Single oral dose toxicity study of α-cypermethrin in rats

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ABSTRACT

Objective: To evaluate the acute effect of α-cypermethrin (α-CP) on antioxidant activities, oxidants, biochemical and histopathological changes at LD₅₀ dose level.

Material and Methods: α-CP at single LD₅₀ (145 mg/kg) dose was administered orally to Wistar rats while controls received an equal volume of the vehicle, DMSO. The antioxidants, oxidants, biochemical and histopathological changes in some visceral organs were studied following α-CP.

Results: A single LD₅₀ dose of α-CP increased the malondialdehyde (MDA) level and decreased the activities of catalase (CAT), superoxide dismutase (SOD) and glycogen in the liver. It also increased the serum aminotransaminases (AST, ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities, and blood glucose level. It produced some cytotoxic effect on the lungs, liver, stomach, intestine, testes and cerebellum. The vehicle DMSO may have influenced the determination of LD₅₀ of α-CP in rats.

Conclusion: A single, oral LD₅₀ dose of α-CP decreased antioxidant status, and altered biochemical parameters, which correlated with histopathological changes in rats.

KEY WORDS: Acute toxicity, insecticide, pyrethroid.

Introduction

Cypermethrin is a synthetic pyrethroid with potent insecticidal property. The technical grade cypermethrin is the racemic mixture of 8 isomers (four cis and four trans isomers). Two stereoisomers are termed α-isomer of cypermethrin, which is believed to be the most active isomer, and is known as α-cypermethrin. Alfa-cypermethrin (α-CP) is extensively used not only as an ectoparasiticide in animals, but also in agriculture and public health programs. Some of the toxic actions of α-CP have been reported earlier, but the effects of oral administration of α-CP on blood biochemistry and histology of some tissues in rats have not been studied. It has been reported that the vehicle used for α-CP has a great influence on the LD₅₀ probably by influencing absorption. The oral LD₅₀ values in rats were 79 mg/kg (5% in corn oil) and 40-80 mg/kg (10% in corn oil). But the LD₅₀ value of α-CP for rats with dimethylsulfoxide (DMSO) as a vehicle has not been studied. Therefore, the present study was undertaken to determine the oral LD₅₀ of α-CP in DMSO and to know the acute effect of α-CP on the antioxidant status, biochemical parameters and histopathology of the lung, liver, stomach, kidney, testis and cerebellum following a single dose in rats.

Material and Methods

Pesticide

Alfa-cypermethrin (α-CP; >99% pure, Gharda Chemicals Ltd. Mumbai).

Animals and experimental design

Ninety healthy adult Wistar rats of both sexes (equal sex ratio; weighing about 200 g) were divided into nine equal groups (I to IX) each consisting of ten animals. All rats were kept under controlled conditions of temperature (22±1°C) and humidity (60±5%). They were given pellet food (Amrut feeds Ltd., Pune, India) and drinking water ad libitum. A twelve-hour day and night cycle was maintained in the animal house. The experimental protocol met the national guidelines on the proper care and use of animals in laboratory research. The Institutional animal ethics committee approved the experimental protocol.
Groups I - VI were used for the determination of LD$_{50}$ of $\alpha$-CP. Group VII served as control group for LD$_{50}$ determination and Group VIII was used as an experimental group for biochemical, antioxidants and histopathological study. Group IX served as control for Group VIII. The animals were fasted overnight and $\alpha$-CP was administered orally by gavage after dissolving in DMSO (1 ml) as stated above. The animals were observed for respiratory and CNS symptoms, behavioral patterns and mortality. The LD$_{50}$ was determined by graphical method. $\alpha$-CP was administered orally to the animals of Group VIII at 145 mg/kg and Group IX animals were dosed an equal volume of DMSO only (1 ml). The control group for LD$_{50}$ determination (Group VII) and the control group for study of $\alpha$-CP-induced changes in different parameters (Group IX) are separate because they were experimented on different days.

Biochemical profile

Out of 10 animals in Group VIII (experimental), four animals died during the 48 h observation period. The remaining six animals of Group VIII and all the animals of Group IX were killed under ether anesthesia after 48 h of treatment and blood was collected after severing the neck vessels in two sets of test tubes. One set was kept under refrigeration (4°C) for separation of serum and utilized for the estimation of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) activities, total protein (TP), globulin and albumin (GLB, ALB) levels. The blood of another set of test tubes (with a mixture of potassium oxalate and sodium fluoride as anticoagulant) was used for estimation of glucose. The liver was dissected out and washed in physiological saline. The liver homogenate was used for the estimation of catalase (CAT), super oxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and glycogen.

