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CYPERMETHRIN TOXICOSIS IN THE CHICKS OF DOMESTIC FOWL, *GALLUS DOMESTICUS*: HAEMATOLOGICAL STUDIES

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Abstract: Cypermethrin was administered orally to two groups of chicks @ 250 mg/kg body weight (strong dose, only once) and 150 mg/kg body weight/day (weak dose) for a total duration of 40 hours and 12 days, respectively. A group of five chicks were dissected at the intervals of 5, 10, 20 and 40 hours of toxicant feeding in strong dose (short term) experiment and at 3, 6 and 12 days durations in weak dose (long term) experiment. Blood samples were collected and processed for various haematological parameters which did not show any severe abnormality in both strong and weak dose experiments, except some changes in Hb and MCHC contents. The Hb content increased significantly by 18% within 5 hours, followed by decreasing trend. At 10 hours cypermethrin treatment increase was 16% which was reduced to 6% at 20 hours while at 40 hours significant decrease of 11% was observed. MCHC content raised by 13% and 14% at 5 and 10 hours of insecticide feeding. The only parameter, that showed significant change in both experiments was WBC count, which was increased by 75%, 48%, 86% and 62% at 5, 10, 20 and 40 hours in short term experiment, while in long term treatment 28% rise was observed at 6 days insecticide treatment. All other compounds such as RBC, PCV, MCV and MCH remained unaffected in both treatments.

Key words: Insecticides, pyrethroids, cypermethrin, haematology, blood cells, erythrocytes, leukocytes, RBC, WBC, haemoglobin, haematological indices, poultry, birds.

INTRODUCTION

The pyrethroids are third generation insecticides, playing very significant role in controlling insect pests of agriculture, vetrinary medical and house hold importance (Class and Kintrup, 1991; Lee and Clark, 1998; Martinez-Torres *et al.*, 1998). Most of the early developed pyrethroids are unstable in air and light (Elliott *et al.*, 1973, 1978). This property restricts their use particularly against pests of field crops regardless of their other favorable properties. The modern and more stable pyrethroid insecticides are not only more effective in controlling insect pest population but are also more harmful towards mammals and other non target systems.

Cypermethrin is one of these pyrethroids which has been extensively used to control wide range of insect pests. Most of the initial work performed with these insecticides was on toxicity, metabolism, metabolic fate and excretion in target and non target animal systems (Kaneko *et al.*, 1987; Herzberg, 1988; Akhtar *et al.*, 1989;

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Gupta, 1990; Hodgson and Levi, 1992, 1996; Hodgson *et al.*, 1995). These pesticides and their residues, due to their stability produced variety of harmful effects in non target living systems (Mugambi *et al.*, 1989). Substantial amount of work has been published on the effects of pyrethroids on the central nervous system which is the principal site of action of these toxic compounds in the animal system (Staatz *et al.*, 1982; Akhtar *et al.*, 1985, 1987; Hutson and Stoydin, 1987; Saleh *et al.*, 1987; Reddy *et al.*, 1991).

Childhood cancers in North America, according to 31 studies review, was also due to paternal occupational exposure with pesticides (National Research Council, 1993; Daniels *et al.*, 1997). It has also been reported that in addition to nervous system (Saunder and Harper, 1994), these pesticides induce their toxic effects on other tissues and systems of the body (Kagan *et al.*, 1986; Guguen-Guillouzo *et al.*, 1988; Ansari and Kumar, 1988; Shakoori *et al.*, 1991, 1992b, 1994). Cypermethrin and deltamethrin induced chromosomal abberations in human lymphocytes have been reported (Dolara *et al.*, 1992; Osman *et al.*, 1995). Several workers have studied and reported their effects on liver, kidney and other tissues systems of the animals. Similar type of work with pyrethroids and other insecticides has already been reported from this laboratory in chick, rat and rabbit (Shakoori *et al.*, 1988, 1990, 1992a; Ali *et al.*, 1994, 1997). The toxicity of these compounds may vary depending upon the route of administration and if the route is oral, as is the case in the present study, generally, the blood and its various cellular components will the first target of insecticide in the body.

The main objective of the present study is to investigate the harmful effects of cypermethrin on the blood and its cellular components in the chick model.

