

# Evaluation of toxicological and behavioral symptoms on deltamethrin treated albino rats

## Abstract

**Background:** Pesticides are one of the most alarming toxic substances that are deliberately added to our environment. Deltamethrin, a Type-II synthetic pyrethroid used to control a variety of insects in agriculture and domestic environments. Its extensive commercial use has led to inescapable concern over the potential adverse effects on the human health. The present study investigated the deleterious effects on behavior in albino rats, including motor coordination abilities and overall activity.

**Methods:** Deltamethrin was administered intraperitoneal in adult wistar albino rats (250-270 gm), in the dose of 0.5 mg/kg/ body weight for a month. All rats were observed for behavioral and toxicological symptoms. Body weight and rectal temperature were measured. The open field behavior, social interaction test, landing foot splay, and forelimb and hindlimb grip test for neuromuscular function were done.

**Result:** Experimental rats showed sign of mild to moderate variety of behavior and toxicological symptoms characterized by decreased food intake and salivation, motor incoordination, looping, weaving etc. Deltamethrin treated rats showed statistical significant decrease in body weight. There was decreased locomotor and rearing frequency but increased duration of immobility. The social interaction was decreased and large foot angle was seen in treated rats. Delayed onset of reflexes was also observed.

**Conclusion:** The present study, thus, shows a great deal of variability in the toxicity and motor coordination in deltamethrin exposed rats.

**Keywords:** deltamethrin, pyrethroids, insecticides, behavior pattern, open field behavior, social interaction test, landing foot splay, motor coordination, toxicity

Volume 5 Issue 1 - 2018

Anil Kumar

Department of Anatomy and Neurobiology, Oman Medical College, Oman

**Correspondence:** Anil Kumar, Assistant Professor, Department of Anatomy and Neurobiology, Oman Medical College, Sohar, Al tareef, Postal code: 321, P. O. Box: 391, Sultanate of Oman, Tel +968-95393982, +968 22314761, Email anil@omc.edu.om, virgo.8977@gmail.com

**Received:** December 22, 2017 | **Published:** February 23, 2018

**Abbreviations:** gm, grams; °C, degree Celsius; cm, centimetres; i.p, intraperitoneal; mg, milligrams; Kg, kilograms;  $\mu$ , mean; S.D, standard deviation; min, minutes; GABA, gama amino butyric acid; AChE, acetylcholinesterase.

## Introduction

Deltamethrin, a type-II synthetic pyrethroid with potential insecticidal property is extensively used as used as an ectoparasiticide in crop protection and public health programme.<sup>1</sup> Extensive studies on animals revealed that deltamethrin are particularly toxic to neurons, acting directly on the axon by interference with sodium channel gating mechanism that underlines the generation and conduction of nerve impulses.<sup>2</sup>

Recently, toxicity has been reported in experiments on mice, rat, rabbit and guinea pig via dermal, oral and inhalational routes. These were in form of, excessive salivation, impaired limb function, ataxia, loss of righting reflex, lethality, paraesthesias, choreoathetosis, tremors, rarely paralysis and convulsions.<sup>3-6</sup> Deltamethrin administered during period of major organogenesis reduced the average weight of the live fetuses.<sup>7</sup> A few occupational hazards in human beings during manufacturing and handling are transient cutaneous and mucous membrane irritation, itching, dizziness, abnormal facial sensations and allergic reactions.<sup>8</sup>

The indiscriminate and injudicious use of pesticides poses threat to human health. As pesticide residue concentration enter food chain and exceeds the maximum limit, ill effects are produced.<sup>9</sup> Humans are increasingly exposed to pesticides at all stages of life due to changed lifestyle but there is dearth of studies on the behavior changes caused by deltamethrin exposure. Hence the objective of this study was to determine deleterious effects on behavior in albino rat, including motor/coordination abilities and overall activity due to deltamethrin exposure.

