BIOCHEMICAL TARGETS AFFECTED BY SUBLETHAL DOSES OF CYPERMETHRIN IN MICE

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Abstract

The effects of repeated sublethal doses of cypermethrin (0.25 mg/Kg/day) for 14 days on some haematological, biochemical and immunological parameters in male mice were investigated 1 hour, 24 hours, 1,3 and 5 weeks after the last dose. No significant changes were recorded in hemoglobin content (Hb), haematocrit value (Hct) and red blood cells count (RBC's). Only white blood cells count (WBC's) significantly increased 3 and 5 weeks after the last dose.

The activities of the tested enzymes; adenosine triphosphatase (ATPase), carboxylesterase (CaE), glutathione S-transferase (GST), acid phosphatase (AcP), alkaline phosphatase (AlP), alanine transaminase (AlT) and aspartate transaminase (AsT) in treated choshen organs of male mice were increased gradually. Cypermethrin caused a pronounced direct suppressive effect on cellular immunity as illustrated by lymphocyte proliferation to mitogens and phagocytic activity. Also, humoral immunity as indicated by plaque forming cells (PFC) and haemagglutinating titre were suppressed. The suppressive effect of cypermethrin on immune response was time dependent.

INTRODUCTION

In Egypt, intensive agriculture requires the import and application of large quantities of pesticides. The long term application of these pesticides has resulted in pesticide residues accumulating in soil, water, and in the general environment, thereby posing a serious threat to public health in Egypt (Selim and El-Sebae 1995). Cypermethrin, a member of a synthetic pyrethroid family of pesticides, is used to control a variety of important insects; particularly lepidoptera in cotton, fruit and vegetables. The world-wide use of pesticides makes it urgent to know as much as possible about the effects of pesticides and their degradation products on humans and animals. Blood parameters are sensitive to exposure to many toxic substances including insecticides (Bhatia and Kaur, 1994). Changes in some biochemical parameters such as an increase in amino acid levels and total serum proteins, as well as

changes in the activity of some enzymes have been attributed to disturbances of liver functions (El-Gendy et al. 1986).

The immune system is important for defense against a variety of organic insults. It is a highly evolved system and is distributed throughout the body. The cellular units of this system primarily lymphocytes and macrophages, are found in most organs, and are regulated by a variety of multiple control processes. (Luster et al., 1987). The immune system as a traget of chemical toxicants, has only recently gained concen and importance (Bick, 1982). This has been brought about by increasing awareness of safety with chemical substances and also by growing knowledge in the field of immunology (Smith et al. 1996).

The present study aims to evaluate the possible effects of cyperimethrin on haematological, immunological and biochemical targets in mice.

MATERIALS AND METHODS

Animals: Male Swiss albino mice strain (*Mus musculus*), 6-8 weeks old were obtained from the High Institute of Public Health, Alexandria University.

Insecticide: Cypermethrin, 98% was obtained from Shell Chemical Co.

Animals treatment: The animals were divided into two groups, the first one was daily treated with 0.25 mg/kg of cypermethrin for 14 days. The second group was daily treated with corn oil and used as control. Five animals from each group were decapitated after 1 hour, 24 hours, 1, 3 and 5 weeks of the last treatment.

Haematological tests: Blood samples were collected in anticoagulated tubes for haematological parameters; haemoglobin content, haematocrit value, RBC's and WBC's counts (Dacie and Lewis, 1984).

Enzyme activities determination: Liver and kidney of treated and untreated mice were homogenized and centrifuged. The supernatants were used for the assay of the following enzymes; ATPase was measured according to Koch et al. (1969), CaE was determined by the method of Verschoyle et al. (1982). GST was assayed by the colorimetric method of Vessey and Boyer (1984), AcP and AlP were determined by the method of Bessey et al. (1946) and AsT and AlT were measured by the method of Reitman and Frankel (1957). Total protein was determined according to Lowry et al. (1951).

