

EFFECT OF MEDIAN LETHAL DOSE OF DELTAMETHRIN ON BEHAVIOR AND MEMORY OF FEMALE ALBINO RATS

Gulshan and R. Sudesh*

Department of Zoology, Maharshi Dayanand University, Rohtak, Haryana, India

* Author for Correspondence: sudesh_zoology@rediffmail.com

ABSTRACT

This study evaluated the effect of deltamethrin on the female albino rats. For this animals were divided into four groups. Group-I, II, and III were given 200mg/kg, 400 mg/kg and 800mg/kg dose of deltamethrin respectively. Group-IV was untreated and act as control. Zero Mortality in group-I, 50% mortality in group-II and 100% mortality in group-III was observed. All the symptoms of poison were seen in dose treated animals. In group where rats survived showed sign of mild to moderate variety of behavior and toxicological symptoms like decreased food intake, sudden jumping etc. The results of poison tests have significant changes mainly nausea, vomiting, rash, redness near the mouth, itching on body, trouble breathing, dilated pupils, shaking of body, dyspnea, salivation and convulsion. Results further indicate that deltamethrin markedly impaired learning function and significantly affect the behavior and memory in albino rats.

Keywords: Albino Rats, Deltamethrin, Behavior, Memory Ability, Mortality

INTRODUCTION

Pesticides are substances which may be organic or inorganic in nature and are used to control the different type of pests and weeds. Along with these benefits pesticides also have a bad effect on human and other animals (US Environmental Protection Agency, 1989). Since deltamethrin is one of the most suitable insecticide for agriculture. This is extremely used to eliminate or prevent variety of pests. It is also an allergen and may cause asthma in some people (Bouwman *et al.*, 2006). This is able to affect the cognitive power of organism (Viel, 2015). When deltamethrin exposed to the new born infant it drastically affect learning, memory, startle, LTP, norepinephrin (Pitzer *et al.*, 2019). High dose of deltamethrin during pregnancy cause defect in the cognitive ability of rats (Zhang *et al.*, 2018). This chemical is able to induce the Parkinson's disease which is a multifactorial disease (Souza *et al.*, 2018). Ability to find food and other things depends upon the learning & memory of animals, experimental models are used to study pathological effect of different toxic substance on physiology, learning and memory of animals. Maze is a best way for evaluate defect in learning and memory. There are different types of maze used by researchers to study the effect of substance on brain. When test animal enter in a wrong arm which had no reward in past, this is known as reference memory error (Hyde *et al.*, 2000). When a rat/ mice enter in an arm only one time this is known as working memory. When a rat visit enter arm twice this is known as working memory error (Tarragon *et al.*, 2012).

Present study was carried out to observe effect of LD 50 of deltamethrin on learning and memory ability in female albino rats.

MATERIALS AND METHODS

Animals and their groups

Female Albino rats *Rattus rattus norvigicus* were taken for present experiment. 16 female rats taken from animal house MDU Rohtak and each of them weighted from 125g-220g. Four groups-I,II,III,IV of females were made on the basis of their weight. They were kept in cages specific for laboratory rats under normal conditions and proper supply of food and tap water. Food includes 60% of laboratory food and 40 percent of vegetables like carrot, cabbage, cauliflower, potato, spinach, cucumber, etc. Proper access of

food and water were granted to the rats. Before starting the experiment the rats of that specific group were fasted overnight and next day in morning procedure was carry out. Group-IV was considered as control.

Chemicals

Deltamethrin is the chemical which was used in the present study. Its chemical formula is $C_{22}H_{19}Br_2NO_3$ and molecular mass is 505.21g/mol, density is 1.5g/cm³. Boiling and melting point of deltamethrin is 300 °C and 98 °C.

Methodology

The experiment was started on Oct. 6, 2020.

Dose administration: Three doses 200mg/kg, 400 mg/kg and 800mg/kg were selected for the present study. Deltamethrin dose were given orally to rats by following procedure of Hamdani *et al.*, 2017. All females were kept on fast for overnight. Next day in the morning females were carried out from the cage to on experimental table and enough time was given them for relaxation. Dose was administered with the help of cannula. A small exposure to the chloroform is necessary for the easy dose transfer. Rats were kept under observation for 48 hours. LD50 was calculated following Nagarjuna and Jacob (2009).

Group-I females were given dose of 200mg/kg.

