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CYPERMETHRIN INDUCED ANAEMIA IN MALE RABBITS

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ABSTRACT

The effects on haematology of 48 rabbits (24 of each sex) were studied by administering cypermethrin (CY) intraperitoneally. In each sex, animals were divided into four equal groups A, B, C and D. The animals in groups B, C and D received low (50 $mg.kg^{-1}$ b. wt.), medium (100 $mg.kg^{-1}$ b. wt.) and high (150 $mg.kg^{-1}$ b. wt.) CY doses, respectively in mustard oil. Group A served as control and each animal received equivalent volume of mustard oil. The animals received five CY treatments (d 1, 8, 15, 22 and 29). Blood samples with anticoagulant from all animals were collected prior to experiment (day 0) and after every treatment for haematological studies. The total erythrocyte count (TEC), haemoglobin (Hb) concentration and packed cell volume (PCV) were analyzed at each experimental period and erythrocyte indices were calculated. During the experiment, significant increase in mean corpuscular volume (MCV) and significant decrease in TEC, Hb concentration and mean corpuscular Hb concentration (MCHC) were found in the male rabbits treated with CY especially at higher doses, while the decrease in these parameters in the female rabbits was non-significant at all dose levels and time periods. Thus, it was concluded that macrocytic hypochromic anaemia was induced in the male rabbits due to CY treatment, but not in the females at the dose levels administered during the study.

Key words: Rabbits, cypermethrin, anaemia**.**

INTRODUCTION

Pesticides have become omnipresent contaminants of our environment and have been found in water, soil, air and both human and animal tissues all over the world (Anwar, 1997). Silent Spring, the book written by Rachel Carson, facilitated the ban of the pesticide DDT in 1972 in the United States and foretold of the poisoning of the planet by man (Paull, 2007). Since then, many countries have devised policies to reduce pesticide use. However, data (1992–2003) of EU statistics show that consumption of pesticide did not decrease (Bjørling-Poulsen *et al*., 2008). Among the severe pesticide poisoning (3 million cases) and deaths (220,000) through out the world, about 99% are reported from the third world countries (Tinoco and Halperin, 1998).

Major share of total pesticide usage is in the developing countries as compared to developed world (Anwar, 1997). Various classes of insecticides include organophosphate, organochlorine, carbamate and pyrethroid. Pyrethroids use has increased much for the last 10 years (Wolansky *et al*., 2006). The incidence of major outcomes and fatalities attributable to pyrethroids are considerably less than organophosphates (Sudakin, 2006), however, their pathological effects have been encountered in experimental studies in different animals (Manna *et al*., 2004; Khan *et al*., 2009).

If ectoparasites are not treated, consequences appear in the form of blood loss, reduced birth weight, behavioral changes such as excessive scratching and decreased weight gain and decreased milk production up to 15-25% per animal per year (Kakar and Kakarsulemankhel, 2009). Cypermethrin (CY), a pyrethroid is used commonly in Pakistan for the control of ectoparasites, which may have health risk to livestock as well as human beings. None of the preparations available in the market are manufactured locally (Ahmad *et al*., 2009). Scanty information about the type of toxicity due to CY in animals of the region is available (Shah *et al*., 2007; Aslam *et al*., 2009). Contentious reports about the effects of CY on haematological parameters are available. No CY effect has been reported on Hb concentration in rats (Mansee, 1998), while in sheep decreased Hb has been reported (Yousef *et al*., 1998). Similarly, significantly decreased TEC, Hb, PCV and MCHC with increased MCV in CY treated goats (Khan *et al*., 2009) and broilers (Sharaf *et al*., 2009) have been reported. Some workers, however, reported significantly increased Hb, TEC and PCV in mice treated with pyrethroids (Luty *et al*., 2001; Haratym-Maj, 2002). This study was tailored and performed to explore whether CY induces anaemia in rabbits or not, and, whether response of the pesticide under study is sex dependent or not.

