BIFLEX INDUCED HAEMATO-IMMUNOMODULATORY CHANGES IN MICE *(MUS MUSCULUS)*

NAJMA ARSHAD, SAIRA MAHMOOD, ANWAR MURTAZA, ZAHEER IQBAL AND M. ARSHAD

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore (NA, SM, AM), Poultry Research Institute, Rawalpindi (ZI) and Department of Zoology, Government College, Lahore (MA).

Abstract: Study was designed to find affects of Biflex, a pyrethroid insecticide on Haemato-immuno modulations of (Mice) (*Mus musculus*). Male mice were taken for experimental study and divided into experimental and control groups. A daily dose of 96.80 mg/kg of body weight/day of Biflex was administered along with feed for a period of four weeks and its effects were monitored on some haematological and immunological parameters at one-week interval. Results showed a significant depletion ($P<0.001$) in spleen to body weight ration of 49.35%, 50.41% and 46.61% after II, III and IV weeks of treatment, respectively. In haematological studies significant ($P<0.05$) elevations of 82.5% and 57.2% were observed in WBCs count following 3 & 4 weeks of treatment respectively. MCHC levels increased ($P<0.05$) by 17.5%, 24.7% and 26.47% at I, III and IV weeks, whereas PCV level depleted by 15.21%, 12.27%, 24.31% and 32.60% after I-IV weeks, respectively. Moreover, MCV also reduced by 10.95%, 15.04% and 19.23% after II, III and IV weeks of exposure. In serological studies total protein estimation, abumin and globulin levels were considered, but no significant changes could be recorded throughout the experimental period.

Key words: Biflex, haemato-immunomodulation, mice, liver, spleen, thymus.

INTRODUCTION

Environmental pollution has become a matter of great concern for world community. Human activities and industrial developments are polluting the environments to a great extent. The deleterious effects of pyrethroids on health are not clandestine.

Immune system is essential for health and survival. It defends body from pathogen through complex interaction of white blood cells and blood serum molecules. The fundamental characteristic of adaptive immune system which has evolved in vertebrates is its ability to recognize and subsequently destroy foreign and potentially harmful antigens. The selective advantage which immune system confers is its capacity to resist infections and possibly malignant diseases. Bradely (1995) mentioned that pesticide exposure significantly reduces resistance to bacterial viral and parasitic infections and promotes tumor growth in many animal species. There is an increasing awareness from rodents study that a variety of drugs and environmental chemicals have the potential to unintentionally impair component of immune system including, spleen,

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thymus, busra of fibricious etc. (Gleichmann *et al.*, 1989; Wright *et al.*, 1991; Rodgers, 1995).

Thymus is one of the primary lymphoid organ. Abnormalities in thymic weight and thymic atrophy following insecticidal exposure have been reported by various authors (Kendall and Ritter, 1991; Esser, 1994; Institoris *et al.*, 1995). Spleen serves as a biological sieve where macrophages mature and interact with T and B lymphocytes Tests for pesticides induced abnormalities on spleen weights indicate its effects on macrophage development, humoral and cell mediated immunity. Abnormalities in spleen weight, lymphocytes counts & number of splenic cells, as a consequence of pesticide exposure have also been reported by many workers (Wright *et al.*, 1991; Varshneya *et al.*, 1992; Siroki *et al.*, 1994; Ladics *et al.*, 1994; Bernier *et al.*, 1995, Abdel Nasser,1995).

Synthetic pyrethroids have become more popular as effective pesticides because they have high toxicity to wide range of insects including resistant strains (Elliott, 1977). Their low toxicity for non-target organisms and rapid biodegradability has encouraged the wide spread use of these synthetic insecticide for an effective pest control (Casida *et al.*, 1971; Hayes Jr., 1975; Huston, 1979, Walker, 1981; Cole *et al.*, 1987; Edwards *et al.*, 1987). However, it is possible that during the pyrethroid metabolism, reactive oxygen species (ROS) may be generated and thus produce oxidative stress in intoxicated organisms (Yoshida and Gershwin, 1993; Kale *et al.*, 1999). Inhibitory effects of pyrethroids S-bioallethrin on proliferation of basophils from atopic and non-atopic subjects have been reported by Diel *et al.* (1998). Cypermethrin is another synthetic pyrethroids that has been found to have toxic effects on male and female rat hepatocytes (El. Tawil & Abdul Rehman, 1997).

