Repeated dose toxicity of alfa-cypermethrin in rats

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The present study was performed to investigate the subacute effect of α-cypermethrin (α-CP) in rats. Alfa-cypermethrin a synthetic pyrethroid insecticide, dissolved in dimethyl sulfoxide (DMSO) and oral LD₅₀ was investigated after administering orally different doses in rats and was determined as 145 mg/kg. Other groups of rats were given repeated daily oral dose (1/10 LD₅₀) of α-CP for 30 days. The animals were sacrificed on 31st day. Activities of various enzymes, cytochrome P450 and b5 contents in liver, hepatic antioxidant status, tissue residue concentration, haemogram and pathological changes were studied. It increased the serum aminotransaminases (AST, ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities and blood glucose level significantly. α-CP decreased RBC count, PCV and Hb level significantly. It significantly decreased cytochrome P450 in liver. Residues were present in different tissues. It increased malondialdehyde (MDA) level, while decreased the activities of catalase (CAT), superoxide dismutase (SOD) and glycogen level in liver significantly. Mild to moderate histological alterations were observed in lungs, liver, stomach, kidneys, testes and cerebellum. So repeated daily oral doses of α-CP at 1/10LD₅₀ altered the biochemical parameters, decreased cytochrome P450 content, antioxidant status, which correlated with histopathological changes of tissues.

Key words: α-CP, cytochrome P450, cytochrome b5, antioxidants, tissue residue concentration, histopathology, rat

Introduction

Cypermethrin is a synthetic pyrethroid with potent insecticidal property. The technical grade cypermethrin is a racemic mixture of 8 isomers (four cis and four trans isomers). Two stereoisomer is termed α-isomer of cypermethrin, which is believed to be the most active isomer, and is known as α-cypermethrin (α-CP) [20]. Alfa-cypermethrin is extensively used as an ectoparasiticide in animals, and as insecticides in crop production and public health programme [20]. Some of the toxic actions of α-CP have been reported earlier [20], but reports on tissue residue level and effects after repeated daily oral administration of α-CP on cytochrome P450, cytochrome b5, antioxidant status, blood biochemistry, and histology of some tissues in rats are not available. It has been recorded [1] that the vehicle has a great influence on the LD₅₀, probably by influencing absorption. The oral LD₅₀ values for rats were 79 mg/kg (5% in corn oil) [20] and 40-80 mg/kg (10% in corn oil) [20]. But the report of LD₅₀ value of α-CP for rats in presence of dimethylsulfoxide as a vehicle is not available. Therefore, the present study was undertaken to determine the oral median lethal dose of α-CP dissolved in DMSO and to investigate the subacute toxicity (30 days) of α-CP.

Materials and Methods

Materials

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

Animals and experimental design

Ninety [90] adult Wistar rats of both sexes (equal sex ratio; weighing about 200 ± 20 g) were divided into nine equal groups (I to IX) each containing ten [10] 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 the laboratory research. The Institutional Animal Ethics Committee approved this experimental protocol.
The animals were grouped as follows:

Groups | Treatment
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Group-I | DMSO (1 ml) + α-CP at the dose of 100 mg/kg b.wt.
Group-II | DMSO (1 ml) + α-CP at the dose of 125 mg/kg b.wt.
Group-III | DMSO (1 ml) + α-CP at the dose of 150 mg/kg b.wt.
Group-IV | DMSO (1 ml) + α-CP at the dose of 175 mg/kg b.wt.
Group-V | DMSO (1 ml) + α-CP at the dose of 200 mg/kg b.wt.
Group-VI | DMSO (1 ml) + α-CP at the dose of 225 mg/kg b.wt.
Group-VII | DMSO (1 ml) (Control for Group-I to VI).
Group-VIII | DMSO (1 ml) + α-CP at the dose of 14.5 mg/kg (1/10 LD₅₀) b.wt. × 30 days.
Group-IX | DMSO (1 ml) × 30 days (Control for Group-VIII)

Groups I-VI were used for determination of LD₅₀ of α-CP. Group VII served as control for groups I-VI. The animals were fasted overnight and α-CP was administered orally after dissolving in DMSO (1ml) as stated above. The animals were observed for respiratory and CNS symptoms, behavioral changes and death. LD₅₀ was determined as per the method of Miller and Tainter (9). Group VIII was used for short-term toxicity study. Group IX served as control for Group VIII. α-CP was administered orally to the animals of group VIII at 14.5 mg/kg b.wt. and group IX animals were dosed equal volume of DMSO only (1 ml) daily for 30 days. On the 31st day group-VIII and control group (group-IX) were sacrificed under halothane anesthesia by severing the neck vessels aseptically.