Histopathology

The lung, liver, stomach, kidney, testis and cerebellum were fixed in 10% neutral buffered formalin. Sections of 3-5 mm thickness were stained with hematoxylin and eosin (H&E) for histological examination.

Statistical analysis

All the values were expressed as mean $\pm$ SEM. Statistical analysis was done using SPSS 7.5. The statistical significance of differences between the two means was assessed by unpaired Student’s ‘t’ test. A difference at $P<0.05$ was considered statistically significant.

## Results

$\alpha$-CP did not produce any gross effect on the central nervous system at 100 mg/kg. However, at higher doses ranging from 125 to 225 mg/kg, it produced signs of CNS stimulation followed by prolonged depression (for 20-24 h). Initially, the animals exhibited chewing, licking, salivation and then CNS depression. A somewhat variable sequence of motor symptoms then developed involving occasional pawing, or burrowing, coarse whole body tremor associated with movement of legs, gradual development of hind limb extensor tone and an increase in startle response. Finally, choreoathetosis (sinuous writhing) developed, and the animals exhibited slow twisting or writhing movement of the neck and tail. When the symptoms progressed, choreoathetosis became continuous and the righting reflex was gradually lost. Violent twisting movements sometimes lifted the body from the floor in severely affected animals and these were classed as severe athetosis. At the terminal stage, animals showed labored breathing, gasping and death. The data for the determination of LD$_{50}$ were 100, 125, 150, 175, 200 and 225 mg/kg and the probit values were 3.04, 4.75, 5.25, 5.25, 6.28 and 6.96 respectively. The acute oral LD$_{50}$ value was calculated as 145 mg/kg-body weight.

Biochemical profile

The effect of $\alpha$-CP on certain biochemical parameters is summarized in Table 1. $\alpha$-CP significantly ($P<0.05$) increased the activities of serum AST, ALT, ALP and LDH. It decreased the activities of liver CAT, SOD and increased the MDA level.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10)</th>
<th>$\alpha$-CP treated (n=6)$^*$</th>
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<tbody>
<tr>
<td>Liver</td>
<td></td>
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<tr>
<td>MDA (nmol/mg protein)</td>
<td>0.26 $\pm$ (0.06)</td>
<td>0.27 $\pm$ (0.06)</td>
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<tr>
<td>SOD (activity per mg protein)</td>
<td>0.52 $\pm$ (0.01)</td>
<td>0.51 $\pm$ (0.01)</td>
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<tr>
<td>CAT (activity per mg protein)</td>
<td>0.41 $\pm$ (0.02)</td>
<td>0.38 $\pm$ (0.01)</td>
</tr>
<tr>
<td>GSH (µ mol/mg protein)</td>
<td>1.45 $\pm$ (0.02)</td>
<td>1.43 $\pm$ (0.01)</td>
</tr>
<tr>
<td>Glycogen (mg%)</td>
<td>7.94 $\pm$ (0.84)</td>
<td>3.15 $\pm$ (0.64)$^*$</td>
</tr>
<tr>
<td>Blood glucose (mmol)</td>
<td>3.50 $\pm$ (0.38)</td>
<td>12.72 $\pm$ (1.54)$^*$</td>
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<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/ml)</td>
<td>60.83 $\pm$ (5.85)</td>
<td>73.11 $\pm$ (5.66)$^*$</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>11.33 $\pm$ (0.98)</td>
<td>19.33 $\pm$ (2.52)$^*$</td>
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<tr>
<td>ALP (U/ml)</td>
<td>82.36 $\pm$ (4.58)</td>
<td>112.40 $\pm$ (6.41)$^*$</td>
</tr>
<tr>
<td>LDH (U/ml)</td>
<td>48.66 $\pm$ (1.26)</td>
<td>106.33 $\pm$ (9.80)$^*$</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>8.25 $\pm$ (0.79)</td>
<td>8.28 $\pm$ (0.51)</td>
</tr>
<tr>
<td>ALB (g/dl)</td>
<td>4.53 $\pm$ (0.37)</td>
<td>4.59 $\pm$ (0.22)</td>
</tr>
<tr>
<td>GLB (g/dl)</td>
<td>3.71 $\pm$ (0.13)</td>
<td>3.78 $\pm$ (0.18)</td>
</tr>
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</table>

Values are mean $\pm$ SEM. * 4/10 animals died. † $P<0.05$ significantly different from control.
but non-significantly, and without appreciable change in the GSH level. The blood glucose level was significantly increased whereas liver glycogen was significantly decreased. Serum ALB, GLB and total protein levels were not appreciably altered.

