Research Article

Impact of cypermethrin on blood profile, tissue redox parameters and the observation of histopathological changes in the liver of Rabbit (*Oryctolagus cuniculus*)

Farzeen Saeed^{1*} and Razia Iqbal¹

1. Department of Zoology, University of Gujrat, Punjab, Pakistan

*Corresponding author's email: saeed.frzn@gmail.com

Citation

Farzeen Saeed and Razia Iqbal. Impact of cypermethrin on blood profile, tissue redox parameters and the observation of histopathological changes in the liver of Rabbit (*Oryctolagus cuniculus*). Pure and Applied Biology. Vol. 11, Issue 2, pp468-482. <http://dx.doi.org/10.19045/bspab.2022.110046>

Received: 03/05/2021

Revised: 16/07/2021

Accepted: 27/07/2021

Online First: 26/08/2021

Abstract

The present study was conducted to examine the effects of Cypermethrin on the blood profile, tissue redox parameters and the histopathological changes in the liver of rabbit. 36 male rabbits were randomly divided into three treatment groups and orally administered concentrations of Cypermethrin was provided to them per unit of body weight; Treatment group A; T1 (300mg/kg), Treatment group B; T2 (600mg/kg), Treatment group C; T3 (900mg/kg) and group D; T0 (Control group). The blood profile of these rabbits showed that there was a noteworthy decrease in the Hb, RBCs, Hct, MCV, MCH and MCHC of the rabbits. The number of platelets and WBCS increased as the concentration of Cypermethrin increased. The catalase activity also increased in a dose dependent manner with the highest activity seen at the chemical concentration of 900mg/kg. Histopathological studies showed that Cypermethrin caused necrosis, congestion, vacuolization and tissue degeneration in the livers of almost all the treatment groups. On the basis of these results, it was concluded that Cypermethrin is not safe for animals, so finding alternatives to these pesticides is a prerequisite to prevent toxicity in animals and humans alike.

Keywords: Cypermethrin; Histopathological; Necrosis; Pyrethroids; Tissue redox; Toxicity

Introduction

Pesticides have not only been used to combat pests but have also played a major role in the amelioration of crop yields, worldwide. Pesticides have been devised to kill pests but a large amount of these pesticides and their residues become a part of non-targeted species and their environments, ultimately causing harm to their overall health [1].

Pyrethroids are the pesticides that have been synthetically produced belonging to

two classes i.e. Type 1 and Type 2 which are set apart from one another on the basis of their chemical properties. Cypermethrin, a type 2 pyrethroid has shown incredible insecticidal properties making it one of the best agricultural pesticides [2]. It is a neurotoxin with a rapid activity rate to cause tissue redox damage [3]. Cypermethrin is sprayed on various crops to control insect pests of apple, pears, cabbage, cotton, oats, potatoes and wheat [4]. Cypermethrin has always been

successful in protection of crops throughout the world. But studies conducted in the last few years have shown that it is recognized to negatively impact the non-targeted species including animals and humans, alike.

Cypermethrin enters the body via the inhalation of the chemical spray or by coming in direct contact with the skin. Being lipophilic in nature, it accumulates under the skin, in the liver, kidneys, ovaries and brain [5]. Being a neurotoxin Cypermethrin affects the central nervous system of the insects [6]. It makes the sodium channels open up and prolongs the depolarization of the cell membrane, directing the release of augmented levels of the neurotransmitters. The unending firing of the neurons and the emission of neurotransmitters ultimately causes their reduction. This depletion in turn decreases the activity of amino butyric acid receptor after subsequent convulsions and the body stays in a state of disordered CNS [7].

The most common symptoms of Cypermethrin exposure are dizziness, nausea, vomiting, fatigue, headache and abnormal facial sensations. Hematological, biochemical and physiological systems have been reported to be affected by this chemical [8].

Cypermethrin affects the kidneys by causing inflammation in the renal tubes, shrinkage of glomeruli, hemorrhage and disintegrated Bowman's capsule [9].

It affects the lungs by causing cellular deposition in the extracellular matrix and subsequent reduction in the alveolar pockets. Cypermethrin deposition also leads to hyperplasia, necrosis and pycnosis due to the destruction of alveolar cells and inflammation of lung tissues [10].

Cypermethrin also causes a drastic increase in the LPO production in fishes and boosts up the formation of Reactive Oxygen Species and modification of antioxidant or liberated oxygen. Radical hunting enzyme systems thereby deteriorate the redox parameters [11].

