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***Research Article** (Open Access)*

PATHOLOGICAL EFFECT OF DELTAMETHRIN ON BEHAVIOR AND MEMORY OF ALBINO RATS

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ABSTRACT
A pathological study of deltamethrin in albino rats was carried out. The aim of the present study was to investigate the acute oral toxicity (LD50) of deltamethrin and its effect on the memory of male albino rats. For this four groups were made. Animals of Group-I, II, and III were given 200mg/kg, 400 mg/kg, and 800mg/kg doses of deltamethrin respectively while group-IV was act as a control group. Eight radial arm maze task (8-RAM) was used to assess memory in form of working and reference memory. The result shows that exposure to LD50 dose of deltamethrin is likely to affect working and reference memory functions in male rats. In conclusion, it was reported that deltamethrin can lead to memory impairment in mammals.

***Keywords**: Acute Toxicity, Deltamethrin, Memory Impairment, Pathological Study*

INTRODUCTION
Deltamethrin is a synthetic insect repellent that is used to control the different types of pests and weeds. Along with these benefits pesticides also have a bad effect on humans and other animals. There are two classes of pyrethroids; Type1 (cause ataxia and hyperactivation) and type II (more toxic). Type I pyrethroids do not have a cyano group and hence they are less toxic than cyano group-containing type II pyrethroids. The addition of the cyano group enhanced the insecticidal properties of Type II pyrethroids e.g. Deltamethrin. Oxidative damage caused by deltamethrin is reported in various experimental animals (Chandra *et al.*, 2013). Deltamethrin can easily cross the lipid bilayer as it is lipophilic in nature and can pass the selective semi-permeable boundary of the blood-brain barrier. It performs an oxygen-based toxicity mechanism in which they cause oxidative damage in the nerve cells. The neurotoxic effect is one of the most lethal effects of deltamethrin. It mainly targets the hippocampus and its effects can be seen on the sodium channel present in axons. Also, Long term uses of pesticides impose toxic effects on the organs of the body even in humans (Hamadache *et al.*, 2016). It decreases learning and memory performance in Y-maze test (Aziz *et al.*, 2001) as well as affects the emotional state in an open field and forced swimming tests (Lazarini *et al.*, 2001). Some of the toxic actions of deltamethrin have been recorded earlier (WHO, 1989) but the data related to the determination of oral acute toxicity (LD50) and the effect of sub-lethal dose on behaviour of adult rats is scarcely available. Oral LD50 value of deltamethrin for rats were 52mg/kg (in peanut oil), 67mg/kg (in polyethylene glycol 200) (WHO, 1990) and 150 mg/kg (in dimethyl sulphoxide). Based on previous data, the aim of the present study was determined to study the oral median lethal dose of deltamethrin and to evaluate the effect of sublethal dose of deltamethrin on the behavior of albino rats by using a radial arm maze (RAM).

MATERIALS AND METHODS
***Pesticide*:** Deltamethrin 2.8 % E.C. was purchased from the local market, Rohtak, Haryana, India.
***Animals*:** Adult male Wistar albino rats (150-200g) were obtained from the animal house of DFSAH, LUVAS, Hisar, Haryana. Animals were housed in plastic polypropylene cages under controlled conditions with LD 12:12 hours cycle and temperature 250C with proper ventilation and air conditioning. They were provided with a standard laboratory pellet diet with free access to drinking water. The rats were acclimatized under the laboratory condition for one week before the commencement of experimental work. The experimental protocol met the national guidelines on the proper care and use of animals in laboratory research. The institutional animal ethics committee (IAEC) approved the experimental protocol.

***Experimental design*:** Male albino rats were divided into 4 groups. Each group contain 2 rats as follow:
Group IV was remained unexposed and considered as a control group.
Experimental groups I, II and III was treated with deltamethrin.
Dose-1 of 100mg/kg/BW (Body Weight), Dose-2, 200mg/kg/BW and Dose-3, 400mg/kg/BW of albino rats respectively.
These groups were further used for the determination of LD50 of deltamethrin for male albino rats. The animals were kept without food overnight and deltamethrin was administered orally by gavages method. The volume of the dose given to rats was according to their size and weight. The animals were observed at an interval of 2hrs up to 24 hrs and monitoring was continued for a period of 14 days for respiratory, behavioral changes and death. After 24 hrs the numbers of deceased rats in each group were counted, and the percentage of mortality was calculated by using the method of Wilbrandt (1952). Animals that were alive after the determination of LD50 were used for the study of learning and memory behavior.