Immunological Studies: The lymphocytes were separated from the mice spleen using the method described by Erwin et al. (1987).

a. Cellular Immunity: Splenocyte proliferation to mitogens were measured in a 3-day microculture assay using the T-cell mitogen; Phytohaemagglutinin (PHA) and B-cell mitogen; lipopolysaccharide (LPS) as described by Anderson et al. (1979). The microcultures were pulsed with 1μCi of tritiated thymidine (sp. act. 5 Ci/mmol, Amersham). The data were represented as stimulation index (SI); that is mitogen-stimulated thymidine incorporation divided by thymidine incorporation in non stimulated controls.

Phagocytic activity was measured using acridine orange and expressed as a percentage positive phagocytic index (PI).

b. Humoral Immunity: The humoral immunity was assessed in this work by studying the binding capacity of antigen-antibody by plaque forming cell (PFC) per 10^6 viable lymphcytes as described by Cunningham (1973) and antibody titres in serum by the method of Hundson & Hay (1976). Serum antibody titres were expressed as the reciprocal of the highest dilution showing agglutination.

Statistical Analysis: Results are expressed as mean ± standard deviation (SD). Data were subjected to one way analysis of variance (ANOVA) by Student's "t" test. The accepted level of significance was $P < 0.05$.

RESULTS AND DISCUSSION

I. Haematological studies: Table 1 presents the blood picture of male mice exposed to sublethal doses of cypermethrin for 14 days. The data showed non significant alteration in Hb content, Hct value and total erythrocyte count. Gradual increase in WBC's count was noted and reached to a significant level 3 and 5 weeks after finishing treatment. The high increase of leukocytes may be due to the inflammatory response induced as a defence mechanism. This result was in full agreement with Bhatia and Kaur, 1994.

II. Biochemical Studies: The in vivo effect of multiple sublethal doses of cypermethrin on tested enzymes were investigated and presented in table 2. The data indicated that the ATPase activities of treated kidney mice were higher than untreated ones. This activation was highly significant, this data was paralleled with El-Sebae et al. (1985) who reported that ATPase was the more sensitive enzyme to

Table. 1. Effect of daily sublethal dose of cypermethrin (0.25 mg/kg) for 14 days on basic haematological parameters of male mice.

Animal group	Time after last dose	Hb (g / dl)	Hct (%)	RBC's ($x10^6/\mu l$)	WBC's ($x10^3/\mu l$)
Control		14.2 ± 0.7	40.0 +0.9	5.9 ± 0.6	7.6 ± 1.2
Treated	1 hour	14.0 ± 0.4	39.6 ± 1.0	5.8 ± 0.3	7.8 ± 2.0
	24 hours	14.6 ± 1.0	40.6 ± 2.0	6.1 ± 0.2	7.8 ± 3.8
	1 week	14.7 ± 0.8	40.5 ± 1.3	6.1 ± 0.2	8.5 ± 2.6
	3 weeks	12.8 ± 0.4	39.5 ± 3.1	5.9 ± 0.8	9.6 ± 3.2*
	5 weeks	14.7 ± 1.0	40.5 ± 1.0	6.0 ± 0.3	9.6 ± 3.0*

(*) : Significantly different from control. ($P < 0.05$).
Hb : Haemoglobin content.
Hct : Haematocrit value.
RBC's : Red blood cells.
WBC's : White blood cells.

Table. 2. Effect of repeated doses of cypermethrin for two weeks on different enzymes in the chosen organs of male mice related to time after last dose.