Bifenthrin being a contact insecticide is used to provide effective control to a wide range of insects including termites. It is an insecticide and acaricide which affects the nervous system and cause paralysis in insects. It is highly toxic to fish and aquatic organisms. The US EPA has classified bifenthrin as toxicity class II. Evidence of mutagenic effects from exposure to bifenthrin are incondusive. Studies of mouse white blood cells were positive for gene mutation. However, other tests of bifenthrin mutagenic effects, include Ames tests and studies in live rat bone marrow cells. Moreover, mesenchymal tumors of mouse urinary bladder have also been reported as a carcinogenic effect of bifenthrin (Butler *et al.*, 1997).

Keeping the effective use of befenthrin as a potential insecticides, against insecticide, study was designed to evaluate its effects on non-target organisms. It was administered @ 96.80 mg/kg of body weight / day for one month along with food. Its effects were monitored by using different haematological and serological parameters like Hb contents, RBCs count, leukocyte count, packed cell volume and haematological indices, serum protein, albumin and globulin contents. Albumin / globulin ratio was also estimated.

MATERIALS AND METHODS

Maintenance of animals

Male mice (*Mus musculus*) were obtained from VRI (Veterinary Research Institute), Lahore and kept in an isolated room of Animal House in Department of Zoology, Punjab University, Lahore. The animals were maintained at 20-25°C in natural photoperiod. Chick feed No.4 was purchased from the local market (Hi tech Shadman Lahore). Mice were provided with this feed and water *ad libitum*.

In the beginning 10 animals were selected at random and their mean body weight was considered for preparing dose of insecticide (Biflex) which was given to animal by mixing in their feed.

Animals were divided into two groups:

a: Control group: Animals in this group were fed on uncontaminated feed i.e. free from any insecticide.

b: Experimental group: Animals in experimental group were reared on insecticide contaminated feed.

Dose preparation and quantification:

A packing of 1 liter (2.5% w/w Bifinthrin) was purchased from local market. About 5 ml of liquid insecticide was mixed in 100 gm of this feed, was offered to experimental group. The dose of insecticide was determined by taking into account the quantity of feed consumed by animals.

Blood Sampling and Study Procedures:

Blood was collected at 0, 7, 15, 21, 28 days of treatment. For sampling, 5 animals were selected at random from each group, and blood samples were taken out from all animals under mild anesthesia. About 1 ml of blood was drawn from heart with the help of disposable plastic syringes of 1 cc half of this was used for haematological studies. Rest of blood was kept in sterilized Eppendorf vials for serological study. Serum was separated in clean vial and was stored at -20°C for serology.

After drawing blood, visceral organs of mice were exposed and spleen, thymus, kidney and liver were dissected out for further analysis and placed in different vials containing Bouin's fixative. For haematological studies Hb, RBCs, WBCs, PCV were determined using standard procedures (Dacie and Lewis, 1991) and haematological indices like MCV, MCH and MCHC were calculated after (Swarup *et al.*, 1986). Serum proteins were estimated by using Biuret method (Henry *et al.*, 1974). Serum albumin was determined by Bromocresol green reagents method (Doumas *et al.*, 1971) using commercial kit of Randox Laboratories Limited, U.K. Serum globulin was estimated by indirect method i.e., by subtracting the albumin value from total protein value. Albumin /

globulin ratio was estimated by dividing albumin contents from globulin contents. All the data so obtained was tabulated and subjected to statistical analysis using Student's 't' test in SPSS software.

RESULTS

Tables I to III are showing the changes on different haematological and immunological parameters due to continuous exposure of Bifenthrin (Biflex termiticide) through contaminated feed (96.80 mg/kg of body weight/day) at one week interval for a period of 4 weeks. The comparison has been made with respective control groups.

Body weight and organ to body weight ratio

Adverse effects of treatment were observed on spleen weight. It was found to be significantly low ($P<0.001$) after II, III and IV weeks of exposure (Table I). Body weight, thymus, liver and kidney weights did not show any measurable differences from control groups.

