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Research article

Serological effects of cypermethrin on the kidneys of rabbit (*Oryctolagus cuniculus*)

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Abstract

Cypermethrin a commonly used synthetic pyrethroid insecticide, has become a big concern due to its harmful impact on animals. In this study, cypermethrin's (CY) serological effects were studied on the kidneys of rabbit (*Oryctolagus cuniculus*). Twenty rabbits were divided into four groups including three experimental (G_2, G_3 & G_4) and one control group (G_1). The three experimental groups were exposed to 500mg/kg (G_2), 1000mg/kg (G_3), and 1500mg/kg (G_4) of dose of cypermethrin respectively, every day for fifteen days. Blood sampling was done on the 5th, 10th, and 15th day of the experiment, the serum was separated from blood samples and two parameters including urea and creatinine were studied. The results indicated that both Urea and Creatine values were significantly different ($p \leq 0.05$) in all three experimental groups that were given cypermethrin treatment compared to the control group. In conclusion, cypermethrin is highly dangerous for mammals including rabbits when exposed to high doses.

Keywords: Cypermethrin, rabbit, urea, creatinine, serological

1. Introduction

Although insecticides are essential to agricultural productivity, their toxicity raises some health risks for people and other animals [1]. Similar to this, organophosphorus insecticides are widely used for a variety of agricultural and public health practices [2]. These pesticides cause toxicity in mammals by inhibiting the activity of acetylcholinesterase, which causes acetylcholine to accumulate in synaptic junctions and causes cholinergic toxicity through excessive stimulation of postsynaptic cells [3].

Since pyrethroids are extremely stable substances, repeated ingestion can result in systemic toxicity. The pyrethroids enter the body through the skin and enter the peripheral sensory nerves. Class II pyrethroids, which include cypermethrin (CY) and malathion, are the most widely used pyrethroids [4]. Numerous pyrethroids alter biochemistry and physiology in addition to causing hematological modulations [5]. A powerful, all-purpose pesticide, cypermethrin is widely sprayed on cotton, vegetables, and other crops as an insecticidal spray. Similarly, Ecofleece and other veterinary products are based on cypermethrin, which is commonly used for dipping or spraying food animals. Additionally, Pakistan uses cypermethrin extensively [6,7].

Rabbits are considered important livestock and are being raised in more and more nations across the world [8]. These animals have been purposefully released to provide meat and fur. However, the main obstacles to the sustainability of rabbit farming are illnesses [9]. Hematological studies are thought to be the primary method for selecting animals that are genetically resistant to specific diseases and environmental conditions. They are also helpful in ecological and physiological research to comprehend the relationship of blood characteristics to the environment [10,11]. Hematological parameter changes are frequently used to assess the body's overall health and identify stress brought on by dietary, pathological, and environmental factors [12].

Cypermethrin has been shown in studies to cause itching, restlessness, shaking of the head, and other nervous disorders in rabbits, as well as affecting hormones, testicular weights, sperm concentration, and motility [13]. According to a different study, cypermethrin is unsafe to use and negatively impacts the health of female rabbits [7]. Additionally affecting animals' nervous

systems, cyclomethrin is regarded as a stomach poison [14]. Because the liver enzymes that break down cypermethrin in the body are not fully developed in newborns, it has been discovered that newborn animals are more susceptible to cypermethrin than adult animals [15]. It results in early embryonic demise [16].

The kidney is the body's principal organ and plays a variety of biological roles. It is essential for growth, development, health, and illness [17]. The kidneys process blood plasma and eliminate urine, which is essential for the body's removal of xenobiotics, which include drugs and drug products. Estimating certain metabolic waste products (like urea and creatinine), which are entirely eliminated by the kidneys, can reveal important information about the health of people with nephrotic kidneys [18].

It is commonly known that the kidneys are essential for helping mammals remove pesticide residues from their bodies. Cypermethrin damages the kidneys by dissolving Bowman's capsule, shrinking glomeruli, inducing hemorrhage, and inflaming the renal tubes [19]. It has an effect on the lungs by reducing the alveolar pockets and causing cellular deposition in the extracellular matrix. Because cyclomethrin destroys alveolar cells and inflames lung tissues, it also causes hyperplasia, necrosis, and pycnosis [20]. Additionally, cypermethrin dramatically increases fish LPO production, promotes the emergence of reactive oxygen species, and modifies antioxidant or liberated oxygen. Thus, the redox parameters are deteriorated by radical hunting enzyme systems [21].

