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Assessment of Comparative Hemotoxicity of Cybil and Fenvalerate in *Rattus norvegicus*

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The agriculturists, in their bid to obtain quantitative and qualitative improvement of food products, have been using pesticides indiscriminately. The increase in food and fiber products has become more dependent on the use of synthetic chemicals, particularly pesticides. The use of such pesticides on land and water has posed potential health hazard not only to livestock and wildlife but also to fishes, birds, mammals and even to human beings.

Synthetic pyrethroids have emerged as a major class of useful agricultural insecticides. Synthetic pyrethroids are chemical analogues of natural pyrethrins, which are derived from the flower, *Chrysanthemum cinerarifolium.* The most commonly used synthetic pyrethroids are cybil, fenvalerate, deltamethrin, allethrin, λ-cyhalothrin etc.

In the present investigation the synthetic pyrethroids cybil and fenvalerate have been selected to investigate their haemotoxic potential in albino rats after acute (1 day) and subacute (7, 14 and 21 days) treatment.

MATERIALS AND METHODS

Albino rats, (*Rattus norvegicus*) ranging in weight from 120-130 gm with an average of 125 ± 2.36 gm and body size ranging 15-16 cm with an average of 15.5 ± 0.24 cm from an inbred colony representing both the sexes were selected for experimentation. The rats were kept in polypropylene cages at the 20 ± 5°C temperature, 50 ± 5% relative humidity and 12 hrs/day photoperiod. Rats were fed on rat feed obtained from Hindustan Antibiotic Ltd., (Pune), and water was provided *ad libitum.*

Cybil [α-cyno-3-phenoxybenzyl-3- (2,2, dichlorovinyl) 2,2- dimethyl cyclopropane carboxylate] was obtained from Bayer India Ltd, Bombay and fenvalerate [α-cyno-3-phenoxybenzyl, -2- (4-chlorophenyl) 3-methyl butyrate] from Hindustan Antibiotic Ltd, Pune. The acute oral LD_{50} of both pyrethroids was determined separately on albino rats. The pyrethroids were dissolved in

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coconut oil of pharmaceutical quality and introduced by gavage tube. The data were analysed by probit analysis (Finney 1971) for LD_{50} determination. (Table 1). Rats from the control set were given coconut oil alone.

Sixty four albino rats were divided into two groups of 32 rats each. The first group of 32 albino rats included the treatment groups for acute (1day) and subacute (7,14 and 21 days) studies for fenvalerate and cybil with 16 rats in each. The second group of 32 rats served as control for fenvalerate and cybil with 16 rats in each for various time intervals. The doses were introduced orally through gavage for 1,7,14 and 21 days. The doses were selected on the basis of LD_{50} (Table 1) The selected sublethal dose of 1/5 LD_{50} was given to rats. The acute and subacute doses for cybil were 129 mg/kg and 6.14 mg/kg of body weight respectively and for fenvalerate 389 mg/kg and 18.5 mg/kg of body weight respectively.

Four rats were taken out after 1,7, 14 and 21 days from control and treated sets and rats were anaesthesized by chloroform. The blood was collected directly from cardiac puncture by sterilized needles and stored in vials having anticoagulant (EDTA). Hemoglobin concentration (Hb.Conc.) was estimated by Sahli's method as outlined by Wintrobe et al. (1981). Total erythrocyte count (TEC) and total leukocyte count (TLC) were conducted using the Improved Neubaur hemocytometer (Dacie and Lewis, 1975). Packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) determined by Wintrobe's method (Wintrobe and Landsberg, 1985).

Statistical significance between experimental and control values were calculated according to Fisher's student 't' test.(Fisher, 1950)

RESULTS AND DISCUSSION

Both cybil and fenvalerate showed dose-dependent toxicity. Parker et al. (1984) and Desi et al. (1986) also observed similar dose-dependent mortality in dogs and rabbits after fenvalerate and cypermethrin intoxication, respectively.

On the basis of LD_{50} values shown in (Table 1) cybil has been found to be more toxic than fenvalerate. The findings in the present investigation gain support by the observations made by Qadri et al. (1987) and Institoris et al. (1999 b) who estimated LD_{50} of cybil to be 669 mg/kg of body weight and 554 mg/kg body weight in rats respectively. The LD_{50} of fenvalerate was 1949 mg/kg and is in accordance to Saxena and Sharma (2000) as they reported LD_{50} of fenvalerate as 1991 mg/kg of body weight. These differences between the oral LD_{50} of cybil and fenvalerate are presumably a consequence of structural variation in both pyrethroids.

