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EFFECT OF CYPERMETHRIN ON CLINICO-HAEMATOLOGICAL PARAMETERS IN RABBITS

M. KAMAL SHAH, A. KHAN, F. RIZVI, M. SIDDIQUE[1] AND SADEEQ-UR-REHMAN[2]

Department of Pathology, [1]Department of Microbiology, University of Agriculture, Faisalabad;
[2]Department of Animal Health, Faculty of Veterinary Sciences and Animal Husbandry,
NWFP Agricultural University, Peshawar, Pakistan

ABSTRACT

The aim of this study was to know the clinical and haematological changes in female rabbits following the treatment with cypermethrin (CY). In this study, 24 female rabbits were divided randomly into four equal groups A, B, C and D. Groups A, B and C were injected intraperitoneally CY @ 25, 50 and 75 mg/Kg b. wt., respectively, while the group D served as control and received normal saline. Rabbits in each group received four treatments at an interval of five days. Blood samples with EDTA were collected after two days of each treatment for haematological studies. All the animals were monitored twice daily for clinical signs. Signs like increased urination, licking of legs, jerky movements, ataxia, incoordination, staggering gait and dizziness persisted for 30 to 90 minutes after each dose. Data analysis revealed that CY affected the haematological parameters in dose dependent manner. There was significant ($P<0.05$) decrease in RBC counts, Hb concentration and PCV in treated animals indicating that rabbits were suffering from anaemia. There was significant ($P<0.05$) increase in TLC, lymphocytes and MCV in all treated groups. On the basis of these results, it was concluded that CY is not safe for animals in high doses.

Key words: Rabbits, cypermethrin, clinical signs, haematology.

INTRODUCTION

Pyrethroid insecticides have been used in agricultural, veterinary and home formulations for more than 40 years and account for approximately one-fourth of the worldwide insecticide market (Shafer *et al.,* 2005). The WHO recommends that pesticides of acute toxicity (class Ia or Ib) should not be used in developing countries (Plestina, 1984). However, in Pakistan pyrethroid insecticides including class II (cypermethrin) are extensively used. Pesticide market in Pakistan comprises 88% insecticides, 11% herbicides and one percent fungicides (Husain, 2002). These pyrethroids are photostable with a half-life of 8-16 days in direct sunlight. In soil and water, their half-life is as long as 56 and 100 days, respectively.

In spite of wide range of effectiveness, cypermethrin (CY) is not free from side effects. Signs like muscular tremors, ataxia, weakness of limbs, convulsions, coma and death from respiratory depression have been reported after ingesting high doses of CY, while its dermal contact in the facial area may cause a subjective sensation of tingling or numbness (Sandhu and Brar, 2000).

Haematological values are widely used to determine systemic relationship and physiological adaptations including the assessment of general health condition of animals. Most studies on the effects of pyrethroids are confined to reporting biochemical and physiological changes, and little attention has been paid to the haematological modulations induced by these pesticides (Atamanalp *et al.,* 2002), except a few reports in rabbits (Yousef et al., 2003) and Wistar rats (Sayim *et al.,* 2005).

Although enough information is available about CY toxicity from different regions of the world, yet little work has been accomplished on CY toxicity especially in relation to clinical signs/symptoms and haematological changes in experimental animals under local conditions. This paper describes the above mentioned parameters as affected by CY treatment in rabbits.

MATERIALS AND METHODS

This study was carried out on 24 adult, apparently healthy female rabbits procured from the local market at Faisalabad, Pakistan. These animals were kept under similar management conditions. Green seasonal fodder and water were available *ad libitum* around the clock. After five days of acclimatization, rabbits were randomly divided into four equal groups A, B, C and D. Rabbits of groups A, B and C were injected intraperitoneally cyprmethrin (CY) @ 25, 50, and 75 mg/kg b. wt., respectively. Animals of group D served as control and received normal saline. Each group

received 4 injections of respective treatment at 5 days interval. Experiment continued for 17 days.

The animals in each group were monitored for clinical signs and behavioral alterations twice daily (morning and evening) till the end of experiment. Blood samples with anticoagulant (EDTA) from all animals were collected from jugular vein two days after each treatment i.e. days 2, 7, 12 and 17 after the start of treatment. These blood samples were analyzed for haematological parameters including red blood cells (RBC) counts, haemoglobin (Hb) concentration, packed cell volume (PCV), total leukocyte counts (TLC) and differential leukocyte counts (Benjamin, 1978). Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were also computed.

