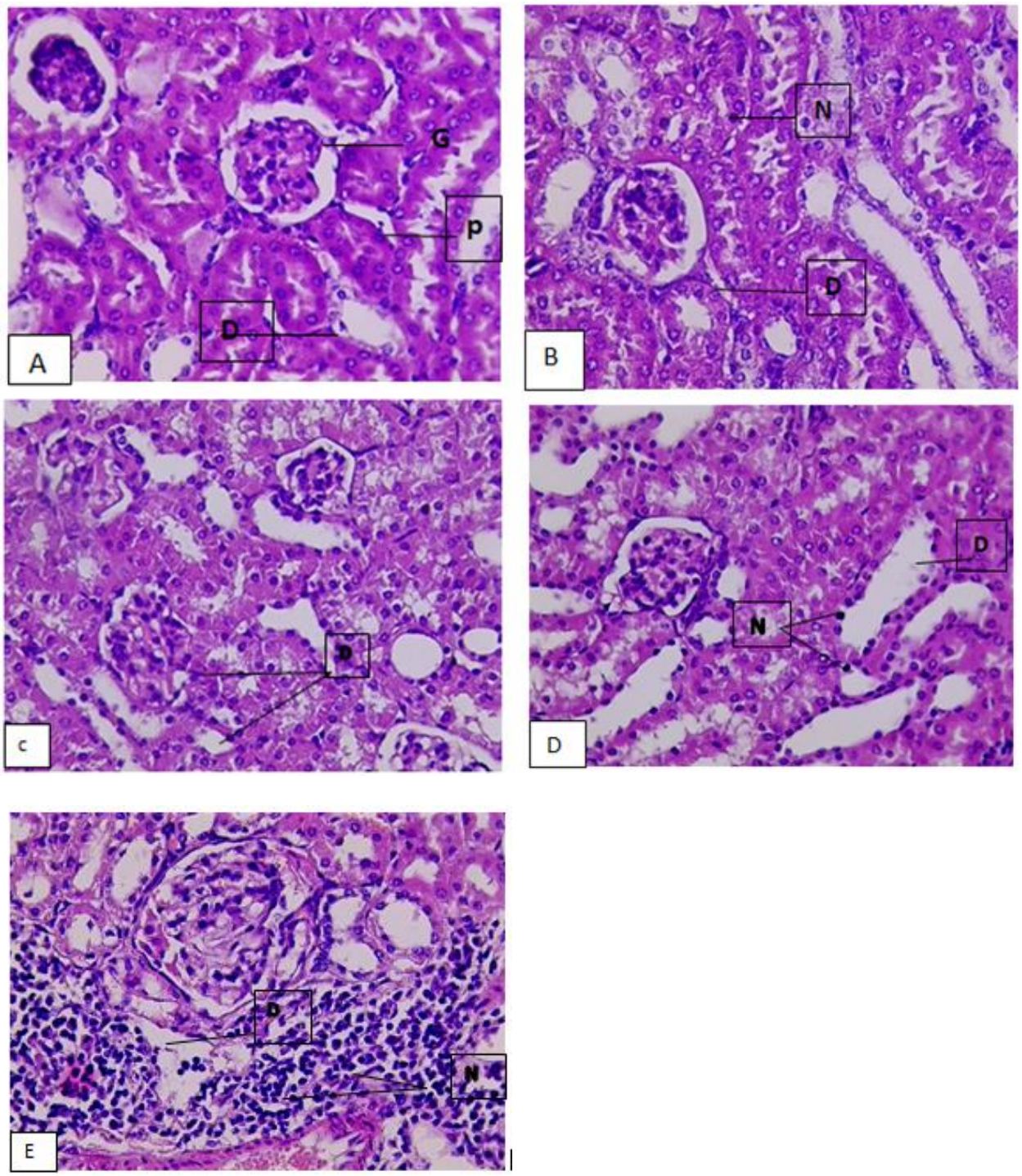

Figure 2. Section of kidney (A) control showing normal structure of renal tubule and glomeruli (G); (B): 1.75 mg/kg for 2 weeks treated animal showing mild degeneration of renal tubular epithelium. (C) 3.5 mg/kg of for 2 weeks showed an increase of degeneration of cells and presence of necrosis because of condensed chromatid cells of few cells with inflammatory cells. (D) 1.75 mg/kg for 4 weeks showing moderate degeneration of renal tubular epithelium with necrosis and heavy inflammatory cell infiltration with pyelonephritis. (E) 3.5 mg/kg for 4 weeks showing severe degeneration of renal tubular epithelium with necrosis and heavy inflammatory cell infiltration with pyelonephritis. (H&E)(x40).

