Study of some toxicological aspects of Alfa-cypermethrin in rabbits

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Abstract

This study was conducted to evaluate the immediate alterations that occur in rabbits after acute toxicity with single oral dose of cypermethrin. Twenty one rabbits were randomly divided into three equal groups: treated groups (A1), (A2) and control group used in this study. Groups (A1), (A2) received orally cypermethrin in dose of (25, 50 mg/kg body weight) respectively, While control group received normal saline at same time. Most obvious clinical sings in the treated groups were Restlessness, salivation, licking of legs and face, frequent urination, muscular tremor, incoordination, and ataxia appeared in dose dependent manner. In addition, significantly increased in respiratory rate and heart rate in treated groups compared with control group and appeared petechial hemorrhage and congestion in the conjunctiva in treated groups. The blood samples were taking to evaluate some of blood parameters includes (RBC counts, WBC counts, PLT counts, Hb concentration, PCV, RBCs indices (MCV, MCH, MCHC, RDW -CV and RDW - SD), PDW, MPV, P-LCR and some biochemical study as ALT and AST. Two rabbits from each group were killed to evaluate the histopathological alteration in liver. The statistical analysis revealed that there were significant decreased in RBC counts and Hb concentration ($P<0.05$) in treated groups (A1), (A2) as compared with control group. The PCV percentage, platelet counts and MCHC values revealed significant decreased ($P<0.05$) between group (A2) and control group. The RDW-SD, RDW-CV valves were significantly increased ($p<0.05$) between group (A1) and control group. Other parameter studied did not show significant differences between treated groups and control group. The statistical analysis revealed that the levels of ALT and AST were significantly elevated ($P<0.05$) in (A1 and A2) groups as compared with control group. In gross appearance there were petechial hemorrhage and erosion of liver parenchyma. Histopathological study revealed that there were engorgement of hepatic vein with presence of extravasated RBCs between the hepatocytes in group (A1), while group (A2) the hepatocytes undergo atrophy and severe necrosis and destruction with infiltration of polymorphnuclear leukocytes and engorgement of some portal trait with bile secretion.

Introduction

Pyrethrin and pyrethroid insecticides are effective against a variety of insect pests on pets, livestock and other animals and are readily available for home, farm and garden use (1). They are available in shampoos, sprays, powders, flea collars, dips, spot-on, pour-on, (2, 3).Cypermethrin is a type II pyrethroid (synthetic pyrethrins) that contain an Alpha- cyano group increases insecticidal potency (1). Cypermethrin is toxic to nervous system it also suppresses the immune system and classified as possible human carcinogen because it causes an increase in the frequency of lung tumors in female mice (4).All animals may exhibit paresthesia following application of apyrethrin or pyrethroid product. In cattle may become restless and act uncomfortable twitch the skin on their backs after application of a pyrethroid pour-on product (1). While clinical signs like increased urination licking of legs, jerky movement, ataxia, incoordination, staggering and dizziness have been reported in rabbits after injected intraperitoneally cypermethrin (5).Long-term feeding studies with laboratory animals have shown that cypermethrin causes adverse effects; in mice it caused reduced weight gain, mild anemia and increases liver weight (4). This project was designed to investigate symptoms and alterations in some blood parameters and some biochemical parameters after induced of single oral dose of cypermethrin and study the gross and histopathological lesion in liver due cypermthrin toxicity.