Haematological studies:

A significant ($P<0.05$) increase of 82.5% and 57.2% was recorded in leukocyte count in 3rd and 4th week, respectively. Packed cell volume was found to be significantly low throughout experimental period. The reduction was 15.21%, 12.27%, 24.31% and 32.6% in 1st – 4th week of treatment. In haematological indices, mean cell volume was found to be decreased by 10.95%, 15.04% and 19.23% in 2nd to 4th week, respectively. A significant ($P<0.05$) elevation of 17.5%, 24.7% and 26.47% in mean cell hemoglobin concentration (MCHC) was recorded in 1st, 3rd and 4th week of insecticidal exposure (Table II).

Immunological Studies:

Total protein, albumin and globulin concentration and albumin to globulin ratio were taken into consideration but no measurable differences could be recorded in any of above mentioned parameters throughout the experimental period (Table III).

DISCUSSION

Efforts are continuously being made in the field of pesticide research to formulate the insecticides of minimum toxicity to non-target organisms. New formulations are being prepared and launched in the field for termite and other pest control. Bifenthrin is one of these newly launched termiticides. The objective of our present study was to find its haemato-immuno toxicity in mammals.

Spleen is the sole lymphatic tissue specialized to filter blood. It has a number of non-immunologic and immunologic functions. A reduction in spleen weight following Bienthrin exposure was observed. Abnormalities in spleen weight, reduction in its cellularity, reduced lymphocyte reaction and their responses in spleenocyte, as a consequence of insecticidal exposure has been reported by Hiroyuki (1990), Wright *et*

al. (1991), Varshneya *et al.* (1992), Tryphonas *et al.* (1997) and Burnnass *et al.* (1998). No adverse effects could be observed on liver, kidney, thymus and body weight.

In haematological studies leukocyte counts were found to be elevated. Leukocytes constitute a special system for combating the different infectious and incoming toxic agents. These are sensitive indicators of immune status of body. The increase in the total leukocyte count indicate that bifenthrin can either induce lympho-proliferation or can sensitize certain cell subset. An increase in leukocyte due to pesticide toxicity has also been reported by Areechon and Plump (1990). Casale *et al.* (1993), Tryphonas *et al.*(1997) and Yoo *et al.* (1997).

Hemoglobin concentration, total erythrocyte counts, packed cell volume (PCV) and haematological indices are important indicators of anaemia. Present study show a pronounced decrease in packed cell volume and mean cell volume (MCV) and significant elevation in mean cell hemoglobin concentration (MCHC). It is indicating a breakage or shrinkage in red blood cells, which is also evident from PCV and MCV but probably as a compensatory mechanism MCHC increased, therefore, hemoglobin level and mean cell hemoglobin remained unaltered. All of these findings are indicating that bifenthrin possess haematotoxic potential. Our findings are in agreement with McMurry *et al.* (1999) and Waterfield *et al.* (1997).

Various tests were performed with blood serum to monitor immunotoxic effects of Bifenthrin. Globulin level and albumin to globulin ratio are important indicators of humoral immune system. No significant alteration could be observed in any of the serological parameters selected for the study (Table III). All of these results indicate that bifenthrin may disturb cell mediated immune responses but may not influence humoral immune responses. Similar findings have been reported by Pande *et al.* (1995), Khurana *et al.* (1996), Thaker *et al.* (1996) and French *et al.* (1999).

Table 1: Biflex induced changes on body and organ weight:

Parameters	0 week		1st week		2nd week		3rd week		4th week	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Body wt. (g.)	25.95 ±0.88	25.49 ±0.74	26.90 ±1.67	26.90 ±0.67	28.76 ±0.67	27.33 ±0.67	25.20 ±0.42	25.30 ±0.45	25.53 ±0.89	23.83 ±0.73
Spleen wt. (g.)	0.698 ±0.01	0.689 ±0.01	0.706 ±0.00	0.600 ±0.08	0.672 ±0.02	0.360 ±0.02***	0.716 ±0.04	0.355 ±0.01***	0.680 ±0.01	0.363 ±0.01*
Thymus wt. (g.)	0.866 ±0.05	0.843 ±0.03	0.950 ±0.01	1.240 ±0.08	0.870 ±0.05	1.070 ±0.04	0.780 ±0.10	0.980 ±0.07	0.880 ±0.08	1.048 ±0.05
Kidney wt. (g.)	1.39 ±0.07	1.28 ±0.05	1.59 ±0.15	1.59 ±0.08	1.35 ±0.07	1.54 ±0.10	1.3040 ±0.02	1.3120 ±0.13	1.1820 ±0.02	1.2910 ±0.05
Liver wt. (g.)	5.422 ±0.18	5.195 ±0.08	5.770 ±0.07	6.125 ±0.20	5.146 ±0.15	4.950 ±0.28	5.350 ±0.14	4.580 ±0.09	5.090 ±0.06	5.050 ±0.25