Enzyme	Organ	Enzyme activity** (mean + S.D)					
		Control	1 hour	24 hours	1 week	3 weeks	5 weeks
ATPase	Kidney	18.3±2.1	20.3±2.4	24.1±0.7*	24.7±3.5*	23.3±4.2*	25.7±5.3*
CaE	Kidney	391± 9.0	425±12.0	443±14.0	463±13.0*	501±17*	521±21*
GST	Liver	78 ± 18	82.0±21	88.0±7.0	89.0±16.0	86.0±12.0	84.0±15.0
AcP	Kidney	9.9±1.6	10.9±2.3	12.6±1.4*	13.7±2.3*	16.3±2.3*	18.0±2.0*
AlP	Kidney	8.7±1.4	8.7±2.3	8.9±2.3	9.1±1.0	9.9±3.0	10.6±2.6*
AsT	Liver	6.8±1.4	7.6±2.3	8.3±0.9*	8.6±1.1*	8.9±1.3*	9.2±0.8*
AlT	Liver	7.3±1.5	7.0±1.3	7.9±2.2	8.3±1.3	8.7±1.2	9.0±1.8*

* : Significantly different from control ($P< 0.05$)

**: The activity of tested enzymes expressed as following:-

ATPase : µmoles Pi/mg protein / hr.

CaE & GST : OD/ gm protein / min

AcP & AlP : µmole *p*-nitro phenol/ mg protein/ min

AsT & AlT: 10^3 Units / mg protein.

the tested synthetic pyrethroids.

The obtained results indicated that the carboxylesterase activity of treated animals was significantly increased compared with untreated ones. CaE is thought to play a role in the detoxification of some pesticides. Our results go in line with the results of El-Gendy (1990).

The hepatic GST was insignificantly activated in treated mice. Cypermethrin stimulated AcP & Alp with a percentage activation reaching up to 82.5 & 22.4 in kidney after 5 weeks, respectively. The elevated AcP activity may be associated with the cell disintegration resulting from pesticide treatment, thus suggesting prenecrotic changes in tissues (Saigal et al. 1982).

AsT and AlT activities were activated in liver of treated animals. The disruption of transaminases from the normal values denote biochemical impairment and lesions of tissues and cellular function because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential functions (Tordior and Van Heemstra Lequin 1980).

III. Immunological Studies

a. Cellular immunity

Lymphocyte proliferation: The data in Table 3 show a gradual decrease in the level of stimulation index to PHA and LPS in treated mice which reached its maximum after 5 weeks. The results clearly indicated that there was a time dependent decline in SI. These data suggest that pesticides can interfere with DNA synthesis and inhibit the mitogen-induced lymphocyte transformation which is correlated with depressed cell mediated immunity. The present results were disagreement with Bhatia and Kaur 1994 and Smith et al. 1996 who observed marked depression of the cellular immunity in the animals treated with a variety of pesticides.

Phagocytic activity: From table 3 it is clear that the percentage positive phagocyte index decreased by time in a steady manner till the end of experiment.

Macrophages are known to regulate cell mediated immunity through controlling lymphocyte blastogensis and antigen presentation to these lymphocytes (Nelson, 1976). The immunosuppression noticed in our study are inagreement with the results of many investigators (Smith et al. 1996).

Table. 3. Effect of sublethal doses of cypermethrin (0.25 mg/kg/day) for 14 days on cellular and humoral immunity.

Animal group	Time after last dose	Cellular Immunity SI [1]			Humoral Immunity PFC/10^6 cells	Humoral Immunity Antibody titre ($\log_2$)
		PHA	LPS	% PI [2]		
control		2.4 ± 0.18	3.6 ± 0.35	43.76 ± 0.7	82.7 ± 0.7	7.7 ± 0.6
Treated	1 hour	2.2 ± 0.24	3.4 ± 0.19	42.6 ± 0.4	80.3 ± 0.3	6.6 ± 0.5
	24 hours	1.7 ± 0.31 *	3.0 ± 0.23	40.3 ± 0.5	68.2 ± 0.5	5.6 ± 0.4 *
	1 week	1.4 ± 0.23 *	2.4 ± 0.34 *	24.6 ± 0.2 *	37.6 ± 0.4 *	2.0 ± 0.5 *
	3 weeks	0.86 ± 0.20 *	2.0 ± 0.21 *	19.5 ± 0.3 *	30.6 ± 0.5 *	1.0 ± 0.4 *
	5 weeks	0.66 ± 0.03 *	1.9 ± 0.18 *	9.2 ± 0.4 *	21.0 ± 0.2 *	0.5 ± 0.3 *

