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SHORT COMMUNICATION

IMMUNOTOXIC RESPONSES OF CYPERMETHRIN, A SYNTHETIC PYRETHROID INSECTICIDE IN RATS

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Abstract : The study was undertaken to evaluate immunotoxic effects of cypermethrin administered orally (in ground nut oil) to male albino rats at dose levels (mg/kg) of 0 (control), 5, 10, 20 and 40 once daily for 90 days. Cypermethrin administration produced a significant leucopenia at 40 mg/kg on day 90. A dose dependent decrease ($P > 0.05$) in delayed type hypersensitvity reaction was noticed on day 61 post treatment. Humoral response as evidenced by serum haemagglutinin and haemolysin titres did not show any definite pattern on day 90. However, a significant decrease in spleen weights and significant increase in adrenal weights was recorded in rats receiving the highest test level. Total body weights and liver weights did not show any significant change with any of the dose level studied. Results of the study reveal that low doses (5 and 10 mg/kg) did not have any adverse effect on the immuno-competence of rats.

Key words : cypermethrin synthetic pyrethroids rats

INTRODUCTION

Considerable interest has arisen in immunotoxicological evaluation of agro-chemicals and other environmental chemicals in view of their immunosupressive action (1-4). Some of these chemicals, especially, insecticides are encountered by the organisms over long periods of time through food chain. Continuous low level exposure to such chemicals may increase the susceptibility of the host to various diseases due to immunity breakdown. It has, therefore, become important to screen new environmental chemicals with respect to their immunotoxic potential.

Cypermethrin, a recent synthetic pyrethroid insecticide, is extensively used for plant protection (5) and for the control of ectoparasites of domestic animals (6).

In the present studies, we report the effect of oral administration of cypermethrin on induced humoral and cellular immune response in male albino rats.

METHODS

Thirtyfive male albino rats of wistar strain, weighing 150-190 g, obtained from the Small Animal House of the College were used in these studies. The animals were kept on a balanced diet. Feed and water were provided *ad libitum.* The animals were divided into 5 groups of 7 animals each. The animals of group nos. II, III, IV and V were administered technical grade cypermethrin (Bharat Pulverising Mills, Bombay) in groundnut oil orally at the dose levels (mg/kg) of 5, 10, 20 and 40 once daily in the morning for 90 days. The insecticide solution was administered (ml/kg) as 0.5, 1, 2 and 4 percent

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solution to group nos. II, III, IV and V respectively by tuberculin syringe. The animals of Group I were administered isovolumetric amount of ground nut oil and served as experimental control.

The experimental studies were undertaken as per the following schedule :

Elapsed days	*Activities conducted*
—15	Rats received from Animal House were caged individually and adjusted to the control diets.
0	Diets assigned and body weights taken.
48	Animals shaved in the flank region and given intradermal injections of 0.1 ml of tuberculin. (B.P. Division, IVRI, Izatnagar, India).
60	Animals challenged with tuberculin (0.1 ml) given intradermally.
61	Skin sensitivity to tuberculin was determined by measuring skin thickness with Vernier Callipers and values expressed as mm change in skin thickness (7).
78	Sheep blood collected, red cells washed thrice with normal saline solution and animals immunised with 0.5 x 10^8 cells/ml suspension/100 g body weight intraperitoneally.
90	Body weights recorded. Blood samples (without anticoagulant) were collected by cardiac puncture. Serum was separated and titrated for haemagglutinin and haemolysin titres against sheep red blood cells (SRBC) (7). Whole blood samples were collected (using EDTA sodium salt as anticoagulant) and total leucocyte count done using improved Neubauers counting chamber.

Statistical evaluation : The data were analysed by analysis of variance and mean values of various treatments were compared with control values by critical difference (8). A pre-determined P=0.05 level was used for specifying a significant difference.

RESULTS AND DISCUSSION

The observations of the study revealed a significant leucopenic response at the level of 40 mg/kg on day 90. Delayed type hypersensitivity reaction, measured to evaluate cellular immune response, revealed a decrease by 24% and 27% in rats receiving cypermethrin at the dose levels of 20 and 40 mg/kg, respectively on day 61 post treatment. The decrease in cellular response was dose dependent though the values were not significant statistically at P=0.05. On the other hand, no definite pattern was noticed in the humoral immune response as evidenced by mean serum haemagglutinin titres and haemolysin titres against sheep RBC on day 90 post treatment (Table I).

