Repeated dose toxicity of deltamethrin in rats

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ABSTRACT

Objective: To investigate the short-term toxicity of deltamethrin in rats.

¹Department of Pharmacology, Materials and methods: Deltamethrin was dissolved in dimethyl sulphoxide and the oral LD₅₀ was calculated to be 150 mg/kg. Then groups of rats were given repeated daily oral dose (1/10 LD₅₀) of deltamethrin for 30 days. The animals were killed on 31st day. Activities of various enzymes, cytochrome $P_{_{450}}$ and $b_{_5}$ contents in liver, hepatic antioxidant status, tissue residue concentration, haemogram and pathological changes were studied.

Results: Deltamethrin increased the serum aminotransaminase, alkaline phosphatase, lactate dehydrogenase activities and blood glucose level significantly. Deltamethrin decreased PCV and Hb level and the liver cytochrome P450 content significantly. Considerable amount of residues were present in different tissues. It increased malondialdehyde level, while decreased the activities of catalase, superoxide dismutase, reduced glutathione and glycogen levels in liver significantly. Mild to moderate histological alterations were observed in lungs, liver, stomach, kidney, testes and cerebellum.

Conclusion: Repeated dose toxicity studies of deltamethrin at 1/10 LD₅₀ altered the biochemical parameters; decrease cytochrome P450 content, antioxidant status, which correlated with histopathological changes with considerable amount in tissue residues.

KEY WORDS: Biochemical parameters; LD₅₀; tissue residue.

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Introduction

Deltamethrin is a synthetic pyrethroid with potent insecticidal property. The technical grade deltamethrin comprises of eight stereomeric esters (four cis and four trans isomers) of the dibromo analogue of chrysanthemic acid, 2,2dimethyl-3-cyclopropanecarboxylic acids. Deltamethrin is extensively used as an ectoparasiticide in animals and as insecticides in crop production and public health programme.^[1] Some of the toxic actions of deltamethrin have been reported earlier.^[1] but reports on tissue residue level and effects after repeated daily oral administration on cytochrome P450, cytochrome b₅, antioxidant status, blood biochemistry and histology of some tissues in rats are scarcely available. It has been recorded that the vehicle has a great influence on the LD_{50} , probably by influencing absorption. The oral LD_{50} values of deltamethrin for rats were 52 mg/kg (in peanut oil) and 67 mg/kg (in polyethylene glycol 200).^[1] The dimethyl sulphoxide (DMSO) is not used in the commercial formulation, but it is one of the vehicles in which alpha-cypermethrin (α -CP) is dissolved whenever it is used. The DMSO is used as a solvent for any organic compounds including α -CP. Yet the LD₅₀ of deltamethrin in DMSO (vehicle) has not been determined in rodents. Therefore, the present study was undertaken to determine the oral median lethal dose of deltamethrin dissolved in DMSO and to investigate the sub-acute toxicity (30 days) of deltamethrin in DMSO in rats.

Materials and methods

Pesticide

Deltamethrin (>99% pure) was obtained from Gharda Chemicals Ltd., Bombay.

Animals and experimental design

Fifty-four adult Wistar rats of both sexes (equal sex ratio; weighing about 200 ± 20 g) were divided into nine equal groups (I-IX) each consisting six animals. All rats were kept under controlled conditions of temperature (22±1 °C) and humidity $(60\pm5\%)$. They were given pellet food (Amrut feeds Ltd., Pune, India) and drinking water ad libitum. A twelve-hour day and night cycle was maintained. 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 the experimental protocol.

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Groups	Treatment*	Dose (mg/kg)			
Group-I	DMSO + deltamethrin	100			
Group-II	DMSO + deltamethrin	125			
Group-III	DMSO + deltamethrin	150			
Group-IV	DMSO + deltamethrin	175			
Group-V	DMSO + deltamethrin	200			
Group-VI	DMSO + deltamethrin	225			
Group-VII	DMSO (control for Groups I-	VI)			
Short-term toxicity (30 days treatment)					
Group-VIII	DMSO + deltamethrin	15			
Group-IX	DMSO (control for Group-VII	()			

*For all the groups, DMSO was adjusted to a final volume of 1 ml/rat.

