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Effect of single oral dose of alphamethrin on immune response of chicks

S.P. Singh[1], L.D. Sharma[2] and R.S. Chauhan[3]
[1,2] Department of Pharmacology & Toxicology;
[3] Department of Pathology, College of Veterinary Sciences,
G.B. Pant University of Agriculture & Technology, Pantnagar-263145, India.

ABSTRACT

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Eight week old, 18 white leghorn male chickens were randomly and equally divided into three groups and administered single oral doses of alphamethrin @ 0, 100 and 200 mg/kg b.wt., respectively, to study humoral and cellular immune responses. Humoral immune response was examined by evaluating antibody titres by ELISA against bovine serum albumin (BSA, Fraction V used as antigen). On 14 days post administration of insecticides, antibody titres were significantly ($P < 0.01$) declined in both the dose groups of alphamethrin as compared to untreated control. Cellular immune response was assayed by delayed type of skin hypersensitivity (DTH) using dinitrofluorobenzene (DNFB), macrophage function test (MFT) and lymphocyte stimulation test (LST). A significant ($P< 0.01$) decrease in the skin hypersensitivity to DNFB was observed in the high dose (200 mg/kg) group. A significant ($P<0.01$) suppression in the phagocytic activity of macrophages of high dose group was observed. A significant ($P<0.01$) reduction in lymphocyte stimulation to Con A was observed at the both dose levels. Results indicate an immunosuppressive effect of alphamethrin following acute toxicity in chickens.

Key Words : Alphamethrin, immunosuppression, humoral and cellular immune response, poultry.

INTRODUCTION

Various chemical agents including synthetic pyrethroid insecticides after short and long term exposure in animals alter the immune system of the body (Stelzer and Michael, 1984; Desi *et al.*, 1986; Carbonell *et al.*, 1989). Alphamethrin, a synthetic pyrethroid, has been widely used in agriculture and animal husbandry practices. The immunopathological studies of alphamethrin in calves showed significant immunosuppression (Chauhan and Agrawal, 1999). However, its immunotoxic effects are not well studied in poultry. Therefore, this study was undertaken to evaluate the effect of alphamethrin on immune response of chicks.

MATERIALS AND METHODS

Eight weeks old 18 white leghorn male chickens procured from Poultry Research Centre of the University, were randomly divided into three equal groups. The birds were immunized with BSA

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(40 mg/kg b.wt.) i/v at day 0. Alphamethrin (10% EC, Stop India) was administered in single oral doses of 0, 100, 200 mg/kg b. wt. to each group, respectively. Feed and water were given *ad libitum* during the study. Blood samples were collected from wing vein of each bird at 7 and 14 days interval in heparinised sterilised syringe for separation of lymphocytes and in other test tube without anticoagulant for serum separation. Following immunological parameters were evaluated:

Antibody titers were measured at 7 and 14 days intervals by employing ELISA as described by Miers *et al.* (1983) with minor modifications (Chauhan, 1995).

Cellular immune response in chicken was examined by measuring delayed type hypersensitivity (DTH) reaction, lymphocyte stimulation (LST) and macrophage function test (Chauhan, 1998).

Statistical analysis of data was done by one way analysis of variance test using Statis Version 3, M/S Glyde Soft, Glasgow, U.K.

***Table 1** : Effect of single oral administration of insecticide alphamethrin on immune response of chicks.*

Group	Dose (mg/kg)	Interval (days)	
		7	14
		ELISA values* (Mean ± SEM)	
Control	0	7.508±0.809	21.983±0.572 ^{p}
Alphamethrin	100	6.561±0.463	20.708±2.067
	200	5.564 ±0.440	14.068±2.445^{p}
		LST DOD(Mean ±SEM)	
Control	0	0.418±0.028 ^{ap}	0.452±0.038^{pq}
Alphamethrin	100	0.259±0.031^{p}	0.275±0.038^{p}
	200	0.140±0.030^{a}	0.267±0.044^{q}
		Skin Thickness mm (Mean ±SEM)	
Control	0		0.965±0.129^{p}
	100		1.233±0.057
	200		0.547±0.039^{p}
		Macrophage function test (%) (Mean ±SEM)	
Control	0		69.0±2.14^{a}
Alphametrhin	100		70.3±2.69
	200		66.7±1.66^{a}

*ELISA value = OD of test serum sample/OD of negative control.
Means bearing common superscripts a (P>0.01) and p or q (P<0.05) differ significantly when compared vertically.

RESULTS

Antibody titre in sera of chickens given 200 mg of alphamethrin was significantly suppressed on the 14th day post challenge with antigen. These values were 14.068 in high dose group of

alphamethrin, in comparison to 21.983 ± 0.572 in untreated control after 14 days of administration of antigen. Cellular immune response in insecticide treated chickens was assayed by measuring the thickness of skin (mm) against DNFB after 14 days of sensitization. A significant ($P< 0.05$) reduction in the thickness of the skin of group III revealed immunosuppressive effect of alphamethrin in chickens.

LST was employed to study cellular immune responses and was represented by delta OD. A significant ($P <0.01$) reduction in lymphocyte stimulation was reported from all the treated groups as compared to control at 7 and 14 days post intoxication. The delta OD was found to be 0.275 ± 0.038 and 0.267 ± 0.044 in chickens of alphamethrin treated groups respectively as compared to 0.418 ±0.028 in controls. *In vitro* phagocytic activity of macrophages was evaluated by NBT technique and expressed as % NBT positive cells showing phagocytic activity. A significant ($P < 0.01$) suppression in phagocytic activity of macrophages of high dose groups of alphamethrin was observed. An average of 69.0 ±0.214 percent NBT positive cells were recorded in comparison to 66.7±1.66 in controls.

DISCUSSION

The results indicate an immunosuppressive potential of alphamethrin on humoral and cellular immune response in chickens. Reduction in the antibody titre could be attributed to hampered proliferation and activation of B-lymphocytes, responsible for biosynthesis of immunoglobulins. Leucocytopenia and lymphopenia with lowered serum globulin level was reported in alphamethrin intoxicated chicks (Singh, 1997). Chauhan and Agrawal (1999) reported immunosuppression in calves due to alphamethrin affectiing both the wings of immune system i.e. humoral and cellular.

A significant decrease in delayed type skin hypersensitivity to DNFB, lymphocyte stimulation to mitogen concavalin A and macrophage activity was observed in alphamethrin treated chicks in this study. Suppression of DTH, lymphocyte stimulations and macrophage function indicates suppression of cellular immune response by alphamethrin following its single oral dose administration in chickens. Suppression in cellular immune response observed in chickens might have occurred on account of hinderance in the differentiation, maturation and activation of T-lymphocyte and macrophages or due to their lysis. Decrease in calcium level has been demonstrated to be the basis of immunotoxicity by poly aromatic hydrocarbons. Alterations in the Ca^{++} dependent pathways, brought about by Ca^{++} metabolism leading to improper activation of B- and T-lymphocytes, is probably the cause of immunotoxicity (Cambier *et al.*, 1994; Weiss and Littman, 1994; Holsapple *et al.*, 1996). Another possible effect of pesticide on lymphokines and cytokines which may interfere with the maturation, differentiation and activation of lymphocytes and macrophages. Tamang *et al.* (1988) reported immunosuppression in mice and goats as a result of lymphocytolysis caused by cypermethrin.

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