*=P<0.05. **=P<0.01***=P<0.001

Table II: Biflex induced changes in haematological parameters.

Parameters	0 week		1st week		2nd week		3rd week		4th week	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Hb (g/dl)	14.66 ± 0.03	14.90 ±0.20	14.60 ±0.81	13.40 ±0.68	14.70 ±0.16	12.29 ±0.78	14.70 ±0.21	14.80 ±0.30	15.30 ±0.60	13.60 ±0.90
RBCs ($x10^6$/µl)	8.57 ±0.11	8.51 ±0.07	8.73 ±0.34	7.70 ±0.49	8.61 ±0.37	8.55 ±0.40	8.37 ±0.39	7.17 ±0.27	8.56 ±0.27	7.35 ±0.32
WBCs ($x10^3$µl)	5.20 ±0.29	4.97 ±0.41	4.37 ±0.56	4.02 ±0.35	5.76 ±0.37	6.56 ±0.58	4.80 ±0.21	8.76 ±0.57*	5.05 ±0.12	7.93 ±0.20*
PCV (%age)	44.60 ±0.67	44.60 ±0.67	46.00 ±0.57	39.00 ±0.71***	44.00 ±1.52	38.60 ±1.76*	44.00 ±1.52	33.30 ±1.20***	46.00 ±0.57	31.00 ±1.00***
MCV (fl)	52.10 ±0.50	52.03 ±0.47	52.70 ±2.25	52.20 ±2.50	51.10 ±1.19	45.50 ±0.90*	52.50 ±0.67	44.60 ±0.33**	52.50 ±1.18	42.60 ±1.36**
MCH (pg)	16.89 ±0.43	17.50 ±0.23	16.60 ±1.64	16.03 ±0.81	17.10 ±0.70	13.73 ±1.67	17.50 ±0.77	18.00 ±2.31	17.90 ±0.46	17.20 ±2.22
MCHC (pg)	32.38 ±1.04	33.38 ±0.04	30.30 ±1.42	35.63 ±1.82*	33.43 ±0.92	32.00 ±3.43	33.42 ±0.92	41.66 ±4.05*	33.36 ±1.59	41.90 ±6.30*

*=P<0.05. **=P<0.01***=P<0.001

Table III: Biflex induced changes on some immunological parameters.

Parameters	0 week		1st week		2nd week		3rd week		4th week	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Protein (g/dl)	5.91 ±0.34	5.65 ±0.38	6.02 ±0.30	5.06 ±0.28	5.27 ±0.21	5.84 ±0.41	6.43 ±0.14	7.26 ±0.52	5.28 ±0.14	6.75 ±0.20
Albumin (g/dl)	3.53 ±0.05	3.38 ±0.10	3.62 0.38	3.26 ±0.34	3.44 ±0.13	3.56 ±0.38	3.52 ±0.13	3.87 ±0.19	3.18 ±0.15	3.98 ±0.17
Globulin (g/dl)	2.91 ±0.43	2.67 ±0.42	3.18 ±0.45	2.37 ±0.43	2.07 ±0.26	2.26 ±0.17	3.48 ±0.53	3.73 ±0.42	2.46 ±0.05	2.49 ±0.19
Albumin/ Globulin (ratio)	1.21 ±0.11	1.23 ±0.12	1.25 ±0.08	1.81 ±0.05	1.37 ±0.01	1.65 ±0.10	1.17 ±0.04	1.55 ±0.04	1.34 ±0.08	1.63 ±007

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