Apparatus

Radial arm maze (to study learning and memory)

Standard operating procedure No. 12 reviewed by IACUC was followed to construct the Radial Arm Maze (Wenk, 2004). The central octagonal cardboard material was used to construct the 8 arms radial maze in the laboratory, a platform was constructed 40 cm in diameter, and eight arms each of 87cm long and 10cm wide radiating from the central platform were made the distance between the radiating arms was 12-15cm approximately. A wall 12cm high surrounds the central platform consisting of 8 doors open to every 8 arms. At the end of each arm, a small food cup was placed in such a way that the food bait present in it was not visible to the rats from the central platform of the RAM.

RAM TASK (Olton and Samuelson, 1976)

Acclimatization phase: - This is also known as the familiarization phase as well. In this phase, we familiarize the rat with the environment of the maze so it was able to feel comfortable in the maze during the experiment. This session was set for 5 days.

***Training period*:** - This period starts after acclimatizing phase. There were three stages.

First stage

On the first five days, the food was placed in all arms. Each rat was given a time of 2 minutes to get the bait. The rat was kept at the central platform before starting the session and allowed to find the reward. The sequences of their entries into the arms were noted. The performance of each rat was analyzed by calculating the working memory error (re-entries to the arm).

Second stage

The food was placed in alternate arms. Only four arms were baited namely 2, 4, 6, and 8 i.e., alternate arms. Each rat was freely allowed for 2 minutes daily for seven consecutive days to find the bait kept in the food cup at end of each arm. The sequences of arms visited by the rats were observed. The performance of each rat was analyzed by calculating the working memory error – Entries into baited arm were considered as correct entries. An entry into an un-baited arm was considered as a reference memory error. Re-entries into a baited arm were taken for working memory error

Third stage

In this stage food was not provided at all, so all arms were without food. By all these, it becomes possible to evaluate the learning and memory of rats by considering the working memory and reference memory. The error in these memories is referred to as working memory error and reference memory error.

Calculation of LD50 or median lethal dose toxicity. Welbrandt method was followed for the calculation of LD50.

$LD_{50} = D_m - \sum \frac{Z \times d}{n}$

D_m = dose which kill all the animals in the group
Z = the mean of dead animals in two successive groups
D= constant factor, mean of dose in the two successive groups
N = number of animals of each group
Σ = sum of all (z x d)

RESULT AND DISCUSSION

Deltamethrin cause no more significant effect on the behavior of albino rats at Dose-1, 100 mg/kg. But the animals are given Dose-2, 200mg/kg behavioral changes such as hyperexcitability, convulsions, tremor, loud sounds, sudden jumps, quarreling, high epilepsy were observed and also rats got injured due to continuous grooming. The body movements after one hour were reduced and after three hours they were freeze means nobody's movement was shown by them. Further, no convulsions were recorded after 6 hours of exposure and no death occur in this group. The animals ate the lab food and again became sleepy i.e. very few activities were seen in them at that time. After 24 hours they showed normal behavior.

However, at a higher dose of 400mg/kg, it produced more convulsions with tremor, salivation, and hyperexcitability. At a terminal stage, the animal shows a deep breath, mouth and eye swells, gasping, and finally died after 3hrs of dose administrated.

Welbrandt method used for calculation of LD_{50} –

$LD_{50} = D_m - \sum \frac{Z \times D}{n}$

LD_{50} =400– 300/2

= 400 – 150 = 250 mg/kg/BW

The LD_{50} value of deltamethrin on male rats was calculated to be 250mg/kg/BW.

Behavioural study using RAM

Radial Arm Maze task was performed to check the performance of deltamethrin-induced rats.

During the first stage training period, fewer errors were made by control rats as compared to both deltamethrin-induced rats. The percentage of incorrect entries of control rats were 2.5% only (Table-1) while incorrect entries of dose-1 and dose-2 rats treated rats were recorded higher up to 10% and 20% respectively (Table-2).