Data thus collected were subjected to two-way analysis of variance for groups and days of treatment. Different group means were compared by Duncan's multiple range test.

RESULTS

Five to 10 minutes after the administration of cyprmethrin (CY) dose, the animals started showing clinical signs like itching, restlessness, muscular tremors, jerky movements, skin scratching, salivation and increased urination. Three animals (50%) remained off feed for 16-24 hours in group C. Nervous signs consisted of incoordination, ataxia, staggering gait and dizziness and persisted for about 30 to 90 minutes. These signs appeared in CY treated rabbits in dose dependent manner.

In all CY treated groups, RBC counts decreased significantly ($P<0.05$) on 12 and 17 days of experiment compared to control group (Table 1). There was also a decrease in Hb concentration on day 12 and 17 in groups B and C, while PCV decreased on day 12 in groups B and C and day 17 in group C only, compared to control group. Dose dependant gradual decrease in RBC counts, Hb concentration and PCV in all the treated groups was observed throughout the experiment.

Groups A and D showed non-significant difference in RBC counts, Hb concentration and PCV among various experimental days. In group B, significantly ($P<0.05$) higher RBC counts were observed at day 2 compared to days 7 and 17, however, no difference in RBC counts was observed in this group between days 7 and 12. In group C, significantly ($P<0.05$) lower RBC counts were observed at day 17 compared to day 2, however, no differences in RBC counts were observed in this group between day 2 and 12 and day 7 to 17 (Table 1). In groups B and C, significantly ($P<0.05$) lower Hb concentration was observed on day 17 compared to day 2, however, no difference in Hb concentration was observed in these groups at day 7 and 12. Significantly ($P<0.05$) higher PCV was observed at day 2 in group B compared to other experimental days. Significantly ($P<0.05$) lower PCV in group C was observed at day 17 which was non-significantly different than PCV at day 12.

MCV values were significantly ($P<0.05$) higher at day 17 in all treated groups than control group. However, MCV was significantly ($P<0.05$) higher in group C at day 7 and in group A at day 12 compared to control group. Non-significant difference was observed in MCH and MCHC values in all treated groups compared to control group at various experimental days (Table 1).

Total leukocyte counts were significantly ($P<0.05$) lower at day 2 in group C compared to control rabbits (Table 2). TLC on various experimental days revealed significant!y ($P<0.05$) higher TLC at day 17 in groups A and B at day 17 compared to all other experimental days. The leukocyte counts in group C were significantly ($P<0.05$) higher at days 12 and 17 than at days 2 and 7.

Significantly ($P<0.05$) higher percentage of neutrophils was observed in group C at day 2 than other experimental days. A decreasing trend was observed from day 2 to 17 in neutrophils of groups B and C (Table 2). Significantly ($P<0.05$) higher lymphocytes were observed at day 7 in group B, at day 12 in groups B and C and at day 17 in all treated groups compared to control group (Table 2). Dose dependant gradual increase in TLC and lymphocyte counts whereas decrease in neutrophil counts, was observed in all the treated groups throughout the experiment.

DISCUSSION

Pesticides exposure poses a serious risk to all domestic animals, to the environment and the public health (Ohcme and Mannala, 2001). The widespread use of pyrethroid insecticides in agriculture, veterinary and public health applications has acted as a stimulus for the study of their toxicity to non-target organisms. Cypermemrin (CY) is most widely used pyrethroid. In terms of acute toxicity, CY specifically is a moderately toxic material by dermal absorption or ingestion.

Clinical signs of CY toxicity in rabbits started 5-10 minutes after CY administration and persisted for 30-90 minutes in all treated groups in the dose dependent manner in the present study. Skin irritation, itching, skin scratching, licking of legs and other body parts were observed in CY treated rabbits in dose dependent manner. Skin irritation has also been reported

Table 1: Erythrocyte indices in rabbits treated with different concentrations of cypermethrin