200mg/kg dose was calculated with the help of numerical algorithm and this is refilled in injection. 100 ml bottle has 2.8 gram of deltamethrin so,

$$\begin{aligned} 2.8 \text{ gram deltamethrin} &= 100\text{ml} \quad (\text{change gram in milligram}) \\ 2800 \text{ milligram deltamethrin} &= 100 \text{ ml} \\ 1 \text{ milligram deltamethrin} &= 100\text{ml}/2800 \end{aligned}$$

$$\begin{aligned} 200 \text{ milligram deltamethrin} &= 100\text{ml}/2800 \times 200 \\ &= 1/28 \times 200 \text{ ml} \\ &= 7.11\text{ml/kg} \end{aligned}$$

Group- II Animals received 400 mg/kg dose

$$\begin{aligned} 1 \text{ milligram deltamethrin} &= 100\text{ml}/2800 \\ 400 \text{ milligram deltamethrin} &= 100/2800 \times 400 \text{ ml} \\ 400 \text{ milligram deltamethrin} &= 0.036 \times 400 \text{ ml} \\ &= 14.2/\text{kg}. \end{aligned}$$

For further confirmation group-III animals were given 800mg/kg dose

$$\begin{aligned} 1 \text{ milligram deltamethrin} &= 100\text{ml}/2800 \\ 800 \text{ milligram deltamethrin} &= 100/2800 \times 800 \text{ ml} \\ 800 \text{ milligram deltamethrin} &= 28.5 \text{ ml/kg} \end{aligned}$$

Radial Arm Maze was used to test learning and memory of albino rats

This is a maze made for rat; have 8 arms radiating from the center. This is greatly used for detecting the behavior, learning and memory of the animal. Different phases were used in this experiment.

Acclimatization phase: This is also known as familiarization phase as well. In this phase rats were familiarized with the environment of the maze so that they feel comfortable in maze during experiment. This phase was last for 2 days.

Training Period 1: during this period all arms were baited in the end, in bowl, so there were maximum 8-baits in one session. All arms were at 45° to each other. The rat was kept hungry before experiment. Rats were placed at the central platform and free access to all arms. The time to explore the arm was fixed 2 minute for each rat. The number and series by which rat explore the arms was noted. Their entry sequence is used to calculate working memory error. After each session the rat got free access to food. So by these readings we can calculate and evaluate that the percentage of correct entry and the percentage of incorrect entries in control and deltamethrin treated rats.



Figure 1: Dose administrations in rat

Training Period 2: This session was started with a gap of one week. All the setup of maze was same as previously. The arrangement of bait was changed here. This time bait was placed in 2, 4, 6, 8 arms. All baited arms are refilled between rats. The time given to each rat was same as in previous experiments i.e. 2 minute to each rat. There were two types of memory work in this training period, working memory and reference memory. These memories and their errors are very helpful during evaluating the learning and memory defect in rat.

Testing period: Along with the same setup of maze the arrangement of bait is changed this time as well. There was no bait is placed now in arms. The time given to each rat was same as in previous experiments its 2 minute to each rat. This lasted for only 3 days and the reading were taken in series. So, these values tell about long term memory of normal rat. The testing period was the last step in comparison in between memory persistent capacity of these rats.

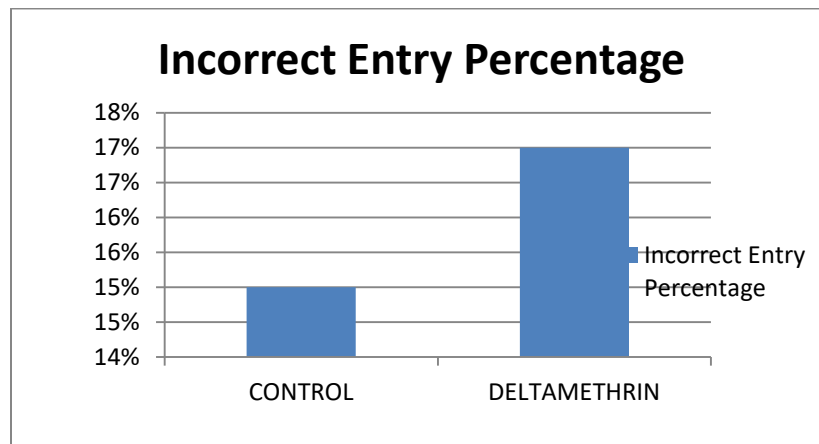


Figure 2: Incorrect entries attempted by control and treated group

RESULTS AND DISCUSSION

The experiment was started on first week of Oct. 2020 and lasted till Nov 2020. Doses given to rats were 200mg/kg, 400mg/kg, and 800mg/kg. Different symptoms of poison were seen in group –I like nausea, vomiting, rash, redness near the mouth, itching on body, trouble breathing, dilated pupils, shaking of