The Result of the second training period showed that the working memory error reference memory error of control rats was less than that of dose-administered rats. The percentage of working and reference memory errors of control rats were 7.14% and 21.4% respectively (Table-3) while The percentage of working memory errors of dose 1and dose-2 rats were recorded higher up to 10.7% and 21.4%. This percentage was further increased in case of reference memory errors in dose-1 and dose-2 treated rats and recorded higher up to 39.2% and 46.4% respectively (Table-4). In the third stage testing period, the percentage of reference and working memory errors of control rats was 16.65 and 8.33 (Table-5). While the percentage of reference memory errors of dose-1 and dose-2 treated rats were recorded higher 41.6% and 50% respectively. The percentage of working memory errors of dose 1 and 2 rats were also remained higher (25% and 33.3%) Table-6.

All observation tables show that Control rats make fewer errors in each task of radial arm maze as compared to deltamethrin induced rats which means that deltamethrin affects the learning capabilities and behavior of the rats. The toxicity of deltamethrin depends upon the dose induced as conferred by calculating the median lethal dose toxicity. This pyrethroid pesticide affects the learning behavior of rats leading them to learn poorly as compared to control rats.

Table 1: Order of arms visited by control male rats during First training session (5 days)

Control male rats				
Days	Sequence of arms visited	Arms entered Every time	Correct entries	(incorrect entries) Re-entries into arm
1 2 3 4 5	2,8,1,7,6,1 7,8,4,6,2 2,8,7,4,3 7,8,6,5,4 7,8,4,6,2,3	8th arm	5 5 5 5 6	1 0 0 0 0
	Total arms = 40 arms		% Correct entries 26/40 x 100 = 65%	% Incorrect entries 1/40 x100 = **2.5%**

Table 2: Order of arms visited by the male rats (100mg/kg and 200mg/kg) during the first training phase (5 days).

Rats with Dose 100mg/kg and 200mg/kg)								
Days	Sequence of arms visited by rats		Arms entered Every time		Correct entries		(incorrect entries) Re-entries into arm	
	Dose 1 100mg/kg	Dose 2 200mg/kg	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
1 2 3 4 5	7,8,1,3,4,1 3,1,2,7,6,4,3 7,8,1,5,4,3,4,1 ,6 2,3,6,7,8,5 7,3,2,1,6	5,3,8,1,3,4 6,7,1,2,4,5,2,8,1, 3 2,4,5,8,1,2,4 7,1,2,2,8,1 3,4,7,1,6,8,1	7^{th}	8^{th}	5 6 7 6 5	5 8 5 4 6	1 1 2 0 0	1 2 2 2 1
Total arms = 40 arms					%Correct entries		% Incorrect entries	
					29/40 ×100 = 72.5%	28/40 ×100 = 70%	4/40 ×100 **= 10%**	8/40 × 100 **= 20%**

Table 3: The sequence of entries in radial arm maze by control rats during the second training phase (7 days)

Days	Arms visited [Comma missing]	Most visited arm	Correct entries	Re-entriesto baited Arms	Entries in Unbaited Arm
1 2 3 4 5 6 7	1, 8, 4, 2 2, 6, 8, 2 5, 6, 8, 2 7, 3, 4, 5, 6 8, 6, 2, 4 7, 2, 4, 6, 8, 2 7, 4, 6, 2, 8	8^{th} arm	3 3 3 2 4 4 4	0 1 0 0 0 1 0	1 0 1 2 0 1 1
Total arms need to be visited =28			%Correct entries= 23/28×100= 82.14%	**Working memory error 7.14%**	**Reference memory error 21.4%**

Deltamethrin is a harmful class II pyrethroid pesticide. The toxicity of deltamethrin depends upon the concentration of dose and the method of dose administration.