Parameters/ Groups	Experimental days			
	2	7	12	17
Erythrocytes counts (10^{12}/L)				
A	4.83 ± 0.17	04.74 ± 0.19	04.71 ± 0.19A	04.50 ± 0.14A
B	4.92 ± 0.08a	04.57 ± 0.13bc	04.63 ± 0.04Aab	04.27 ± 0.07Ac
C	5.13 ± 0.21a	04.65 ± 0.16ab	04.62 ± 0.12Aab	04.26 ± 0.07Ab
D	5.14 ± 0.13	05.18 ± 0.25	05.43 ± 0.17B	05.58 ± 0.31B
Hb. Concentration (g/dL)				
A	11.56 ± 0.19	11.53 ± 0.31	11.23 ± 0.29AB	10.09 ± 0.46AB
D	11.33 ± 0.34a	10.86 ± 0.35ab	10.43 ± 0.09Aab	10.02 ± 0.27Ab
C	11.26 ± 0.42a	11.07 ± 0.33ab	10.83 ± 0.30Aab	09.97 ± 0.40 Ab
D	11.70 ± 0.37	11.96 ± 0.30	12.13 ± 0.34B	12.03 ± 0.53B
Packed cell volume (%)				
A	38.50 ± 0.85	36.17 ± 0.83	37.00 ± 0.82AB	35.67 ± 0.84AB
B	38.17 ± 0.54a	35.67 ± 0.84b	35.33 ± 0.33Ab	34.50 ± 0.81ABb
C	38.67 ± 1.23a	36.67 ± 1.20a	35.17 ± 0.95Aab	32.33 ± 1.17Ab
D	38.67 ± 1.58	38.00 ± 1.5	38.50 ± 1.09B	38.50 ± 1.5B
Mean corpuscular volume (fl)				
A	79.33 ± 1.45	76.83 ± 2.26AB	77.50 ± 1.48A	78.17 ± 1.35A
B	75.67 ± 1.45	77.67 ± 1.41AB	76.00 ± 0.58AB	77.50 ± 1.45A
C	74.00 ± 3.05	78.50 ± 1.71A	76.00 ± 2.79AB	77.00 ± 2.38A
D	76.33 ± 1.58a	71.33 ± 2.09Bab	70.67 ± 0.84Bab	68.00 ± 0.84Bb
Mean corpuscular haemoglobin (pg)				
A	24.17 ± 0.70	23.83 ± 0.79	24.17 ± 1.19	23.17 ± 0.40
B	22.00 ± 0.52	23.33 ± 0.76	22.50 ± 0.43	22.50 ± 0.72
C	21.50 ± 0.81	23.50 ± 0.62	23.00 ± 1.00	23.17 ± 1.14
D	22.83 ± 0.98	22.17 ± 0.48	22.33 ± 0.56	20.50 ± 0.88
Mean corpuscular haemoglobin concentration (g/dL)				
A	30.50 ± 0.62	31.33 ± 0.62	30.00 ± 0.45	29.67 ± 0.80
B	29.00 ± 0.89	30.00 ± 0.77	29.50 ± 0.56	29.17 ± 0.54
C	28.67 ± 0.33	29.67 ± 0.88	30.50 ± 0.43	29.67 ± 0.95
D	30.33 ± 0.92	31.17 ± 1.01	31.33 ± 0.59	31.33 ± 0.76

Groups A, B and C were given cypermethrin (CY) @ 25, 50 and 75mg/kg b. wt. intraperitoneally (i/p). Group D (control) was given equivalent volume of normal saline. Values (mean ± SE) of each parameter with different capital letters within columns and small letters within rows differ significantly ($P<0.05$).

previously with CY treatment in rabbits (Handerson and Parkinson, 1981). Incoodination, muscular tremors, jerky movements, ataxia, staggering gait and dozziness were also observed in CY treated rabbits in dose dependent manner. Similar nervous signs like mild tremors, hyperexcitability or depression (Manna *et al*., 2004), grinding of teeth, hyperaesthesia, opisthotonus, spastic paralysis (Tamang *et al*., 1991), muscle twitching, staggering gait, sunken eyes and thick eye discharge (Jagvinder *et al*., 2001) have previously been reported. Pyrethroids modify the gating characteristics of voltage-sensitive sodium channels in mammalian and invertebrate neuronal membranes (Eells *et al.,* 1992) to delay their closure which produces a protracted sodium influx. Itching, restlessness and head shaking seem to be nervous signs produced by CY by the mechanism of blockage of sodium channels. Pyrethroids and DDT exert similar action on the neuron system through the modulation of the function of voltage-gated sodium channels (Narashi, 2001) and cause membrane depolarization, leading to discharges from sensory neurons.

The decrease in RBC counts observed with CY treatment could be due to haemolysis as a result of type II pyrethroid which causes haemorrhages and reduced erythropoiesis (Mandal *et al.,* 1986). In internal haemorrhages, some erythrocytes are absorbed by lymphatic vessels (autotransfusion) particularly in haemorrhages in body cavities. Remaining RBCs are lysed or phagocytosed (Latimer *et al.,* 2004). Various authors have reported similar results with the treatment of CY in rats (Manna *et al.,* 2004), sheep (Yousef *et al.,* 1998), rabbits (Yousef *et al.,* 2003; Basir, 2005) and goats (Faridi, 2005).

