Original Paper

Acute Oral Toxicity and Histopathological Studies of Cypermethrin in Rats

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ABSTRACT
Cypermethrin is a synthetic pyrethroid insecticide used to control pests in domestic, industrial and agricultural situations. This study was carried out to investigate acute oral toxicity and histopathological changes of cypermethrin in albino rats. As per Finney's probit analysis method, at 48 h, LD_{50} value of cypermethrin in rats was found to be 205 mg/kg bw by gavage method. Behavioural changes were mostly in the form of motor signs. On exposure to sublethal doses (41 mg/kg bw) of cypermethrin as single dose, double dose and multiple dose, mild to severe histological changes in duodenum, lungs and testes could be demonstrated by haematoxylin and eosin staining.

Key Words: Cypermethrin; LD_{50}; Histopathology; Rat

Introduction

Cypermethrin is a composite synthetic pyrethroid, a broad spectrum, biodegradable insecticide, and a fast-acting neurotoxin with good contact and stomach action. It is used to control many pests, including moths, pests of cotton, fruit and vegetable crops. Consistent with its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain.[1] Some of the toxic actions of cypermethrin have been reported earlier,[2] but histological studies in rats are scarcely available.

It has been suggested that the lethal dose of cypermethrin depends upon the vehicle used, percentage of corn oil, cis and trans isomers, isomers ratio, and purity of cypermethrin itself.[2] Coombs et al (1976) found that the acute oral LD_{50} value of cypermethrin for rats was 251 mg/kg bw (cis:trans isomers ratio 40:60; 5% in corn oil) and 303 mg/kg bw (40:60; 5% in dimethylsulfoxide).[3] The LD_{50} value of cypermethrin was 367 mg/kg bw (90:10; corn oil) and 891 mg/kg bw (40:60; corn oil) for female rats.[4] Yet the LD_{50} of cypermethrin with 92% purity, cis and trans ratio 40:60, corn oil and 48 h duration of acute oral toxicity and systemic cytotoxicity have not been determined in rodents. Therefore, the present study was undertaken to determine the oral median lethal dose of cypermethrin dissolved in corn oil, and to investigate the systemic cytotoxicity of sublethal doses of cypermethrin as single dose, double dose and multiple doses in albino rats.

Materials and Methods

Pesticide: Technical grade cypermethrin (92% purity; cis:trans ratio 40:60) was obtained from Tagros Chemicals India Limited, Chennai.

Animals and Experimental Design: 100 adult, healthy wistar strain albino rats (70±5 days, 175±10 g) were obtained from the Indian Institute of Science (Bangalore, India) breeding colony, and provided with standard commercial pellet feed (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum.* The rats were housed at a well-regulated 12 h light/dark schedule and temperature of 28±2 ºC.

The animals were divided into eight equal groups (I to VIII) each consisting of ten rats for acute oral toxicity, and another four equal groups (IX to XII) each consisting of five rats for histopathological studies. The experi-

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mental protocol met the national guidelines on the proper care and use of animals in the laboratory research. The institutional animal ethics committee approved the experimental protocol.

Group I-VII was used for determination of LD_{50} of cypermethrin. Group VIII served as control for Group I-VII. The rats were fasted overnight and cypermethrin was administered orally by gavage method after dissolving in corn oil (0.2 ml). The rats were observed for respiratory and central nervous system symptoms, behavioural changes and death, and then LD_{50} was determined by Finney's (1971) probit analysis method.[3]

The histopathological study component comprised four groups (IX-XII) consisting of five rats each. 1/5 LD_{50} value (41 mg/kg bw) was selected as sublethal dose and administered as single, double and multiple dose with one day interval. The first group of rats was considered vehicle controls-corn oil. To the second group of rats, single dose of cypermethrin (i.e., on 1st day) was administered (41 mg/kg bw). Double doses (82 mg/kg bw) were given with 48 h interval to the third group of rats on 1st and 3rd day. To the fourth group of rats, multiple doses (164 mg/kg bw) were given with 48 h interval, i.e., on 1st, 3rd, 5th and 7th day. After 48 h, both control and experimental rats were sacrificed and portions of duodenum, lung and testes were collected in 10% formalin solution for histopathology.

Histopathology: Small pieces of duodenum, lung and testis were isolated from both control and cypermethrin-treated rats. They were gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and debris adhering to the tissues. They were fixed in 10% neutral buffered formalin for 24 h. The fixative was removed by washing through running tap water overnight. After dehydrating through a graded series of alcohols, the tissues were cleared in methyl benzoate and embedded in paraffin wax. Sections were cut at 6 micron thickness and stained with haematoxylin and counter stained with eosin (dissolved in 95% alcohol).[6] After dehydration and clearing, sections were mounted with DPX and observed under microscope.