## Materials and methods

### Animals

The rats were procured from the animal house of University College of Medical Sciences (UCMS) and Guru Teg Bhadur (G.T.B) Hospital, Delhi. Male and female albino rats, weighing 250-270 gm, and about 90 days old were used. The animals were housed in propylene cages (32 X 40 X 18 cm) under controlled temperature (22-24°C), in a 12-hour light; 12-hour dark schedule, with free access to food and water. The experiments were carried out according to the national regulations and rules for animal welfare and guidelines of Institutional Animal Ethical Committee (IAEC), UCMS and G.T.B. Hospital, Delhi. The rats were acclimatized for one week before starting the treatment.

## Experimental design

All animals were provided with normal feed and water ad libitum during the study period. The animals were divided into two groups (I and II) as control and experimental respectively. Each group contains 10 animals. In the present study, deltamethrin was administered intraperitoneal (i.p.), in the dose of 0.5 mg/kg/ body weight for a month in adult wistar albino rats. Adequate dilutions were made with peanut oil to achieve test concentrations. The test concentrations were calculated from the percentage of active ingredients of commercial formulations. Control rats also received the similar amount of peanut oil (without any pesticide) through intraperitoneal injection. Body weights of all animals were recorded at an interval of 10 days.

## Toxicological symptoms

All the rats (control and experimental) were observed closely during the study period for appearance of behavioral changes and toxic symptoms. The nature, degree, and time of occurrence of toxic symptoms were carefully recorded.

## Behavioral changes

Physical parameters of general behavior were assessed daily. Traditional analyses of drug induced behavior start off with a ready list of arbitrarily defined behavioral acts (such as sniffing, head down, nose-poking, or rearing). The objective of those analyses is to discover the frequency, duration, and sequence of the acts during the course of the drugs action.<sup>10,11</sup>

## Open field studies

The open field apparatus was based on that described by Broadhurst.<sup>12</sup> The device is round arena (96 cm in diameter) surrounded by a 25 cm high wall, painted white, and subdivided into 25 parts by black stripes. During the experiments, 40 Watt white bulb placed 72 cm above the floor provided continuous illumination of the arena. Handheld counters and stopwatches were employed to score locomotion (number of floor units entered), rearing frequency (number of times an animal stood on its hind paws) and immobility (total time without spontaneous movements). Rats were individually placed in the center of the open-field arena and behavioral parameters were observed for 3 minutes. To minimize the possible influences of circadian changes on open field behavior, control and experimental animals were alternated. The device was cleaned with a 5% alcohol/water solution before placing the animals in it to eliminate the possible bias caused by odors left by the previous rats. Control and experimental rats were intermixed, and the tests were conducted between 8:00 AM and 12:00 PM. The animals were previously maintained for at least 90 minutes in access rooms under the same conditions as the test room.

## Social interaction test

The social interaction test was performed in the open field apparatus. Two days before the test, each rat was individually subjected to 10 min familiarization sessions in the test arena. On the next day, the rats were paired by weight (i.e., no more than 10 gms difference), and social interaction was observed for 10 min. Two hours after the administration of deltamethrin (experimental rat) or peanut oil (control rat), the paired rats were placed in the center of the

test arena to evaluate social interaction. The total time (in seconds) spent by the test pairs in active social interaction (i.e., sniffing, following, grooming, kicking, boxing, biting, and crawling under or over the partner) was recorded for 7.5 min. The apparatus was washed with a 5% ethanol solution prior to each behavioral test. Control and experimental rat pairs were intermixed, and the tests were conducted between 8:00 AM and 12:00 PM.

## Rectal temperature

Rectal temperature was measured with a thermometer (Rat Rectal Temperature Probe) connected to a metallic material sensor lubricated with Vaseline. The first measurement was taken prior to and 120 min after the respective treatments, at the deltamethrin peak of effects on open field behaviors. The thermometer was cleaned with a 5% ethanol solution prior to each temperature measurement. Control and experimental rats were intermixed, and the measurements were taken between 8:00 AM and 12:00 PM to prevent interference caused by circadian variations.