Materials and methods

Twenty one rabbits were used in this study. After seven days of acclimatization the rabbits were randomly divided into three equal groups: treated group (A1), treated group (A2) and control group, with seven rabbits in each group. Rabbits of groups (A1) and (A2) were received oral cypermethrin in dose of (25, 50 mg/kg body weight) respectively, while control group received normal saline at same time. Treated animals were monitoring at least once a day to recording the suspected clinical signs the study continued for ten days. The blood samples with anticoagulant were taking to evaluate some of blood parameters include (RBC counts, WBC counts, PLT counts, Hb concentration, PCV, RBC indices (MCV, MCH , MCHC , RDW – CV(RBC distribution width-CV) and RDW – SD(RBC distribution width-SD)) , PDW(Platelet distribution width) , MPV (mean platelet volume), P-LCR(large platelet ratio) used automated hematology analyzer KX-21 N (Sysmex corporation, Kobe, Japan). Then, blood samples with anticoagulant centrifuged immediately after collection for separation of plasma and utilized for the estimation of aspartate aminotransferase (AST) and alanine aminotransferase(ALT) (6,7). Two rabbits from each group were killed to evaluate the histopathological alteration in liver. Tissue samples taken from liver were fixed in 10% buffered formalin and using routine methods and section of 5 micro meter thickness were cut and stained with hematoxylin and eosin (8, 9). Statistical analysis was done using SPSS (version10); the data were analyzed statistically using one way ANOVA with LSD to establish significant differences among groups.

Results

In present study, the onset of clinical signs after 5-10 minute While the nervous signs recorded at 50 minute. Most obvious clinical signs in group (A1) were, restlessness, salivation, licking of legs and face, little incoordination, Ataxia, muscular tremor finely depression while in group (A2) the same clinical signs appear but more severely Table (1). Congestion of conjunctiva and petechial hemorrhage (Figure1a). In addition, the recorded significant increase in heart rate and respiratory rate at a level ($p<0.05$) between (A1), (A2) groups and control group Table (2) Theses clinical signs were subsides during (90) minute. The statistical analysis revealed that there were significant decreased in RBC counts and Hb concentration ($P<0.05$) in treated groups (A1), (A2) as compared with control group. The PCV percentage and MCHC values were revealed significant decreased ($P<0.05$) between group (A2) and control group and also the MCHC values significantly differences between (A1) and (A2) groups. The RDW-SD and RDW-CV valves was significantly increased ($p<0.05$) between group (A1) and control group, and also RDW-CV the was significant differences between treated groups. While the WBC counts revealed non significant decreased in treated groups compared with control group. The platelet counts significantly decreased at level ($P<0.05$) between treated group (A2) and control group, While the PDW, MPV revealed non significant increased in treated groups compared with control group (Table 3). The statistical analysis revealed that the levels of ALT and AST were significantly elevated ($P<0.05$) in (A1 and A2) groups as compared with control group (Table 3). In gross appearance there were petechial hemorrhage and erosion of liver parenchyma and patchy pale areas in liver parenchyma (Figure 1b). Histopathological study revealed that there were engorgement of hepatic vein with presence of extravasated RBCs between the hepatocytes in group (A1) (Figure 2), while group (A2) appeared necrosis of hepatocytes with infiltration of polymorphnuclear leukocytes (Figure 3).The hepatocytes undergo atrophy and severe necrosis and destruction (Figure 4 and 5) and engorgement of some portal trait with bile secretion (Figure 6).

Table (1): The clinical signs that occur after cypermethrin toxicity in rabbits.

N	Groups / Clinical signs	A1	A2
1	Conjunctiva	Mild conjunctival congestion	Severe conjunctival congestion and petechial hemorrhage.
2	Nervous signs	Ataxia, incoordination, muscular tremor, finely Depression.	Severe ataxia, incoordination unable to move and muscular tremor finely depression
3	Abnormal behavior	Restlessness, Salivation, licking of face and legs.	Restlessness, Profuse salivation,licking of face and legs, frequent urination,grunting sound.

A1: represent group that treated with 25 mg/kg cypermethrin
A2: represent group that treated with 50 mg/kg cypermethrin

Table (2): The clinical assessment of rabbits suffering from cypermethrin toxicity. The results represent Mean ±SE.

N	clinical assessment	A1 Group	A2 Group	Control Group
1	Heart Rate	198.29±7.85 A	198.57±22.44 A	124.57±3.62B
2	Respiratory Rate	175.43±11.16 A	185.71± 25.93 A	83.00± 4.71B

Means within a row with different letter differ significantly ($p<0.05$) between groups.

