Original Paper

Alteration of Haematological Profiles due to Cypermethrin Toxicosis in *Rana hexadactyla*

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ABSTRACT

Cypermethrin is a synthetic pyrethroid insecticide with low mammalian toxicity but high insecticidal activity. Following exposure to sublethal concentration of cypermethrin (1/10 LC_{50} 1.63mg/l) in frogs, the haematological profiles were investigated over 7 days and 30 days. The results revealed statistically significant decrease of red blood cells, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and neutrophils, and increase of the other white blood cells, including lymphocytes, monocytes, and eosinophils.

Key Words: Cypermethrin, Frog, *Rana hexadactyla*, Haematology

Introduction

Synthetic pyrethroid pesticides account for over 30% of the global pesticide use.[1] These compounds have gained popularity over organochlorine and organophosphate pesticides due to their high effectiveness against target species,[2] relatively low mammalian toxicity,[3] and rapid biodegradability.[4] Cypermethrin is a composite synthetic pyrethroid, and is a broad spectrum, biodegradable insecticide, with rapid neurotoxicity. It is used to control many pests, including moths, and pests of cotton, fruit, and vegetable crops.

In human medicine, investigation of haematological parameters is necessary for clinical diagnosis of a disease or pathological conditions. Haemopoietic and leukocytic systems are two dynamic systems which react quickly to environmental changes, and facilitate the maintenance of homeostasis by an organism. Haematological and biochemical profiles of blood can therefore provide important information about the internal milieu of the organism.[5]

Alteration in the haematological profiles can be affected by sex,[6] altitude,[7] pesticides,[8] drugs, and metals,[9] food additives,[10] dyes,[11] industrial effluent chemicals[12] and parasitic infestation.[13] The circulatory system serves as the primary target site for most pesticides. The aim of this study is to study the effect of cypermethrin on the haematological profiles in frogs over a 7-day and 30-day cypermethrin exposure period. It is well known that farmers, pesticide applicators, industrial workers, and other pesticide users are exposed to pesticides such as cypermethrin repeatedly.

Materials and Methods

Healthy frogs (*Rana hexadactyla*), were collected from their natural habitat, in and around Tirupati, with a mean weight of 50±5gm. They were housed in glass tanks partially filled with wet soil, and covered with wire mesh. They were acclimatized to laboratory conditions for one week prior to the experiment, with water temperature maintained at 27± 2ºC, pH of 7±0.1, and light period of 12 h. They were fed earthworms and cockroaches *ad libitum* to prevent starvation. The animals were starved for 24 h before they were exposed to the pesticide (cypermethrin). Each frog was examined for signs of abnormality or parasitic infestation, and if anything was

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detected, they were eliminated from the study. Technical grade cypermethrin (92% purity; *cis:trans* ratio 40:60) was obtained from Tagros Chemicals India Limited, Chennai. It was dissolved in acetone to study the subacute and subchronic effects.

One tenth of the LC_{50} value (0.163 mg/l) was selected as sublethal concentration, and the frogs were exposed for 7 days and 30 days, with one day interval, while the control frogs were exposed only to acetone. At the end of the experiment (7 days and 30 days), ten frogs of each group were anaesthetized with anaesthetic ether, and blood samples were drawn from the conus arteriosus of each animal using a heparinised syringe. The blood was collected in a vial containing ethylenediamine tetra acetic acid (EDTA). Blood samples were analyzed with regard to the following parameters: red blood cell (RBC) and white blood cell (WBC) counts,[14] haemoglobin (Hb),[15] haematocrit (Hct),[16] differential count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Statistical analysis

Statistical analysis was performed using the SPSS/PC+ statistical package. Significant differences between control and experimental groups were determined using 't' test. The level of significance was established at $p<0.05$ or $p<0.01$.

Results

The results of haematological profiles of the control and experimental frogs involved in this study on cypermethrin toxicity are mentioned in **Table 1**. The experimental frogs exposed to cypermethrin showed statistically significant ($P<0.01$) decrease in RBC, Hb, PCV, MCV, MCH, MCHC, and neutrophil count, while there was an increase of the other WBC, including lymphocytes, monocytes, and eosinophils, when compared to control animals. No statistically significant difference was noted in the percentage of basophils.

Discussion

Exposure of frogs to cypermethrin in the present study showed remarkable haematological alterations. Cypermethrin is said to have two modes of action on blood cells. It induces oxidative stress,[17] and as a hydrophobic compound, it accumulates in cell membranes and disturbs the membrane structure.[18] The decrease in RBC count, Hb, Hct, MCV, MCH and MCHC levels observed in this study is due to the disruptive action of cypermethrin.