Dose-dependent frequency and incidence of hemorrhages in renal tubules, various stages of degeneration, cast deposition, and increased urinary spaces were observed in rabbits exposed to CY at different doses. These are all considered to be mild histological lesions [22]. It was discovered that the rabbits given CY had pink homogeneous tubular casts in their kidneys and fat deposition and necrosis in their livers [23]. Male New Zealanders receiving diazinon treatment Conversely, rabbits resulted in leucocytic infiltrations in the kidneys and liver, hypertrophy of glomeruli, and degeneration of renal tubules [24].

Because pyrethroid insecticides like cypermethrin are so widely used in both agricultural and residential settings, there is growing concern about their use. Given that animal kidneys are the primary target organs for many xenobiotics, research into the potential negative effects of CY on these systems is particularly appealing given the growing use of CY in agriculture.

The current study is therefore planned to examine the potential serological effects of cypermethrin on the kidneys of rabbits. Blood sampling was done, and renal tests were performed on the serum, indicating the levels of Urea and Creatine due to exposure to cypermethrin. The results of this study can provide significant values and references in further exploring the impact of CY on the renal system of animals.

2. Materials and Methods

All the experimental set-ups were arranged at the Toxicology Laboratory of the Zoology Department, Government Post Graduate Islamia College (W), Cooper Road, Lahore.

2.1. Experimental animals

For this study, a total of twenty rabbits belonging to the local species *Oryctolagus cuniculus* were selected. For this purpose, adult rabbits of weight (approx. 800-1000g) were purchased from the local market in Lahore, Pakistan, and were transported to the Animal House of Zoology Department in perforated nets. All the rabbits were scrutinized for infections. The rabbits were divided into four groups including three experimental (G2, G3 & G4) and one control group (G1), thus each group contained 5 rabbits.

2.2. Experimental Protocol

Group 1 was designated as a control and fed with no cypermethrin. Group 2 animals were fed with 500 mg/kg cypermethrin, Group 3 with 1000 mg/kg, and Group 4 with 1500 mg/kg cypermethrin.

The housing of animals

Rabbits were kept in properly aerated, clean, and dry hutches. The dimensions of each hutch were 100×60×60 cm. The hutches were equipped with plastic containers for water. These cages were kept properly clean. Cleaning was done with

Alcohol twice a day, daily. During the acclimatization period, they were given regular diet and water. They were protected from severe climatic conditions such as heat and rain. Prior to experimentation, animals were given four days to acclimatise to the new laboratory settings. Carrots, veggies, fruit pulp, green feeder, Berseem fodder (Trifolium alexandrium), and drinking water make up the base diet.

2.3. Toxicant used

In this study, a toxicant known to be somewhat toxic to mammals [23]—cyclomethrin (CY)—was employed. Its molecular formula is C22 H19 C12 NO3, and its chemical formula is (R, S)-alpha-cyano 3-phenoxybenzyl-2,2-dimethyl (1R,1S)-cis, trans2, 2dichlorovinyl) cyclopropane carboxylate.

2.4. Toxicant doses

In this study, a unique pattern of selecting doses was based on LD50 (300 mg/kg B.W) of cypermethrin reported by Secaucus [25].

2.5. Weight Determination

All rabbits' weight was determined with an electric balance (SHIMADZU BX-300). Initial body weight per animal was measured and recorded at the start of the experiment. This practice was continued throughout the experiment. Every time before blood sampling, the body weight of animals was recorded.

2.6. Blood sampling

Blood sampling was done on the 5th, 10th and 15th day of the experiment. Blood samples were collected from the marginal ear vein of rabbits from each group (control group and experimental group) after the treatment. The sampling was done with an interval of 5 days. Xylene was applied to the ear vein before drawing the blood to make the vein more prominent. The site of the vein puncture was cleaned properly with alcohol to avoid infections. Butterfly tubes with sterilized syringes were used for blood sampling. Nearly, 5ml of blood was drawn out, and after extracting the blood was transferred into properly labeled test tubes immediately.