MATERIALS AND METHODS

Experimental animals and protocol

Forty eight apparently healthy, adult rabbits of almost the same age (female and male, 50:50) were procured from the local market. They were kept under similar management conditions. The animal room temperature was maintained at 25-27°C throughout the

study. Drinking water was available *ad libitum.* The green fodder Barseem (*Trifolium alexandrinum*) was offered in the morning and evening. After 5 days of acclimatization, male and female rabbits were separated and assigned to the three CY treatments based on initial live weight. In each sex, group A served as control and each animal in the control group received equivalent volume of mustard oil. Animals in groups B, C and D received intraperitoneally low (50 $mg.kg^{-1}$ b. wt.), medium (100 $mg.kg^{-1}$ b. wt.) and high (150 $mg.kg^{-1}$ b. wt.) dose of CY, respectively dissolved in mustard oil on days 1, 8, 15, 22 and 29 of the experiment.

Haematology

Blood samples from all animals were collected prior to (d 0) and after every treatment on the next day. Blood samples were used for haematological studies including haemoglobin concentration, packed cell volume and total erythrocyte count. The cyanome-themoglobin method was used to estimate haemoglobin concentration, whereas microhaematocirt method was used for PCV and cell counts were manually executed using the improved Neubauer haemocytometer (Benjamin, 1978). Erythrocyte indices including mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the formula described by Khan (2008).

Data analysis

The collected data were analyzed using analysis of variance (ANOVA) and mean values were compared on a personal computer using the Minitab statistical software package. The significance level was $P<0.05$.

RESULTS

The results of different haematological parameters in male and female rabbits are presented in Tables 1 and 2, respectively. In males, TEC significantly ($P<0.05$) decreased in groups C and D as compared to A (control) at 2nd week and in group C only at 4th week. However, TEC significantly increased in group B at 3rd week of the treatment (Table 1). Variation in TEC observed in treatment groups of female rabbits (compared to control) was non-significant at all periods (Table 2). A significant increase in PCV was observed in male group B at first week, whereas a non-significant difference throughout the experiment was observed in female rabbits. The Hb concentration was significantly ($P<0.05$) decreased at 3rd weak as compared to control in all male treatment groups (Table 1), whereas it significantly increased in groups B and C at 2nd week in females (Table 2).

In the male rabbits, MCV increased significantly ($P<0.05$) at 1st week in groups B and C, 2nd week in groups C and D and 4th week in all treated groups. The MCH also significantly increased at 1st week in groups B and C and 4th week in group C, but significantly decreased at 3rd week in groups B and D in male rabbits. In the females, MCV and MCH of any group did not differ significantly from control at any period (Table 2).

In the males, MCHC was significantly lower at 1st week in groups B and D and 3rd week in groups C and D but significantly higher at 2nd week in groups B and D than control. In the females, MCHC was significantly lower at 1st week in group D, but significantly higher at 2nd week in groups B and C than control. From the above results it was interpreted that macrocytic hypochromic anaemia was induced by CY in males, but not in female rabbits.

DISCUSSION

The results of the present study indicated that cypermenthrin (CY) induced macrocytic hypochromic anaemia in male rabbits but not in female rabbits. Previously, sex related difference in haematological response to pyrethroid treatment has been reported in mice. Luty *et al*. (2001) reported that irrespective of the dose, the deltamethrin and fenvalerate stimulated erythropoiesis and synthesis of Hb in male Swiss mice, while in female mice the administration of deltamethrin (25 $mg.kg^{-1}$ b. wt.) resulted in anaemia. In contrary to the findings of the present study, Haratym-Maj (2002) reported anaemia in female mice, but not in males at CY doses 5 $mg.kg^{-1}$ b. wt. He also reported that anaemia developed in female mice at low CY doses (5 $mg.kg^{-1}$ b. wt.), whereas at high CY doses (25 $mg.kg^{-1}$ b. wt.) no anaemia was observed. He alleged that female mice were principally susceptible to poisoning, particularly with low doses of pyrethroids used for long time.