Results

Cypermethrin did not produce any gross effect at 150 mg/kg bw. However, at higher doses ranging from 175 to 250 mg/kg (**Table 1**), 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

Table 1 Mortality of Albino Rats Exposed to Different Concentrations of Cypermethrin at 48 h (expressed both in percent and probit kill)

Groups	*Concentration mg/kg bw	Log Conc.	No. of Animals		Percent Kill	Probit Kill
			Exposed	Dead		
Group I	150	2.1761	10	–	–	–
Group II	175	2.2430	10	1	10	3.72
Group III	190	2.2788	10	3	30	4.48
Group IV	205	2.3118	10	5	50	5.00
Group V	220	2.3424	10	7	70	5.52
Group VI	230	2.3617	10	9	90	6.28
Group VII	250	2.3979	10	10	100	8.09
Group VIII	(Control for Groups I-VII)					

**For all groups, corn oil was adjusted to a final volume of 0.2 ml/rat*

floor in severely affected animals, which were cases of severe athetosis. At the terminal stage, animals showed laboured breathing, gasping and death. The graphical representation of percent mortality versus log concentration and probit mortality versus log concentration of cypermethrin showed a typical sigmoid curve (**Fig A**) and a straight line (**Fig B**) respectively, which are in agreement with the principle of probit analysis. The acute oral LD_{50} value of cypermethrin was calculated as 250 mg/kg body weight. Sublethal dose of cypermethrin produced less vigorous symptoms. In general, the potency of behavioural changes was low with sublethal doses.

Gross Pathology: At postmortem examination, the rats demonstrated bloated stomach with severe haemorrhages in both stomach and intestine. Haemorrhages were also seen in lungs. No gross changes were discernible in other visceral organs.

Histopathology: In single-dose cypermethrin administration, hypertrophy of goblet cells was observed (**Fig C**). In double-dose administration, necrotic changes at the tips of villi, hypertrophy of goblet cells and infiltration were observed (**Fig D**). Under multiple-dose administration, congestion in the submucosa, fragmentation of villi, heavy infiltration, and necrotic changes in epithelial and submucous glands were observed (**Figs E & F**).

In single- and double-dose cypermethrin administered rats, infiltration and widening of interalveolar septal changes were observed in the lungs (**Figs G & H**). Under multiple-dose administration, haemorrhage, infiltration,

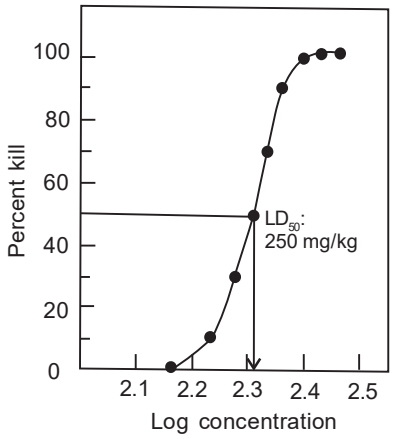

Fig A: Sigmoid "graded response" curve showing mortality of albino rats against log concentration of cypermethrin for 48 h exposure

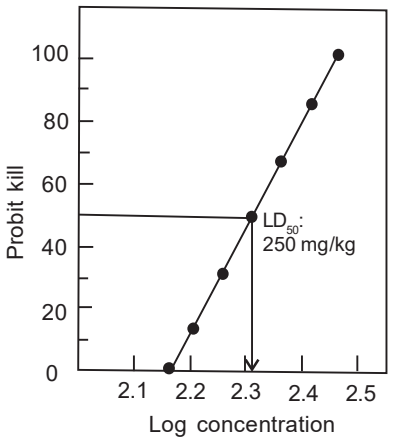

Fig B: Probit regression line showing mortality of albino rats against log concentration of cypermethrin for 48 h exposure

Fig C to H – Photomicrographs (H & E 200x)

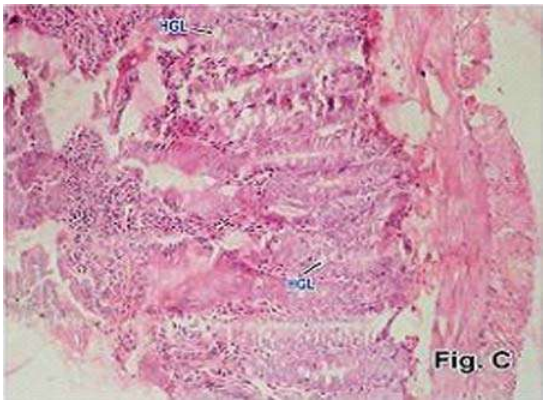

Fig C: Single dose cypermethrin administered rat duodenum showing hypertrophy of goblet cells (HGC).