Table (3): Hematological and biochemical parameters in rabbits treated with different doses of cypermethrin.

N	Groups / Parameters	A1 Group	A2 Group	Control Group
1	WBC 10^3 / µL	5. 38 ± 0. 73	4.27 ± 0.38	6. 05 ± 1. 04
2	RBC 10^6 / µ L	5.06 ± 0.17 A	4.84 ± 0. 27 A	5.78 ± 0.27 B
3	Hb g/dL	10.49 ± 0.48 A	9. 86 ± 0.58 A	12. 07 ± 0.47 B
4	PCV %	34.03 ± 1.39 AB	33.51 ± 1.91 A	39.03 ± 2. 01 B
5	MCV fL	67.13 ± 0.84	69.39 ± 1.48	67.46 ± 0.73
6	MCH pg	20. 67 ± 0.58	20.39 ± 0.39	20.91 ± 0.35
7	MCHC g/dL	30.81 ± 0.57 B	29.43 ± 0.11 A	31.06 ± 0.45 B
8	RDW-SD fL	37.13 ± 1.41 A	35.20 ± 1.03 AB	33.87 ± 0.53 B
9	RDW-CV %	14.74 ± 0. 85 A	12.70 ± 0.44 B	12.89 ± 0.44 B
10	PLT 10^3/ µ L	449.43 ± 120.53 AB	289.29 ± 79.94 A	602.57± 83.09 B
11	PDW fL	10.67 ± 1.25	8.73 ± 0.37	8.43± 0.32
12	MPV fL	7.77 ± 0.37	7.40 ± 0.30	7. 21 ± 0.29
13	P-LCR %	13.09 ± 3.58	9.19 ± 1.45	9.91 ± 2.42
14	ALT U/L	68.37 ± 2.60 A	71.31 ± 14.70 A	33.71 ± 1.45 B
15	AST U/L	54. 71 ± 1.23 A	61.31 ± 7.78 A	25.95 ± 0.42B

Values represent Mean ±SE.
Means within a row with different letter differ significantly ($p<0.05$) between groups.

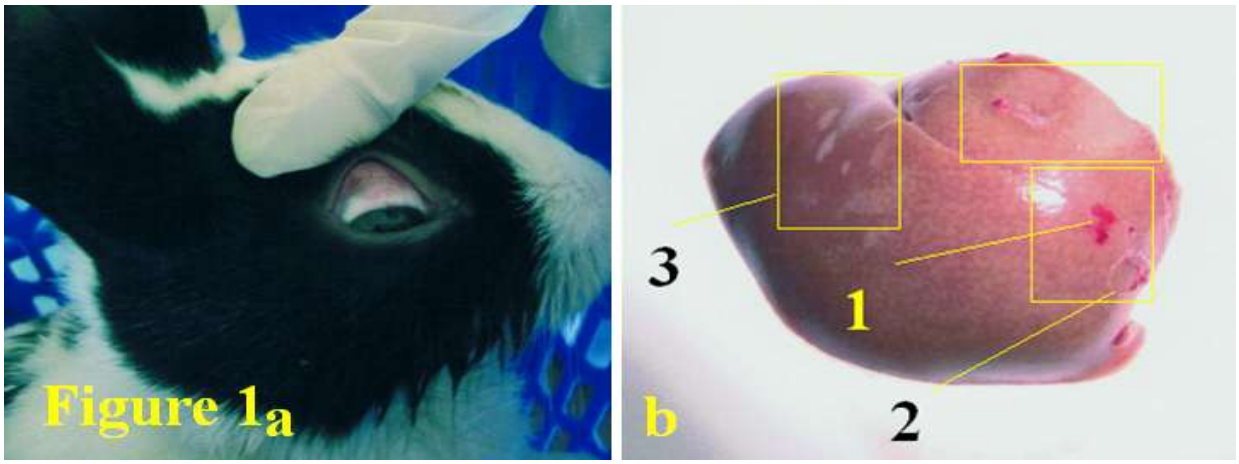

Figure (1 a): Congestion of conjunctiva and petechial hemorrhage. (b):Petechial hemorrhage (1), erosion in liver parenchyma(2) and patchy pale areas in liver parenchyma(3).