Keeping in mind, the neurotoxic properties

of Cypermethrin and its effects in the body of animals, the current research work was designed to determine its effects on the blood profile, tissue redox parameters and histopathological changes in the liver of rabbit, *Oryctolagus cuniculus*.

Materials and Methods

Selection and handling of animals

36 male rabbits *Oryctolagus cuniculus*, weighing approximately 1.5 kg, were chosen as the research animals. They were acquired from Ghazi Road, Lahore Institute of Veterinary Research Lahore, Punjab, Pakistan. The rabbits were kept in a Zoology lab under monitored conditions. Cleanliness and hygiene of the rabbits was thoroughly checked and animal waste was removed daily to prevent disease or stress in the rabbits. Light settings i.e. 12 h light/dark cycles and temperature setting at 21 to 25°C with careful food and water provision three times a day [12].

Dosage design

The rabbits were separated into four groups on the basis of the Cypermethrin doses to be provided to them. They were Treatment group T₁ (300mg/kg), Treatment group T₂ (600mg/kg), Treatment group T₃ (900mg/kg) and Group D (T₀) i.e. Control group. Each group consisted of 9 rabbits. These groups were treated in their separate enclosures in defined conditions. Moreover, doses were administered with the rabbit food, *ad libitum*.

To minimize the chances of mistakes or inaccuracy, three replications were made for each treatment were. The doses were given regularly to the groups for 44 days, early in the morning. On days 11, 22 and 44, samples were taken two hours after the supply of dose [13].

Collection of blood samples

To avoid stress while collecting blood samples, rabbits were handled carefully and in a calm way, since anxiety can affect the blood profile test. Blood was taken out of the jugular vein so the fur of the rabbit was initially shaved off and exposed region was washed with 70% isopropyl alcohol.

The samples were then collected from the jugular vein of all rabbit groups T₁, T₂, T₃ and T₀. A 5 ml syringe was attached to a butterfly catheter and then attached with an insulin syringe to collect samples in the EDTA tubes. Blood was taken carefully to avoid the blood cell breakdown in the syringe. Silver nitrate was used to stop the bleeding from the site [14].

Hematological tests

EDTA tubes containing EDTA anticoagulants were used for the performance of CBC (complete blood count). These tubes were shaken in order to prevent the chance of blood clotting. All samples were properly labeled. Semi-automatic Chemistry Analyzer (Labomed BAM-100 TS) were used to measure the CBC (Hb, RBCs, HCT, MCV, MCH, MCHC, Platelets and WBCs) of rabbits. Samples were assessed after 24 hours for the best results.

Tissue collection in rabbits for histopathological studies

The rabbits were anesthetized using chloroform. Liver tissues were surgically removed and then thoroughly washed with water and dipped in 0.085 saline solution for the elimination of traces of blood [15]. Organs were then cut into tiny pieces to allow maximum saturation of the chemicals inside the tissue samples. Histopathological method [16] was used to

study the changes in the liver samples of rabbits. The tissue was fixed by dipping in Bouin's fixative for 17 hours and then washed with tap water for an hour to allow the removal of Bouin's fluid from the tissues. They were dehydrated in a series of ascending alcohol concentrations; 70%, 90% and 100% for 15, 15 and 30 minutes respectively. The tissues were then thoroughly washed with xylene. Blocks were made with paraffin wax and were mounted on Microtome (SHIBUYA by optical. Co LTD) to make 6µm sections of the tissues. Water, which had adhered to the tissue sections was removed by a 40°C water bath after which the sections were placed on slides and deparaffinized by washing with xylene. Dehydration in descending manner of alcohol grading was done and Haematoxyline blue and eosin yellow solutions were the selected stains for preparing permanent tissue slides. Coverslips were placed over the slide which were then observed at x100 magnification under Stereo Microscope with a digital camera (FRL Lx400), attached to the system and images were captured for future comparisons.

Measurement of catalase activity

Simple spectrophotometric method [17] was used to measure catalase activity at 550-nm band due to the maximum absorbance on this wavelength (Table 1).

