Nature & Life

https://natureandlife.co.in/

VOLUME:1, ISSUE:1

Comparative Toxicity of Cybil and Hafen 20EC on Red Cell Indices of albino rat

Submitted by Dr. Vibha Tomar Assistant Professor
Meerut College, Meerut
E-mail : vibha25vs@gmail.com

Abstract

Acute and sub-acute toxicity of Cybil and Hafen 20EC (the synthetic pyrethroids) were studied separately on red cell indices of albino rat by administering oral dose to albino rat and observing them for one day for acute and 7, 14 and 21 days for sub-acute treatment. In this investigation acute dose of Cybil and Hafen 20EC was 129 mg/kg and 389 mg/kg of body weight respectively and for sub-acute treatment doses's were 6.14 mg/kg and 18.5 mg/kg of body weight respectively. Both the pyrethroids (synthetic pyrethroid) showed dose dependent toxicity and Cybil was found to be more toxic than Hafen 20EC base on LD_{50} values (i.e. LD_{50} of Cybil was 643 mg/kg of body weight and LD_{50} of Hafen 20EC was 1949 mg/kg of body weight) (TABLE 1). Both the pyrethroids caused significant alterations in red cell indices, i.e MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration) but in a different way. Different haematoxic effect of both the pyrethroid have been attributed to their different chemical structure.

Key Words : Toxicity, Synthetic Pyrethroid, Cybil, Hafen 20EC

INTRODUCTION

The indiscriminate use of pesticide posed potential health hazard to human beings. Synthetic pyrethroids are among the most potent pesticides that are known today and have been widely used in agriculture in different parts of the world. In addition to extensive agricultural use, the synthetic pyrethroid are widely available in our homes in the form of mosquito aerosol, mosquito vapour mats, mosquito coils etc. Although these insecticides cannot be considered highly toxic to mammals, their use indoor, in enclosed and poorly ventilated areas has resulted in signs and symptoms of toxicity to humans.

Synthetic pyrethroids are chemical analogues of natural pyrethrins, which are derived from the flower, *Chrysanthemum cinerarifolium*.The most commonly used synthetic pyrethroids are Cypermethrin (Cybil), fenvalerate (Hafen 20EC), deltamethrin, allethrin, λ- cyhalothrin, ß-cyfluthrin etc.

Considering the paucity of information on the effect of pyrethroids on blood and widespread use of these compounds, it is necessary that pyrethroid – blood interaction be exposed further.

Keeping this in view, in the present investigation the synthetic pyrethroids, Cybil and Hafen 20EC 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

Experimental Compounds

Cybil [α-cyno-3-phenoxybenzyl-3-(2,2, dichlorovinyl) 2,2 – dimethyl cyclopropane carboxylate] was obtained from Bayer India Ltd., Bombay and Hafen 20EC [α-cyno-3- phenoxybenzyl-2-(4-, chlorophenyl) 3–methylbutyrate] from Hindustan Antibiotic Ltd., (Pune). The acute oral$LD_{50}$of both prethroids were determined separately on albino rats. The prethroids were dissolved in coconut oil of pharmaceutical quality and introduces 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.

Experimental Animal

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 ± 5º 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*.

Experimental Design

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 (1 day) and subacute (7, 14 and 21 days) studies for Cybil and Hafen 20EC with 16 rats in each. The second group of 32 rats served as control for Cybil and Hafen 20EC 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 acute and sub-acute doses for Cybil were 129 mg/kg and 6.14 mg/kg of body weight respectively. The acute and sub-acute doses for Hafen 20EC were 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 and outlined by Wintrobe et al. (1981). Total erythrocyte count (TEC) were conducted using the Improved Neubaurhemocytometer (Dacie and Lewis, 1975) and Packed cell volume (PCV) were 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).

RESULT AND DISCUSSION

In the present investigation, both the experimental compounds are Type-II pyrethroid which produce CS-syndrome (Barnes and Verschoyle 19740. The oral administration of both pyrethroid, i.e. Cybil and Hafen 20EC showed dose-dependent toxicity, which is in accordance to earlier findings in albino rat after fenvalerate intoxication (Saxena and Sharma 2000) and Parker et al. (1984) & Desi et al. (1986) after cypermethrin intoxication) in dogs and rabbits respectively.

In the present investigation, the absolute value or red cell indices (MCV, MCH, MCHC) of albino rat have been evaluated.

On the basis of LD_{50}values shown in (TABLE 1) cybil has been found to be more toxic than Hafen 20EC. The findings in the present investigation gain support by the observations made by Qadir et al. (1987) and Institoris et al. (1999b) 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 Hafen 20EC 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.