body, dyspnea, lacrimation, salivation, piloerection, and convulsion. Mortality at 200 mg/kg was zero so shifted to the double dose (400 mg/kg) to next group-II. All the symptoms of poison were seen in rats of this group as in group-I along with this 50% death of animals were observed. Hence the observed LD50 was 400 mg/kg dose of deltamethrin for female albino rat. To confirm further the value of LD50 800mg/kg dose was given to group-III rats. Here 100% mortality was recorded within 24hrs. This confirmed that 400mg/kg dose act as LD50 in this study. Learning and memory ability of female albino rats 8 radial arm maze was used in survived group-II animals. Calculations of Reference memory error and working memory errors were in first training phase, the incorrect arm chosen by control group is 15% and the treated rat showed 17%. So it was concluded that the attempt to choose arms of treated group was not as accurate as of control group.

In second training phase where alternate arms were baited, two types of errors were calculated one was working memory error and second was reference memory error. The working memory error was recorded 17.85% in control group and 21.42 % in deltamethrin treated group. The reference memory error was also recorded high 53.57 % in deltamethrin treated group than 28.57 % in control.

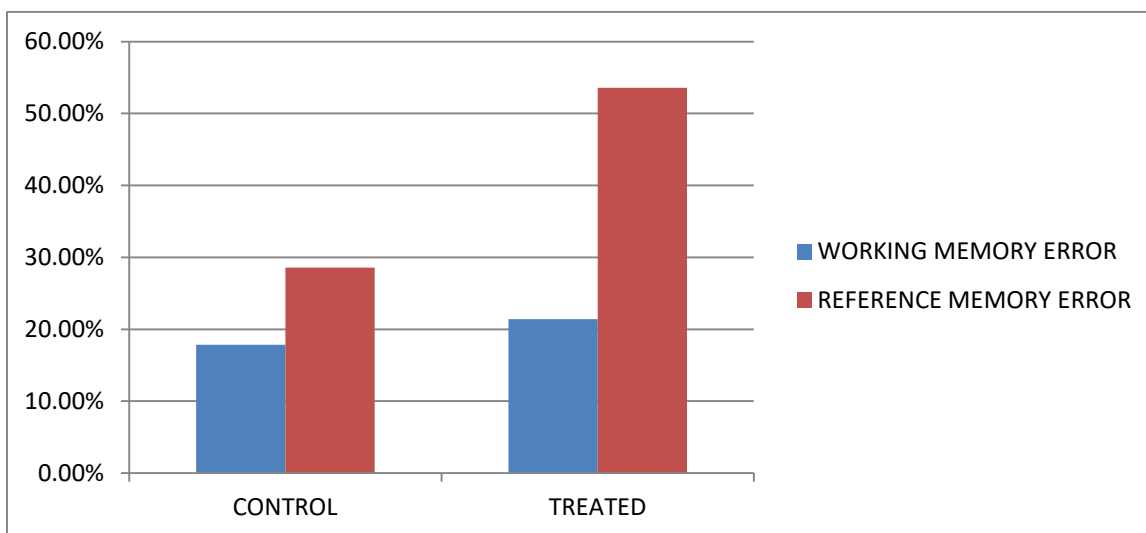


Figure 3: Working and reference memory error of control and treated group in second phase of training

In Third Training Phase which was the last phase where no arm was baited and the error occurs at a large scale. The working memory error was increased from 16 % in control to 41.66 % in deltamethrin treated rats and the reference memory error was raised from 16% to 58%.

The error calculation showed that the deltamethrin has negative effects on the hippocampus and striatum of the rat and affects learning and memory. Present pioneer study showed that deltamethrin effect learning and memory of female albino rats. This all showed that their effect on human brain is also great (Sharma *et al.*, 2012). Side effects of deltamethrin were also studied by Hussain *et al.*, 1994. There were different type of effects including reproductive, immunological and neurotoxicological effect (Issam *et al.*, 2009).