## Landing foot splay

Gait is measured with the footprint test which indicating peripheral nerve damage (neuropathy). The animal is released from a height, and the distance between the hind feet as the animal lands is recorded. Splay (spread of limbs out and apart) values were generally measured in millimeters (mm). Foot splay was apparently not related to body weight, since the values did not increase over time in spite of the increasing weights of the rats.<sup>13,14</sup>

## Forelimb and hindlimb grip test for neuromuscular function

The animal's ability to hang with forelimb, the length of time it does hang, and its activity while hanging was observed. Muscle strength was also measured using the grip strength meter (Columbus Instruments International Company, USA). It is employed in assessing neuromuscular function by sensing the peak amount of force an animal applies in grasping specially designed pull bar assemblies. Metering is performed with precision force gauges in such a manner as to retain the peak force applied on digital display.<sup>15</sup>

## Statistical studies

Quantitative observations in all the rats were done in both the groups and the data was tabulated and statistically analyzed by independent sample "t" test. But body weight in the experimental and control rats was tabulated and statistically analyzed by Tukey's test. Statistical analysis was performed using SPSS 11.5 software.  $P < 0.001$  was considered as a significant level.

## Results

All the control rats were normal, healthy and active during the treatment period. However, rats treated with dose of deltamethrin appeared weak and less active. Animals included in the study survived well throughout the period of thirty days. The rats treated with deltamethrin showed decrease body weight gain as compared to control rats. The mean body weight of the animals in control group was  $178.5 \pm 7.47$ gms before the onset of the experiment and

182.50±8.57gms on the last day (Table 1). Whereas the mean body weight of the animals in experimental group at the beginning of the experiment was 181.50±6.25gms while on the last day the weight was 166.00±8.09gms. The decrease in the body weight in the experimental

rats after administration of deltamethrin was found to be statistically significant ( $p < 0.001$ ) by Tukey's test when compared with the control animals (Table 1).

**Table 1** Comparison of body weight (gm) in control and experimental rats

	Group	Mean ( $\mu$ )	S.D	p-value (one way ANOVA)	Significance (Tukey's test at 5% level)
Before the experiment	Control	178.5	7.47	>0.001*	The groups were not statistically significant
	Experimental	181.5	6.25		
Last day of experiment	Control	182	8.57	<0.001*	Experimental group was statistically significantly different control groups
	Experimental	166	8.09		

\*p value  $\leq 0.001$  means data are statistically significant

$\mu$ , mean; S.D, standard deviation; gm, grams

In this study, Control rats were more active as compared to treated rats. It was observed that physical parameters were affected in deltamethrin treated rats as compared to control rats. Seemingly unrelated acts as the sniffing, keeping head down, jumping, circling, rotating, looping (somersaulting from the cage top) and weaving (pacing to and fro over the same point, with frequent rears when turning) during the course of experiment.

During the course of the experiment, after receiving the first dose of deltamethrin the rats became hyperactive which was likely represented by sneezing, shivering, groaning, and excessive salivation which lasted for half an hour. On subsequent days, the animals appeared sluggish; there was a loss of appetite associated with loose faeces and occasional vomiting.

The open field test showed that the experimental rats decreased locomotor frequency in the 120 minutes session compared with the Control group. Results suggested that locomotor activity decreased with regard to locomotor frequency. Also the test showed decreased rearing frequency in the 120 minutes session in the deltamethrin treated animal compared with the control group of rat. Interestingly, With regard to the duration of immobility, significant effects were found. The decreased locomotor and rearing frequencies were followed by an increased duration of immobility. There were no differences in the amount of time spent in the center between control and treated groups, indicating that both treatment groups were aware of the bounds of the chamber and could see the surrounding area.