The variation in LD_{50}values may be due to the fact that the toxicity of chemical is found to be dependent on number of factors such as vehicle used, species, sex and age of experimental animal, temperature, humidity and social atmosphere etc.

These differences between the oral LD_{50} of Cybil and Hafen 20EC are presumably a consequence of structural variation in both prethroids.

Table 1

Oral Toxicity of Cybil and Hafen 20EC to albino rats depicting variance and fiducial limit

Experimental Rat	Test Compound	Regression Equation	LD_{50} (mg/kg)	Variance	Fiducial limit
Rattus norvegicus	cybil	y = 0.6741 + 1.543x´	643	0.034	0.742 (+) 0.544 (-)
	Hafen 20EC	y = 18.2232 + 7.048x´	1949	0.002	1.972 (+) 1.927 (-)

y = expected probit x´= log dose

It is observed that Haemoglobin concentration (Hb. Conc.), Total erythrocyte count (TEC) and Packed Cell Volume (PCV) decreased after Cybil intoxication. Whereas, Haemoglobin concentration (Hb. Conc.) and Packed Cell Volume (PCV) were increased and Total erythrocyte count (TEC) decreased after Hafen 20EC intoxication.

In the present investigation, Mean Corpuscular Volume (MCV) is an average volume of a single red cell. Cybil intoxication caused a significant reduction in MCV (Table 2) after acute and sub-acute treatment and is an indication of Microcytic anaemia in which RBC become smaller, spherical, pale and can easily be broken down. MCV can also be reduced in haemolytic anaemia due to inadequate erythropoiesis. These findings is in accordance to earlier findings of Mandal and Lahiri (1985), Saxena and Saxena (1997) and Institoris et al. (1999). Whereas, are in contrast to the findings of Shakoori et al. (1988).

Table 2

Effect of sublethal doses of Cybil on red cell indices of albino rat after acute (1 day) and sub-acute (7, 14 and 21 days) treatment

Cybil treated

PARAMETERS *	CONTROL	ACUTE TREATMENT	SUB ACUTE TREATMENT		
		1 day	7 days	14 days	21 days
MCV (fl)	63.70 ± 0.32	59.38 ± 0.48[a]	52.93 ± 0.47[b]	55.89 ± 0.06[b]	60.46 ± 0.14
MCH (pg)	17.33 ± 0.54	16.29 ± 0.05	16.59 ± 0.5	16.30 ± 0.47	16.12 ± 0.45
MCHC (%)	27.21± 0.05[1]	27.44 ± 0.03	31.35 ± 0.05[b]	29.18 ± 1.0	26.66 ± 0.03[b]

[Values are mean + SE from 4 rats]

[a] and [b] indicate statistical significance at P<0.01 and P<0.001 respectively, in comparison to control group. Values without superscript are statistically non significant.

On the other hand, Hafen 20EC intoxication results significant increase (Table 3) in Mean Corpuscular Volume (MCV) after acute and sub-acute treatment and is an indication of Macrocytic anaemia in which RBC become larger in size. In these cases, reticulocytosis is present and the rapid re-generation in the bone marrow leads to formation of macronormoblasts leading to formation of macrocytes. Increase in MCV has also been reported by Ali and Shakon (1981) in rabbits after Malathion intoxication and Rajini et al. (1987) in albino rats after primiphos – methyl administration. Institoris et al. (1999) had also observed non-significant decrease in MCV after permethrin administration in rats.

MCH (Mean Corpuscular Haemoglobin) is the average amount of haemoglobin contained in a single red cell. Cybil administration caused reduction in MCH (Table 2). Reduction in MCH may be due to Microcytic anaemia and can be co-related with reduction in Hb. Concentration and TEC. Wintrobe reported MCH decreases in microcytic anaemia.

While Hafen 20EC induced enhancement in MCH after acute and sub-acute treatment. Induction in MCH may be due to macrocytic anaemia in which large RBC contained increased amount of haemoglobin. In Macrocytic anaemia, DNA synthesis is defective and leads to a state of unbalanced cell growth in which synthesis of RNA and protein continues while DNA synthesis gets retarded. Thus cytoplasmic components especially haemoglobin synthesized in excessive amount during the delay between cell division and may be the probable reason of increased MCH. Caballo et al. (1992) reported that the cell cycle of the Chinese hamster ovary was effected by Fenvalerate administration. Increased Hb. Concentration may also be due to erythropoietin stimulation, which accelerates Hb. Synthesis in erythrocyte precursor without shortening the time between cell division or altering the number of divisions. (Wintrobe et al. 1981).