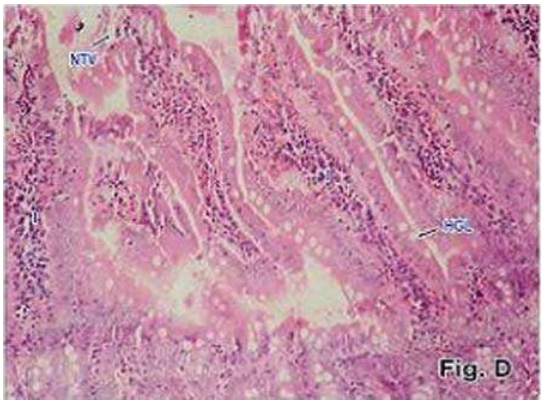

Fig D: Double dose cypermethrin administered rat duodenum showing infiltration (I), hypertrophy of goblet cells (HGC) & necrotic changes at the tips of villi (NTV)

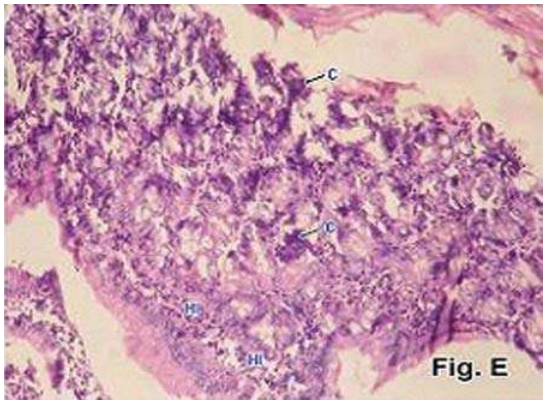

Fig E: Multiple dose cypermethrin administered rat duodenum showing congestion (C) & heavy infiltration (HI)

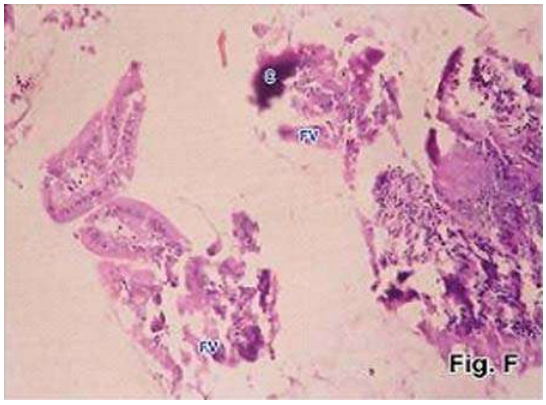

Fig F: Multiple dose cypermethrin administered rat duodenum showing congestion (C) & fragmentation of villi (FV)

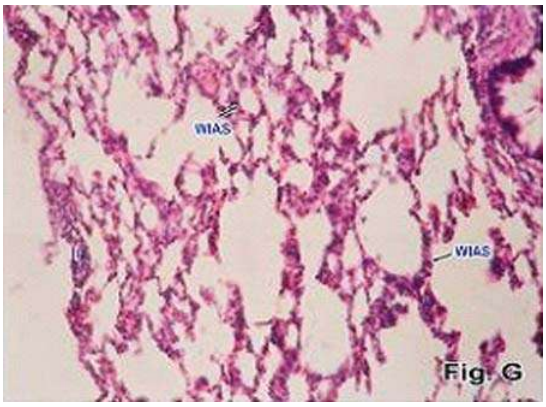

Fig G: Single dose cypermethrin administered rat lung showing infiltration (I) & widening of interalveolar septa (WIAS)

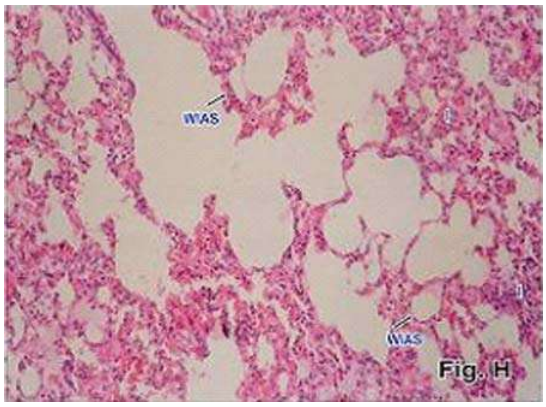

Fig H: Double dose cypermethrin administered rat lung showing infiltration (I) & widening of interalveolar septa (WIAS)

widening of interalveolar spaces, and rupture of interalveolar septa were observed (**Fig I**).

In single-dose cypermethrin administered rats, the testes did not show any marked pathological changes. In double-dose administration, congestion and degenerative changes in seminiferous tubules were observed (**Fig J**). Under multiple-dose administration, increased amount of intertubular connective tissues, degenerative changes, congestion, reduced number of spermatids, clumped spermatozoa, and increased size of lumen in seminiferous tubules were observed (**Fig K**). The duodenum, lungs and testes showed different histopathological changes in the form of a dose- and time-dependent manner.