4. Discussion

4.1 Effect on behavioral and hematological parameters

Study of (15) said weight loss that is statistically significant is continuing to follow 7 days of therapy with 300 mg/kg of (CYP) in male rats. (16) found that the combination of chlorpyrifos and (CYP) significantly inhibits plasma fluid and neural activity, leading to a devastating effect on the neurological system. Furthermore, the exposure at 3.5 mg/kg resulted in a propensity for exposed mice to lose their grip more quickly than some other animals after four weeks. Various studies looking at the risk of cypermethrin (CYP) in mice have indicated that it accelerates salivation, poor coordination, muscular trembling, and seizures.

These signs of toxicity indicate that the target for this compound is the central nervous system in mammals (17, 18) as observed in the present study. Also, intermittent diarrhea, decreased feed intake, and thick eye discharge, whereas higher doses displayed mild to moderate toxicities with diarrhea, decreased feed intake, loss of body weight, dyspnea, ataxia, eye discharge, and salivation. This study backs up what other researchers have said about two different syndromes: pawing, burrowing, salivation, whole-body tremor to choreoathetosis, hypothermia, and decreased motor activity for the type I pyrethroid permethrin; and aggressive sparring behavior, fine to whole-body tremor, hyperthermia, and decreased motor activity for the type II pyrethroid cypermethrin. In addition, (19) reported that permethrin produced decreased grip strengths, increased resistance to capture, increased reactivity to a click stimulus, and induced head and forelimb shaking and agitated behaviors. Although there were similarities in some effects (e.g., decreased motor activity), the pesticides differed significantly in their overall behavioral profiles as well as in terms of severity and the time course of effects.

This reduction in values of TEC, PCV, and HB indicates depressed erythropoiesis. (20) observed reductions in the erythrocyte count, PCV, and hemoglobin concentration. Also, (21) reported a significant dose-dependent decrease in TEC, hematocrit, thrombocyte, and MCH values. (22) found that when male mice were given lower doses of cypermethrin (1.75 mg/kg body weight), the number of white blood cells in their blood increased. In treated mice, the same author reported a significant increase in leukocytes (23).

Our results align with the findings that suggest a disruption in erythropoiesis or an increase in blood cell destruction (11). We noted a decrease in WBC (CYP) in treated mice compared to the control group. The literature indicates that CYP does not have an immunosuppressive effect on mice, but further examination of more parameters is necessary to fully understand this subject (12, 24). Consequently, it became evident that cypermethrin induced a negative alteration in certain hematological parameters. Indeed, researchers have extensively investigated alterations in hematological parameters caused by pyrethroids, but they have paid very little attention to morphological changes induced by CYP.

4.2 The histopathological study

The changes in **Figure (1A)** these outcomes are in line with those of (25), who noted significant neuronal injury and glial cell growth after receiving repeated oral dosages of CYP. Numerous pesticides have been linked to hypoxia, hypoglycemia, and/or impairment to cell ion homeostasis, all of which have been documented to result in varied alterations in the brain after repeated exposure (26). After 30 days of pesticide exposure, organometry experiments on the liver likewise indicated a considerable rise in the relative proportions weight to the extent of 37.82% (for females) and 37.89% (for males) (27). According to (28), the increased drug-

metabolizing multiple enzyme complex and operational hypertrophy of the smooth endoplasmic reticulum were likely to blame for the increased relative weight of the liver.

Regarding the kidney the observed results are in accordance with (24), who reported glomerular and tubular necrosis in broiler chicks treated with oral doses of fenvalerate. In such cases, leakage of lysosomal enzymes may occur, thereby causing cell necrosis and renal damage. A smaller dose of CYP in both male and female rats caused only a slight disruption of the hepatic laminae, while a greater dose resulted in necrosis of the hepatic cells, with pyknotic nuclei, and expansion of the sinusoids with severely damaged hepatic laminae. The discovered results concur with those of (27), who described the varied hepatotoxic impact of CYP in rabbits. Animals exposed to several hepatotoxicants showed signs of fatty deterioration and hepatocyte necrosis.

5. Conclusions

The current study aims to investigate the hazardous effects of cypermethrin on pathohistological alterations of critical organs, as well as its toxic role on blood parameters in albino mice. In conclusion, cypermethrin and lead induced toxicity in Swiss albino mice by causing statistically significant changes in selected biochemical and histological parameters depending on the dose.

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Conflict of Interest

"The authors declare that they have no conflicts of interest."

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Ethical Clearance

This research was subjected to ethical considerations and was approved by the Committee of Animal Ethics (Ref.CSEC/1122/0149) at the University of Baghdad College of Science approved this protocol

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