TEC decreased significantly after cybil (Table 2) and fenvalerate (Table 3) administration. The decrease in TEC may be due to the toxic effect of pyrethroids

on the blood forming organ, which in turn causes a decrease in the erythropoiesis. Qadri et al. (1987) and Shakoori et al. (1988) reported similar decreases in TEC after cypermethrin intoxication in the chicken and albino rat, respectively, with acute anemia noted as the probable reason. In fenvalerate treated rats, TEC increased significantly after 21 days. Increased production of erythrocytes might be due to megaloblastic anemia because megalocytosis (macrocytosis) frequently follows intense erythropoietin-mediated stimulation of red cell production (Wintrobe et al. 1981)

Table 1 Oral Toxicity of Cybil and fenvalerate to albino rats depicting variance and fiducial limit.

Experimental Rat	Test Compound	Regression Equation	LD_{50} (mg/kg)	Variance	Fiducial limit
Rattus norvegicus	1. Cybil	y = 0.6741+1.543x`	643	0.034	0.742(+) 0.544(-)
	2. Fenvalerate	y = 18.2232+7.048x`	1949	0.002	1.972 (+) 1.927 (-)

y = expected probit
x`= log dose

Hemoglobin concentration (Hb. Conc) decreased in cybil (Table 2) treated rats. This decrease in Hb. Conc. may be due to the decrease in RBC count because Hb is an integral part of the RBC and/or to hypohaemoglobinemia. Decreases in Hb. Conc. have also been observed by Qadri et al. (1987), Khan and Ali (1993) and Saxena and Saxena (1997) after cypermethrin, pesticides, and cybil administration in chickens, factory workers and albino rats, respectively.

Fenvalerate intoxication caused enhancement in Hb. Conc. (Table 3) after acute and subacute treatement. Increased Hb Conc. may be due to megaloblastic anemia, in which DNA synthesis is defective and leads to a state of unbalanced cell growth, thus cytoplasmic components, especially hemoglobin, are synthesized in excessive amounts (Wintrobe et al. 1981). Caballo et al. (1992) reported that the cell cycle of the Chinese hamster ovary was affected by fenvalerate administration.

Intoxication of cybil (Table 2) and fenvalerate (Table 3) separately induced leukocytosis after acute and subacute treatment. Leukocytosis in some cases may be due to a protective reaction in which leukocytes protect the body when xenobiotic substances invade. Increased leukocyte count may also be found in leukemia in which uncontrolled abnormal proliferation of haemopoietic cells leads to progressive infiltration of the bone marrow in which a large number of immature forms are produced. These immature forms ultimately escape into the peripheral blood leading to very high leukocyte count.Similar increases in total leukocyte

Table 2- Effect of sublethal doses of cybil on haematological parameters of albino rat after acute (1 day) and subacute (7,14 and 21 days) treatment.

PARAMETERS*	CONTROL	CYBIL TREATED			
		ACUTE	SUBACUTE		
		1day	7days	14days	21days
TEC (million/mm^3)	6.75 ± 0.04[1]	6.40 ± 0.05[b]	6.55±0.01[b]	6.50 ± 0.05[b]	6.45 ± 0.05[b]
Hb. Conc. (gm/l)	11.70 ± 0.05[1]	10.43 ± 0.05[b]	10.87 ± 0.30[b]	10.60 ± 0.41[b]	10.40 ±0.03[b]
TLC (X10^3/mm^3)	7.10 ± 0.05[1]	8.29 ± 0.01[c]	7.13 ± 0.05	8.90 ± 0.05[c]	8.20 ± 0.05[c]
PCV (%)	43.00 ± 0.47[1]	38.00 ± 0.94[a]	34.67 ± 1.44[a]	36.33 ± 0.02[b]	39.00 ±0.47[b]
ESR (mm/hr)	3.00 ± 0.47[1]	4.33 ± 0.27	4.66 ± 0.27[a]	4.33 ± 0.98	5.66 ± 0.72

*Abbreviations used. TEC = Total erythrocyte count, Hb. Conc = Hemoglobin Concentration, TLC = Total Leukocyte Count, PCV = Packed Cell Volume, ESR = Erythrocyte Sedimentation rate.
1- Mean± SEM, Student 't' Test $P<0.05^a$ $P<0.01^b$ $P<0.001^c$

Table 3 - Effect of sublethal doses of fenvalerate on haematological parameters of albino rat after acute (1 day) and subacute treatment (7,14 & 21 days)

