Original Article

Acute Oral Toxicity and Histopathological Study of Combination of Endosulfan and Cypermethrin in Wistar Rats

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ABSTRACT

Background: Endosulfan, a neurotoxic organochlorine insecticide and cypermethrin, a synthetic pyrethroid insecticide used to control pests in domestic, industrial, and agricultural situations. **Materials and Methods:** The present study was carried out to investigate the acute oral toxicity, behavioral and histopathological changes of combination of endosulfan and cypermethrin in albino rats. According to Miller and Tainter analysis method, at 48 h, LD50 value of combination of endosulfan and cypermethrin (ratio 1:1) in rats was found to be 691.83 mg/kg bw by oral gavage. **Results:** When combination of both these pesticides was administered orally at concentration of 103.72 mg/kg bw, 172.95 mg/kg bw and 207.50 mg/kg bw, respectively, as a single dose, no significant changes in behavior of rats was observed, neither in dosed nor in control group of rats. Combination of endosulfan- and cypermethrin-treated rats showed mild histopathological changes in liver and kidney in group IV (207.50 mg/kg BW) as compared to the control. However, no significant changes were observed in brain and small intestine at either dose of combination of endosulfan and cypermethrin with respect to control. **Conclusion:** Thus, the present study, first of its kind in India, demonstrated the oral toxicity, behavioral, and histo-architectual alterations after induction of combination of endosulfan and cypermethrin at acute doses in Wistar rats.

Key words: Behavioral, cypermethrin, endosulfan, histopathological studies, LD_{50}, rat

INTRODUCTION

Pesticides have become an area of intense research due to its diverse properties and related effects. The demand for pesticide products and the concentration that they make towards agriculture efficiency are clear, but the volume of production indicates that the potential for misapplication and accidental exposure is very high. Besides being beneficial for increased crop yield and in vector control program, it has resulted in the manifestation of several health-related problems. Up to 90% of the pesticides used, never reach the intended targets,[1] as a result, many other organisms sharing the same environment as pests due to which humans are accidentally poisoned. Widespread use of insecticides in animal husbandry and agriculture for many years lead to their contamination in the food chain and the environment.[2] Organochlorine and synthetic pyrethroids are extensively used in agriculture to control crop pests and in livestock to control parasites and ectoparasites.[3] The combination of endosulfan and cypermethrin is now a day extensively used by the farmers in India in a large variety of agriculture use. The combination of these two pesticides may have additive, synergistic, potentiative, or antagonistic effect.[4] Cypermethrin is synthetic pyrethroid, and primarily used as an insecticide. It acts as a fast neurotoxin for insects. It is easily degraded on soil

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and plants, but can be effective for weeks when applied to indoor inert surface. Exposure to sunlight, water, and oxygen accelerates its decomposition. Cypermethrin, a synthetic chemical, is similar to the pyrethrins in pyrethrum extract (comes from the chrysanthemum plant).[5] It is widely used as insecticide in developing countries in controlling pests.[6] On the basis of different behavioral, neurophysiologic, and biochemical profiles, cypermethrin is identified as synthetic type II pyrethroid, possess a cyano group, and produce a more complex syndrome, including clonic seizures while, type 1 pyrethroid may cause mainly hyper excitation and fine tremors.[7] Cypermethrin is effective against broad range of pests of cotton, fruits and vegetables, and ectoparasites of domestic animals. The oral LD_{50} for cypermethrin in rats is 250 mg/kg (in corn oil) or 4123 mg/kg (in water). Endosulfan, a neurotoxic organochlorine insecticide of the cyclodine family of pesticide, is used as a broad-spectrum non-systemic, contact and stomach insecticide,[8] and also acaricide against insect pests on various crops. Because of high toxicity, instability, insolubility in water,[9] high potential for bioaccumulation, and environmental contamination, a global ban on the use and manufacture of endosulfan is being considered under the Stockholm Convention.[10] Technical endosulfan is a mixture of stereoisomer, designated "ά" and "ß", in a 7:3 ratio. The half–life of endosulfan in water and in most of fruits and vegetables is reported to be 3-7 days.[11] Acute oral LD_{50} of endosulfan is 80-110 mg/kg, and its chronic poisoning results from food residue and cause kidney and liver damage. Endosulfan persists in the environment and bioaccumulates in animals and plants, leading to instances of food contamination and eventually dietary exposure in humans.[12] This study, therefore, concurrently evaluates the effects of endosulfan and cypermethrin, at acute lethal doses on survival, behavior, and histopathology of the adult Wistar rats. The study has been designed to identify the acute toxic effects of the combination of these pesticides, which are widely used in India, and to contribute to the knowledge of the effects of this combination of pesticides on Wistar rats.