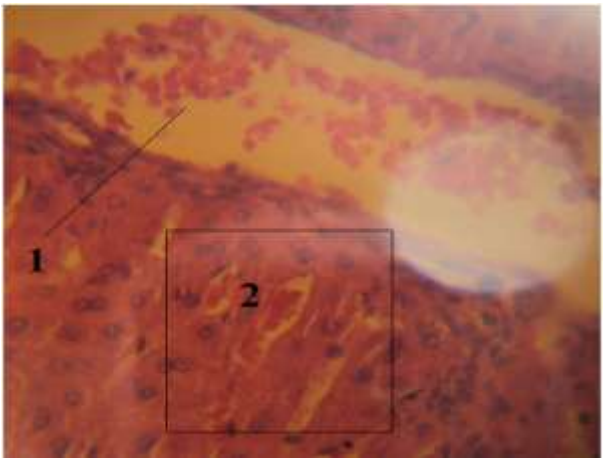

Figure (2) group A1: Engorgement of hepatic vein (1) and extravasated RBCs between the hepatocytes (2). (H&E) 400X.

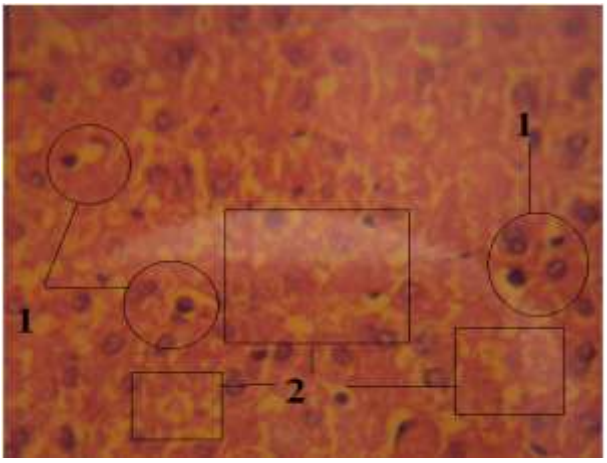

Figure (3) group A2: Infiltration of some polymorphnuclear leukocytes (1) and necrosis of hepatocytes (2). (H&E) 400X.

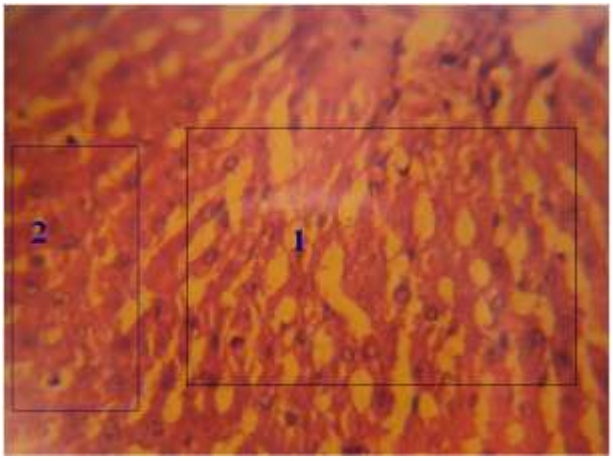

Figure (4) group A2: atrophy of hepatocytes (1), while (2) normal hepatocytes. (H&E) 400X.