**Hematological analysis**

Blood was collected in three sets of test tubes from the severed neck vessels of each animal. Blood smears were prepared for differential leukocyte count. One set was kept under refrigeration (4°C) for separation of serum and utilized for estimation of activities of aspartate transaminase (AST) [16], alanine transaminase (ALT) [16], lactate dehydrogenase (LDH) [1], alkaline phosphatase (ALP) [7] and total protein (TP) [3], globulin (GLB) and albumin (ALB) [17]. The blood of another set of test tubes having mixture of potassium oxalate and sodium fluoride as anticoagulant was used for estimation of glucose [18]. Blood in the 3rd set of test tubes was heparinized and used for RBC, WBC counts and measuring PCV and haemoglobin level.

**Biochemical analysis**

Portions of lungs, liver, stomach, kidney, stomach, testes and cerebellum were collected in 10% formalin solution for histopathology. One portion of liver was washed in physiological saline, homogenized and the homogenate was kept for estimation of catalase activity (CAT) [8], levels of reduced glutathione (GSH) [6], malondialdehyde (MDA) [15], glycogen [13] and tissue protein [10]. Another portion of liver was collected in ice-cold 1.15% KCl, homogenized within 10 min, centrifuged, microsomal pellets were separated and used for estimation of superoxide dismutase (SOD) [12], cytochrome P450 and b5 [14] contents by DB-UV-Vis spectrophotometer.

Animal was sacrificed and the liver was perfused in situ with homogenizing buffer A (Tris-HCL + EDTA + BHT) by single pass injection through the portal vein and dissected out, placed in ice cold KCl (1.15%). All the subsequent steps in the preparation of microsomal fraction were carried out at 0-4°C. Then the liver was minced and mixed with 4 volumes of buffer A and homogenized in a mechanically driven Teflon glass homogenizer (Remi RQ 127 A). The homogenate was centrifuged at 10000 x g in an automatic high-speed cold centrifuge (Hitachi-SCR 20B) by using the rotor RP-20-2 for 30 min. The supernatant was recentrifuged at 105,000 x g for 1 hr in an automatic preparative ultracentrifuge (Hitachi 70 P-72) using rotor RP-65T to yield microsomal pellet. Microsomal pellet was suspended in buffer B (Pot. Pyrophosphate + EDTA + BHT) and homogenized with four passes of mechanically driven Teflon glass homogenizer (Remi RQ 127A), and again centrifuged at 104,000 x g for 1 hr. The supernatant fraction was decanted and the microsomal pellet was resuspended in a minimum volume of buffer C (Tris-Hcl + EDTA + Glycero) and stored at −20°C till further use. The pellet was used for estimating SOD activity and cytochrome P450 and b5 levels.

**Residue level determination**

The tissue residue levels of α-CP in brain, lungs, liver, heart, kidney and testes were estimated by the method of Marei et al. [11].

Tissues (2 g) were extracted for 4 min with acetonitrile (25 ml) and anhydrous sodium sulfate (0.5 g) using a homogenizer. The extract was filtered through anhydrous sodium sulfate (0.5 g) and the tissues were re-extracted twice with acetonitrile (1st by 25 and 2ndly by 12 ml). The extract was clarified by centrifugation and filtered through anhydrous sodium sulfate. The combined acetonitrile extracts were concentrated to 20 ml and partitioned with hexane (2 × 10 ml). The hexane phases were discarded and the acetonitrile phase was evaporated to dryness using a rotary vacuum evaporator at 40°C. The volume was finally made up to 5 ml with acetone for GLC estimation.

A stock solution of 1 mg per litre of α-CP (analytical grade > 99%) was prepared as an external standard. The retention times of α-CP was 13.5 min. The data were