MATERIALS AND METHODS

Pesticide

Cypermethrin 92.21% pure (liquid) and endosulfan 95.80% pure (solid) were obtained from Hindustan Insecticides Limited (A govt. of India Enterprises), Mansa Road, Bathinda, India. The oral LD_{50} of cypermethrin is 187-326 mg/kg in male rats and 150 to 500 mg/kg in female rats.[13,14] The oral LD_{50} of endosulfan for rats is 80 mg/kg bw.[15] The oral LD_{50} of combination of cypermethrin and endosulfan is 691.83 mg/kgbw (in DMSO), calculated by pilot study conducted in the department according to Miller and Tainter method.[16]

Animals

The protocol was approved by the animal ethics committee of All India Institute of Medical Sciences. This experiment was conducted on 24 Albino rats (Wistar strain, Male) aged 20-21 weeks, weighing between 200-250 gm obtained from Central Animal Facility (CAF) AIIMS, New Delhi, India. They were divided into 4 groups (I-IV) each consisting of 6 animals [Table 1]. All rats were kept under controlled condition of temperature at 25 ± 2°C, relative humidity 50 ± 15%. A 12 hours light and 12 hours dark cycle was maintained in the animal house. For feeding, unlimited supply of drinking water along with conventional laboratory diets were used. Individual animals were identified by marking with picric acid. Twelve hours before the behavioral testing, the rats were deprived of food to enhance their motivation to perform the test. Animals were observed twice for over-toxicity, morbidity, and mortality. The animals were acclimatized to the laboratory conditions 5 days prior to the test. Body weight was taken on the day of acclimation, before dosing and before sacrification.

Animals of Gp I were given 0.5 ml DMSO, and Gp II, III, and IV were given a single oral dose of combination of cypermethrin and endosulfan (0.5 ml) orally using 22-gauge oral feeding canula [Table 1].

Behavioral parameters

The elevated plus maze is a behavioral assay for rodents, and it has been validated to assess the anti-anxiety effects of substances. Foot fault is basically used for locomotor assessment, and memory retention deficit was evaluated by step through passive avoidance apparatus. Elevated plus maze, foot fault, and passive avoidance were performed on Gp I, II, III, and IV before induction of doses and after acute dosing. Elevated plus maze was performed before the foot fault and passive avoidance test.

Elevated plus maze test

The elevated plus maze is a behavioral assay for rodents, and it has been validated to assess the anti-anxiety effects of substances. Acquisition, and retention of memory processes were evaluated as described by Reeta *et al.*,[17] and Monika *et al*.[18] In brief, rats were placed individually at the end of one open arm facing away from central platform, and initial transfer latency was noted. The rat was allowed to explore the maze for another 5 min and then returned to its home cage. After 24 hours, retention transfer latency was noted.

Table 1: Animal Grouping

Groups	Treatment	Dose (mg/kg)
I	DMSO (Control)	-
II	DMSO+CP+Endo	103.72
III	DMSO+CP+Endo	172.95
IV	DMSO+CP+Endo	207.50

Foot fault test

Foot fault is basically used for locomotor assessment. Each rat was placed onto an elevated grid (57 cm × 44 cm with 3.5 cm × 3.5 cm grid opening) and allowed to explore for 1 minute to assess forelimb and hindlimb use during locomotion. The number of times each forelimb and hind limb was used and the number of slips made with each forelimb and hind limb were recorded.