The social interaction was decreased in experimental group compared with the control group (Student t test,  $p < 0.001$ ). Deltamethrin treatment did not alter the rectal temperature in any of the groups (32.0–36.2°C).

The Landing foot splay analysis test showed the experimental rat had a larger foot angle than the control rat. Experimental rats were more agitated and tended to move slowly down the walkway.

The animal's ability to hang with forelimb, the length of time it does hang, and its activity while hanging was observed. There were significant reductions in male forelimb and hindlimb hanging time (Seconds) with experimental rat compared to control group.

Delayed onset of certain reflexes was also observed in rats exposed to deltamethrin.

## Discussion

Deltamethrin at a dose of the dose of 0.5 mg/kg/ body weight produced varying degree of mild to moderate toxic symptoms and behavior changes in albino rats. It was observed that the rats became hyperactive immediately after receiving the first dose of deltamethrin. On subsequent days, the animals appeared sluggish; there was a loss of appetite associated with loose faeces and occasional vomiting. Deltamethrin is known to be neuropoisonous acting on the axons in the peripheral and central system by interacting sodium channels.<sup>16</sup> Chesterman et al.<sup>16</sup> observed vomiting, liquid feces, uncoordinated body movements, tremors and abnormal reflex responses in dogs after administration of oral deltamethrin dissolved in polyethylene glycol in gelatin capsules and attributed these symptoms to a dose related dysfunction of the autonomic nervous system. The signs of toxicity observed in rats after deltamethrin administration included salivation and choreoathetosis, a writhing type of toxicity and have been designated as the choreoathetosis / salivation syndrome toxicity.<sup>17-20</sup>

Clark & Brooks<sup>21</sup> have reported a toxicity of Type-II syndrome, particularly choreoathetotic writhing and associated it with unusually high concentration of plasma noradrenaline and adrenaline. Similar observations were seen by Parkin et al.<sup>22</sup>; Carbaral et al.<sup>23</sup>; Bateman<sup>4</sup>; Narahashi<sup>5</sup>; Soderlund<sup>6</sup> in rats after administration of deltamethrin. Wu et al.<sup>24</sup> administered deltamethrin in corn oil, intraperitoneally in male Sprague Dawley rats and suggested DNA fragmentation elicited by deltamethrin induced degeneration and apoptotic cellular death in rat brain, suggesting an important role played by apoptosis in neurotoxicity of deltamethrin. However, Sayim et al.<sup>25</sup> and Mokhtar et al.<sup>26</sup> reported that these symptoms have been attributed to a reduced activity of brain acetyl cholinesterase (AChE) and ischemia of the brain tissue resulting in such cerebral stroke signs and symptoms. Whereas Manna et al.<sup>27</sup> explained it to be a result of significantly low Gamma amino butyric acid (GABA) concentrations in the brain tissue. Chen et al.<sup>28</sup> has suggested mitochondria mediated apoptosis of the nerve cells in the rat brain to be the cause of these toxic effects.

There was a statistically significant decrease in the body weight in deltamethrin treated rats which may have resulted due to the hyperactivity, excessive salivation, loss of appetite, diarrhea and the occasional vomiting observed in these animals. This decrease in body weight in experimental rats is in accordance with the findings of Kavlock et al.<sup>29</sup> who reported a twenty percent (20%) reduction in the body weight in female rats. Madsen et al.<sup>30</sup> and Elbetieha et al.<sup>31</sup> also reported a similar reduction in body weight in male Fisher and Sprague–Dawley rats respectively after oral administration of deltamethrin. Similar findings were seen when deltamethrin was administered dissolved in propylene glycol intraperitoneally in Wistar albino rat by Patro et al.<sup>32</sup> However our findings are in contrast with the observations of Sayim et al.<sup>25</sup> and Varshneya et al.<sup>33</sup> who did not observe a significant change in the body weight of the animals after administration of a Type-II pyrethroid, cypermethrin. The difference in observation of the body weight might have resulted due to the different routes and doses of administration used in our studies.