Groups I–VI were used for determination of LD₅₀ of deltamethrin. Group VII served as control for Groups I-VI. The animals were fasted overnight and deltamethrin was administered orally after dissolving in DMSO (1 ml) as stated above. The animals were observed for respiratory and CNS symptoms, behavioural changes and death and then LD_{50} was determined.^[2] Group VIII was used for repeated dose toxicity study. Group IX served as control for Group VIII. Deltamethrin was administered orally to the animals of Group VIII at 15mg/kg, body weight (b.w.) and Group IX animals were dosed equal volume of DMSO only (1 ml/day) for 30 days. On the 31st day, the animals were killed under halothane anesthesia by severing the neck vessels aseptically. Blood was collected in three sets of test tubes from 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),^[3] alanine transaminase (ALT),^[3] lactate dehydrogenase (LDH),^[4] alkaline phosphatase (ALP)^[5] and total protein (TP),^[6] globulin and albumin (GLB, ALB).^[7] The blood of another set of test tubes having mixture of potassium oxalate and sodium fluoride as anticoagulant was used for estimation of glucose.^[8] Blood in the third set of test tubes was heparinized and used for RBC and WBC counts and measuring PCV and hemoglobin level. All the absolute values were calculated according to Dacie and Lewis.^[9]

Tissue

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),^[10] levels of reduced glutathione (GSH),^[11] malonyldialdehyde (MDA),^[12] glycogen^[13] and tissue protein.^[14] Another portion of liver was collected in ice-cold 1.15% KCl, homogenized within 10 min, centrifuged, microsomal pellets separated and used for estimation of superoxide dismutase (SOD),^[15] cytochrome P_{450} and $b_5^{[16]}$ contents by DB-UV-Vis spectrophotometer.

Preparation of liver microsomal fraction

Animal was killed 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 four volumes of buffer A and homogenized in a mechanically driven Teflon glass homogenizer (Remi RQ 127 A). The homogenate was centrifuged at 10000 g in an automatic high-speed cold centrifuge (Hitachi-SCR 20B) using the rotor RPR 20-2 for 30 min. The supernatant was recentrifuged at 105000 g for 1 h in an automatic preparative ultracentrifuge (Hitachi 70 P-72, Japan) using rotor RP-65T to yield microsomal pellet. Microsomal pellet was suspended in buffer B (potassium pyrophosphate + EDTA + BHT) and homogenized with four passes of mechanically driven Teflon glass homogenizer (Remi RQ 127A) and again centrifuged at 104000 g for 1 h. The supernatant fraction was decanted and the microsomal pellet was resuspended in a minimum volume of buffer C (Tris-HCl + EDTA + glycerol) and stored at -20 °C till further use. The pellet was used for estimating SOD activity and cytochrome P_{450} and b_5 levels.

Residue level

The tissue residue levels of α -CP in brain, lungs, liver, heart, kidney and testes were estimated by the method of Marie *et al.*,^[17] modified by Mandal *et al.*,^[18]

Tissue sample preparation

Tissues (2 g) were extracted for 4 min with acetonitrile (25 ml) and anhydrous sodium sulphate (0.5 g) using a homogenizer. The extract was filtered through anhydrous sodium sulphate (0.5 g) and the tissues were re-extracted twice with acetonitrile (first by 25 and then by 12 ml). The extract was clarified by centrifugation and filtered through anhydrous sodium sulphate. The combined acetonitrile extracts were concentrated to 20 ml and partitioned with hexane (2 x 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.

Calibration

A stock solution of 1 mg/l of deltamethrin (analytical grade >99%) was prepared as an external standard. The retention time of deltamethrin was 5.25 min. The data were recorded in a HP 3392A integrator.

Apparatus

A Hewlett Packard (USA) model 5890A gas chromatograph coupled with a 3392 A (HP) integrator and equipped with a 63Ni electron capture detector was used for analysis of deltamethrin.

Histopathology

Small pieces of lungs, liver, stomach, kidney and cerebellum were fixed in 10% neutral buffered formalin and testis in Bouin's fluid. Sections of 3–5 mm thickness were cut and stained with hematoxylin and eosin (H&E) for observation under light microscope.

Statistical analysis

All values are expressed as mean \pm SEM. Statistical analysis was done using SPSS 10.1. Statistical significance between two means was assessed by Student's 't' test at P < 0.05.