Table 2: Leukocyte indices in rabbits treated with different concentrations of cypermethrin

Parameters/ Groups	Experimental days			
	2	7	12	17
Total Leukocyte counts (10^9/L)				
A	06.60 ± 0.21ABa	06.52 ± 0.77a	07.31 ± 0.29ab	07.48 ± 0.32b
B	06.67 ± 0.21 ABa	06.98 ± 0.29a	07.52 ± 0.44ab	07.98 ± 0.38b
C	06.30 ± 0.16Aa	06.55 ± 0.15a	07.62 ± 0.25b	07.98 ± 0.43b
D	07.19 ± 0.3B	06.99 ± 0.35	06.87 ± 0.34	06.91 ± 0.33
Neutrophils (%)				
A	25.82 ± 1.69AB	25.83 ± 1.70	23.76 ± 1.89AB	20.83 ± 1.22AB
B	28.88 ± 1.14Aa	21.37 ± 2.34b	20.28 ± 0.72Ab	16.90 ± l.l0Ab
C	25.03 ± 2.19ABa	23.86 ± 1.87ab	19.53 ± 1.99Aab	16.29 ± 3.00Ab
D	21.43 ± 1.19Ba	24.87 ± 0.817ab	28.38 ± 2.46Bb	26.26 ± 1.66Bab
Lymphocytes (%)				
A	70.62 ± 1.61	67.60 ± 1.95AB	69.31 ±1.42AB	71.21 ± 0.87A
B	65.93 ± 2. 85a	74.51 ± 2.70Ab	71.55 ±1.34Aab	79.20 ± 0.47Bb
C	67.82 ± 2.21a	69.15 ± 1.25 ABa	73.53 ± 2.90Aab	78.06 ± l.85Bb
D	68.23 ± 2.20	66.12 ± 1.25B	62.16 ± 2.61B	65.86 ± 2.45C

Values (mean ± SE) of each parameter with different capital letters within columns and small letters within rows differ significantly (P<0.05).

Haemoglobin concentration significantly decreased in medium and high dose treated rabbits in the present study. Reduction in Hb contents could be due to the impaired biosynthesis of haem in bone marrow (Shakoori *et al.,* 1992), increased rate of destruction or reduction in rate of formation of RBCs. The decrease in red blood cells and haemoglobin contents could also be due to the disruptive action of the pesticides on the erythropoietic tissue as a result of which the viability of the cells might be affected. Alterations in the haematological parameters were brought about by CY as an anemia due to decreased synthesis of red blood cells (Morgan *et al.,* 1980). The decrease in PCV in medium and high dose treated rabbits was obviously contributed by the decreased cellular counts in blood after CY treatment. In addition, the reduction in blood parameters (PCV, Hb and RBC) may be attributed to hyperactivity of bone marrow (Tung *et al.,* 1975), leading to the production of red blood cells with impaired integrity which were easily destroyed in circulation by reticulo-endothelial system. Shakoori *et al.* (1990) suggested that decrease in RBC counts is either an indicative of excessive damage to erythrocytes or inhibition of erythrocytes formation in rabbits.

Mean corpuscular volume (MCV) significantly increased, while MCHC decreased non-significantly in all treated groups compared to control group in the present study. The increase in MCV and decrease in MCHC indicate macrocytic and hypochromic anemia (Barger, 2003), probably due to the increased activity of bone marrow and deficiency of some hemopoietic factors. Increased MCV may also be observed in regenerative anemia due to hemolysis and haemorrhages. The increase in MCV indicates macrocytosis (Latimer *et al.,* 2004)

Leukocyte counts and lymphocytes increased, while neutrophils decreased significantly in all treated groups compared with control. The increase in WBC may be indicative of activation of defense and immune system of the body (Yousef *et al.,* 2003). This might result in an increase in release of WBC from bone marrow storage pool into the blood. Pathological leukocytosis may have resulted due to chemical, acute haemorrhages and acute hemolysis. Leukocytosis may have occurred due to resistance of the animal for localization of the inflammatory response (Benjamin, 1978). Severe haemorrhages in lungs and liver encountered in the present study may be the possible cause of leukocytosis (Latimer *et al.,* 2004). The lymphocytosis may have resulted due to excitement, fear, pain and apprehension encountered by the rabbits by the treatment (Benjamin, 1978).

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