Locomotion frequency measured as distance travels, immobility or resting time and rearing in the open field has been used as an index of both arousal and “emotionality”. The decrease or absence of movement within the apparatus normally indicates a reduction in arousal or an increase in the level of emotionality.<sup>34–37</sup> Present study showed reduced locomotors activity but not motor coordination. In this respect, the most important response to increased emotionality in the open field is freezing behavior, with a consequent decrease in locomotion frequency parallel to an increase in immobility. Thus, it is possible that the decreased locomotion frequency and the increased immobility observed were consequences of high levels of emotionality induced by the exposure to the pyrethroid.<sup>38</sup> Bhattacharya & Mitra<sup>39</sup> reported that the decreased open field activity after deltamethrin administration may have been a consequence of increased “anxiety” and not motor behavior in general. In this respect, decreased locomotors and rearing frequencies were observed with an increased duration of immobility. However, the lack of effects on motor coordination supports the hypothesis that deltamethrin does not interfere with motor function but interferes with emotional parameters.

In the social interaction test, deltamethrin reduced the time spent engaged in social interaction. These results suggest that deltamethrin induces anxiety like behavior. This conclusion is based on the results with anxiolytic and anxiogenic drugs used in the social interaction test, i.e., a reduction in the time engaged in social interaction indicates an anxiogenic effect of the drug, whereas an increase in this parameter indicates an anxiolytic effect.<sup>40–42</sup>

Thermoregulation in rats is an important factor for evaluating the toxic effects of pyrethroids. One of the effects of Type-I pyrethroid exposure is hyperthermia, possibly attributable to extensive muscular activity.<sup>43</sup> In contrast, Type-II pyrethroids, such as deltamethrin, can produce hypothermia.<sup>44</sup> There was no modification in rectal temperature, suggesting that the present doses do not induce severe toxicity.

The Landing foot splay analysis test was the test done to measure coordination. The experimental rat had a larger foot angle than the control rat. This could indicate that control rat is not as stressed during the test and walked normally. Experimental rats were more agitated and tended to move slowly down the walkway. As a result,

foot placement may be abnormal and an increased foot angle could indicate slower and unnatural movement.<sup>45</sup>

It was observed that control rats had taken more time for hanging as compared to experimental rats and their grip became sharp on alternate days but experimental rats took more days for sharp hanging grip. It was also observed that control rats had sharp negative geotaxis and palmer grasp as compared to treated rats.<sup>46</sup> Deltamethrin treated rats exhibited significant deficits in motor abilities, coordination, and overall activity, as measured by footprint analysis and open field indicate that exposure to deltamethrin can have long-lasting motor and cognitive consequences.

## Conclusion

Even though all these above-reported findings and the available bulk of literature seem to be sometimes and somewhere controversial, integrated approaches combining biochemical markers in combination with neurophysiological and behavioral assays could represent a valuable methodological approach by which human neurotoxicity assessment may become more focused and understanding the cellular mechanisms and the behavioral outcomes of deltamethrin will help reduce the morbidity and dysfunction associated with deltamethrin exposure by creating avenues of prevention and treatment.

## Acknowledgements

I would like to express my profound gratitude to Dr. Mahindra Nagar, Dr. Kamlesh Khatri and Dr. Veena Bharihoke who enlightened my period of work and invaluable guidance and continuous inspiration. Also like to thanks Mr. Laxman Singh for their assistance and technical support.

## Conflict of interest

None of the authors has conflict of interest to declare. No source of support in form of grants.