Table 1. Procedure for measurement of catalase activity

Reagents	Test	Standard	Blank
Liver tissue	1000 µl	----	----
Distilled water (Blank)	----	1000 µl	3000 µl
Hydrogen peroxide	2000 µl	2000 µl	----
The contents in the tubes were rotated with a vortex and then kept at 37 °C for 2 minutes. The subsequent reagent was poured in thereafter:			
Working solution	6ml	6ml	6ml
The tubes were rotated in the vortex for 5 seconds and then incubated for 10 min in the dark. Absorbance was recorded at 550 nm against the blank Reagent			

Statistical analysis

SPSS (Statistical Package for Social Science) 21 was used for the analysis of data. In addition, standard error means

(±S.E.M) was calculated. Additionally, variation among groups was determined by using ANOVA.

Results and Discussion

The study revealed that Cypermethrin had a notable impact on the male rabbits. The outcome displayed that the hematological parameters including Red Blood Cells (RBCs), Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) declined in a dose dependent style. Red blood cells may be reduced due to the formation of hypoxic conditions as a result of Cypermethrin exposure. This could lead to increased rate of RBC destruction or a decreased rate of RBC formation. This reduction in the RBC formation could be a result of the non-availability of hemoglobin content in the cellular medium [18]. It is deduced from prior studies that HCT was minimized as a result of erythroblast and the levels of MCV and Hb declined due to microcytic anemia or hypoxic conditions. The reduction in hemoglobin level may be due to two reasons; either an increase in the hemoglobin destruction rate or a decrease in the hemoglobin synthesis rate [19]. Anemia could have been caused because of erythrocyte destruction, induced by

seepage of Cypermethrin into the RBCs. So, reduced number of erythrocytes lower the levels of hemoglobin and hematocrit [20]. However, an increase in the Platelets and the White Blood cells showed that the natural immunity system of the rabbits was activated, trying to cope with the injury caused by the chemical.

Catalase is a crucial antioxidant enzyme which neutralizes hydrogen peroxide [21, 22]. Decreased concentration of purple disubstituted quinine complex in the present study was associated with an increased catalase activity. Highest peaks were formed at 550nm due to the maximum absorbance. These peaks were used to measure the catalase activity. The increased catalase activity showed elevated oxidative stress in the rabbit liver samples as the concentration of the chemical increased.

The histopathological findings of liver samples of rabbits at days 11, 22 and 44 suggested that there was an increasing trend in the damage caused by Cypermethrin with increasing concentration. The maximum results were observed in doses 900mg/kg at 11th, 22nd and 44th days (Table 2).

Table 2. Effect of different dosage of cypermethrin on blood profile of rabbit at 11th, 22nd and 44th day

Concentration Used					
Parameters	Duration	T0 Control	T1 0.4g/kg BW	T2 0.6g/kg BW	T3 0.7g/kg BW
WBCs (x103/ μ l)		10.50 $\pm$ 0.05 ^a	11.00 $\pm$ 0.11 ^b	11.76 $\pm$ 0.08 ^c	11.90 $\pm$ 0.05 ^c
RBCs (x103/ μ l)		5.90 $\pm$ 0.01 ^d	5.45 $\pm$ 0.01 ^c	5.40 $\pm$ 0.01 ^b	4.26 $\pm$ 0.01 ^a
Hb (g/dL)		10.34 $\pm$ 0.08 ^d	9.26 $\pm$ 0.01 ^c	8.36 $\pm$ 0.07 ^b	8.22 $\pm$ 0.12 ^a
HCT (%)		48.93 $\pm$ 0.14 ^c	42.50 $\pm$ 0.20 ^b	42.10 $\pm$ 0.5 ^b	41.80 $\pm$ 0.11 ^a
MCV (fL)		68.00 $\pm$ 0.57 ^c	58.30 $\pm$ 0.11 ^b	57.91 $\pm$ 0.10 ^b	55.30 $\pm$ 0.11 ^a
MCH (pg)		24.03 $\pm$ 0.88 ^d	21.50 $\pm$ 0.28 ^c	20.00 $\pm$ 0.28 ^b	18.50 $\pm$ 0.28 ^a
MCHC (g/dL)		48.26 $\pm$ 0.08 ^d	39.26 $\pm$ 0.14 ^c	36.60 $\pm$ 0.11 ^b	31.00 $\pm$ 0.11 ^a
Platelets (x103/ μ l)	11th Day	335.0 $\pm$ 2.88 ^a	360.00 $\pm$ 5.77 ^b	390.0 $\pm$ 5.77 ^c	420.00 $\pm$ 5.77 ^d
WBCs (x103/ μ l)		10.50 $\pm$ 0.05 ^a	11.80 $\pm$ 0.11 ^b	12.40 $\pm$ 0.11 ^b	12.53 $\pm$ 0.20 ^c
RBCs		5.90 $\pm$ 0.01 ^d	5.41 $\pm$ 0.01 ^c	5.31 $\pm$ 0.05 ^b	4.58 $\pm$ 0.01 ^a