Results

Deltamethrin did not produce any gross effect at 100 mg/ kg. However, at higher doses ranging from 125 to 225 mg/kg,

Table 1

Effects of deltamethrin on certain biochemical parameters in serum and blood of rats after daily oral administration at 15 mg/kg for 30 days

Parameters	Control	Deltamethrin
Serum		
ALP activity (IU/I)	66.01±1.95	135±5.10*
AST activity (IU/I)	58.21±1.98	68.50±0.61*
ALT activity (IU/I)	10.89±0.85	21.83±0.92*
LDH activity (IU/I)	47.45±2.12	53.83±0.78*
TP (g/dl)	6.89±0.88	6.80 ± 0.08
ALB (g/dl)	4.25±0.33	4.05 ± 0.19
GLB (g/dl)	2.59 ± 0.39	2.75 ± 0.14
Blood glucose (mmol/l)	3.69 ± 0.30	$6.14 \pm 0.34^*$
Haemogram		
RBC x 10 ¹² /I	9.29±0.78	7.45 ± 0.38
WBC x 10 ⁹ /I	10.34±0.95	9.13±1.92
Neutrophil x 10 ⁹ /I	2.98 ± 0.09	3.05 ± 0.12
Eosinophil x 10 ⁹ /I	0.12±0.01	$0.06 \pm 0.00^{*}$
Basophil x 10 ⁹ /I	0.03 ± 0.00	$0.06 \pm 0.00^{*}$
Lymphocyte x 10 ⁹ /I	6.42±0.08	$4.78 \pm 0.26^*$
Monocyte x 10 ⁹ /I	0.41 ± 0.03	$0.20 \pm 0.02^*$
PCV	0.37 ± 0.007	$0.31 \pm 0.001^*$
Hemoglobin (g/l)	99.5±0.05	85.0±7.3*

Values are mean±SEM, n = 6 in each group. *P < 0.05 in comparison with control.

it produced signs of CNS stimulation followed by prolonged depression. Initially the intoxicated animals exhibited chewing, licking and salivation, which was followed by CNS depression. A variable sequence of motor symptoms developed that involved occasional pawing, or burrowing, coarse whole body tremor associated with movement, gradual development of hind limb extensor tone. Finally, choreoathetosis (sinuous writhing) developed and the animals exhibited slow twisting or writhing movement of neck and tail. Violently twisting movements sometimes lifted the body from the floor in severely affected animals, which were cases of severe athetosis. At the terminal stage, animals showed laboured breathing, gasping and death. The acute oral LD_{50} value of deltamethrin was calculated as 150 mg/kg body weights.

Biochemical and hematological profile

Effect of deltamethrin on certain blood and liver biochemical and antioxidants parameters are summarized in Tables 1 and 2, respectively. Deltamethrin significantly (P < 0.05) increased the activities of serum AST, ALT, ALP and LDH while the liver cytochrome P₄₅₀ content and activities of CAT, SOD and GSH level were decreased and MDA level was increased significantly (P < 0.05) without any significant alteration of cytochrome b₅ content in liver. Blood glucose level was significantly (P < 0.05) increased and liver glycogen was significantly (P < 0.05) decreased. Serum ALB, GLB and total protein levels were not altered significantly. Deltamethrin decreased PCV, Hb level and counts of lymphocyte, monocyte and eosinophil, whereas basophil count was increased significantly (Table 1).

Tissue residue

The tissue residual concentration of deltamethrin following

Table 2

Effects of deltamethrin on certain biochemical parameters in liver of rats after daily oral administration at 15 mg/kg for 30 days

Parameters	Control	Deltamethrin treated (30 days)
CAT activity (U/mg protein)	0.32 ± 0.03	0.07±0.01*
SOD (U/mg protein)	0.42 ± 0.03	$0.13 \pm 0.02^*$
MDA (nmol/mg protein)	0.19 ± 0.02	2.47±0.16*
GSH (µmol/mg protein)	1.61 ± 0.06	$1.49 \pm 0.02^*$
Glycogen (mg %)	8.12±0.46	5.74±0.35*
P ₄₅₀ (nmol/mg microsomal protein)	2.38 ± 0.05	1.71±0.09*
b ₅ (nmol/mg microsomal protein)	1.32±0.08	1.50±0.15

Values are mean \pm SEM, n = 6 in each group. *P < 0.05 in comparison with control.

repeated oral administration for 30 days were 0.92 ± 0.01 , 0.74 ± 0.05 , 0.10 ± 0.01 , 0.09 ± 0.01 , 3.00 ± 0.12 and 0.15 ± 0.02 ppm in liver, brain, testis, kidney, lungs and heart, respectively. The concentration of deltamethrin was maximum in the lungs.