## References

1. McGregor DB. Pesticide residues in food: deltamethrin. International agency for research on cancer: Lyon, France; 2000.
2. Dorman DC, Beasley VR. Neurotoxicology of pyrethrin and pyrethroid insecticides. *Vet Hum Toxicol.* 1991;33(3):238–243.
3. Clark JM. Effects and mechanism of action of pyrethrin and pyrethroid insecticides. In: Chang LW, Dyer RS, editors. *Handbook of neurotoxicol.* New York: Marcel Dekker; 1995:511–546.
4. Bateman DN. Management of pyrethroid exposure. *J Toxicol Clin Toxicol.* 2000;38(2):107–109.
5. Narahashi T. Neuroreceptors and ion channels as the basis for drug action: past, present, and future. *J Pharmacol Exp Ther.* 2000;294(1):1–26.
6. Soderlund DM, Clark JM, Sheets LP, et al. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology.* 2002;17(1):30–59.
7. Bhaumik A, Gupta PK. Teratogenicity of decamethrin in rats. *Indian Vet J.* 1990;2:213–219.
8. O'Malley M. Clinical evaluation of pesticide exposure and poisonings. *Lancet.* 1997;349(9059):1161–1166.

9. McLachlan JA. Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals? *Endo Rev.* 2001;22(3):319–341.
10. Elunwood EH. Amphetamine psychosis: description of the individuals and processes. *J Nerv Ment Dis.* 1967;144(4):273–283.
11. Norton S. Amphetamine as a model for hyperactivity in rat. *Physiol Behav.* 1973;11(2):181–186.
12. Broadhurst PL. Experiments in psychogenetics. In: Eisenk HJ, editor. *Experiments in personality.* London: Routledge and Kegan paul; 1960:31–71.
13. Kulig BM, Lammers JHCM. Assessment of neurotoxicant induced effects on motor function. In: Tilson HA, Mitchell C, editors. *Neurotoxicology. Target organ series in toxicology.* New York: Raven Press; 1992:147–179.
14. Schallert T, Whishaw IQ. Two types of aphagia and two types of sensorimotor impairment after lateral hypothalamic lesions: Observations in normal weight, dieted, and fattened rats. *J Comp Physiol Psychol.* 1978;92(4):720–741.
15. Meyer OA, Tilson HA, Byrd WC, et al. A method for the routine assessment of fore-and hindlimb grip strength of rats and mice. *Neurobehav Toxicol.* 1979;1(3):233–236.
16. Chesterman H, Heywood R, Perkin CJ, et al. Oral toxicity study in Beagle dogs. 1977.
17. Barnes JM, Verschoyle RD. Toxicity of new pyrethroids insecticide. *Nature.* 1974;248(5450):711.
18. Ray DE. An EEG investigation of decamethrin-induced choreoathetosis in the rat. *Exp Brain Res.* 1980;38(2):221–227.
19. Ray DE, Cremer JE. The action of decamethrin (a synthetic pyrethroid) on the rat. *Pestic Biochem. Physiol.* 1979;10(3):333–340.
20. Verschoyle RD, Aldridge WN. Structure–activity relationship of some pyrethroids in rats. *Arch Toxicol.* 1980;45(4):325–329.
21. Clark JM, Brooks MW. Role of ion channels and intraterminal calcium homeostasis in the action of deltamethrin at presynaptic nerve terminals. *Biochem Pharmacol.* 1989;38(14):2233–2245.
22. Parkin PJ, Quesne Le PM. Effect of a synthetic pyrethroid deltamethrin on excitability changes following a nerve impulse. *J Neurol Neurosurg Psychiatry.* 1982;45(4):337–342.
23. Cabral JRP, Galendo D. Carcinogenicity studies with deltamethrin in mice and rats. *Cancer Lett.* 1986;49(2):147–152.
24. Wu A, Liu Y. Apoptotic cell death in rat brain following deltamethrin treatment. *Neurosci Lett.* 2000;279(2):85–88.
