Toxic Impact of Synthetic Pyrethroid; Beta - Cyfluthrin on Differential Leukocyte Count (DLC) of Albino Rats

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Abstract

In this study hemotoxic potential of synthetic Pyrethroid; Beta-Cyfluthrin has been evaluated on albino rats. Albino rats were selected randomly from inbred colony. Experiment designed to evaluate acute as well as sub - chronic toxicity. One day for acute and 7, 14, 21 days for sub – chronic toxicity. Dose of Beta-Cyfluthrin was selected on the basis of LD_{50} value i.e. 726mg/kg b.wt. (Table I).

Introduction

Pesticides are toxic chemicals designed to kill invading pests. Each pesticides is intended to kill a certain type of harmful organism, but due to indiscriminate use, a large amount of pesticides reach places other than their intended target. Ideally , the pesticides used should be toxic to the target organisms only, being biodegradable and ecofriendly to some extent. (Roselle et al.2008)

It has been reported that only about 0.1% of the pesticides reach the target organism and the rest bulk of these pesticides pollute the surrounding environment. Because pesticides exist in nature, they now enter the food chain and can enter the human body through food , especially fruits and vegetables, contaminated water or contaminated air.

In this study negative effect of insecticide (Beta-Cyfluthrin) on Differential Leukocytes Count (DLC) of albino rats have been observed, would be helpful in approaching the possibility of toxicity of these commonly used pesticides

Materials and Methods

Material -synthetic Pyrethroid; Beta Cyfluthrin was procued from Bayer India Ltd., Bombay.

Experimental Animal Albino rats (representing both the sexes) selected randomly from inbred colony and of average body weight 145 gm were housed in air conditioned room at 25±5°C temperature and relative humidity 60±5% with a 12 hour/day photoperiod. Animals were fed on a synthetic pellet diet (Hindustan Liver Animal Feed, India) and water *ad. libitum* throughout the period of study.

LD_{50} of Beta-Cyfluthrin – The acute oral LD_{50} of Beta-Cyfluthrin was determined by the probit analysis, log. Dose/ probit regression line method (finney, 1971) on adult albino rats using five doses and four animal per doses.

Selection of Dose - Dose were selected on the basis of LD_{50} (Table I)

Design of Experiment for Acute and Sub - Chronic Study-

The rats were divided into the two sets, one acute and one subchronic set consisting of five and twenty rats respectively. The control sets were rum simultaneously for acute and subchronic examination with five and twenty rats respectively.

The rats from acute set (were given a sub lethal doses of Beta – Cyfluthrin) for 1 day (24 hour) and rats from subchronic set for 7, 14, 21 days orally through gavage. The rats were scarified and then blood samples were collected after 7, 14, and 21 days of treatment.

The rats from control set for acute and subchronic treatment were given a vehicle treatment only using a similar amount (i.e. acute and subchronic dose of Beta – Cyfluthrin) of diluent i.e. coconut oil orally through gavage.

Preparation of Vials

EDTA (Ethylene Dinitrilo Tetro Acetic acid) vials were prepared by adding two to four drops of 2% EDTA solution in vials. Vials were then dried in an oven at 60-80°C. These vials prevent coagulation of blood and maintain the cell volume and the cell size within normal range.

Collection of Blood

The rats were anaesthetized by chloroform and the blood samples were collected from the dissected rats by cardiac puncture and stored in a vials.

Procedure for Differential Leukocyte Count (DLC)

Five thin blood smear were prepared of each animals for DLC. After drying, the smear stained with Leishmans stain for about four minute. The twice volume of distilled water added on the stain. After ten minutes the slide were washed with the water, till it appear pink colour.

The different types of Leukocyte were examined under oil emersion lens of microscope.

Calculation

The hundred cells were counted by Battlement method.

The percentage of each type of leukocyte was calculated by the following formula:

Percentage of cell type

$$= \frac{\text{Number of Cell type}}{\text{Total number of leukocyte}} \times 100$$

Table- I

LD_{50} of Beta – Cyfluthrin

Experiment Animal	Test Compound	Regression Equation	LD_{50} (ml/kg b. wt.	Variance	Fiducial Limit
Rattus norvegicus	Beta Cyfluthrin	Y=7.359+4.35x'	0.726	0.007	0.751(+) 0.751(-)

Result

In this study Differential leukocyte count (DLC) of the Control and treated (acute and subchronic) animals are observed.

Granulocyte Profile (After Beta-Cyfluthrin administration)

Neutrophils- In control set showed no significant increase while in acute (1 day) treatment, it showed significant ($P<0.01$) increase.Neutrophil count showed significant ($P<0.02$) to very highly significant ($P<0.01$) increase after subchronic treatment (i.e. 7, 14, 21 days)

Eosinophils – Showed significant ($P<0.05$) increase after acute (1 day) treatment while non-significant ($P<0.05$) increase after subchronic (7, 14, 21 days) treatment.

Basophils – Have not been found in control and treated albino rats in this study.