Histopathology

It is evident that α–CP produced hemorrhages in the lungs (Figure 1). Congestion and hemorrhages were found in the liver (Figure 2). In the stomach it produced desquamation and necrosis of the epithelium (Figure 3). The section of testis revealed edema between seminiferous tubules, vacuolation and hyalinization in the tubules (Figure 4). Congestion and hemorrhages in the meninges (Figure 5) and cerebellum were observed. The postmortem findings of rats (died within 48 h) showed bloated stomach with hemorrhages in the stomach and intestine. Hemorrhages were also seen in the lungs. No other changes were discernible in other visceral organs.

Figure 1: Section of rat lungs showing hemorrhages (H), and infiltration of mononuclear cells (arrows) after single oral administration of α-cypermethrin at 145mg/kg. (H&E, 100 ×)

Figure 2: Section of rat liver showing congestion, hemorrhages and necrosis (N) after single oral administration of α-cypermethrin at 145 mg/kg. (H&E, 400 ×)

Figure 3: Section of rat stomach showing desquamation and necrosis (N) of epithelial cells after single oral administration of α-cypermethrin at 145 mg/kg. (H&E, 100 ×)

Figure 4: Section of rat testes showing hyalinization (H), vacuolation within the tubule after single oral administration of α-cypermethrin at 145 mg/kg. (H&E, 100 ×)

Figure 5: Section of rat cerebellum showing congestion (C) in the meningeal vessel after single oral administration of α-cypermethrin at 145 mg/kg. (H&E, 400 ×)
Discussion

The pattern of the motor signs after α–CP administration is strongly suggestive of CNS toxicity. Animals showed a sequence of the signs of toxicity, and a sinuous writhing movement. The acute oral LD₅₀ value of α–CP in DMSO was 145 mg/kg, which is higher than the LD₅₀ value determined using other vehicles like corn oil. This suggests that the vehicle DMSO reduced the toxicity of α–CP in rats. Activities of SOD, CAT, GSH, and MDA levels in the liver reflect the oxidative status and the serum enzymes like AST, ALT, and ALP represent the functional status of the liver. Chemical-induced cellular alterations vary from simple increase of metabolism to death of cell. The increase or decrease of enzyme activity is related to the intensity of cellular damage. Therefore, increase of transaminase activity along with the decrease of activity of free radical scavengers may be the consequence of α–CP induced pathological changes of the liver. The severe hyperglycemia may be due to the effect of an increase in the catecholamines level, which causes glycogenolysis, and this may be the reason for a significant decrease in liver glycogen. The decreased CAT and SOD activities and increased MDA level in the liver as well as increased serum AST, ALT and ALP levels suggest that α–CP causes hepatic damage which may be through free radicals. α–CP undergoes metabolism in the liver via esoteric and oxidative pathways by the cytochrome P450 microsomal enzyme system which results in oxidative stress producing depletion of the activity of CAT, SOD, and the glycogen level, and increased level of MDA leading to hepatic necrosis. Blood lactate was not measured in this study, but increased activity of LDH may indicate a shift towards anaerobiosis resulting in enhanced production of lactic acid, which may be a cause for the convulsions. The antioxidant status and biochemical changes correlated with histopathological changes of tissues are in agreement with the study by Giray et al. In conclusion, the present study demonstrated that oral LD₅₀ of α–CP dissolved in DMSO in rats was 145 mg/kg. In acute toxicity study, single dose oral LD₅₀ of α–CP in rats increased the levels of liver MDA, and serum AST, ALT, ALP, LDH, glucose and decreased the activities of SOD and CAT and the glycogen level. It produced moderate cytotoxic effects in the lungs, liver, testis and the least effect in the cerebellum, stomach and intestine. The pathological changes correlated with the altered enzyme activities.

References