CYPERMETHRIN INDUCED CHANGES IN BIOCHEMICAL CONSTITUENTS OF PLASMA OF FEMALE ALBINO RATS

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ABSTRACT

Cypermethrin is a broad spectrum, biodegradable pyrethroid insecticide, with enhanced stability and considerable low mammalian toxicity. The present investigation was performed to investigate the sub acute effects of cypermethrin in female rats. Cypermethrin was dissolved in vegetable oil and administered orally (50mg/kg body weight daily) to female albino rats for 15 and 30 days to evaluate the alterations in levels of various plasma constituents and enzymes. Group of rats receiving similar amount of distilled water and vegetable oil served as control rats. Bioconstituents like proteins and creatinine showed significant decrease in plasma after two weeks of cypermethrin treatment, however, plasma urea levels initially decreased after two weeks of treatment and then increased significantly after four weeks in treated rats. Activity of enzymes viz; phosphatases (acid phosphatase (ACP) and alkaline phosphatase (AKP)), transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and lactate dehydrogenase (LDH) were enhanced in plasma of cypermethrin treated rats as compared to control rats. These changes observed in the levels of various enzymes and proteins, urea and creatinine in plasma could be sensitive index of the toxic effects of pesticide.

Key words : Cypermethrin, Enzymes, Proteins, Urea, Creatinine, Female albino rats.

INTRODUCTION

Pesticides are being extensively used in agriculture and public health to control insects, weeds, animals and vectors of diseases (McLachlan 2001). Pesticides may cause toxicity through several different mechanisms: direct damage to structure of cells, interference with biochemical processes necessary for normal cell function and biotransformation resulting in toxic metabolites (Colborn 1998). Synthetic pyrethroids are used preferentially in place of organophosphates and organochlorines because they are highly effective for wide range of insects and exhibit low toxicity to mammals, birds and undergo rapid biodegradability (Muthuviveganandavel et al. 2008). Cypermethrin, a synthetic pyrethroid is a broad spectrum insecticide and fast acting neurotoxin with good contact and stomach action (Vijverberg and Vanden 1990; Tao et al. 2008). Prethroids (tetramethrin, sumithrin, deltamethrin, fenevalerate) cause decrease in total serum proteins and increase in transaminases and alkaline phosphatase enzymes in rats (Abu-El-Zahab et al. 1993; Muthuviveganandavel et al. 2008). Alpha cypermethrin increase the serum ALT, AST, AKP, LDH and blood glucose level in male albino rats (Manna et al. 2004). Information regarding the effects of cypermethrin in female rats is scarce and not well defined. Therefore, the present investigation was performed to investigate the sub acute effects of cypermethrin in female rats.
study was undertaken to investigate the sub-chronic effects of cypermethrin on biochemical parameters of plasma in female albino rats.

**MATERIALS AND METHODS**

Commercial formulation of cypermethrin (Rallis India Limited) having 25% EC was used for the present study. All chemicals used in this study were of analytical grade. Glass distilled water was used for preparation of all reagents. The test concentration of cypermethrin was calculated from the percentage of active ingredient of commercial formulation of cypermethrin. Adequate dilutions were made with vegetable oil to achieve the test concentration of 50 mg/kg (1/5th of LD$_{50}$).

Sexually mature female albino rats, 3 months of age, weighing 110-150 gm were procured from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The animals were housed in groups of two rats per cage. The rats were maintained under controlled conditions of temperature (22±2°C) and provided with standard diet containing pelleted food supplemented with presoaked black gram and water *ad libitum*. The rats were acclimatized for 10 days before using them for experimentation. Their ovarian cyclicity was checked by daily microscopic examination of vaginal smears. Rats showing regular cyclicity were selected for the present investigation. Rats were divided into two groups. One group of rats (8 rats) were fed orally through oral intubation with cypermethrin (50 mg/kg body weight daily) for two weeks while the second group of rats received the same dose for four weeks continuously. Control rats (in two groups of 8 each) received same amount of vegetable oil/distilled water. The body weights of control as well as treated rats was taken before the start of the treatment and then on the day of sacrifice. On the completion of experiment, the rats were sacrificed and organs like liver and kidney were excised and weighed. The experimental protocol met the National guidelines on the proper care and use of animals in the laboratory research. The Institutional Animal Ethics Committee (IAEC) approved this experimental protocol.