According to Wang, after treatment with deltamethrin of rats, there is increase in permeability and mitochondrial swelling in hippocampus and striatum. Ca^{+} ion presence in large amount is responsible for this phenomenon (Wang *et al.*, 2017). Acetylcholine is only neurotransmitter molecule present in hippocampus and striatum region of brain. This is not synthesized by the help of amino acids. The choline gets degenerated in Alzheimer disease (Songlin *et al.*, 2015). Decrease in acetylcholine leads to impaired memory, dramatical reduction in concentration and impairment in recognition. If the acetylcholine is present in large amount then Acetylcholinesterase come in action and reduce TNF α as well. Deltamethrin,

dramatically increases level of acetylcholine and then acetylcholine is degraded to choline. This affects the proper conduction of Ach which affect largely the learning memory. These effects are reflected in change in storage of memory, learning and memory of rats (Bhutada *et al.*, 2010).

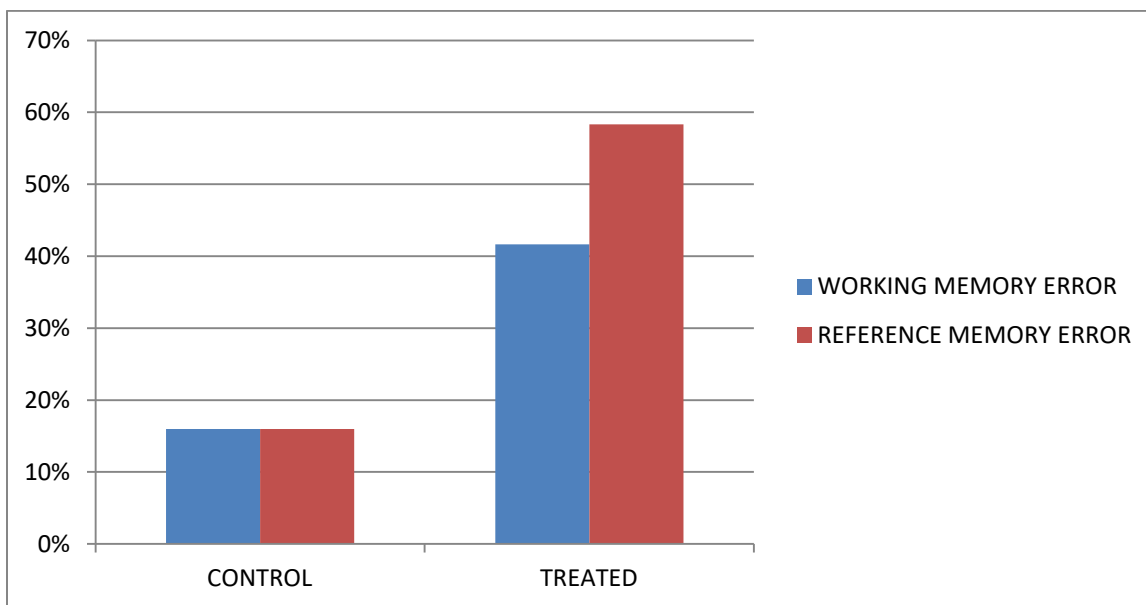


Figure 4: Working and reference memory error of control and treated group in third phase of training

Different types of psychological problems are also occurring by this, like anxiety, fear, nervousness, improper thinking, and short-term memory loss. Because of increase in ROS in mitochondria, there is a large effect on locomotors activity, exploration urge, memory, learning and memory was observed. Stress more affects the memory of animal and makes it memory deficits. In stressed situations, the performance of rat is quite low. Hippocampus is a region that deals with the depression and anxiety. Deltamethrin mostly affect that area in brain (Wang *et al.*, 2017). Deltamethrin prone to attack the area of brain cells in hippocampus and striatum. This is due to alteration in mitochondrial membrane, metabolism and neurotransmitter. This leads to deficits in learning and memory (Gasmi *et al.*, 2017).

So, we can say that the dose of a pesticide must be below that recommended by experts otherwise a very harmful effect of that substance can be seen on animal body.

CONCLUSIONS

In conclusion, these results demonstrated that intoxication with Cypermethrin induced significant impairment of memory functions. These results further support the hazards of pesticide use and show the importance of minimizing pesticide use or discovering new safe pesticides.

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