MATERIALS AND METHODS

Experimental animals and their maintenance

Fifty two, one day old broiler chicks were obtained from Coccation Breeders, Shadman market, Lahore. They were kept in the cages (125 cubic feet size)in the animal house of Zoology Department under cotrolled temperature conditions 20 ± 1°C. During this period the chicks were fed on commercial poultry feed, purchased from the local market. The feed and water was provided to the animals *ad libitum*. The chicks were allowed to acclimatize for about two weeks before dose administration.

Toxicant used

Cypermethrin as Ripcord, 100 EC, [α-cyano-3-phenoxybenzyl 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate], a highly active synthetic pyrethroid and a product of Shell, was used for the experiment.

Administration of toxicant

Two sublethal doses (weak and strong) of cypermethrin were administered keeping in view the LD_{50} values of the insecticide against chicks.

A strong dose of cypermethrin was administered orally only once to chicks at a dose of 250 mg/kg body weight for a total duration of 40 hours. In another experiment,

a weak dose of insecticide was administered @ 150 mg/kg body weight/day for a total period of 12 days.

Experimental procedure

In short term experiment, a group of twenty chicks with almost same body weight and age, after weighing, were administered with strong oral dose (250 mg/kg body weight) of cypermethrin. After the stipulated periods of 5, 10, 20 and 40 hours a group of five birds were anaesthetized and dissected for sampling. A group of four chicks processed similarly, except insecticide treatment was used as control.

In Long term experiment, thirty chicks after weighing, were divided into three groups of ten animals (six treated and four control). Weak dose (150 mg/kg body weight/day) of cypermethrin was administered orally to six animals in each group. The blood samples were collected at 3, 6 and 12 days intervals from control and treated groups with the help of sterlized syring and quickly transferred to EDTA coated tubes with gentle rotation for further studies.

Methodology for haematological studies

The haemoglobin (Hb) content of the blood was estimated according to vanKampen and Zijlstra (1961), the packed cell volume (PCV) was analysed by microhaematocrit method of Strumia *et al.* (1954), while the red blood cells (RBC) and white blood cells (WBC) were counted according to the routine visual haemocytometer method (see, Dacie and Lewis, 1984). The above haematological values were also used for computing different haematological indices *i.e.*, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) by the relationships mentioned in Dacie and Lewis (1984).

RESULTS

Cypermethrin, administered as sublethal doses @ 250 mg/kg body weight (strong dose) for 40 hours (short term) duration produced many significant changes in haematology of chicks (Tables I-II). The Hb content showed 18% and 16% increase, respectively after 5 and 10 hours of cypermethrin administration, while the effects were reversed to normalization when treatment was extended upto 20 hours and decreased significantly by 11% at 40 hours cypermethrin treatment (Tables I-II). In weak dose (long term) experiment at 150 mg/kg body wt./day treatment, although 17% and 12% increase was found at 3 and 6 days which was statistically non-significant. The PCV and MCV showed 36% and 25% increase, respectively at 6 day uninterrupted insecticide feeding daily, the latter value was statistically nonsignificant (Tables III and IV).

The leukocytes (WBC count) show special sensitivity to cypermethrin in both short and long term treatments. A prominent but irregular increase was noticed in WBC count which was 75% , 48%, 86% and 62% at 5, 10, 20 and 40 hours after continuous insecticide feeding (Tables I-II). In weak dose treatment, WBC count did not show any change upto 3 day toxicant feeding, however, on increasing the duration of treatment, 28% and 20% rise was noticed at 6 and 12 day treatments, respectively (Tables IV-VI).

Amongst the haematological indices, MCHC was the only component which exhibited 13% and 14% significantly higher values when compared with control after 5 and 10 hours insecticide feeding (Tables I-II). In long term experiment MCHC was decreased by 22% following 6 day cypermethrin administration (Tables IV and VI). All other haematological parameters, such as RBC count, MCV and MCH, tested for evaluation of cypermethrin toxicity were remained undisturbed.

Table I: **Effect of cypermethrin (250 mg/kg body weight) on some haematological parameters of chick, *Gallus domesticus*, administered for a total period of 40 hours.**