**Biochemical profile**

One day after the last dose i.e. after two and four weeks, the animals (control as well as treated) were mildly anaesthetized using chloroform and the blood was collected directly from heart in heparinized vials. Plasma was separated from blood by centrifuging the blood at 3000 rpm for 15 minutes. The supernatant was collected and used for biochemical estimations. Quantitative estimations of the levels of various biochemical constituents like proteins (Lowry et al. 1951), urea and creatinine (Hawk 1988) and enzymes like phosphatases ACP and AKP (Bessey et al. 1946), aminotransferases ALT and AST (Bergmeyer 1974), and lactate dehydrogenase(LDH) (King 1965) was carried in plasma.

**Statistical analysis**

All the values are expressed as Means ± SE. The data were analyzed on computer using “Analysis of Variance (ANOVA)” as a Statgraphics statistical package, to evaluate the significance levels between the parameters studied. “P” value of 0.05 was selected as a criterion for statistically significant differences.

**RESULTS AND DISCUSSION**

Mean changes in body weight, liver and kidney weights are summarized in Table 1. At the end of the experiment, the net body weight gain in all the treated rats was significantly less at both two and four weeks as compared to control rats. Liver weight decreased slightly in the treated rats as compared to control rats. Kidney weight of rats in experimental group increased slightly throughout the experimental period when compared to control rats (Table 1). The prolonged and indiscriminate use of cypermethrin is reported to cause both acute and chronic toxicity in non-target species including humans (Aldridge 1990; Cantalamessa 1993). Body weight gain of rats in experimental groups was less as compared to control rats. This reduction in body weight was significantly less at both two and four weeks as compared to control rats.
weight gain is a clear indication of general toxicity (Elbeitha et al. 2001). Previous workers have also observed significantly lower body weight gain in cypermethrin treated rats and rabbits (Aldana et al. 2001; Elbeitha et al. 2001). In a repeated dose 28-day oral toxicity study, significant changes in liver weight was also observed in rats treated with higher doses of cypermethrin (Yavasoglu et al. 2006). Cypermethrin also caused slight increase in kidney weight of treated rats after two and four weeks which are in agreement with the previous workers where long term feeding of cypermethrin have shown increase in kidney weights (Elbeitha et al. 2001). Most toxic chemicals are metabolized in liver or excreted out through kidneys and these processes may alter their function (Bansal et al. 2007).

Treatment of rats with cypermethrin caused changes in the plasma levels of proteins, urea and creatinine (Table 2). The amount of proteins decreased in the plasma of treated rats after two weeks of treatment as compared to both the control rat groups. Cypermethrin treatment when continued for four weeks did not further decrease the amount of proteins as compared to two weeks treatment. Reduction in protein content can partially be attributed to the decreased level of protein synthesis as a result of degenerative changes in tissues of cypermethrin treated rats and is thus toxic at cellular levels. Abu-El-Zahab et al. (1993) also stated that total serum proteins were decreased in animals treated with various pyrethroids (tetramethrin, sumithrin).

Table 1: Mean changes in body weight, liver and kidney weights of rats in control and treated groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>After Two Weeks</th>
<th>After Four Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control I</td>
<td>Control II</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>116.60 ± 13.60</td>
<td>125.0 ± 0.0</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>140.0 ± 14.70</td>
<td>150.0 ± 2.35</td>
</tr>
<tr>
<td>Weight change (g/100g b.w.)</td>
<td>20.50 ± 0.80</td>
<td>20.00 ± 0.90</td>
</tr>
<tr>
<td>Liver weight (g/100g b.w.)</td>
<td>4.61 ± 0.28</td>
<td>4.62 ± 0.66</td>
</tr>
<tr>
<td>Kidney weight (g/100g b.w.)</td>
<td>0.32 ± 0.01</td>
<td>0.36 ± 0.01</td>
</tr>
</tbody>
</table>

a Significantly different (P<0.05) between a group. All the values are Mean ± SE of 6 animals in each groups.

Table 2: Effect of cypermethrin treatment on levels of proteins (mg/100 ml), urea and creatinine (mg/ dL) in plasma of control and treated female albino rats after two and four weeks of treatment.