(x103/ μ L)					
Hb (g/dL)		48.93 $\pm$ 0.14 ^c	41.95 $\pm$ 0.02 ^b	41.88 $\pm$ 0.01 ^b	41.40 $\pm$ 0.11 ^a
HCT (%)		36.21 $\pm$ 0.57 ^a	37.77 $\pm$ 0.61 ^a	38.07 $\pm$ 0.89 ^b	39.67 $\pm$ 0.04 ^b
MCV (fL)		68.00 $\pm$ 0.57 ^c	58.00 $\pm$ 0.05 ^b	57.30 $\pm$ 0.11 ^b	54.80 $\pm$ 0.05 ^a
MCH (pg)		24.03 $\pm$ 0.88 ^d	19.00 $\pm$ 0.28 ^c	17.50 $\pm$ 0.28 ^b	16.00 $\pm$ 0.28 ^a
MCHC (g/dL)		48.26 $\pm$ 0.08 ^d	38.73 $\pm$ 0.08 ^c	33.20 $\pm$ 0.05 ^b	27.60 $\pm$ 0.05 ^a
Platelets	22th Day	335.0 $\pm$ 2.88 ^a	400.00 $\pm$ 5.77 ^b	430.0 $\pm$ 5.77 ^c	460.0 $\pm$ 5.77 ^d
WBCs (x103/ μ L)		10.50 $\pm$ 0.05 ^a	12.60 $\pm$ 0.11 ^b	12.80 $\pm$ 0.05 ^c	13.31 $\pm$ 0.08 ^c
RBCs (x103/ μ L)		5.90 $\pm$ 0.01 ^d	5.39 $\pm$ 0.01 ^c	4.91 $\pm$ 0.04 ^b	4.45 $\pm$ 0.05 ^a
Hb (g/dL)		10.34 $\pm$ 0.08 ^d	8.77 $\pm$ 0.04 ^c	7.94 $\pm$ 0.03 ^b	7.20 $\pm$ 0.11 ^a
HCT (%)		48.93 $\pm$ 0.14 ^c	41.70 $\pm$ 0.05 ^b	41.71 $\pm$ 0.01 ^b	40.96 $\pm$ 0.13 ^a
MCV (fL)		68.00 $\pm$ 0.57 ^c	57.60 $\pm$ 0.11 ^b	56.80 $\pm$ 0.11 ^b	53.93 $\pm$ 0.14 ^a
MCH (pg)		24.03 $\pm$ 0.88 ^d	17.50 $\pm$ 0.28 ^c	16.00 $\pm$ 0.28 ^b	14.50 $\pm$ 0.28 ^a
MCHC (g/dL)		48.26 $\pm$ 0.08 ^d	37.50 $\pm$ 0.11 ^c	32.20 $\pm$ 0.11 ^b	22.93 $\pm$ 0.08 ^a
Platelets	44th Day	335.0 $\pm$ 2.88 ^a	440.0 $\pm$ 5.77 ^b	480.0 $\pm$ 5.77 ^c	520.0 $\pm$ 5.77 ^d

The (Figs. 1-8) for hematological parameters exhibited a pronounced difference in the values expressed by the treatment groups in comparison to the control group. Statistical analysis also indicated that at different concentrations of Cypermethrin (300, 600 and 900mg/kg) the values were lower than normal range. The maximum reduction was seen at 900mg/kg at 44th day with values Hemoglobin (7.20 $\pm$ 0.11), RBCs (4.45 $\pm$ 0.05), HCT (40.96 $\pm$ 0.13), MCV (53.93 $\pm$ 0.14), MCH (14.50 $\pm$ 0.28) and MCHC (22.93 $\pm$ 0.08) respectively. Platelets and the White Blood Cells increased in a dose dependent manner and were above the normal level in comparison with the control group with the mean values at 520.0 $\pm$ 5.77 and 13.31 $\pm$ 0.08 at 44th day, respectively. The results suggested that maximum change was observed in treatment group T3 due to the highest concentration (i.e. 900mg/kg) of Cypermethrin administered to it. These findings were compatible with those of [23] who used Cypermethrin at concentrations 25, 50 and 75 mg/Kg respectively. The