Gross pathology

At post-mortem, rats showed bloated stomach with severe hemorrhages in both stomach and intestine. Hemorrhages were also seen in lungs. No gross changes were discernible in other visceral organs.

Histopathology

Deltamethrin produced congestion and emphysema in lungs (Fig. 1). Congestion and fatty changes were found in liver (Fig. 2). In stomach, it produced desquamation and necrosis of the epithelium. Kidneys showed congestion of the blood vessels (Fig. 3). The section of testis showed edematous fluid accumulation between the tubules and vacuole formation within the tubules (Fig. 4). Congestion and hemorrhages were also seen in intracerebellar vessels of the cerebellum (Fig. 5).

Discussion

The motor signs, following deltamethrin administration is strongly suggestive of central nervous system involvement. The acute oral LD₅₀ value of deltamethrin in DMSO was found to be 150 mg/kg, which is higher than the LD_{50} values of deltamethrin determined using other vehicles like peanut oil. This suggests that the vehicle DMSO reduced the toxicity of deltamethrin in rats. Activities of SOD and CAT and GSH and MDA levels in the liver reflect the oxidative status and the serum enzymes like AST, ALT, ALP represent the functional status of the liver.^[19] Increase of transaminase activity along with the decreased of content of free radical (0, -) scavengers are probably the consequence of deltamethrin induced pathological changes in liver and other visceral organs. Increased catecholamine level^[20] causes glycogenolysis and this may be a reason for significant decrease in liver glycogen leading to hyperglycemia. Like the present findings, prolonged oral administration of cypermethrin and fenvalerate produced leucopenia and lymphocytopenia in rats.^[21] The decreased activities of CAT. SOD and GSH level and increased MDA level in liver as well as increased serum AST, ALT, ALP activities

Figure 1. Photomicrograph of rat lungs showing congestion (C) and emphysema (E) of alveoli after consecutive daily oral administration of deltamethrin at 15 mg/kg for 30 days (H&E, 100x)



Figure 3. Photomicrograph of rat kidney showing congestion (R) of the blood vessels after consecutive daily oral administration of deltamethrin at 15 mg/kg for 30 days (H&E, 100x)



suggest that deltamethrin causes hepatic damage. The pathogenesis may be through free radical (0, -) formation. Deltamethrin undergoes metabolism in the liver via hydrolytic ester cleavage and oxidative pathways by the cytochrome P_{450} system.^[22] This may cause decreased content of P_{450} in liver (Table 2). It may results in oxidative stress leading to depletion of activities of CAT, SOD and levels of GSH and glycogen and increased level of MDA. These results may cause hepatic degeneration and necrosis. Alfa-one (α -1) an antitrypsin, normally present in serum, tissue fluids and macrophages is a major inhibitor of proteases (particularly elastase) secreted by neutrophils during inflammation and results in the degradation of elastin that introduce to formation of emphysema. High concentration of deltamethrin in lungs may have caused inflammation leading to progressive emphysema in the present study. In testes, vacuole formation and oedematus fluid accumulation were observed, which may cause testicular degeneration due to the presence of deltamethrin. The present status of antioxidants and status of biochemical changes correlated with histopathological changes of tissues,

Figure 2. Photomicrograph of rat liver showing fatty changes (F) and congestion (C) of the blood vessels after consecutive daily oral administration of deltamethrin at 15 mg/kg for 30 days (H&E, 100x)



Figure 4. Photomicrograph of rat testes showing edematous fluid (F) accumulation between the tubules and vacuole (\star) formation within the tubule after consecutive daily oral administration of deltamethrin at 15 mg/kg for 30 days (H&E, 100x)



Figure 5. Photomicrograph of rat cerebellum showing congestion (C) of the blood vessels tubule after consecutive daily oral administration of deltamethrin at 15 mg/kg for 30 days (H&E, 100x)



which corroborated with the findings of Giray.^[22] In conclusion repeated short-term toxicity at $1/10 \text{ LD}_{50}$ dose for 30 days decreased antioxidant status as well as increased the transaminase activity resulting in damage to important organs.

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