One trial passive avoidance task

Memory retention was evaluated by a step through passive avoidance apparatus (Ugo Basile, Italy) according to the method described earlier.[19] On the acquisition trial, each rat was placed in the lighted chamber. After 30 s of habituation, the guillotine door separating the light and dark chambers was opened, and the initial latency to enter the dark chamber was recorded. The guillotine door was closed immediately after the rat enters the dark chamber, and an electric foot shock (0.2 mA) was delivered to the floor grids for 0.2 s. The animal was removed after 10 s from the dark chamber. After 24 h, retention latency was measured in the same way as in the acquisition trial, but foot shock was not delivered, and the latency time was recorded up to a maximum of 600 s.[18]

Histopathology

The liver, kidney, brain, and intestine were removed, and their weight were recorded, and brain/body weight ratios, liver/body weight ratios, and kidney/body weight ratios of each animals were calculated. Then, they were fixed in 10% neutral buffered formalin and processed for paraffin embedding. Sections of 4-6 µm thickness were cut and stained with hematoxylin and eosin (H and E)[20] and observed under light microscope for histopathological changes.

Statistical analysis

All the values were expressed as mean + SEM. Comparisons were made between control and treatment groups using SPSS 16 using one-way ANOVA, followed by Bonfrroni test. A value of $P < 0.05$ was considered as statistically significant.

RESULTS

Effect on cognitive impairment

Elevated plus maze

There was no significant difference in the initial transfer latencies as well as retention transfer latencies amongst the groups as well as within the group as compared to control group as revealed by Bonferroni *post hoc* test, thus showing no anxiety-related changes in the treated groups [Figure 1].

Foot fault test

There was no significant difference in the initial transfer latencies and retention transfer latencies. *Post hoc* analysis showed that there were no differences among treated groups [Figure 2].

Passive avoidance test

There was no significant difference in the initial transfer latencies and retention transfer latencies amongst the groups as well as between the groups as compared to control group as revealed by Bonferroni *post hoc* test. Therefore, no significant effect on memory was seen [Figure 3].

Gross pathology

Post–mortem examination of rats reveals bloated stomach with hemorrhages in stomach, lungs, and intestine. No gross changes were discernible in other visceral organs.

Histopathological observation

Combination of endosulfan- and cypermethrin-treated rats showed hepatic congestion and hemorrhage in

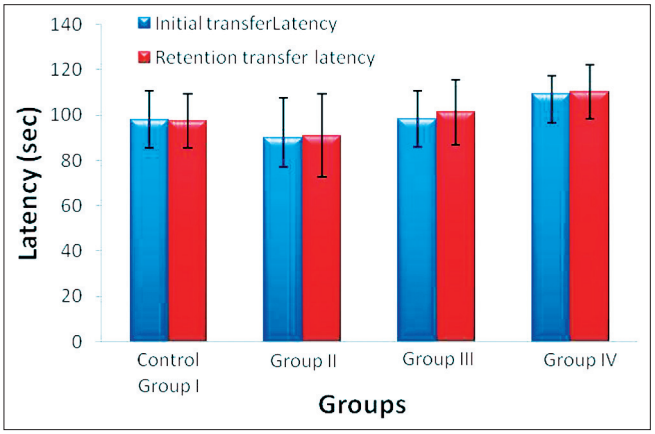

Figure 1: Effect of combination of endosulfan and cypermethrin on initial and retention transfer latencies in elevated plus maze test. Data represent mean ± SEM (n=6), *P<0.05 as compared to control group

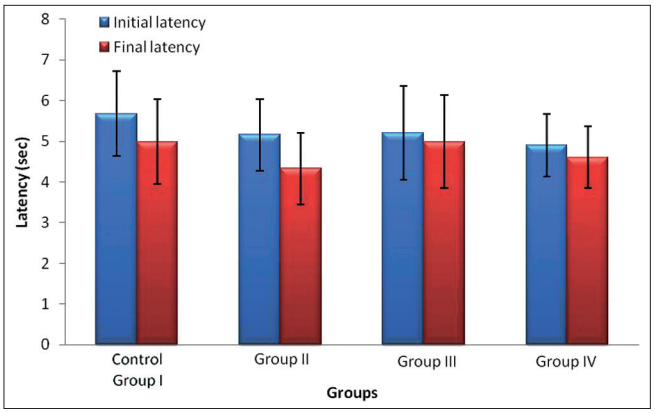

Figure 2: Effect of combination of endosulfan and cypermethrin on initial and retention transfer latencies in foot fault test. Data represent mean ± SEM (n=6), *P<0.05 as compared to control group