Parameters[b]	Control	Cypermethrin treatment (hours)			
		5	10	20	40
	(n=4)	(n=5)	(n=5)	(n=5)	(n=5)
RBC count ($x10^6$ cells/μl)	2.397[a] ±0.154	2.434 ±0.164	2.454 ±0.147	2.492 ±0.053	2.290 ±0.137
Hb content (g/dl)	9.26 ±0.32	10.93* ±0.52	10.75** ±0.16	9.85 ±0.25	8.27* ±0.22
PCV (%)	37.77 ±1.84	39.42 ±1.97	38.52 ±1.54	38.73 ±1.03	36.94 ±1.14
MCV (fl)	158.72 ±8.90	163.68 ±9.36	158.10 ±6.34	155.90 ±6.61	163.35 ±9.92
MCH (pg)	39.07 ±2.48	45.43 ±2.68	44.28 ±2.11	39.62 ±1.50	36.50 ±1.85
MCHC (g/dl)	24.59 ±0.58	27.76** ±0.49	28.04* ±0.93	25.47 ±0.70	22.46 ±0.94
WBC count ($x10^3$ cells/μl)	21.70 ±1.70	38.00** ±2.71	32.10* ±3.46	40.30** ±3.07	35.10** ±2.49

[a]Mean ± SEM, Student's `t' test; *$P<0.05$; **$P<0.01$.

[b]**Abbreviations used:** RBC, red blood cells; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells; fl, femtolitre = 10^{-15} litre; pg, picogram = 10^{-12} gram.

Table II: Percent increase (+) or decrease (-) in different hematological parameters of chick, *Gallus domesticus*, after cypermethrin administration (250 mg/kg body weight) for a total period of 40 hours.

Parameters[b]	Cypermethrin treatment (Hours)			
	5 (n=5)	10 (n=5)	20 (n=5)	40 (n=5)
RBC	+1.54	+2.38	+3.96	-4.46
Hb	+17.98*	+16.00**	+6.30	-10.78*
PCV	+4.36	+1.98	+2.54	-2.21
MCV	+3.13	-0.39	-1.78	+2.92
MCH	+16.30	+13.35	+1.41	-6.56
MCHC	+12.86**	+14.00*	+3.57	-8.67
WBC	+75.12**	+47.93*	+85.71**	+61.75**

[a]Student's `t' test; *P<0.05; **P < 0.01.

[b]For abbreviations see Table I.

Table III: Effect of Cypermethrin (150 mg/kg body weight/day) on some hematological parameters of chick, *Gallus domesticus*, administered for a total period of 3 days.

Parameters[b]	Cypermethrin treatment (days)	
	Control (n=4)	Treated (n=6)
RBC count (x10^6 cells/μl)	1.707±0.111[a]	1.780±0.050
Hb content (g/dl)	13.10±1.96	15.36±1.82
PCV (%)	29.21±2.52	33.15±0.87
MCV (fl)	181.49±11.87	184.03±2.12
MCH (pg)	76.36±8.79	82.97±10.96
MCHC (g/dl)	44.55±4.50	44.07±5.10
WBC count (x10^3 cells/μl)	37.13±1.59	40.67±1.12

[a]Mean ± SEM, Student's `t' test.

[b]For abbreviations see Table I.

Table IV: **Effect of Cypermethrin (150 mg/kg body weight/day) on some haematological parameters of chick, *Gallus domesticus*, administered for a total period of 6 days.**

Parameters[b]	Cypermethrin treatment (days)	
	Control (n=4)	Treated (n=6)
RBC count (x10^6 cells/μl)	2.178±0.095[a]	2.358±0.185
Hb content (g/dl)	11.93±1.30	13.34±1.88
PCV (%)	27.46±2.48	37.31±2.67*
MCV (fl)	126.97±20.88	159.05±17.21
MCH (pg)	55.80±8.21	57.45±7.98
MCHC (g/dl)	46.31±3.52	36.26±2.43*
WBC count (x10^3 cells/μl)	35.25±1.45	45.10±3.01*

[a]Mean ± SEM, Student's `t' test; *P<0.05.

[b]For abbreviations see Table I.

Table V: **Effect of Cypermethrin (150 mg/kg body weight/day) on some hematological parameters of chick, *Gallus domesticus*, administered for a total period of 12 days.**

Parameters[b]	Cypermethrin treatment (days)	
	Control (n=4)	Treated (n=6)
RBC count (x10^6 cells/μl)	2.338±0.082[a]	2.218±0.068
Hb content (g/dl)	11.90±0.38	11.39±0.54
PCV (%)	39.39±1.92	34.64±1.00
MCV (fl)	168.38±4.48	156.33±3.21
MCH (pg)	50.92±0.68	51.70±1.25
MCHC (g/dl)	30.33±1.16	32.87±1.16
WBC count (x10^3 cells/μl)	20.88±1.48	25.97±1.08*

[a]Mean ± SEM, Student's `t' test.

[b]For abbreviations see Table I.