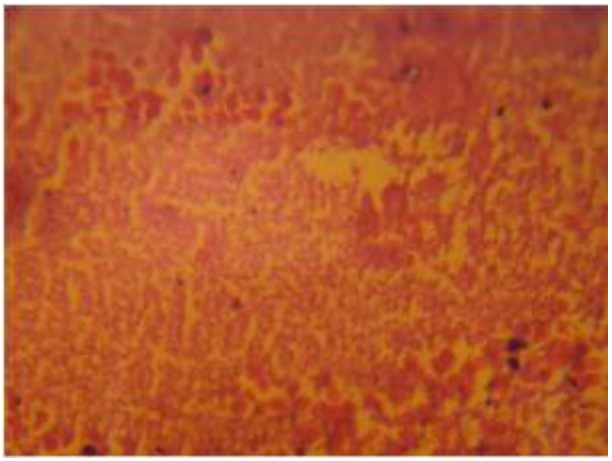

Figure (5) group A2: severely destructed hepatocytes (H&E) 400X.

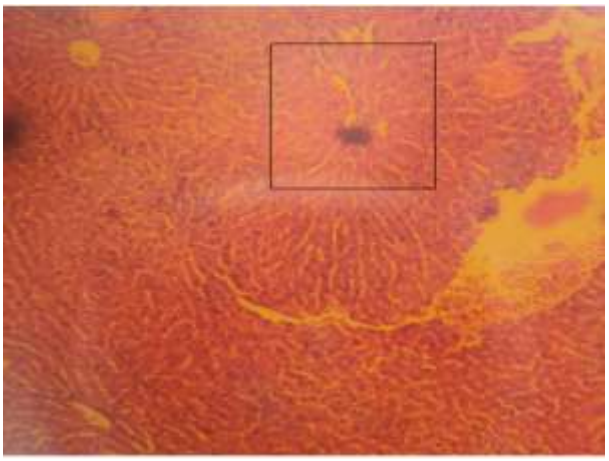

Figure (6) Group A2: engorgement of portal trait with bile secretion. (H&E stain).100X (left figure) 400X (right figure).

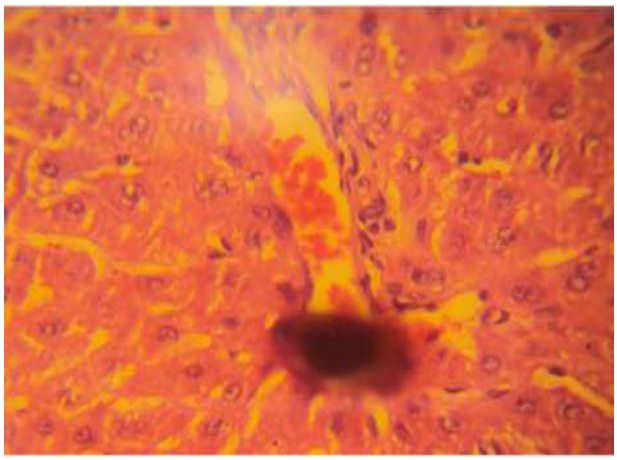

Figure (6) Group A2: engorgement of portal trait with bile secretion. (H&E stain).100X (left figure) 400X (right figure).