liver [Figure 4a] and medullary congestion in kidney [Figure 5a] in group IV (i.e. 207.50 mg/kgbw) as compared to control [Figures 4b and 5b]. However, no significant changes were observed in brain [Figure 6a] and small intestine [Figure 7a] at either dose of combination of endosulfan and cypermethrin with respect to control [Figures 6b and 7b].

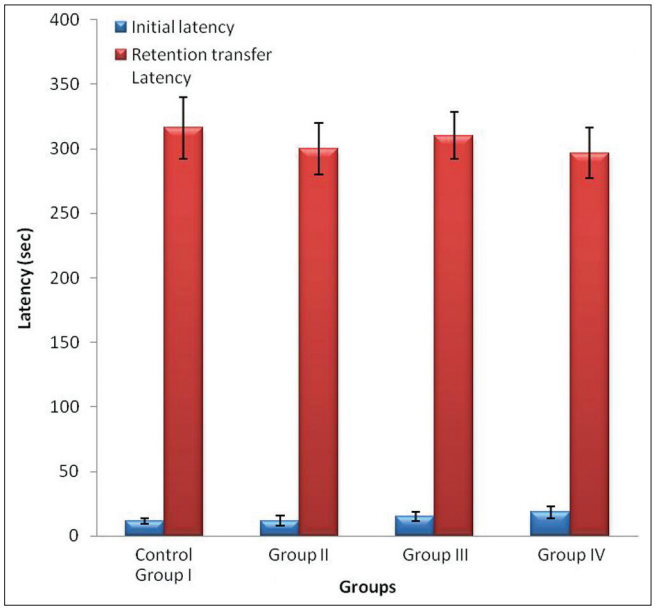

Figure 3: Effect of combination of endosulfan and cypermethrin on initial and retention transfer latencies in passive avoidance test. Data represent mean ± SEM (n=6), *P<0.05 as compared to control group

DISCUSSION

Animals administered with the combination of endosulfan and cypermethrin (Ratio 1:1) at the dose rate of 172.95 mg/kgbw and 207.50 mg/kgbw showed acute cholinergic symptoms viz. occasional pawing, burrowing chewing, licking, salivation, coarse whole body tremors, writhing, hyperactivity to sound and touch, abnormal gait, and development of hind limb extensor tone immediately after dosing, and these symptoms were persisted for 6 hours; similar signs were observed by Nagarjuna *et al*.[21] Rats treated with a synthetic pyrethroid named cyhalothrin revealed signs and symptoms of intoxication that included salivation, liquid feces, and tremors.[22] Organophosphorus insecticides react with acetylcholinesterase at the serine hydroxyl group within the active site of enzyme. In this reaction, serine hydroxyl group is phosphorylated, yielding a leaving group. The phosphorylated acetylcholinesterase is inactivated, blocking acetacholine degradation in the synapse.[23] All organo-phosphorus pesticides potentially have a mechanism of toxicity in common i.e., the phosphorylation of AChE causes accumulation of ACh. This buildup of neurotransmitter at the nerve ending results in signs of intoxication includes restlessness, hyper excitability,