Table 1. Treatment schedule of various groups.

Groups	Treatment
G_1 (Control)	Feed+ water
G_2	Feed + 500mg/kg Cypermethrin + water
G_3	Feed+1000mg/kg cypermethrin+ water
G_4	Feed+1500mg/kg cypermethrin+ water

Note: G_1= Group 1; G_2= Group 2; G_3= Group 3; G_4= Group 4

Table 2: Urea test procedure

	Blank	Calibrator	Sample
Reagent 1	1000µl	1000µl	1000µl
Sample	……..	……	10µl
Calibrator	……	10µl	……
Reagent	200µl	200µl	200µl

Table 3: Creatinine test procedure

	Blank	Calibrator	Sample
Reagent 1	500µl	500µl	500µl
Sample	……..	……	50µl
Calibrator	……	50µl	……
Reagent	500µl	500µl	500µl

2.7. Serum separation

For two hours, the blood sample tubes were left to clot at room temperature [32]. After that, the clotted blood was centrifuged for 15 minutes at 3000 rpm. Following centrifugation, the serum with a light straw colour was separated from the cellular portion of the blood and fibrinogen that accumulated in the bottom of the centrifuge tube. The serum was then moved using a micropipette to sterile serum tubes. Tubes were labeled and sealed for storage at the end. Each rabbit's serum was kept in a freezer at 4°C until additional biochemical measurements were made [32].

2.8. Renal function test (RFTs)

Renal function tests were performed by Kits (Audit Diagnostics, Ireland).

2.9. Principle of Urea test

When urease is present, urea is transformed into ammonia. Then, in the presence of glutamate dehydrogenase (GLDH), ammonia is linked to alpha-ketoglutarate, resulting in the conversion of NADH into NAD. The amount of urea present in the sample directly correlates with the rate of NADH consumption.

Enzymatic determination according to the following reactions.

$$Urea + H_2O \longrightarrow 2NH_3 + CO_2$$

$$2NH_3 + 2\alpha\text{-ketoglutarate} + 2NADH^+ \xrightarrow{GLDH} 2Glutamate + 2NAD^+ + 2H_2O$$

2.10. Test procedure

The test was performed at Wavelength (340nm (Hg 334), Temperature (25 ^oC,30 or 37 ^oC), and Optical path (1cm light path). The reagents were mixed and the change in Optical density (OD/min) was measured between 40-300 seconds.

2.11. Principle of creatinine test

A coloured complex is formed when creatinine and picric acid combine in an alkali solution. The amount of creatinine in the sample directly correlates with the complex formation rate, which is measured.

Creatinine + picric acid alkali sol. ⟶ creatinine-picric acid complex

2.12. Statistical analysis

The standard deviation of the mean value was used to represent all the data. Fourteen versions of SPSS software were used to analyze the data. One-way analysis of variance (ANOVA) was used to analyze the data. After comparing the mean values, values with a significance level of less than 0.05 were deemed important.

3. Results

In the present study, the serological effects of cypermethrin (CY) on the kidneys of rabbits (*Oryctolagus cuniculus*) were studied. The serum was separated from blood samples and two parameters including urea and creatinine were studied.

3.1. Urea

G_1(Control): In group 1 (kept as control), the values of urea on the 5th, 10th, and 15th day of the experiment were 17.45 ± 0.10, 19.49 ± 1.35, 19.87 ± 1.07 mg/dl respectively. There was no significant change ($p<0.05$) in the concentration of urea at different days of the experiment.

G_2 (Experimental): In group 2, urea values on the 5th, 10^{th,} and 15th day of the experiment were 32 ± 1.15, 55 ± 5.77, and 83.33 ± 4.04 mg/dl respectively as shown in Table 4. There was a significant increase ($p<0.05$) in the value of Urea as compared to the control group.

G_3 (Experimental): In the rabbits of group 3, the urea values on the 5th, 10^{th,} and 15th day of the experiment were 46.66±4.04, 68.33±4.04 and 92.33±6.48 mg/dl respectively as shown in Table 4. There was a significant increase ($p<0.05$) in the value of urea as compared to the control group.