Table 3

Effect of sublethal doses of Hafen 20EC on red cell indices of albino rat after acute (1 day) and sub-acute (7, 14 and 21 days) treatment

Hafen 20 EC treated

PARAMETERS *	CONTROL	ACUTE TREATMENT	SUB ACUTE TREATMENT		
		1 day	7 days	14 days	21 days

MCV (fl)	67.56 ± 0.47	75.89 ± 0.70[b]	77.23 ± 0.8[b]	74.84 ± 0.57[b]	67.15 ± 0.6
MCH (pg)	17.50 ± 0.01	18.46 ± 0.8	18.77 ± 0.6[a]	18.90 ± 0.7[a]	18.29 ± 0.7
MCHC (%)	26.00± 0.07	27.44 ± 0.05[b]	24.31 ± 0.81[b]	25.26 ± 0.5[b]	27.24 ± 0.05[b]

[Values are mean + SE from 4 rats]

[a] and [b] indicate statistical significance at P<0.01 and P<0.001 respectively, in comparison to control group. Values without superscript are statistically non significant.

MCHC (Mean Corpuscular Haemoglobin Concentration) is the percentage saturation of red cells with haemoglobin. Cybil intoxication caused increase and decrease in MCHC (Table 2) after 1, 7, 14 days and 21 days respectively. Hafen 20EC causes decrease after 1, 7, 14 days and increase after 21 days (Table 3) in MCHC. Fluctuation in MCHC in both the cases, may be due to inadequate erythropoiesis. The involvement of macrocytic and microcytic anemia in Hafen 20 EC and Cybil intoxication cannot be ignored.

In the present study both the experimental compounds are synthetic pyrethroids but difference in their LD_{50} values may be due to structural differences. The experiment shows that there is a noticeable difference in their toxicity. It seems likely that such a difference originates from a different mode of interaction at molecular level. There is a considerable evidence that all pyrethroids do not act in mammals in the same way. (Miyamoto 1976).

This study shows that the health effects of Cybil and Hafen 20EC; in general, may be more severe than previous toxicological evaluations and are capable of inducing changes in blood and blood forming organs.

REFERENCES

Ali, S.S. and A.R. Shakon (1981) Resistance to malathion toxicity in rabbits as revealed by studies on blood and liver. Pak. J. Zool. 13(1/2) : 269-281

Barnes JM, Verschoyle RD (1974) Toxicity of new pyrethroid insecticide. Nature 248 : (5450) 711

Caballo C, Heromera A, Barrucca C, Santa Maria A, Sanz F, DeLa Pena E (1992) Analysis of cytogenetic damage induced by the pyrethroid insecticide fenvalerate. Teratogen Carcinogen Mutagen 12(6) : 243-249

Dacie JA, Lewis SM (1975) Practical Haematology.J.and A, Churchilli Ltd., London Finney DJ (1971) Probit Analysis. Cambridge University Press, London. pp 303

Fisher RA (1950) Statistical method for research workers, 11th ed. Oliver and Boyd Ltd., Edinburg, U.K. pp 146

Institoris L, Siroki O, Undeger U, Desi I, NagyaMajtenyi L (1999a) Immunotoxicological effects of repeated combined exposure by cypermethrin and heavy metals lead and cadmium in rats. Int J Immunopharmacol 21 (II) : 735-743

Mandal, AS, Lahiri P (1985) Haematological response to sumithion in the blue rock pigeon, Columbia Livia Gemlin. Ind. J Exp. Biol. 23 : 702-7905

Miyamato, J. (1976) Degradation, metabolism and toxicity of synthethic prethroids. Environ Health Perspect 14 : 15-28

Saxena PN, Saxena P (1997) Haemogrammic studies in albino rat after Cybil intoxication. J Environ Biol 18 (4) : 425-428

Saxena PN, Sharma DC (2000) Effect of synthethic pyrethroid on behaviour pattern in Rattus Norvegicus. Proc. Nat. Acad. Sci. India 70(B) : 1

Shakoori AR, Ali SS, Saleem MA (1988) Effect of six months feeding of cypermethrin on the blood and liver of albino rat. J BiochemToxicol3 : 59-71

Wintrobe MM, Landsberg JW (1985) A standardized technique for blood sedimentation test. Amer J Med Sci189 : 102-105

Wintrobe MM, Richard G, Lee Boggs DR, Bithell TC, Foerster J, Athens JW, LukonsJN (1981) Clinical Haematology, 8thEdn. Lea and Febiger, Philadelphia.