Discussion

Pyrethroids tend to have more potent insecticidal properties, tend to be more toxic and more stable in the environment than naturally occurring pyrethrins , Although most pyrethrin and pyrethroid products are applied dermally, the natural grooming behavior of most animals results in oral and possibly inhalational exposures (1). In present study, most obvious clinical signs like Restlessness, salivation, licking of legs and face, frequent urination In addition, the recorded significant increase in heart rate and respiratory rate between treated groups and control group, petechial hemorrhage and congestion in the conjunctiva. The nerves signs like muscular tremor, incoordination, ataxia finely depression in treated groups in a dose dependent manner. Similar clinical signs have been recorded by different works with cypermethrin effects, These included salivation, muscular tremor, ataxia in rabbits (10) and restlessness, salvation recorded in dwarf goats (11), increased urination, licking of legs, jerky movement, ataxia, in coordination has been reported in rabbits (5); salivation, labored breathing and then CNS depression in rats (12); alpha-cypermethrin produce incoordination(13). The poisoning syndromes of type II pyrethroids produced (Choreoathetosis, with salivation) (CS), hyperexcitability and seizures (14, 15, 16). Similarly (4) cypermethrin caused eye irritation. The main effects of pyrethroids are on sodium and chloride channels pyrethroids modify the gating characteristics of voltage sensitive sodium channels to delay their closure (17).The duration of sodium tail currents is much longer for type II pyrethroids the result membrane depolarization predominates in type II pyrethroids , The signs may be attributed to the disagreeable taste of the product or to a tingling sensation on the skin or oral mucous membranes (Paresthesia), is thought to result from direct action of pyrethroids on sensory nerve endings causing repetitive firing of these fiber (1). Type II pyrethroids also decrease chloride currents through voltage- dependent chloride channels and high concentrations can also act on GABA-gated chloride channels (17). The results of the present study indicated that cypermethrin induced anemia the significantly decrease in RBC counts, Hb concentration in treated groups and significantly decrease in PCV percentage in treated (A2) group, While the WBC counts revealed non significant decreased in treated groups compared with control group. Similarly, (5) reported anemia in rabbits because decrease in red blood cell counts and hemoglobin concentration due to the disruptive action of the pesticides on the erythropoietic tissue and might be affected of cell

viability. Similarly, (18) showed that cypermethrin significantly decrease total erythrocyte counts, hemoglobin concentration, and PCV percentage. Similarly, (19) recorded a decrease in RBC counts, hemoglobin concentration and WBC counts this probably explained by effect of cypermethrin on the erythropoiesis, which is agreement with (20) high cypermethrin doses reduced the WBC count in peripheral blood. MCHC significantly decreased in all treated groups compared to control group while MCV non significantly increased in treated (A2) group compared with control. The increase in MCV and decrease in MCHC indicate macrocytic and hypochromic anemia. MCHC valves may be decreased in animals with regenerative anemia while increased MCV valves (Macrocytosis) are usually associated with regenerative anemia; macrocytosis is more likely to occur in response to hemolytic anemia (21). While and MCHC valves significantly difference between treated groups depended to dose used. Which is agreement with (22) showed significant decrease in total erythrocyte counts, hemoglobin concentration, and MCHC valves were found in the male rabbits treated intraperitoneally cypermethrin at higher doses. RDW (the red cell distribution width), RDW-CD and RDW-CV valves as expressed as a percentage of mean erythrocyte volume significantly increased between (A1) and control group. It is often increased in regenerative anemia because reticulocytes and young erythrocytes are larger than mature erythrocytes (21).and also RDW-CV valves significantly difference between treated groups caused by the effects of cypermethrin depend on the dose. The platelet significantly decrease in (A2) group compared with control group, Which is agreement with (23) were recorded significant decrease of platelets which may be effect of pesticides these results could suggest that thrombocytopenia may be associated with disturbed production of platelet due to hypoplasia of bone marrow megacaryocytes. Histopathological study revealed that hepatocytes undergo atrophy and sever necrosis and destruction with infiltration of polymorph nuclear leucocytes dependent manner. Similarly, (24) were marked diffuse necrosis of hepatic tissue in all cypermethrin treated in rats. While the evident that cypermethrin produced hemorrhage and congestion were found in liver, agree with (12). In present study the significantly elevated plasma ALT, AST in treated (A1, A2) groups compared with control group, Is an indicator of tissue damage. Raised AST levels in rabbit can be found in association with liver disease (25).Induced biochemical changes correlate well with histopathological changes in liver. These result agree with (12) were recorded increased the activities of AST, ALT in rats treated with cypermethrin. The (26) were recorded that cypermethrin has toxic effect female hepatocytes and leakage of ALT, AST was significantly increased in a dose depended manner.