G_4 (Experimental): Rabbits of group 4 were kept as experimental groups and were fed with 1500 mg/kg of cypermethrin. Urea values on the 5th, 10th, and 15th day of the experiment were 65± 2.88, 85± 2.88, and 100± 2.88 mg/dl as shown in Table 4.

3.2. Creatinine

G_1 (control**):** In the rabbits of group 1, the values of creatinine on the 5th,10th, and 15th day of the experiment were 0.53± 0.37,0.59± 0.22, 0.58± 0.11 mg/dl. There was no significant change ($p<0.05$) in the concentration of creatinine at different days of the experiment.

Table 4. Urea value (mg/dl) in rabbits following cypermethrin intoxication.

S. No.	Sampling	G1 (Control)	G2 (500mg/kg)	G3 (1000mg/kg)	G4 (1500mg/kg)
1	5th	17.45±0.01	32±1.15	46.66±4.40	65±2.88
2	10th	19.49±1.35	55±5.77	68.33±4.40	85±2.88
3	15th	19.87±1.07	83.33±4.40	92.33±6.48	100±2.88

Table 5. Creatine value (mg/dl) in rabbits following cypermethrin intoxication.

S. No.	Sampling	G1 (Control)	G2 (500mg/kg)	G3 (1000mg/kg)	G4 (1500mg/kg)
1	5th	0.53 ± 0.37	3.2±0.11	46.66±4.40	5.33±0.24
2	10th	0.59±0.22	4.63±0.08	5.7±0.15	6.43±0.12
3	15th	0.58±0.11	7.4±0.11	8.33±0.08	9.56±0.18

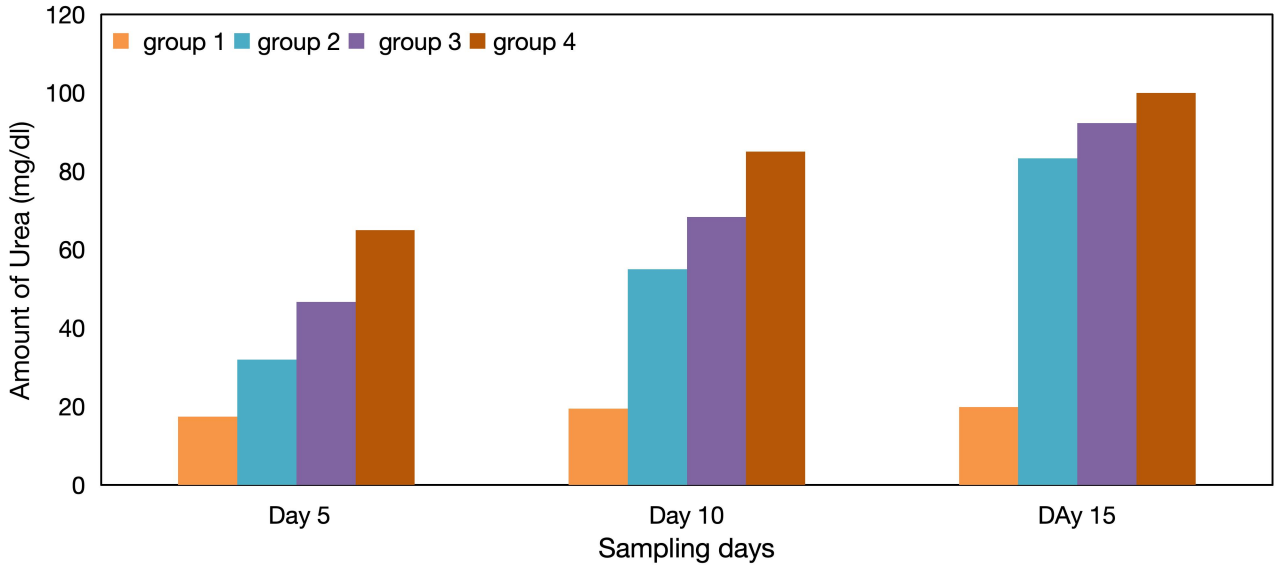

Figure 1. Mean values of urea concentration (mg/dl) in the control and experimental group.