* : Significantly different from control ($P < 0.05$)
1 (SI) : Stimulation Index to mitogens; PHA & LPS
2 (PI) : Phagocytic Index.

b. Humoral Immunity

The effect of mice treatment with cypermethrin for 14 days on the ability of splenocytes to form antibodies was mentioned in table 3. The mean values of PFC showed insignificant difference after 1 hour and 24 hours of last dose when compared with control. Significantly decreased by time reaching its maximum at the end of experiment was recorded. This data was in full agreement with Tamang et al., 1988, who found that the rate of plaque formation in the lymphocyte suspension of cypermethrin treated goats was significantly reduced and the diameter of the plaque was also significantly lower than in that of control animals.

Like the PFC response, serum antibody titres were significantly different from control. It was clear that cypermethrin caused gradual significant decrease in the mean serum antibody titres log base 2 (log2) on time dependent manner, which reached its maximum after 5 weeks of pesticide exposure. These data paralleled those by Desi et al., (1980) who reported that reduced haemagglutinin serum titres were found in animals treated with pesticides compared with control.

This study showed that cypermethrin affected the tested tissue enzymes. Blood WBC's was the only studied blood parameter, that was affected by exposure to the insecticide. Also, cypermethrin caused marked suppression for both cellular and humoral immunity. Our study provide additional evidence that parameters of immune function may serve as sensitive biomarkers in animals and human exposed to environmental contaminants.

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الاهداف البيوكيميائية في الفئران التي تتأثر بالجرعات تحت المميته من مبيد السيبرمثرين

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أن الانتشار الواسع في استعمال المبيدات الحشرية لمكافحة الافات المختلفة جعل من الضروري معرفة مدي تأثير التعرض المتكرر لهذه المبيدات علي الانسان والحيوان. لذلك تناول البحث دراسة تأثير التعرض المتكرر لمبيد السيبرمثرين من عائلة البيريثرويدات المحضرة صناعيا والشائعة الاستعمال علي بعض الاجهزة الحيوية في جسم فئران التجارب.

ويستهدف البحث الي دراسة تأثير مبيد السيبرمثرين علي الجهاز المناعي وما يصاحب ذلك من تغيرات بيوكيميائية لبعض النظم الانزيمية بالاضافة للتأثير علي بعض ثوابت الدم وعلي فترات زمنية متفاوته.

واتضح من الدراسة ان هذا المبيد يؤثر تأثيرا معنويا علي عدد كرات الدم البيضاء وخاصة بعد ٥,٣ اسابيع من المعاملة، بينما لم يكن له تأثير واضح علي كل من مستوي الهيموجلوبين والهيماتوكريت وكرات الدم الحمراء.

واظهر المبيد المختبر تأثيراً معنويا علي كل الانزيمات المختبرة مثل الادينوزين ثلاثي الفوسفاتيز، والكربوكسيل استريز، والجلوتاثيون – س- ترانسفيريز، الفوسفاتيز الحامضي والقلوي، والترنساميناز حيث سبب المبيد تنشيط لهذه الانزيمات المختبرة.

وقد وجد أن المبيد المختبر قلل من وظيفة الجهاز المناعي والخلايا المناعية والتي لها دور مباشر وقوي في حماية الجسم من عديد من الامراض.

وهذا البحث يوضح الاضرار الجسيمة الناتجة عن التعرض المستمر لهذا المبيد، لذلك من الضروري اجراء الفحص الدوري علي الذين يتعرضون لهذا المبيد باستمرار في الحقول والمصانع المنتجة له وكذلك يجب ترشيد استخدام المبيدات مع اتخاذ الاحتياطات الواجبة والطرق المناسبة للوقاية من اخطار التعرض للمبيدات.