AGRANULOCYTE PROFILE (After Beta – Cyfluthrin administration)

1. Lymphocytes- A non significant ($P<0.05$) decrease after acute (1 day) treatment. A significant ($P<0.05$) increase has been noted after subchronic (7, 14, 21 days) treatment.

2. Monocytes – These are the largest cells in the peripheral blood. Monocytes showed very highly significant ($P<0.01$) increase after acute (1 day) treatment. In subchronic treatment, non-significant ($P<0.01$) increase after 7 and 21 days while significant increase after 14 days of treatment.

Animals in control set showed no significant change during the period of study

Table- II

Table - XV

Effect of Bata - Cyfluthrin on differential leukocyte count (DLC) of albino rat after acute (1day) and subchronic (7, 14 & 21 days)

S. No.	Differential leukocyte count (DLC) (%)		No. of Rats	TREATMENTS											
				ACUTE			SUBCHRONIC								
				1 DAY			7 DAYS			14 DAYS			21 DAYS		
				Range	Mean ± SE	SL	Range	Mean ± SE	SL	Range	Mean ± SE	SL	Range	Mean ± SE	SL
1	Neutrophils	C	5	26 - 29	27.4 ± 0.50	P<0.01	26 - 30	28.0 ± 0.71	P<0.02	26 - 31	28.4 ± 0.92	P<0.01	26 - 30	28.0 ± 0.71	P<0.01
		T	5	29 - 31	30.02 ± .37		30 - 40	34.0 ± 1.70		34 - 38	35.6 ± 0.68		35 - 41	37.4 ± 1.29	
2	Eosinophils	C	5	2 - 3	2.2± 0.2	P<0.01	1 - 2	1.6 ± 0.24	P>0.05	1 - 3	2.2 ± 0.37	P>0.05	1 - 3	2.4 ± 0.4	P>0.05
		T	5	3 - 4	3.2 ± 0.2		2 - 4	2.6 ± 0.4		2 - 5	3.6 ± 0.67		3 - 4	3.4 ± 0.24	
3	Lymphocytes	C	5	65 - 69	66.6 ± 0.51	P>0.05	67 - 69	67.8 ± 0.37	P<0.05	65 - 70	68.0 ± 0.87	P>0.05	65 - 69	67.4 ± 0.68	P<0.05
		T	5	60 - 69	65.8 ± 1.56		68 - 72	69.6 ± 0.68		68 - 75	70.6 ± 1.21		69 - 71	69.6 ± 0.4	
4	Monocytes	C	5	3 - 4	3.4 ± 0.24	P<0.001	2 - 4	3.0 ± 0.45	P<0.05	3 - 4	3.6 ± 0.24	P<0.02	3 - 4	3.6 ± 0.24	P<0.05
		T	5	4 - 5	4.2 ± 0.2		3 - 4	3.8 ± 0.2		4 - 5	4.6 ± 0.24		2 - 4	3.0 ± 0.35	

C - Control
T - Treated
SE - Standard Error
SL - Significance Level

Discussion

In mammals white blood cells (WBCs) are of five type: Lymphocytes, neutrophils, eosinophils, basophils and monocytes. Neutrophils and lymphocytes make up the 80% (combined) of WBCs.

Neutrophils are the primarly phagocytic leukocyte and proliferate in response to infections, inflammation and stress (Jain 1993)

Lymphocytes involved in antibody productions (immunoglobulin) and also involve in modulation of immune define. Rest of the leukocytes or associated with defence mechanism against infection.

In the present investigation, DLC showed significant increase (Table II) throughout the treatment. DLC level could serve as marker for severity of pesticide (Beta-Cyfluthrin).

These cells are important to an immunity response and increased in number during an infection or stress like poisoning. Yousef et.al.(2003) observed increase in total leukocyte count in rabbits after isoflavanos and cypermethrin intoxication. Similar result have been observed by Celik et.al.(2009) and Zuhain (2006) after dichlorovas and Sumithion intoxication in rabbits. Neutrophilia observed in this study, which occurs within a few hours after the onset of acute inflammation, this result from a combination of chemical substances that are released from the inflamed tissues, collectively called leucocytosis - inducing factor. This factor carried to the bone marrow, thus causing release of many leukocytes. Eosinophils also increased and gain support by Khan and Ali (1993), Catinot et.al. (1984) reported eosinophilia and granulocytosis in mice after deltamethrin intoxication. Sharma and Saxena (1998) in *Columba livia* after furadon SP_{50} intoxication.These results indicates that Beta-Cyfluthrin has a hemotoxic potential which in turn induced phagocytic activity of blood. Mandal and Lahiri (1985) reported lymphopaenia in experimental animals as an outcome of the possible immune – suppression by pesticide treatment.

Pesticide induced immune suppression is one of the severely emerging, imperfectly understood health risks associated with pesticide exposure

The aim of present is to evaluate, toxic potential of newly synthesized pesticide are one of the major cause of pollution and greatly upset the natural balance. These pesticides must be tested before releasing for commercial use.

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