Discussion

As per Finney's probit analysis method, at 48 h, LD_{50} value of cypermethrin in albino rats was found to be 205 mg/kg bw in the present study. This shows that cypermethrin is moderately toxic to rats, as per US EPA Acute Toxicity Rankings (2002).[7] Behavioural changes due to cypermethrin exposure were found to be similar to those reported for deltamethrin by Manna et al (2005).[8] Motor signs, following cypermethrin administration, are strongly suggestive of central nervous system involvement. However, published experimental work on cypermethrin rats' toxicity is quite limited.

In the present investigation, the obtained LD_{50} value is 205 mg/kg bw. This value is in agreement with LD_{50} value reported previously by Ray (1991)[9] and US EPA (1989).[10] According to these references, the rats LD_{50} value was found to be 150-500 mg/kg bw.

The intestine is a very important location of absorption for toxic compounds.[11] Since the gut is considered to be the main route for absorption of pesticides, it is not surprising that the duodenum showed hypertrophy of goblet

Fig I to K – Photomicrographs (H & E 200x)

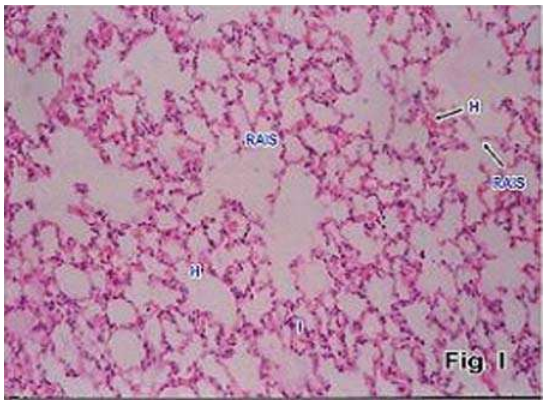

Fig I: Multiple dose cypermethrin administered rat lung showing infiltration (I), haemorrhage (H) & ruptured interalveolar septa (RIAS)

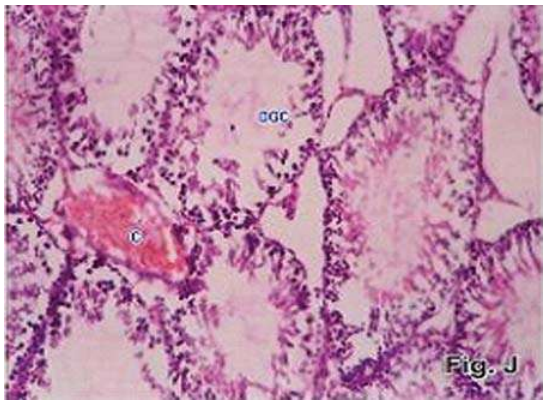

Fig J: Double dose cypermethrin administered rat testis showing congestion (C) & degenerative changes (DGC) in seminiferous tubules

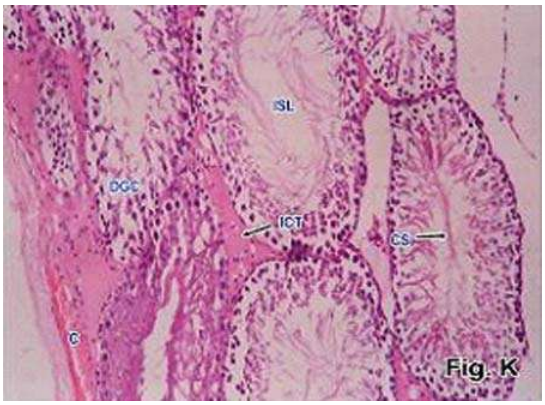

Fig K: Multiple dose cypermethrin administered rat testis showing increased amount of connective tissue (ICT), congestion (C), degenerative changes (DGC) in seminiferous tubules, clumped spermatozoa (CS) & increased size of lumen (ISL) in seminiferous tubules

cells, necrotic changes at tips of villi, infiltration, congestion in submucosa, fragmentation of villi, heavy infiltration, and necrotic changes in epithelial glands due to the presence of high concentration of cypermethrin. In the lung, pathological changes included infiltration, widening of interalveolar septa, rupture of interalveolar septa and haemorrhage due to the presence of cypermethrin. In the testes, congestion, increased amount of intertubular connective tissues, clumped spermatozoa, increased size of lumen in seminiferous tubules, reduced number of spermatids and degenerative changes were observed due to the presence of cypermethrin. The pathological changes observed in the duodenum, lungs and testes in the present study are in agreement with the findings of Velmurugan et al (2007),[12] and Manna et al (2005).[8]

In conclusion, it can be stated that long-term exposure to sublethal doses of pyrethroid pesticides can result in systemic cytotoxicity of various organs.

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