TABLE I : Effect of oral administration of cypermethrin on total leucocyte count, cellular immune response (delayed skin hypersensitivty) and humoral response (haemagglutinin and hemolysin titres) in rats.

Parameter	Control	Cypermethrin (dose levels mg/kg)			
		5	10	20	40
Total leucocyte count ($X10^3$/mm)	11.95 ±0.15	11.76 ±0.17	11.24 ±0.38	10.72 ±0.18	7.88* ±0.20
Skin reactivity to tuberculin (expressed as mm change in skin thickness 24 hrs post challenge)	0.060 ±0.008 (100)	0.055 ±0.005 (91.0)	0.054 ±0.011 (90.0)	0.046 ±0.006 (76.6)	0.044 ±0.007 (73.3)
Haemagglutinin titres (log 2 values)	6.751 ±0.29	7.322 ±0.31	7.489 ±0.31	7.322 ±0.36	7.489 ±0.31
Haemolysin titres (log 2 values)	7.751 ±0.20	8.036 ±0.29	7.989 ±0.33	7.822 ±0.22	7.989 ±0.21

Values are Mean ± SEM (n = 6 to 7); *Significant (P< 0.05).
The figures in parenthesis indicate percent response as compared to control animals.

The body weights of rats remained unchanged during the experimental studies. However, a significant decrease (P<0.05) was observed in spleen weights at the highest dose level. On the contrary to this, weights of adrenals had undergone a significant increase (P<0.05) at the dose level of 40 mg/kg. The weights of adrenals registered an increase in a dose dependent manner.

The levels of insecticide tested in the present study were not grossly toxic as no untoward clinical

symptom or change in body weight could be noticed. The toxic manifestations were only associated with a decrease in spleen weights and an increase in adrenal weights. Immune status of animals, however, reflected a depression of cellular response accompanied by leucopenia. Our findings are, thus, consistent with cellular immunosuppressive action of cypermethrin in mice (9) but are not in agreement to their findings with respect to humoral immune response against sheep RBC in goats. Such a variation in results could be explained on the basis of species variation and different method of testing. The method used by them (9) was based on the enumeration antibody forming cells and measurement of plaque diameter in lymphocyte suspensions of goats whereas our findings are based on the direct measurement of haemagglutinin and haemolysin titres against sheep RBC. Thus, immunosuppressive action of pesticide has little effect on the development of circulating antibody titres (10). It appears that suppression of antibody titres takes place relatively at a higher dose level (11).

TABLE II : Effect of oral administration of cypermethrin on total body weights and weights of internal organs.

Parameter	Control	Cypermethrin treated group (dose levels mg/kg)			
		5	10	20	40
Body weights (g)	203.86 ±22.03	183.71 ±13.08	201.67 ±18.40	212.33 ±21.72	205.67 ±16.82
Liver (g/100 g)	2.94 ±0.18	2.92 ±0.12	2.76 ±0.16	2.74 ±0.20	2.70 ±0.15
Spleen (g/100 g)	0.28 ±0.02	0.25 ±0.01	0.26 ±0.03	0.23 ±0.01	0.21[b*] ±0.02
Adrenals (g/100 g)	0.016 ±0.003	0.024 ±0.003	0.024 ±0.003	0.025[b] ±0.004	0.026[b*] ±0.005

Significant ($P < 0.05$); Values are Mean ± SEM (n = 6 to 7).

There are only few reports (9, 12) on immunosuppression by pyrethroids. The immuno-suppressive action of environmental chemicals can be explained on the basis of functional defect in immuno-competent cells, depletion of responding cell types and alteration in normal hormone levels (13). In this study increased adrenal weight might reflect a state of physiological stress in the body of the rats. Glucocorticoids released from adrenals might play an important role in mediation of depressed immune response (14). Increased weights of adrenals were consistent with the depressed cellular response in our study. The depletion of the lymphocytes from the cortical region of lymph nodes (9) has also been ascribed to the immunotoxic response of cypermethrin. Leucopenic response at the highest test level in the present study further substantiated the immunotoxic potential of this insecticide.

The present studies revealed that Cypermethrin at low doses (5 and 10 mg/kg) did not have any adverse effect on the host defense mechanism. But the threshold level of chemical depends upon the method of testing for immune responses. In conducting such studies, it would probably be more desirable to investigate as many tests as possible and with response observed against variety of antigens (1).

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