Although the mechanism of toxicity of pyrethroids has not been fully explored, various opinions have been put forward. CY can induce oxidative stress in blood cells (Kale *et al*., 1999) or may accrue in cell membranes and disturb structure of membrane (Michelangeli *et al*., 1990) which could lead to lysis of erythrocytes as a result their number would be low in circulation. Low Hb concentration could be due to enhanced Hb destruction or decreased Hb synthesis (Moss and Hathway, 1964). Increased activity of bone marrow or haemolysis could lead to impaired Hb synthesis (Barger, 2003). TEC production is regulated by tissue oxygenation. Tissue receive inadequate oxygen (O_2) if there is an insufficient supply in inspired air, impaired O_2 transport from alveoli into blood stream, hypoventilation, inadequate Hb to carry O_2, decreased arterial O_2 saturation, abnormal blood flow or failure of haemoglobin to release bound O_2 at tissue sites (Helms *et al*., 2006).

Table 1: Haemogram of male rabbits treated at various intervals with cypermethrin

Parameters/ experimental weeks	Groups (Cypermethrin doses; mg.kg^{-1} b. wt.)			
	A (0)	B (50)	C (100)	D (150)
Total erythrocyte counts (10^6/ml)				
0	4.12 ± 0.55	4.54 ± 0.72	3.66 ± 0.72	4.52 ± 0.52
1	4.56 ± 0.44	4.12 ± 0.42	4.30 ± 0.30	4.90 ± 0.79
2	5.65 ± 1.20	5.08 ± 0.66	4.00 ± 0.29*	3.93 ± 0.59*
3	4.16 ± 0.11	4.58 ± 0.19*	4.16 ± 0.10	4.46 ± 0.30
4	5.20 ± 0.90	4.70 ± 0.32	3.88 ± 0.53*	4.62 ± 0.62
5	5.03 ± 0.94	4.74 ± 0.21	4.97 ± 0.13	5.68 ± 1.29
Packed cell volume (%)				
0	42.30 ± 1.08	42.40 ± 4.72	38.40 ± 1.86	42.00 ± 30.41
1	37.00 ± 1.41	41.00 ± 3.23*	39.20 ± 1.72	39.58 ± 2.15
2	43.50 ± 1.66	40.60 ± 1.50	43.00 ± 2.28	40.67 ± 3.45
3	39.50 ± 0.79	38.40 ± 1.02	39.20 ± 0.40	38.20 ± 1.69
4	39.00 ± 1.23	37.70 ± 1.57	38.53 ± 1.46	39.20 ± 2.59
5	40.27 ± 0.75	37.90 ± 2.36	40.50 ± 1.78	39.20 ± 2.80
Haemoglobin concentration (g/dl)				
0	10.51 ± 0.647	10.84 ± 1.36	11.12 ± 0.81	11.54 ± 0.32
1	10.07 ± 1.13	10.04 ± 0.60	10.54 ± 0.27	9.57 ± 0.84
2	9.30 ± 1.41	9.63 ± 0.43	9.19 ± 0.64	9.81 ± 0.56
3	9.90 ± 0.51	8.53 ± 0.62*	7.87 ± 0.38*	8.04 ± 0.67*
4	9.50 ± 0.70	8.41 ± 0.47	9.47 ± 0.88	8.95 ± 0.73
5	9.17 ± 0.56	8.55 ± 0.30	8.91 ± 0.93	8.89 ± 0.46
MCV (fl)				
0	104.65 ± 15.76	91.32 ± 17.80	111.12 ± 32.19	93.80 ± 10.11
1	77.76 ± 3.01	100.18 ± 9.06*	91.48 ± 5.94*	82.35 ± 12.16
2	78.98 ± 12.78	80.97 ± 10.48	107.63 ± 3.37*	105.03 ± 21.75*
3	95.04 ± 3.72	85.42 ± 3.47	94.28 ± 2.59	86.08 ± 8.06
4	76.82 ± 13.16	80.54 ± 6.57*	100.56 ± 12.10*	84.46 ± 8.50*
5	82.26 ± 14.22	80.06 ± 5.40	81.53 ± 1.96	71.80 ± 15.82
MCH (pg)				
0	26.73 ± 5.14	22.60 ± 4.49	29.87 ± 11.48	21.60 ± 2.10
1	22.06 ± 1.87	24.53 ± 2.13*	24.65 ± 2.03*	19.95 ± 3.86
2	20.37 ± 4.23	19.14 ± 2.74	21.68 ± 2.88	21.83 ± 5.17
3	23.80 ± 0.81	19.49 ± 2.76*	20.32 ± 3.34	18.18 ± 2.63*
4	18.82 ± 4.07	17.90 ± 0.53	24.82 ± 4.43*	19.46 ± 2.86
5	18.88 ± 4.33	18.06 ± 0.74	17.93 ± 1.52	16.30 ± 3.62
MCHC (g/dl)				
0	25.44 ± 2.02	24.28 ± 6.13	26.32 ± 2.29	23.14 ± 1.82
1	28.40 ± 2.35	24.52 ± 1.06*	26.96 ± 1.65	24.26 ± 2.70*
2	21.68 ± 2.90	25.12 ± 2.35*	21.38 ± 1.50	24.18 ± 1.25*
3	25.10 ± 1.42	22.99 ± 2.67	20.08 ± 0.77*	21.04 ± 1.62*
4	24.36 ± 1.90	22.38 ± 1.89	24.64 ± 2.76	22.92 ± 1.29
5	22.78 ± 1.55	22.60 ± 1.30	22.00 ± 2.08	22.73 ± 0.59