The dose-related toxic effect of deltamethrin concluded that a high dose of deltamethrin can cause the death of the organism. This data was collected by calculating the LD50 of deltamethrin on adult male rats. Even low doses of pyrethroid pesticide show some of the toxic effects related to learning and behavior of the deltamethrin-induced male rats. LD50 value determination is necessary as it gives a brief about the toxicity of a chemical product. In the present study LD50 value of deltamethrin was found 250mg/kg. A lower value of median lethal dose suggests that the toxicity of a chemical is high whereas the high median lethal dose suggests that the chemical is less toxic. So LD50value of highly

Table 4: Order of the arms visited by 100mg/kg and 200mg/kg dose induced rats during the second training phase (7 days)

Days	Arms visited		Most visited arm		Correct entries		Re-entries to baited Arms		Entries in Unbaited Arm	
	Dose 1 100mg/kg	Dose 2 200mg/kg	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
1 **2** **3** **4** **5** **6** **7**	2,4, 7, 8, 6,2 2, 3, 4, 6,4 4, 3, 8 5,4, 3, 8, 1,2 7, 6, 5, 8,4 4, 3, 8, 4 5, 4, 3, 8,2	2,4,8,3,2,1,7 2, 4, 6, 8, 2 3, 8, 6, 4, 3,6 5,3, 2, 8, 4, 5,4 8, 4, 8, 6, 7 8, 4, 6, 2, 8, 7 5, 3, 2, 8, 4, 5	8^{th}	8^{th}	4 3 2 3 3 2 3	3 4 3 3 3 4 3	1 1 0 0 0 1 0	1 1 1 1 1 1 0	1 1 1 3 2 1 2	3 0 2 3 1 1 3
Total arms need to be visited =28					%Correct entries		**Working memory error**		**Reference memory error**	
					20/28 x100 =71.4 %	23/28x 100= 82.14%	**10.7%**	**21.4 %**	**39.2 %**	**46.4 %**

Table 5: Reference and working memory errors of control rats during the third testing phase

Days	**Arms entering Sequence**	**Reference memory error**	**Working memory error**
Day1 Day 2 Day 3	8, 7, 2, 4, 6 1, 2, 4, 6, 8 8, 2, 6, 8	1 1 0	0 0 1
	Total entries Considered= 12	% error 2/12×100**= 16.65%**	% error 1/12×100**= 8.33%**

Table 6: Reference and working memory error of dose rats during the third testing phase

Days	**Arms entering sequence**		**Reference memory error**		**Working memory error**	
	Dose 1 100mg/kg	Dose 2 200mg/kg	Dose 1	Dose 2	Dose 1	Dose 2
Day 1 Day 2 Day 3	6,7,8,1,2,4,2 4, 5, 8, 2, 6 2, 1, 2, 5, 2	5,4,3,4,6,2 4,3,2,6,4,5 2,3,2,4,6,4,5	2 1 2	2 2 2	1 0 2	1 1 2
Entries considered= 12			% error		% error	
			5/12x100 = **41.6%**	6/12x100 = **50%**	3/12x100 = **25%**	4/12x100 = **33.3%**

toxic insecticides is quite less as compared to less toxic insecticides. The toxicity of a particular chemical on humans is inferred from its median lethal dose calculated on various animals. In the present study, deltamethrin exposed rats show a decrease in memory and learning ability as compared to unexposed rats. It was observed that this decrease in behavior ability was dose-dependent. The exposed group shows higher working and reference memory error as compared to the control group. Deltamethrin at a dose of 0.5 mg/kg/ body weight produced varying degrees of mild to moderate toxic symptoms and behavior changes in albino rats, Kumar (2018). The repeated exposure of the pyrethroid deltamethrin causes stress in the endoplasmic reticulum of the hippocampus and induces a deficit in learning in adult rats (Muhammad *et al.*, 2015). The memory error was noted more in the deltamethrin group when compared to Syringic acid (SA) treated rats (antioxidants) in which there was a rapid decrease in the memory errors (Ogut *et al.*, 2019). Deltamethrin exposure induces spontaneous uncontrolled behavior of rats, convulsion, contraction, and loss of coordination (Gray 1985). Deltamethrin-induced rats have low locomotor activity due to alteration in the oxidants balance system (Husain *et al.*, 1994). The present status of errors of working and reference memory was found supported with the findings of Ogut *et al.*, 2019.

In this study, it was observed that in the testing session, exposed rats showed a higher value of reference memory error which concluded that deltamethrin affects the long-term memory of rats.

CONCLUSION

Present study on Albino rats revealed that deltamethrin is toxic to mammals when given in high doses. It targets the central nervous system of animals and causing impairment in learning and behavior but a high dose of this also causes the death of the target. From time to time studies on such chemicals further gave a holistic approach to reduce their harmful and toxic impacts on the environment and other mammals including human beings.

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