Alterations in haematological profiles were brought about by cypermethrin in the form of an anaemic condition because of decreased synthesis of RBC/increased RBC haemolysis. The results in the present study are in agreement with those found by Morgan et al[19] in the bone marrow. The decline in RBC count, haemoglobin concentration, and haematocrit presumably reflects erythrocyte haemolysis/decreased synthesis of RBC. The decrease in Hb concentration may be because of either an increase in the rate at which Hb is destroyed, or a decrease in the rate of Hb synthesis.[20]

Red cell indicators such as MCV, MCH, and MCHC are dependent on the RBC count, Hb concentration, and PCV values. MCV, MCH and MCHC showed statistically significant decrease in the present study due to destruction of RBC (size and shape), and decrease in Hb synthesis and Hb content.

An increase in lymphocytes and monocytes were noted in cypermethrin-treated frogs when compared to the control group. A clear increase in the number of leukocytes in the blood of frogs poisoned with cypermethrin was the result of stimulation of the process of lymphopoiesis. These results are in agreement with the earlier report of Nagarjuna et al,[8] who demonstrated a similar situation in albino rats exposed to cypermethrin. In male mice poisoned with alpha cypermethrin, a mobilization of the leukocytic system took place, manifested by a statistically significant increase in the number of leukocytes. This increase was particularly evident in the case of monocytes and lymphocytes.[21] Haratym-Maj[7] demonstrated an increase in the number of leukocytes in the blood of animals, irrespective of the pyrethroid applied for intoxication, which may be due to the mobilization of the immunological system, and/or a shift in the leukocytic pool from the spleen to peripheral blood. Increased lymphocyte and monocyte counts have been observed in rats treated with cypermethrin.[8] Based on the results of these studies, it can be presumed that cypermethrin mobilizes leukocytic, lymphocytic and monocytic systems to peripheral blood.

A decreased percentage of neutrophils in the peripheral blood observed in animals poisoned with cypermethrin suggests that neutrophils are involved in phagocytosis during xenobiotic intoxication, as a result of which, some

Table 1 Haemogram of control and cypermethrin-treated frogs

Parameters	Control	7 Days	30 Days
RBC (millions/cmm)			
Mean	0.5341	0.4650**	0.3750**
SD	±0.0102	±0.0105	±0.0138
PC		(-12.94)	(-29.79)
Hb (g/100ml)			
Mean	9.1333	6.9333**	4.6001**
SD	±0.2160	±0.1633	±0.2366
PC		(-24.08)	(-49.63)
PCV (percent)			
Mean	31.0010	24.3333**	18.1666**
SD	±0.8944	±1.2111	±0.7528
PC		(-21.50)	(-41.39)
MCV (µg)			
Mean	580.3767	523.3730**	484.4580**
SD	±14.2221	±25.0978	±10.2609
PC		(-9.82)	(-16.52)
MCH (pg)			
Mean	171.0020	149.1980**	122.8560**
SD	±3.6864	±5.9462	±8.7741
PC		(-12.75)	(-28.15)
MCHC (percent)			
Mean	29.465	28.538^{NS}	25.3660**
SD	±0.3277	±1.2650	±1.9170
PC		(-3.14)	(-13.90)
WBC (thousands/cmm)			
Mean	234462.5000	268566.7000**	290283.3000**
SD	±553.1162	±933.6309	±1479.7520
PC		(-14.54)	(-23.80)
Lymphocytes			
Mean	27.0010	39.3330**	51.8330**
SD	±1.4142	±1.6340	±1.4720
PC		(-45.67)	(-91.97)
Monocytes			
Mean	3.8330	5.6660**	7.5000**
SD	±0.7528	±0.5164	±0.5477
PC		(47.82)	(95.65)
Neutrophils			
Mean	61.1666	43.8330**	28.0000**
SD	±1.4720	±1.9408	±2.2803
PC		(-28.33)	(-54.22)
Eosinophils			
Mean	5.0000	8.3330**	9.6660**
SD	±0.6324	±0.5164	±0.8165
PC		(66.66)	(93.33)
Basophils			
Mean	3.0000	3.0000^{NS}	3.0000^{NS}
SD	±0.8944	±0.8944	±0.8944
PC		(0)	(0)

All the values are mean ± SD of six individual observations.
SD – Standard Deviation PC – Percent change over control NS: Not Significant,
*-Significant (P<0.05), **- Highly Significant (P<0.01)

of the neutrophils were destroyed. Therefore, the neutrophil count consistently decreased during 7 days, as well as 30 days cypermethrin intoxication in frogs in the present study. Studies on humans (males and females) participating in the production of liquid pesticides, have also demonstrated a significant decrease in the number of neutrophils.[22] Haratym-Maj[7] reported a decrease in the number of neutrophils in mice intoxicated with high deltamethrin doses.