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دراسة بعض الجوانب السمية للالفا سايبرمثرين في الارانب

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كلية الطب البيطري/جامعة القادسية

الخلاصة

اجريت هذه الدراسة لتقيم التغيرات الانية التي يحدثها التسم الحاد للسايبرمثرين cypermethrin وبجرعة فموية واحدة ، استخدم احدى وعشرون أرنب قسمت عشوائياً الى ثلاثة مجاميع متساوية ، جرعت مجموعتي المعاملة (A2,A1) جرع فموية من السايبرمثرين (25 ،50 ملغم/كغم من وزن الجسم) على التوالي، بينما جرعت مجموعة السيطرة بالمحلول الملحي الفسلجي في الوقت نفسه ،اكثر العلامات السريرية الواضحة في مجموعتي المعاملة كانت الاستياء وسيولة اللعاب، لعق الاقدام والوجه ، تكرار التبول ، رعشة عضلية وعدم التناسق والترنح في المشي حسب الجرعة المعطاة، بالأضافة الى زيادة معنوية $p<0.05$ في معدل التنفس والنبض في مجموعتي المعاملة مقارنةً بمجموعة السيطرة كذلك ظهور نزف حبري واحتقان في قرنية العين في مجموعتي المعاملة. أخذت عينات الدم لتقيم بعض معاييرالدم والمتضمنة (العد الكلي للكريات الدم الحمراء،العد الكلي للكريات الدم البيضاء، العد الكلي للصفيحات الدموية، تركيز خضاب الدم، حجم الخلايا المضغوطه، قياس ملاحق كريات الدم الحمراء (معدل حجم الكرية، معدل خضاب الكرية ، معدل تركيز خضاب الكرية،معدل انتشار الكرية) قياس معدل أنتشار الصفيحات الدموية، معدل حجم الصفيحات الدموية ومعدل الصفيحات الدموية الكبيرة . وكذلك دراسة بعض التغيرات الكيموحيوية مثل قياس انزيمات الكبد (خميرة الالنين ناقلة الأمين(Alanine aminotransferase(ALT وخميرة الاسبارتيت ناقلة الأمين) Aspartate aminotransferase (AST) . ومن ثم التضحية بحيوانين من كل مجموعة لدراسة التغيرات النسجية المرضية للكبد. اظهرالتحليل الاحصائي انخفاضاً معنوياً $p<0.05$ في العد الكلي للكريات الدم الحمر وخضاب الدم في مجموعتي المعاملة(A2,A1) مقارنة مع مجموعة السيطرة وكذلك لوحظ انخفاضاً معنوياً $p<0.05$ في حجم الخلايا المضغوطة ، العد الكلي للصفيحات الدموية ومعدل تركيز خضاب الدم بين مجموعة المعاملة (A2) ومجموعة السيطرة. اما معدل انتشار كريات الدم الحمرRDW-SD, RDW-CV valves ازدات معنوية $p<0.05$ بين مجموعة المعاملة (A1) ومجموعة السيطرة . اما بالنسبة للمعايير الاخرى المدروسة فلم يظهر أي اختلاف معنوي بين مجموعتي المعاملة ومجموعة السيطرة. اظهرت النتائج الاحصائية زيادة معنوية $p<0.05$ في نشاط خميرة الالنين ناقلة الأمين (ALT) وخميرة الاسبارتيت ناقلة الأمين (AST) بين مجموعتي المعاملة مقارنةً مع مجموعة السيطرة. عيانياً ظهر نزف حبري ، تآكل في متن الكبد ، سجلت تغيرات نسجية مرضية وهي تضخم في الوريد الكبدي وجود كريات دم حمر خارج الاوعية بين الخلايا الكبدية في مجموعة المعاملة (A1)، بينما لوحظ في مجموعة المعاملة (A2) ضمور و تنخر شديد وتحطم في الخلايا الكبدية مع ارتشاح خلايا البيض متعددة الاشكال وتضخم القناة البوبية بافرازات الصفراء.