PARAMETERS*	CONTROL	FENVALERATE TREATED			
		ACUTE	SUBACUTE		
		1day	7days	14days	21days
TEC (million /mm^3)	6.66 ± 0.05[1]	6.53 ± 0.02	6.30 ± 0.13c	6.40 ± 0.05c	6.85 ± 0.02b
Hb. Conc (gm/l)	11.66 ± 0.13[1]	12.00 ± 0.01	11.83 ± 0.07	12.10 ± 0.17	12.53 ± 0.03c
TLC (X10^3/mm^3)	6.94 ± 0.03[1]	7.15 ± 0.02d	8.92 ± 0.04d	9.78 ± 0.04d	7.54 ± 0.02d
PCV (%)	45.00 ± 0.47[1]	49.33 ± 0.27c	48.66 ± 0.72	47.90 ± 0.8	46.00 ± 0.47
ESR (mm/hr)	2.66 ± 0.27[1]	3.33 ± 0.27	3.66 ± 0.27a	2.66 ± 0.27	4.66 ± 0.27c

*Abbreviations used. TEC = Total erythrocyte count, Hb. Conc = Hemoglobin Concentration, TLC = Total Leukocyte Count, PCV = Packed Cell Volume, ESR = Erythrocyte Sedimentation rate.

1- Mean± SEM, Student 't' Test $P<0.05^a$ $P<0.02^b$ $P<0.01^c$ $P<0.001^d$

count (TLC) were reported by Khan and Ali (1993) in factory workers, Siroki et al. (1994) in mice and Sharma and Saxena (1998) in *Columba livia* after pesticides, super cypermethrin forte and furadon SP_{50} intoxication, respectively. Contrary to these findings, Institoris et al. (1999b) observed reduction in TLC after cypermethrin treatment in rats.

The increased erythrocyte sedimentation rate (ESR) in both cybil (Table 2) and fenvalerate (Table 3) treated rats may be due to decreased total erythrocyte count (TEC) because ESR depends on Rouleux formation of erythrocytes. When Rouleux is formed the density of it's mass increases. Thus, with reduced erythrocyte count Rouleux formation decreases which in turn increases ESR. Saxena and Saxena (1997) also reported a significant increase in ESR after cybil intoxication.

PCV decreased after acute and subacute treatment of cybil administration (Table 2). Continuous decrease after acute and subacute treatment may be due to hypochromic microcytic anemia. Reduction in PCV can also be correlated with reduced RBC count.

Qadri et al. (1987) and Institoris et al. (1999a & b) reported reduction in PCV after cypermethrin intoxication in chicken and rats respectively. Siroki et al. (1994) revealed enhancement in PCV of rats following treatment by super cypermethrin.

PCV was found to be increased after oral administration of fenvalerate (Table 3). Increased PCV despite the decrease in RBC may possibly be due to macrocytic anemia in which RBCs become larger and occupy a larger space, which in turn increases PCV. Institoris et al. (1999b) reported enhancement of PCV in albino rats after permethrin intoxication.

In the light of present findings it can be concluded that both the pyrethroids, cybil and fenvalerate, are capable of inducing changes in blood and blood-forming organs, but in a different way because there is considerable evidence that all pyrethroids do not act the same way in mammals.

While both compounds were synthetic pyrethroids, their effect on haematological parameters were different. It was observed that cybil is more toxic or reactive than fenvalerate based on LD_{50} values. (Table 1) These difference may be due to difference in their structure. As the cybil contains a cyclopropane ring (Fig.1 B1), having both cis and trans isomers, which make cybil more reactive. The cyclopropane ring is absent in fenvalerate, probably accounting for its reduced reactivity.

Activity of these complexes was found to depend on the presence of the halogen group. The complexes having a halogen group show greater reactivity than the

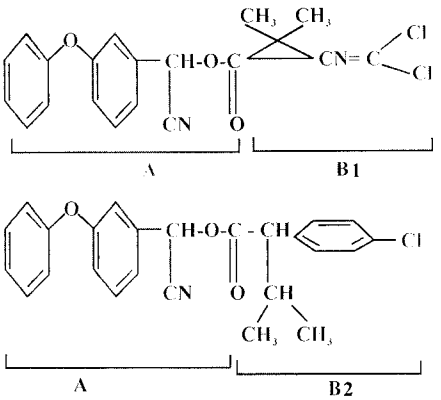

Figure 1. Structural relationship between cybil and fenvalerate

complexes lacking or having lesser number of such groups. Cybil contains two chlorine atoms which accounts for its greater toxicity compared to fenvalerate, with one chlorine atom. Saxena and Crowe (1988) and Saxena and Saxena (1989) observed that halogen groups are an important contributor to this activity for organotin compounds. Low reactivity of fenvalerate may also be due to presence of the chlorophenyl ring. Reductive dehalogenations may occur in the phenyl ring and with only one chlorine atom for fenvalerate attached to the phenyl ring, (Fig.1 B2). Fenvalerate is likely to be less reactive than cybil.

Cybil, also contains a double bond. Because the double bond is a weak bond, cybil is more reactive. It can be interpreted that the different action of both the pyrethorids is probably due to their different structures.

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