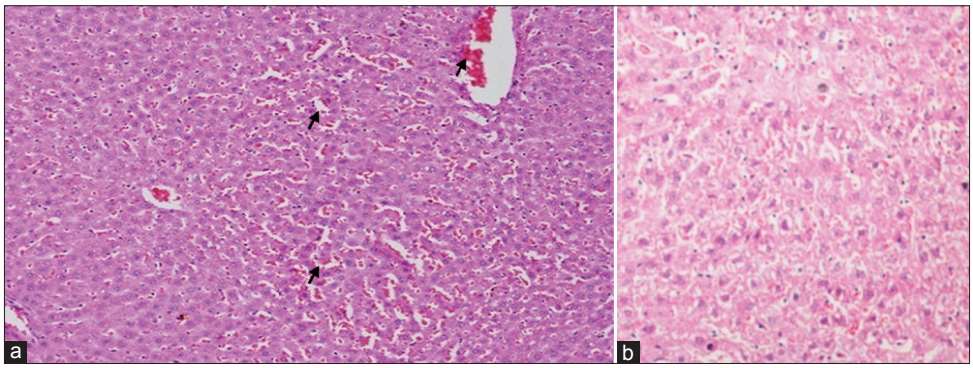

Figure 4: (a) Section of rat liver showing hepatic congestion and hemorrhage after administration of combination of endosulfan and cypermethrin (H and E, ×400), (b) Control

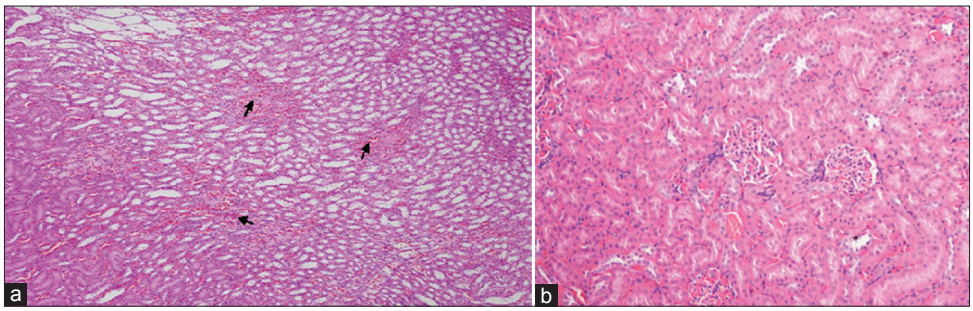

Figure 5: (a) Section of rat kidney showing medullary congestion after administration of combination of endosulfan and cypermethrin (H and E, ×100), (b) Control

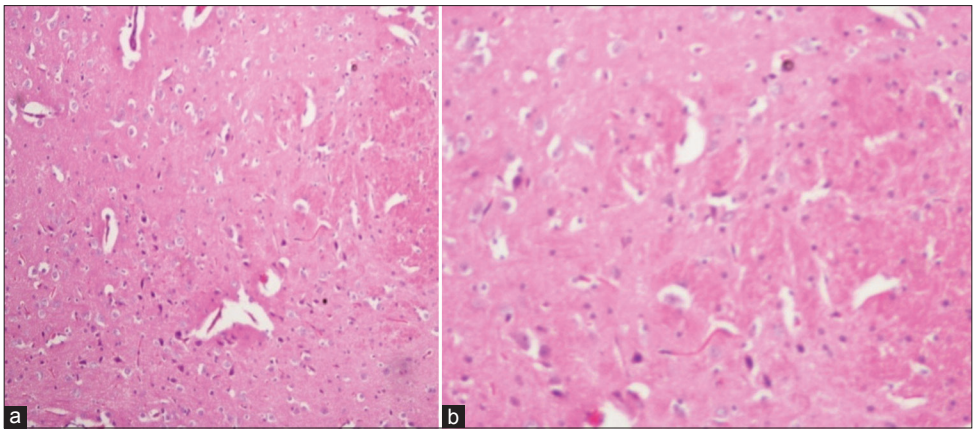

Figure 6: (a) Section of rat brain showing no significant histopathological changes after administration of combination of endosulfan and cypermethrin (H and E, ×100), (b) Control