<table>
<thead>
<tr>
<th>Plasma components</th>
<th>After Two Weeks</th>
<th>After Four Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control I</td>
<td>Control II</td>
</tr>
<tr>
<td>Proteins (mg/100ml plasma)</td>
<td>4.54±0.07</td>
<td>4.47±0.10</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>13.33±0.90</td>
<td>12.65±0.37</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.50±0.17</td>
<td>0.49 ±0.07</td>
</tr>
</tbody>
</table>

a Significantly different (P≤0.05) between a group. All the values are Mean ± SE of 6 animals in each groups.
The level of urea decreased in the plasma of treated rats after two weeks of treatment, however the four weeks treatment of cypermethrin increased the urea concentration in the plasma (Table 2). Cypermethrin treatment for two and four weeks also resulted in significant decrease in the levels of plasma creatinine. Decreased levels of urea and creatinine are indicative of the impairment in kidney functions because of cypermethrin toxicity that resulted in decreased metabolism of physiological processes due to degenerative changes in the various segments of nephrons resulting in concomitant increase of enzyme in extracellular fluid (Abu-El-Zahab et al. 1993; Bansal et al. 2007). Endogenous creatinine is excreted by filtration through the glomerulus and by tubular secretion. Creatinine clearance is an acceptable measure of glomerular filtration rate (Murray et al. 1990). Decreased levels of creatinine may be indicative of impaired kidney tubules (Abu-El-Zahab et al. 1993).

Levels of various enzymes like ACP, AKP, AST, ALT and LDH increased significantly ($P < 0.05$) in female rats after two weeks of treatment with cypermethrin as compared to control groups (Table 3). Non significant change in the enzyme levels was observed after four weeks of treatments. Enzyme activities did not differ significantly in plasma of both control rats. Cypermethrin treatment altered the levels of various plasma enzymes. Increase in the acid phosphatase level is an indicator of tissue damage since acid phosphatase is a lysosomal enzyme and stimulated in case of eminent tissue damage (Kaur and Dhanju 2004; Manna et al. 2004). The rise in serum AKP levels seems to indicate a possible subtle disturbance taking place in hepatic dysfunctions and biliary secretions of animals (Zimmerman et al. 1969; Adeniran et al. 2006; Srivastava et al. 2006; Muthuviveganandavel et al. 2008). The slight decrease in liver weight of treated rats in the present study may also be an indicative of some liver damage. The increase in serum aminotransferases (AST and ALT) activity further suggests that cypermethrin might have caused

**Table 3:** Effect of cypermethrin treatment on acid phosphatase (ACP), alkaline phosphatase (AKP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in plasma of control and treated female albino rats after two and four weeks of treatment.

<table>
<thead>
<tr>
<th>Plasma constituents</th>
<th>Control I</th>
<th>Control II</th>
<th>After Two Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACP ($\mu$moles/mg protein)</td>
<td>1.75±0.05</td>
<td>1.73±0.02</td>
<td>2.30±0.08</td>
</tr>
<tr>
<td>AKP (U/l)</td>
<td>125.00±10.60</td>
<td>123.50±46.32</td>
<td>170.00±6.32</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>136.00±17.50</td>
<td>105.00±8.48</td>
<td>153.00±6.32</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>49.33±5.98</td>
<td>48.00±7.36</td>
<td>56.00±6.36</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>839.00±20.90</td>
<td>679.00±66.67</td>
<td>1253.00±66.67</td>
</tr>
</tbody>
</table>

*Significantly different ($P < 0.05$) between a group. All the values are Mean ± SE of 6 animals in each groups.
hepatic damage and induced pathological changes in liver (Kaur and Dhanju 2004; Manna et al. 2004, 2006; Muthuvivegangandavel et al. 2008). The increased levels of aminotransferases in plasma of rats in present study may also indicate their enhanced metabolic activity, perhaps to meet the stress induced by exposure to the pesticide (Adeniran et al. 2006).

Cypermethrin administered to female albino rats had resulted in elevation in blood plasma levels of LDH (Shakoori et al. 2006). Alpha cypermethrin administered daily orally to rats had also resulted in increased LDH levels in plasma and this increased activity of LDH indicated a shift towards anaerobiosis, resulting in enhanced production of lactic acid, which may be the cause for convulsions (Manna et al. 2004, 2006; Shakoori et al. 2006; Tao et al. 2008).

The overall results of this study showed that oral exposure to cypermethrin introduces significant biochemical alterations in plasma of treated rats which may be indicating an early projection for the disturbances to the body systems and great care should be taken during their application.

REFERENCES