The values bearing asterisk indicate significant ($P<0.05$) difference compared to those of control group.

The toxicity of any compound depends on many factors, such as the chemical and physical form of the compound, route of administration, dose and duration of exposure, time elapsed after exposure, dietary level of the interacting elements, physiological conditions (pregnancy, lactation etc.), nutritional status, age and sex of the exposed individuals (Haratym-Maj, 2002; Khan *et al*., 2009; Aslam *et al*., 2009). Although pyrethroid effect in both sexes of rabbits in any single study have not been investigated previously, but two separate studies undertaken at our department in rabbits are noteworthy. Basir (2005) reported increased TEC and Hb concentration in female rabbits treated with lambda cyhalothrin (a pyrethroid) at 1.0, 4.0 and 8.0 mg.kg^{-1} b. wt intraperitoneally but Shah *et al*. (2007) reported anemia in female rabbits treated with CY (25, 50 and 75 mg.kg^{-1} b. wt., intraperitoneally). The above workers used lower dose levels of pyrethroids than

Table 2: Haemogram of female rabbits treated at various intervals with cypermethrin

Parameters/ experimental weeks	Groups (Cypermethrin doses; mg.kg^{-1} b. wt.)			
	A (0)	B (50)	C (100)	D (150)
Total erythrocyte counts (10^6.mm^{-3})				
0	4.02 ± 0.25	4.18 ± 0.41	4.11 ± 0.13	4.15 ± 0.24
1	3.97 ± 0.49	4.10 ± 0.43	4.47 ± 0.49	4.05 ± 0.80
2	4.46 ± 0.23	4.50 ± 0.33	4.75 ± 0.77	4.80 ± 0.73
3	4.97 ± 0.75	4.83 ± 0.42	4.13 ± 0.42	5.27 ± 0.50
4	4.58 ± 0.16	4.68 ± 0.19	4.58 ± 1.14	4.78 ± 1.18
5	4.80 ± 0.34	4.67 ± 1.06	3.90 ± 0.20	3.63 ± 0.35
Packed cell volume (%)				
0	38.00 ± 2.36	39.17 ± 2.14	41.33 ± 3.78	40.00 ± 1.41
1	39.00 ± 2.19	37.33 ± 3.98	40.33 ± 3.27	40.83 ± 1.47
2	36.80 ± 1.10	37.17 ± 1.33	38.50 ± 2.59	39.17 ± 1.47
3	37.33 ± 2.07	38.50 ± 3.56	36.67 ± 2.88	39.67 ± 2.80
4	38.33 ± 1.53	36.00 ± 0.00	37.33 ± 3.51	37.00 ± 4.00
5	38.00 ± 3.00	35.67 ± 1.16	36.00 ± 3.60	38.33 ± 1.53
Haemoglobin concentration (g/dl)				
0	8.10 ± 0.67	8.85 ± 0.43	8.96 ± 0.73	9.14 ± 0.95
1	8.64 ± 0.98	8.43 ± 0.51	8.40 ± 0.52	7.89 ± 0.76