In conclusion, it can be stated that heavy pyrethroid pesticide use can result in their accumulation in various components of the environment, which can lead to levels that can pose serious threat to non-target organisms.

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REFERENCES

1. Eisler R. Fenvalerate hazards to fish, wildlife, and invertebrates: A synoptic review. In: Report 24, Contaminant Hazards Reviews, US Department of the Interior Fish and Wildlife Service, Washington, DC. 1992. p3.
2. Eillot M, Janes NF, Potter C. The future of pyrethroids in insect control. Ann Rev Entomol 1978; 23: 744-769.
3. Parker CM, Patterson DR, Van Gelder GA, Gordon EB, Valerio MG, Hall WC. Chronic toxicity and carcinogenicity evaluation of fenvalerate in rats. J Toxicol Environ Health 1984; 13: 83-97.
4. Leahey JP. Metabolism and environmental degradation. In: Leahey JP (editor). The Pyrethroid Insecticides. 1985. Taylor and Francis, London. 133-224.
5. Masopust J. In: Clinical Biochemistry (in Czech). 2000. Karolinum, Prague. p832.
6. Haratym-Maj A. Haematological alterations after pyrethroid poisoning in mice. Ann Agri Environ Med 2002; 9: 199-206.
7. Murray Robert K, Granner Daryl K, Mayes Peter A, Rodwell Victor W. In: Harper's Illustrated Biochemistry. International 27th edn, 2007. McGraw-Hill Company Inc.
8. Nagarjuna A, Rajendra Prasad S, Siraj Mohyuddin S, Jayakumar R, Savithri Y, Jacob Doss P. Responses of cypermethrin intoxication-induced stress in hematological profiles of mammalian model albino rat. Asian J Microbiol Biotechnol Environ Sci 2008; 10: 48-51.
9. Sharma JD, Sharma MK, Agrawal P, Sohu D, Jain P. Physiological and biochemical alterations in vital organs of albino rats following fluoride water treatment. Indian J Environ Sci 2006; 10: 125–130.
10. Sharma A, Goyal RP, Chakravarty G, Sharma S. Toxicological studies on the effect of apple green - a permitted food colour, on Swiss albino mice. Indian J Environ Sci 2006; 10: 21–24.
11. Ramulu B, Swarnalata G, Krishnan S. Case report: Hair dye poisoning. J Indian Soc Toxicol 2006; 2: 46–47.
12. Lynch DW, Placke ME, Persing RL, Ryan MJ. Thirteen-week inhalation toxicity of N,N-dimethylformamide in F344/N rats and B6C3F1 mice. Toxicol Sci 2003; 72: 347–358.
13. Arti Saxena, Shakuntala Shukla. Study of changes in haematological parameters in *Labeo calbasu* infected by Trypanosoma at Rewa (MP). Asian J Microbiol Biotech Environ Sci 2006; 8: 111–114.
14. Davidson I, Henry JB. Todd-Sanford's Clinical Diagnosis by Laboratory Methods. 14th edn, 1969. WB Saunders Co. 139–143.
15. Sahli T. In: Seward E Milles (editor). Textbook of Clinical Pathology. 1962. William & Williams and Co. Baltimore. p35.
16. Schalm OW, Jain NC, Caroll EJ. Veterinary Haematology. 3rd edn, 1975. Lea and Febiger, Philadelphia, USA. 45–46.
17. Kale M, Rathore N, John S, Bhatnagar D. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: A possible involvement of reactive oxygen species. Toxicol Lett 1999; 105: 197-205.
18. Michelangeli E, Robson MJ, East JM, Lee AG. The conformation of pyrethroids bound to lipid bilayers. Biochimicaet Biophysica Acta 1990; 1028: 49–57.
19. Morgan DP, Stockdale EM, Roberts RJ, Walter HW. Anemia associated with exposure to lindane. Arch Environ Health 1980; 35: 307–310.
20. Moss JA, Hathway DE. Transport of organic compounds in the mammalian partition of dieldrin and telodrin between the cellular components and soluble proteins of blood. Biochem J 1964; 91: 383–393.
21. Luty S, Latuszynska J, Obuchowska-Przebirowska D, Tokarska M, Haratym-Maj A. Subacute toxicity of orally applied alpha cypermethrin in Swiss mice. Ann Agri Environ Med 2000; 7: 33–41.
22. Klucinski P, Hrycek A, Stasiura-Zielinska H, Kossmann S, Tustanowski J, Friedek D, Kaminska-Kolodziej B. Humoral and cellular immunity rates in chemical plant workers employed in the production of liquid pesticides. Int J Occup Med Environ Health 1996; 9: 103–110.