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 the acute oral toxicity and histopathological changes of cypermethrin in albino rats. According to 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. Behavioral changes in rats after oral administration of cypermethrin showed 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 testis were observed by hematoxylin and eosin staining.

Key words: LD$_{50}$, Behavioral, Histopathological studies, Cypermethrin, Rat.

INTRODUCTION
Cypermethrin is a composite synthetic pyrethroid, broad spectrum, biodegradable insecticide, and a fast–acting neurotoxin with good contact and stomach action. It is used to control 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 (Hall et al., 1980). Some of the toxic actions of cypermethrin have been reported earlier (WHO, 1989), but reports on histology of some tissues in rats are scarcely available. It has been recorded that the vehicles, percentage of corn oil, cis and trans isomers, isomers ratio and purity of cypermethrin, environmental conditions and sex influences the lethal dosage of cypermethrin (WHO, 1989). Coombs et al. (1976) found the acute oral LD$_{50}$ value of cypermethrin for rats as 251 mg/kg bw (cis:trans isomers ratio 40:60; 5% in corn oil) and 303 mg/kg bw (40:60; 5% in dimethylsulfoxide). 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 (FAO/WHO, 1980). Yet the LD$_{50}$ of cypermethrin with 92% purity, cis and trans ratio 40:60, corn oil (commercial grade) 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.

MATERIAL 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: One hundred adult, healthy wistar strain male albino rats (70±5 days, 175±10g) were obtained from the Indian Institute of Science (Bangalore, India). They were 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 ten rats for acute oral toxicity and another four equal groups (IX to XII) each consisting five rats for histopathological studies. 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 the experimental protocol.

Group I-VII was used for determination of \( \text{LD}_{50} \) 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, behavioral changes and death and then \( \text{LD}_{50} \) was determined by Finney’s (1971) probit analysis method.

The histopathological studies were done in four groups (IX-XII) consisting five rats each. One fifth \( \text{LD}_{50} \) value (41 mg/kg bw) was selected as sublethal dose and administered as single, double and multiple dose. The first group of rats was considered vehicle controls-corn oil. To the second group of rats single dose of cypermethrin (i.e., on 1\(^{st}\) 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 1\(^{st}\) and 3\(^{rd}\) day. To the fourth group of rats multiple doses (164 mg/kg bw) were given with 48h interval i.e., on 1\(^{st}\), 3\(^{rd}\), 5\(^{th}\) and 7\(^{th}\) day. After 48 h both control and experimental rats were sacrificed and portions of duodenum, lung and testis tissues were collected in 10% formalin solution for histopathology.

**Histopathology:** Small pieces of duodenum, lung and testis tissues 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, embedded in paraffin wax. Sections were cut at 6m thickness and stained with hematoxylin (Drury and Wallington, 1980) and counter stained with eosin (dissolved in 95% alcohol). After dehydration and clearing, sections were mounted with DPX and observed under microscope.

**RESULTS AND DISCUSSION**

Cypermethrin did not produce any gross visible change 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 and 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 labored 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)

**Table 1. Mortality of albino rats exposed to different concentrations of cypermethrin at 48 h**

(Mortality was expressed both in per cent and probit kill)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration log conc.</th>
<th>Exposed</th>
<th>Dead</th>
<th>Percent Kill</th>
<th>Probit Kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I</td>
<td>150</td>
<td>2.1761</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group – II</td>
<td>175</td>
<td>2.2430</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Group – II</td>
<td>190</td>
<td>2.2788</td>
<td>10</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Group – IV</td>
<td>205</td>
<td>2.3118</td>
<td>10</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Group – V</td>
<td>220</td>
<td>2.3424</td>
<td>10</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Group – VI</td>
<td>230</td>
<td>2.3617</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Group – VII</td>
<td>250</td>
<td>2.3979</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*For all groups, corn oil was adjusted to a final volume of 0.2 ml / rat.*
respectively which are in agreement with the principle of probit analysis. The acute oral LD$_{50}$ value of cypermethrin was calculated as 205 mg/kg body weights. Sublethal dose of cypermethrin produced less vigorous symptoms mentioned above. In general, the potency of behavioral changes was low with sublethal doses.

Gross pathology: In post-mortem examination, rats showed bloated stomach with severe hemorrhages in stomach and intestine. Hemorrhages 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 tip of villi, hypertrophy of goblet cells and infiltration were observed (Fig. D). Under multiple dose administration congestion in submucosa, fragmentation of villi, heavy infiltration, necrotic changes in epithelial and submucous glands was observed (Figs. E and F).

In single and double dose cypermethrin administered rats, the infiltration and widening of interalveolar septa changes were observed in lung (Figs. G and H). Under multiple dose administration hemorrhage, infiltration, widening of interalveolar septa and rupture of interalveolar septa were observed (Fig. I).

In single dose cypermethrin administered rats, the testis 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 increase amount of intertubular connective tissues, degenerative changes, congestion, reduced number of spermatids, clumped spermatozoa, increased size of lumen in seminiferous tubules were observed (Fig. K). The duodenum, lung and testis showed different histopathological changes in the form of a dose- and time- dependent manner.

According to 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 present investigation. This shows that cypermethrin is moderately toxic to rats, according to U.S. EPA Acute Toxicity Rankings (US EPA, 2002). Behavioral changes due to cypermethrin exposure in the present work were similar to those reported for deltamethrin by Manna et al. (2005). The motor signs, following cypermethrin administration were strongly suggestive of central nervous system involvement.
However, published experimental work on cypermethrin toxicity rat is quite limited.

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

The intestine is a very important site of absorption for the toxic compounds (Timbrell, 1991). Since gut is considered to be main route for absorption of pesticide, the duodenum showed hypertrophy of goblet cells, necrotic changes at tip of villi, infiltration, congestion in submucosa, fragmentation of villi, heavy infiltration of lymphocytes, and necrotic changes in epithelial and glands due to the presence of high concentration of cypermethrin. Such changes would definitely result poor absorption of nutrients in experimental animals.

**Fig. H.** Photomicrograph of double dose cypermethrin administered rat lung showing infiltration (I) and widening of interalveolar septa (WIAS). H & E. 200x

**Fig. I.** Photomicrograph of multiple dose cypermethrin administered rat lung showing infiltration (I), hemorrhage (H) and ruptured interalveolar septa (RIAS). H & E. 200x

**Fig. J.** Photomicrograph of double dose cypermethrin administered rat testis showing congestion (C) and degenerative changes in seminiferous tubules (DGC). H & E. 200x

**Fig. K.** Photomicrograph of multiple dose cypermethrin administered rat testis showing increase amount of connective tissue (ICT), congestion (C), degenerative changes (DGC) in seminiferous tubules, clumped spermatozoa (CS) and increased size of lumen (ISL) in seminiferous tubules. H & E. 200x
In lung, pathological changes include infiltration, widening of interalveolar septa, rupture of interalveolar septa and hemorrhage due to the presence of cypermethrin. Such changes would definitely result poor exchange of respiratory gases. In testis, congestion, increase 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. Such changes would definitely result sterility in experimental animals. The pathological changes were observed in duodenum, lung and testis in the present study, which corroborated with the findings of Velmurugan et al. (2007) and Manna et al. (2005). In conclusion, it can be stated that long term exposure to sublethal doses of pyrethroid pesticides can result in systemic cytotoxicity.

ACKNOWLEDGEMENT

Dr. A. Nagarjuna thanks Andhra Pradesh Council of Science and Technology, Hyderabad for the financial support (Young Scientist Fellowship).

REFERENCES