2	8.12 ± 0.48	9.54 ± 1.13*	10.14 ± 0.48*	8.96 ± 0.54
3	8.04 ± 0.62	8.17 ± 0.45	8.14 ± 1.52	7.66 ± 0.57
4	7.92 ± 0.29	7.15 ± 0.24	7.22 ± 0.36	7.30 ± 0.90
5	8.11 ± 0.54	7.55 ± 0.25	7.61 ± 38	7.71 ± 0.95
MCV (fl)				
0	94.68 ± 4.29	94.30 ± 9.47	100.29 ± 6.62	96.65 ± 6.47
1	99.34 ± 10.63	92.16 ± 16.20	90.96 ± 9.91	104.14 ± 20.35
2	82.65 ± 3.89	82.79 ± 3.29	82.84 ± 15.08	83.00 ± 11.26
3	81.50 ± 5.17	82.30 ± 7.39	82.50 ± 12.52	86.43 ± 18.09
4	71.52 ± 2.15	73.80 ± 6.04	86.73 ± 2.15	70.71 ± 14.28
5	85.87 ± 9.66	79.37 ± 19.88	92.26 ± 6.78	106.42 ± 14.38
MCH (pg)				
0	20.18 ± 1.72	21.35 ± 2.63	21.75 ± 1.51	21.98 ± 1.09
1	21.88 ± 2.17	20.68 ± 1.49	18.95 ± 1.69	20.09 ± 4.36
2	18.13 ± 1.13	2.24 ± 2.44	21.81 ± 3.67	19.75 ± 3.16
3	17.54 ± 1.16	17.46 ± 0.87	18.46 ± 4.74	17.05 ± 5.15
4	14.78 ± 0.45	14.69 ± 1.69	16.80 ± 0.35	13.97 ± 3.02
5	16.95 ± 1.72	16.65 ± 3.14	19.53 ± 1.02	21.51 ± 4.66
MCHC (g/dl)				
0	21.30 ± 1.07	22.64 ± 1.47	21.73 ± 1.64	22.87 ± 2.55
1	22.16 ± 2.48	22.77 ± 2.41	20.88 ± 0.94	19.30 ± 1.50*
2	21.94 ± 1.07	25.65 ± 2.74*	26.38 ± 1.20*	22.89 ± 1.39
3	21.65 ± 2.67	21.39 ± 2.39	22.19 ± 3.80	19.45 ± 2.50
4	20.67 ± 0.13	19.87 ± 0.65	19.38 ± 0.86	19.72 ± 0.29
5	21.38 ± 1.54	21.20 ± 1.32	21.21 ± 1.07	20.06 ± 1.69

The values bearing asterisk indicate significant (P<0.05) difference compared to those of control group.

those of our study. They, however, used commercial mixtures, so that the possibility of interference of other compounds (e.g. xylene) cannot be excluded. The present study was designed to use 92% CY in inert solvent (mustard oil). Thus, in this study anaemia was not observed in female rabbits, while anaemia was reported by Shah *et al.* (2007) even at doses lower than the present study.

From the results of this study it was concluded that anaemia (macrocytic hypochromic) was induced in the male rabbits due to CY treatment at all dose levels, but not in the females at the dose levels administered during the study. So, sex is also an important factor for the anaemia development due to CY toxicity in addition to dose administered and duration of the treatment.

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