25. Sayim F, Yavasoglu NUK, Uyanikgil Y, et al. Neurotoxic effects of cypermethrin in Wistar rats: A haematological, biochemical and histopathological study. *Journal of Health Science.* 2005;51(3):300–307.
26. Mokhtar I, Yousef A, Talaat I, et al. Deltamethrin–induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. *Toxicology.* 2006;227(3):240–247.
27. Manna S, Bhattacharyya D, Mandal TK, et al. Neuropharmacological effects of deltamethrin in rats. *Vet Sci.* 2006;7(2):133–136.
28. Chen YL, Casida JE. Photodecomposition of pyrethrin I, phthalthrin, and dimethrin: Modification in the acid moiety. *J Agric Food Chem.* 1969;17(2):208–215.
29. Kavlock RJ, Daston GP, DeRosa C, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA– sponsored workshop. *Environ Health Perspect.* 1969;104 Suppl 4:715–740.
30. Madsen C, Claesson MH, Ropke C. Institute of toxicology, national food agency, soeberg, denmark. immunotoxicity of the pyrethroid insecticides deltametrin and alpha-cypermethrin. *Toxicology.* 1996;107(3):219–227.
31. Elbetieha A, Da'as SI, Khamas W. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. *Arch Environ Contam Toxicol.* 2001;41(4):522–528.
32. Patro N, Mishra SK, Chattopadhyay M. Neurotoxicological effects of deltamethrin on the postnatal development of cerebellum of rat. *J Biosci.* 1997;22(2):117–130.
33. Varshneya C, Singh T, Sharma LD. Immunotoxic responses of cypermethrin, a synthetic pyrethroid insecticide in rats. *Indian J Physiol Pharmacol.* 1992;36(2): 123–126.
34. Ivinskis A. A study of validity of open-field measures. *Aust J Psychol.* 1970;22(2):175–183.
35. Kelley A. Locomotor activity and exploration. *Methods in behavioral pharmacology*, chapter 5, 1993. p. 499–518.
36. Walsh RN, Cummins RA. The open field test: a critical review. *Psychol Bull.* 1976;83(3):481–504.
37. Whimbey AE, Denenberg VH. Two independent behavioral dimensions in open-field performance. *J Comp Physiol Psychol.* 1967;63(3):500–504.
38. Lazarini CA, Florio JC, LEMONICA IP, et al. Effect of prenatal exposure to deltamethrin on forced swimming behavior, motor activity, and striatal dopamine levels in male and female rats. *Neurotoxicol Teratol.* 2001;23(6):665–673.
39. Bhattacharya SK, Mitra SK. Anxiogenic activity of quinine-an experimental study in rodents. *Indian J Exp Biol.* 1992;30(1):33–37.
40. File SE. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide- like drugs. *J Neurosci Methods.* 1980;2(3):219–238.
41. File SE, Hyde JR. Can social interaction be used to measure anxiety? *Br J Pharmacol.* 1978;62(1):19–24.
42. Sanchez C, Arnt J, Costall B, et al. The selective sigma2-ligand Lu 28–179 has potent anxiolytic– like effects in rodents. *J Pharmacol Exp Ther.* 1997;283(3):1323–1332.
43. Hudson PM, Tilson HA, Chen PH, et al. Neurobehavioral effects of permethrin are associated with alterations in regional levels of biogenic amine metabolites and amino acid neurotransmitters. *Neurotoxicology.* 1986;7(1):143–153.
44. Kavlock R, Chernoff N, Baron R, et al. Toxicity studies with decamethrin, a synthetic pyrethroid insecticide. *J Environ Pathol Toxicol.* 1979;2(3):751–765.
45. Gandhi DN, Dhull DK. Postnatal behavioural effects on the progeny of rat after prenatal exposure to methylmercury. *American Journal of Experimental Biology.* 2014;1(1):31–51.
46. Joya, Sangha GK. Development and behavioural toxicity of deltamethrin on *Rattus norvegicus* following gestational exposure. *Journal of Applied and Natural Science.* 2016;8(1):40–45.