results marked a reduction in RBC counts, the concentration of Hemoglobin and PCV in rabbits indicating that they were suffering from anemia. Similar results were reported by [19] who exposed *Cyprinus carpio* at 0.02 ppm of Cypermethrin. They saw a decrease in RBC number and hemoglobin content, suggesting that pesticides have toxic effects on the erythropoietic tissue which result in the reduced viability of the cells. In another research performed by [24], studied the effects of cypermethrin (24 mg/kg body weight) on male rabbits. Their results suggested that cypermethrin reduced hemoglobin (Hb), packed cell volume (PCV) and total erythrocytic count (TEC). Furthermore, [25] reported that a diminution of -5.8% in Hb, -5.5% in HCT and -0.5% in MCV level was seen in male rabbits after cypermethrin exposure. They suggested that both of these chemicals encouraged modifications in hematological indices and are proposed to be neurotoxic with leukemic predisposition for rabbits and other vertebrates including humans.

Figure 1. Effect of Cypermethrin on Hemoglobin of Rabbit at the Time Scale 11th, 22nd and 44th Day

Figure 2. Effect of Cypermethrin on RBCs of Rabbit at the Time Scale of 11th, 22nd and 44th Day

Figure 3. Effect of Cypermethrin on Hematocrit of Rabbit at the Time Scale of 11th, 22nd and 44th Day

Figure 4. Effect of Cypermethrin on MCV of Rabbit at the Time Scale of 11th, 22nd and 44th Day

Figure 5. Effect of Cypermethrin on MCH of Rabbit at the Time Scale of 11th, 22nd and 44th Day

Figure 6. Effect of Cypermethrin on MCHC of Rabbit at the Time Scale of 11th, 22nd and 44th Day

Figure 7. Effect of Cypermethrin on Platelets of Rabbit at the Time Scale of 11th, 22nd and 44th Day

Figure 8. Effect of Cypermethrin on WBCs of Rabbit at the Time Scale of 11th, 22nd and 44th Day

The (Figs. 9-12) clearly displayed that the catalase activity significantly increased in all the treatment groups in a dose dependent manner. However when the results of all the groups were compared, the treatment group C(T3) which was administered 900mg/kg of Cypermethrin showed the highest value for catalase activity at days 11, 22 and 44 with values 23.60 ± 0.11 , 29.00 ± 0.11 and 31.03 ± 0.14 respectively. Catalase causes the breakdown of hydrogen peroxide and releases oxygen. The increased catalase activity showed elevated oxidative stress in the rabbit liver samples as the concentration of the chemical increased. The results coincided with [26] who expressed in their results the elevated catalase activity in the zebra fish was linked to high levels of CAT countenance, an adaptive reaction in fish body to counterbalance the generated reactive species. Similarly, [27] shared their results

which reported an increase in catalase activity after 30 days of chemical exposure to rats. This increased catalase activity allows the scavenging nature of erythrocytes to convert the hydrogen peroxide to molecular oxygen and water. Likewise, [28] studied the effects of cypermethrin on freshwater mussel *Unio elongatulus eucirrus*; suggesting that level of catalase enzyme was raised after Cypermethrin exposure at concentrations 5, 10, 20, 40, 80, and 160 $\mu\text{g/L}$, for 1, 24, 48, 72, and 96h. The active periods of fishes had also shortened concluding that the insecticide cypermethrin has harmful effects not only on non- target aquatic arthropods and fish, but also on mollusks. [29] announced similar results that a diet containing Cypermethrin at concentration 0.02mg/kg/day induced oxidative stress and transformed oxidant-antioxidant status in the cells of not only the pregnant rats but also in their newborns.

