BIOCHEMICAL TARGETS AFFECTED BY SUBLETHAL DOSES OF CYPERMETHRIN IN MICE

KAWTHER EL-GENDY1, NAGAT ALY2, EIMAN RASHWAAN3 AND A.H. EL-SEBAE1

1 Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University, Egypt.
2 Central Agricultural Pesticides Laboratory, Agricultural Research Centre, Dokki, Egypt.
3 Medical Research Institute, Alexandria University, Egypt.

(Manuscript received 2 December, 1997)

Abstract

The effects of repeated sublethal doses of cypermethrin (0.25 mg/Kg/day) for 14 days on some hematological, biochemical and immunological parameters in male mice were investigated. The effects of repeated sublethal doses on some changes in hemoglobin content (Hb), hematocrit (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: adenylate triphosphatase (ATPase), carboxylesterase (CSE), glutathione S-transferase (GST), acid phosphatase (AcP), alkaline phosphatase (AlP), alanine transaminase (ALT) and aspartate transaminase (AST) in treated choslen 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 hemagglutinating 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 target of chemical toxicants, has only recently gained concern 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 cypermethrin 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), Ca²⁺ was determined by the method of Verschoyle et al. (1982), GST was assayed by the colorimetric method of Vessey and Royer (1984), AcP and AIP were determined by the method of Bessey et al. (1946) and AsT and AIT 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 lymphocytes as described by Cunningham (1973) and antibody titre in serum by the method of Hudson & Hay (1976). Serum antibody titre 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 Bhatt 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.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Time after last dose</th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>RBC’s (x10^6/μl)</th>
<th>WBC’s (x10^3/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>1 hour</td>
<td>14.0 ± 0.4</td>
<td>39.6 ± 1.0</td>
<td>5.8 ± 0.3</td>
<td>7.8 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>14.6 ± 1.0</td>
<td>40.6 ± 2.0</td>
<td>6.1 ± 0.2</td>
<td>7.8 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>14.7 ± 0.8</td>
<td>40.5 ± 1.3</td>
<td>6.1 ± 0.2</td>
<td>8.5 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>12.8 ± 0.4</td>
<td>39.5 ± 3.1</td>
<td>5.9 ± 0.8</td>
<td>9.6 ± 3.2*</td>
</tr>
<tr>
<td></td>
<td>5 weeks</td>
<td>14.7 ± 1.0</td>
<td>40.5 ± 1.0</td>
<td>6.0 ± 0.3</td>
<td>9.6 ± 3.0*</td>
</tr>
</tbody>
</table>

(*) : 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.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Organ</th>
<th>Enzyme activity** (mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>ATPase</td>
<td>Kidney</td>
<td>18.3±2.1</td>
</tr>
<tr>
<td>CaE</td>
<td>Kidney</td>
<td>391±9.0</td>
</tr>
<tr>
<td>GST</td>
<td>Liver</td>
<td>78±18</td>
</tr>
<tr>
<td>AoP</td>
<td>Kidney</td>
<td>9.9±1.6</td>
</tr>
<tr>
<td>AIP</td>
<td>Kidney</td>
<td>8.7±1.4</td>
</tr>
<tr>
<td>AsT</td>
<td>Liver</td>
<td>6.8±1.4</td>
</tr>
<tr>
<td>AT</td>
<td>Liver</td>
<td>7.3±1.5</td>
</tr>
</tbody>
</table>

* : 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
AoP & AIP: µmole p-nitro phenol/mg protein/min
AT & AST: 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 AC and AL with a percentage activation reaching up to 82.5 and 22.4 in kidney after 5 weeks, respectively. The elevated AC activity may be associated with the cell disintegration resulting from pesticide treatment, thus suggesting necrotic 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 (Tordjor 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 phagocytic index decreased by time in a steady manner till the end of experiment.

Macrophages are known to regulate cell-mediated immunity through controlling lymphocyte blastogenesis and antigen presentation to these lymphocytes (Nelson, 1976). The immunosuppression noticed in our study are in agreement 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.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Time after last dose</th>
<th>Cellular Immunity SI</th>
<th>Humoral Immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PHA</td>
<td>LPS</td>
</tr>
<tr>
<td>control</td>
<td>2.4 ± 0.18</td>
<td>3.6 ± 0.35</td>
<td>43.76 ± 0.7</td>
</tr>
<tr>
<td>Treated</td>
<td>1 hour</td>
<td>2.2 ± 0.24</td>
<td>3.4 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>1.7 ± 0.31 *</td>
<td>3.0 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>1.4 ± 0.23 *</td>
<td>2.4 ± 0.34 *</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>0.86 ± 0.20 *</td>
<td>2.0 ± 0.21 *</td>
</tr>
<tr>
<td></td>
<td>5 weeks</td>
<td>0.66 ± 0.03 *</td>
<td>1.9 ± 0.18 *</td>
</tr>
</tbody>
</table>

*: Significantly different from control (P < 0.05)
1 (SI): Stimulation Index to mitogen; 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.
REFERENCES


الأهداف البيوكيميائية في القشران التي تتأثر بالجرعات تحت
المئوية من مبيد السيبيرشرين

1. قسم كيمياء مبيدات الاعراق - كلية الزراعة - جامعة الاسكندرية.
2. العمل المركزي لمبيدات - مركز البيولوجيا الزراعية - الدق - جميع.
3. مبيدات الحشرات الطبية - جامعة الاسكندرية - الاسكندرية.

أن الانتشار الواسع في استخدام المبيدات المضادة للقيدام يجب أن يثير واعية
الخصوصي 스ومه، ويكون ذلك تأثير الضرر الثابت لهذا المبيدات على الأكلان والجوان.-
لذلك تناول
البحث بدراسة تأثير التعرض المستمر لبيض السيبيرشرين من عائلة السباعيات المزهرة مخصصاً
والثناea للاستخدام على بعض الأجهزة الحيوية في جسم فراش النحل.

ويستهدف البحث أن بدراسة تأثير مبيد السيبيرشرين على الجهاز التناسلي وما يصاحب ذلك
من تغيرات بيوكيميائية تبعي النظائر الأخزرة بالإضافة إلى تأثيره على بعض توازن الدم وعلى
القناة ونسبة مكثرة.

وتفعال من الدراسة أن هذا الجربور يؤثر بشكل جيد على عدد كرات الدم البيضاء، وخاصة
بعد 6-7 أسابيع من الجراحة، بينما لم يكن له تأثير واضح على كل من مستوي الهيموغلوبين،
والهيماتوكريت وركاز الدم المحرم.

وظهرت النتائج التحليلية ملاحظة على كل الانتسايات المثيرة مثل الانزيمات قتالي
الفسفاتاز، والكوفيوكسل، التستيروصور، والكوفيوكسل لـ- طبابة، الكوفيوكسل المحدد،
والكوفيوكسل مكتشفة حيث تبين أن الانتسايات المثيرة.

posure، والاطروايمين، حيث تبين أن الانتسايات المثيرة.

وفي فضية أن الجربور يتأثر في عدد من واضحة النظائر النباتية، والثقافات المكثرة،
وبالنسبة مكثرة في حماية الجسم من عدد من الاضرار.

وهذا البحث يوضح الاصرار الواسع في استخدام الانتسايات من التعرض المستمر لهذا المبيد، حيث من
الضغويات إلى أن أهمية القشران الذي يثير تأثيره موثوقاً باستشرار في القطر وكثير
المشروع له وكذلك يجب تشريحة استخدام المبيدات مع إشارة الاحتياطات الوقائية والطرق المناسبة
للويفية من اتخاذ الاحتياطات المبيدات.