Table VI: **Percent increase (+) or decrease (-) in different hematological parameters of chick, *Gallus domesticus*, after cypermethrin administration (150 mg/kg body weight/day) for a total period of 12 days.**

Parameters[b]	Cypermethrin treatment (days)		
	3 (n=6)	6 (n=6)	12 (n=6)
RBC	+4.28[a]	+8.26	-5.13
Hb	+17.21	+11.76	-4.29
PCV	+13.48	+35.88*	-12.07
MCV	+1.40	+25.27	-7.16
MCH	+8.66	+2.95	+1.53
MCHC	-1.08	-21.70*	+8.36
WBC	+9.54	+27.94*	+19.76*

[a]Student's `t' test; *$P<0.05$.

[b]For abbreviations see Table I.

DISCUSSION

Following experimental ingestion or accidental exposure of animal by toxic compounds these are generally, assimilated or absorbed through the portal blood or general surface of the body. It is evident that after entry of the toxicant into the body, initially it comes incontact with the blood including its cellular components. The toxicant may induce variable toxicity in the animal systems depending upon the type of chemical, duration of exposure. and amount of material ingested.

In the present experiment, cypermethrin treatment to chicks at two sublethal levels for 40 hours and 12 days, respectively did not produce any severe abnormality in hematological parameters, except some significant increase in Hb (18% and 16%) and MCHC (13% and 14%) at 5 and 10 hours of insecticide administration respectively. Increase in both these components within initial 10 hours, without increase in RBC count, MCV and PCV, is an indication that Hb in the erythrocytes has become more condense, the condition which may be pathological for the organism. The increase in Hb and MCHC may be due to some stimulatory response of cypermethrin on Hb synthesis in the hemopoietic tissue. However, it has also been reported that in birds hypoxia and dehydration may develop due to insecticidal poisoning (Coles, 1986; Campbell, 1988) and rise in Hb during this study can be explained as a compensatory response to restore the oxygen level in the body. Ali *et al.* (1997) in another study on chicks with malathion showed rise in haemoglobin in 12 days and 4 weeks treatment at 400 and 250 mg/kg body wt./day. The findings in the present study differ from earlier studies conducted by Shakoori *et al.* (1990, 1994) and Ali and Shakoori (1994), with another pyrethroid and DDT, an organochlorine compound, bifenthrin in rabbits which showed significant decrease in Hb content, RBC count and MCHC in 30 day study with sublethal doses. Similar decrease was also observed in rats and fish with other pesticides like malathion, aldrin, gamma-BHC (Ali and Shakoori, 1981; Shakoori and Ali, 1985; Reddy and Bashamohideen, 1989). In another study, with oral administration of cypermethrin alongwith feed @ 420 mg/kg/body weight/day for 6 months in rabbits, the Hb and

MCV remained unchanged while MCHC increased just similar to the present study (Shakoori *et al.*, 1988).

The Hb content at 40 hour cypermethrin treatment showed significant decrease although significant increase (upto 18%) was found during initial 10 hours toxicant feeding with no change at 20 hours. This pattern of changes in the present study was indicative of stimulatory effect of cypermethrin during early hours of strong dose treatment (as is the case in long term experiment with comparatively weak doses) while further exposure to toxicant upto 40 hours may lead to inhibition of Hb synthesis.in hematopoietic tissue during this short term experiment. These studies suggested that the effect of cypermethrin on blood cellular components and Hb may be dose- and time-dependent. The unaltered data at 12 day cypermethrin feeding suggest that effect of this toxicant may be neutralized by the induction of drug metabolizing enzymes and other defence systems of the body (Franklin *et al.*, 1980; Hutson, 1981; Cole *et al.*, 1982; Hodgson and Levi, 1992; Hodgson *et al.*, 1995), as a result animals may have developed resistance against this pesticide under the conditions of the present experiment.

These findings were also confirmed by the increase in WBC in both treatments, which were more prominent in 250 mg dose level administered for 40 hours. Increase in WBC is a typical response of vertebrate systems against toxic insult (Ali and Shakoori, 1990). Similar rise in leukocytes was also reported by Ahmad (1988) with sublethal doses of Danitol (a pyrethroid) in fish, which may result in enhancement of detoxification process.

The study suggests that cypermethrin, under the present experimental conditions and at dose levels used in this experiment, is moderately toxic to chicks and effects are reversible on extending the treatment duration, as for as the blood morphological components are concerned.

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