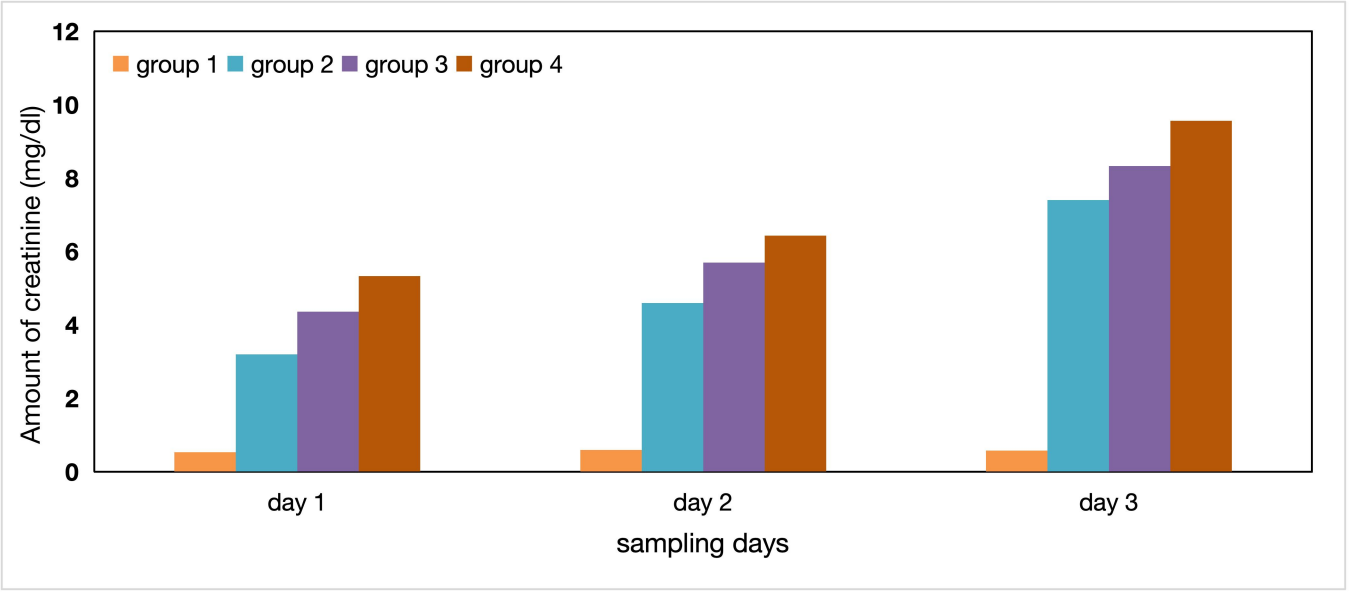

Figure 2. Mean values of creatinine concentrations (mg/dl) in control and experimental groups.

G_2 (Experimental): In group 2 the creatinine values on the 5^{th},$10^{th,}$ and 15^{th} day of the experiment were 3.2± 0.11, 4.63± 0.08, and 7.4±0.11 mg/dl as shown in Table 5. there was a significant increase in the value of creatinine as compared to the control group.

G_3 (Experimental): In group 3. The creatinine values on the 5^{th},$10^{th,}$ and 15^{th} day of the experiment were 4.36± 0.12, 5.7± 0.15, 8.33± 0.08 mg/dl as shown in Table 5. There was a significant increase in the values of creatinine as compared to the control group.

G_4 (Experimental): In the rabbits of group 4, the creatinine values on the 5^{th},$10^{th,}$ and 15^{th} day of the experiment were 5.33±0.24, 6.43±0.12, 9.56±0.18 mg/dl as shown in table 3.2. There was a significant increase in the value of creatine as compared to the control group.

4. Discussion

Despite the possibility of negative health effects, pyrethroids, such as cypermethrin (CY), are still widely used in agriculture and animal husbandry [7]. The use of pyrethroids has significantly increased over the past ten years [22]. The purpose of this study was to investigate the serological effects of cypermethrin on rabbit (Oryctolagus cuniculus) kidneys. Cypermethrin is one of the pyrethroids that the body absorbs most quickly [26]. Due to the fast metabolism of pyrethroids in the body, changes in the animals exposed to them can be considered immediate. Because CY is smaller and lipid soluble, it can easily pass through cell membranes and damage DNA by causing chromosomal injuries as well as the destabilisation and unwinding of the DNA helix [27].