Figure 9. Effect of Cypermethrin on Catalase Activity of Rabbit at the Time Scale of 11th, 22nd and 44th Day

Figure 10. Histopathological changes observed upon Cypermethrin exposure at different concentrations (Control, 300, 600, 900 mg/kg) on 11th Day in the Rabbit Liver (x100 Magnification)

Figure 11. Concentration and Time Dependent Effects of different concentrations of Cypermethrin (Control, 300, 600, 900 mg/kg) on 22nd Day in the Rabbit Liver(x100 Magnification)

Figure 12. Concentration and Time Dependent Effects of different concentrations of Cypermethrin (Control, 300, 600, 900 mg/kg) on 44th Day in the Rabbit Liver (x100 Magnification)

Photomicrograph sections of rabbit livers at day 11. (A) Control group showing the normal histopathological structures present in the liver. (B) Cypermethrin concentration at 300mg/kg showing tissue lysis (diamond), infiltration (arrow) and vacuolation (triangle). (C) Cypermethrin concentration at 600mg/kg showing congestion of cells (arrow) and tissue lysis (triangle). (D) Cypermethrin concentration at 900mg/kg showing tissue degeneration (diamond), congestion (half triangle) and vacuolization (triangle).

Photomicrographs sections of rabbit livers at day 22. (A) Control group showing the normal histopathological structures in the liver. (B) Cypermethrin concentration at 300mg/kg showing necrosis (diamond), infiltration (arrow) and cell coalition (half triangle). (C) Cypermethrin concentration at 600mg/kg showing congestion of cells (arrow), hyperplasia of binary epithelium (half triangle) and vacuolization (triangle). (D) Cypermethrin concentration at 900mg/kg showing necrosis (arrow),

congestion (diamond), vacuolation (triangle) and infiltration (half-triangle).

Photomicrographs sections of rabbit livers at day 44. (A) Control group showing the normal histopathological structures present in the liver. (B) Cypermethrin concentration at 300mg/kg showing infiltration (diamond), necrosis (arrow) and tissue lysis (triangle). (C) Cypermethrin concentration at 600mg/kg showing Portal area with hepatocyte infiltration (diamond), tissue degeneration (half triangle) and vacuolation (triangle). (D) Cypermethrin concentration of 900mg/kg showing tissue lysis (arrow), congestion (diamond) and necrosis (half triangle).

The photomicrograph sections of the rabbit livers showed significant changes that occurred in a dose dependent manner. Tissue degeneration can be seen in all treatment groups. Similarly, the incidence of infiltration, necrosis and vacuolization was a recurrent phenomenon with increasing chemical concentration. The results were similar to that of [30] who

performed a study by using 50, 100 and 150mg/kg of cypemrthin and concluded that Cypermethrin at various doses administered during a certain period of time generated moderate histological lesions in liver of rabbits. Likewise, [9] reported that cypermethrin in 5, 7.5 & 10 ml/kg doses of the body weight of mice demonstrated enlargement of the sinusoidal space, formation of vacuoles in hepatocytes, infiltration and congestion of blood vessels with hemorrhage in hepatic tissues. Similarly, [31] exposed *Clarias gariepinus* (Burchell, 1822) to cypermethrin concentrations (10.05, 20.10 and 30.15 microg/L) for ten days. They observed lesions in the liver tissues of fish characterized by the swelling of hepatocytes, pyknotic nuclei, lipoid vacuoles and necrosis. [32] expressed that at a Cypermethrin diet containing 7.5, 15 and 30 mg/kg body weight; blood congestion in hepatic vein, diffusion of some inflammatory cells, sinusoidal dilatation, diffusion of inflammatory cells, hemorrhage, distortion in the arrangement cells, hypertrophy of hepatocytes, cellular degeneration and necrosis, presence of some pyknotic cells and cytoplasmic vacuolation were observed in liver of rats.

Conclusion

The present study revealed that Cypermethrin caused a pronounced ($P \leq 0.05$) reduction in the Blood profile level in a dose dependent style of male rabbits in comparison to the control group. The tissue redox parameters showed a significant increase. Comparison between the treatment groups and the control group showed that as the concentration level of the chemical increased, so did the catalase activity. It was due to the stress caused by the chemical in the rabbit body systems their body's natural mechanism to control it. Histopathological results presented that there was noteworthy damage caused to the morphology and internal structures of the livers of rabbits in this study. As a result, it is concluded that if the use of Cypermethrin as an insecticide is continued, it will not

only affect the insects and animals that eat those crops but also humans. Therefore, the need of the hour is to look for replacements to these harmful pesticides which do not pose a threat to the living organisms.

Authors' contributions

Conceived and designed the experiments: F Saeed, Performed the experiment: F Saeed, Analyzed the data: F Saeed & R Iqbal, Contributed materials/ analysis/ tools: R Iqbal, Wrote the paper: F Saeed.

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