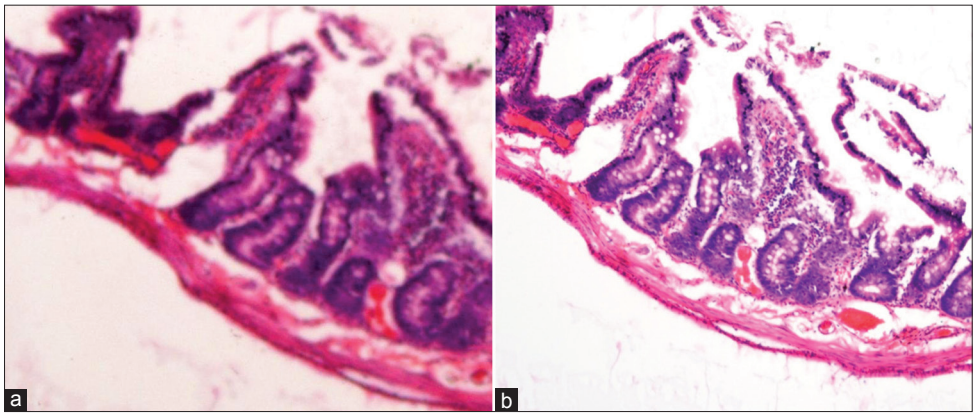

Figure 7: (a) Section of rat intestine showing no significant histopathological changes after administration of combination of endosulfan and cypermethrin (H and E, ×100), (b) Control

tremors, convulsions, and paralysis.[23,24] Single dose of combination of cypermethrin and profenofos induced treatment-related and dose-dependent neurobehavioral alterations; however, no structural/histopathological alterations were apparent.[25]

Intoxication symptoms due to oral administration of combination of endosulfan and cypermethrin in the present work were similar to those reported for deltamethrin by Manna *et al.*[26] and those reported for cypermethrin by Nagarjuna *et al.*[21] Results in this study indicated that the acute exposure (i.e. 24 hrs) of combination of endosulfan and cypermethrin (Ratio 1:1) has no significant effect on behavioral parameters.

Histopathology provides a rapid method to detect effects of irritants in various organs.[27] Animals administered with the combination of endosulfan and cypermethrin (Ratio 1:1) at the dose rate of 207.50 mg/kg bw cause hepatic and medullary congestion, leading to mild pathological change in liver and kidney tissues, which was also observed by Manna *et al.*, in 2004.[26] Different other studies, supporting the results of this present study, showed that malathion and other pesticides induced histopathological alteration in liver and kidney of the experimental animals.[28-32] The most consistent changes were seen in the liver of animals, of all treatment groups, were varying degrees of degenerative changes and vascular changes. These findings were in agreement with those observed in cypermethrin toxicity in rats by Grewal *et al.*,[33] Muthuviveganandavel *et al.*,[34] Yavasoglu *et al.*,[35] Manna *et al.*,[26] and Luty *et al.*[36] All had conducted experiments in albino rats, but the dosage and route of administration of cypermethrin were different. The result of the present study showed that the combination of endosulfan and cypermethrin, even in a single dose, can induce mild damage to liver and kidney as shown in histopathological examination. The similar results are reported for malathion and other pesticides, which indicated that exposed these pesticide lead to induce physiological and biochemical disturbances in experimental animals.[31,37-39]

Intestine is a very important site of absorption for the toxic compounds.[40] In our study, no significant histopathological alteration were seen in intestine at either doses (103.72 mg/kg bw, 172.95 mg/kg bw, and

207.50 mg/kg) of the combination of endosulfan and cypermethrin, but some studies showed hypertrophy of goblet cells, necrotic changes, infiltration, and congestion in the duodenum.[21] Manna *et al.*[26] and Khan *et al.*[41] reported congestion and hemorrhage in the brain of rats intoxicated with single and repeated doses of cypermethrin, but our study showed no histopathological changes in brain of Wistar rats at acute exposure (i.e., 24 hrs) of combination of endosulfan and cypermethrin (Ratio 1:1). In conclusion, the overall results of this study clearly demonstrate that oral administration of combination of endosulfan and cypermethrin at acute doses did not induce any behavioral alterations. The combination leads to slight histopathological changes in liver and kidney and no histopathological alterations in intestine and brain in rats. Further, this study also suggests that the combination of endosulfan and cypermethrin (Ratio 1:1) reduced the toxicity of individual pesticide i.e. endosulfan and cypermethrin in Wistar rats.

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