Waste products of protein metabolism, urea and creatinine are absent from blood due to cellular damage [28]. As a result, these biochemical markers are the most sensitive for identifying renal damage [18]. Hemorrhages, malabsorption, liver failure, and renal and intestinal protein loss are the main causes of decreased serum protein levels [6]. Therefore, the analysis of proteins in animals treated with CY may provide valuable insights regarding hepato-renal toxicity [7].

All three experimental groups in the current study showed a significant increase ($P \leq 0.05$) in their urea and creatinine levels, a classic indication that CY exposure had a negative impact on the kidneys of Oryctolagus cuniculus. Our findings are in line with some earlier research; for instance, an investigation into the histopathological changes caused by CY in rat kidneys found that experimental groups receiving CY had higher levels of urea and creatinine [29]. A different study found that rabbits exposed to CY had biochemical changes in their serum samples as well as a dose- and time-dependent rising trend in their urea and creatinine concentrations [22].

Since renal damage is the only significant factor that raises serum creatinine in mammals, creatinine is more specific to the kidneys [18]. Similar to numerous other metabolic waste products, the majority (> ¾th) of creatinine is eliminated from the body via glomerular filtration, with the remaining portion (<25%) being eliminated through tubular secretion [30]. Therefore, changes in these two mechanisms may be the cause of the elevated creatinine concentrations in the serum [31]. Because the kidneys also excrete urea, urea from the blood is less easily excreted into urine when kidney function is compromised [31].

Blood volume drops along with erythrocytes, which may be the cause of the anemia seen in animals treated with CY [31]. However, white blood cells and other inflammatory cell types rise, increasing the body's susceptibility to infections [18]. The kidneys' capacity to remove waste materials from the blood and filter them out is reduced by changes in the cellular structure of the kidneys. As a result, blood levels of creatinine and urea were raised and clearance values for these substances may have decreased in CY-treated animals [13]. The administration of CY at different doses resulted in moderate histological lesions in the kidneys, as well as elevated levels of urea, creatinine, and other enzymes and lowered protein levels in serum samples. The majority of these modifications were dose- and time-dependent.

Body weight, total leukocyte count, lymphocyte count, serum total protein, serum albumin, serum globulin, antibody titer against sheep red blood cells, and cell-mediated immunity

were all significantly decreased after subchronic exposure to cypermethrin [32]. The study's observed significant increase ($P \leq 0.05$) in urea and creatinine levels is a classic indicator that CY exposure negatively impacted the kidney. The kidneys' capacity to remove waste materials from the blood and filter them out is reduced by changes in the cellular structure of the kidneys. As a result, blood levels of creatinine and urea may have increased and clearance values for these substances may have decreased in rabbits given CY.

5. Conclusion

The present study demonstrated serological effects of cypermethrin on the kidneys of rabbits (*Oryctolagus cuniculus*). For this purpose, three experimental groups were made, and were exposed to various doses of cypermethrin, then serum was separated from the blood of these rabbits and two parameters including urea and creatine were explored. The results indicated that both urea and creatine values were significantly different ($p \leq 0.05$) in all three experimental groups as compared to the control group. Thus, the present study concludes that cypermethrin, which is an organophosphorus insecticide, is highly dangerous for mammals including rabbits when subject to elevated dosage. Based on the detrimental effects of pesticides on non-targeted organisms, proactive measures should be taken to address their use by collective efforts in research, regulation, and education, thereby promoting a more sustainable and healthier environment. Additionally, further studies are to be carried out on diverse model animals for a deeper knowledge of the subject.

Data Availability statement

The data that were analyzed in the present article are available upon justifiable request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Habiba conceived, collected, and analyzed data. Maham Rafiq, Khadeeja Akram, Areeba Sabtain, Shanza Nazar, and Mahnoor Raja wrote the manuscript. Dilawar Hussain and Zahra Hussain read, edited, and approved the final manuscript.

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