Original Article

Determination of Median Lethal Dose of Combination of Endosulfan and Cypermethrin in Wistar Rat

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ABSTRACT

The present study was designed to determine the lethal dose 50 (LD_{50}) of combination of cypermethrin, a pyrethroid, and endosulfan, an organochlorine compound in Wistar rats. LD_{50} is the amount (dose) of a chemical, calculated as per the concentration of chemicals that produces death in 50% of a population of test animals to which it is administered by any of a variety of methods. A single oral dose of combination of cypermethrin and endosulfan were dissolved in dimethyl sulfoxide (DMSO) in a ratio of 1:1 and administered orally at the concentration of 165 mg/kg body weight (b.w), 330 mg/kg b.w, 660 mg/kg b.w, and 1320 mg/kg b.w to experimental animals. LD_{50} was calculated according to the method described by Miller and Tainter (1994) and was observed as 691.83 mg/kg b.w for this combination. Single dose of test article at 165 mg/kg b.w did not reveal any toxic signs or behavioral alterations, hence considered as No observed Adverse Effect level (NOAEL).

Key words: Cypermethrin, endosulfan, LD_{50}, no observed adverse effect level, organochlorine, pyrethroid

INTRODUCTION

Pesticide, a substance or a mixture of substance used against pests in all developmental forms, have become omnipresent contaminants of environment and are also found in water, soil, air, and both human and animal tissues all over the world.[1,2] The pesticides play a very important role in modern agriculture, particularly in controlling harmful pests and insects with less effort and are cost effective. Pesticides have become an area of intense research due to their diverse properties and related effects. Though the demand for pesticide products and their contribution in aiding agricultural efficiency are clear, but the volume of production indicates that the potential for misapplication and accidental exposure is great. Besides, being beneficial for increased crop yield as well as in vector control programmers, it has resulted in the manifestation of several health-related problems.[3] In order to find new insecticides of low mammalian toxicity, less insect resistance, and low persistence, the number of groups of insecticides have been synthesized and synthetic pyrethroid is the result of one of such attempt.[4]

The toxicity of a pesticide is measured in several ways, but, generally, human toxicity is estimated based on the test results on rats and other animal models. A pesticide that is poisonous for rats is not necessarily equally poisonous to humans or other animals. Some pesticides turn out to be fatal after one large dose (acute toxicity), others can be dangerous after small dose or repeated doses (chronic toxicity). The commonly used term to describe acute toxicity is LD_{50}, where LD means lethal dose deadly amount, and the subscript 50 means that the dose is acutely lethal to 50% of the animals to whom the chemical was administered under controlled laboratory conditions. In other words, LD_{50} is the statistically derived

Access this article online

Quick Response Code:

Website: www.toxicologyinternational.com

DOI: 10.4103/0971-6580.111531

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single dose of a substance that produces death in 50% of a population of test animals to which it is administered by any of the methods like oral, dermal, inhalation, or intravenous. Determination of this test examines the relationship between dose and the most extreme response–death. The more potent or toxic the chemical, lower the LD_{50} and smaller the dose needed to cause death. Therefore, a pesticide with an oral LD_{50} of 500 mg/kg would be much less toxic than a pesticide with an LD_{50} of 5 mg/kg. Normally, LD_{50} is expressed as milligrams of substance per kilogram of animal body weight (mg/kgbw). It provides information on health hazards likely to arise from short-term exposure. This data serve as a basis for labeling and classification and also helpful in establishing a dosage regimen in sub-chronic and chronic studies.

The LD_{50} is determined by any accepted methods, e.g. Miller and Tainter,[5] Bliss,[6] Litchfield and Wilcoxon,[7] Finney,[8] Weil,[9] and Thompson.[10]

Previously, single insecticides/pesticides were used in agriculture to control insects, but repeated use of these insecticides has made insects/pests resistant to them. Therefore, to overcome such problems, cocktails of pesticide are prepared by farmers on the suggestion of pesticide sellers in order to get better quality and faster results. These cocktails not only kill the harmful pests but also are very hazardous to both farmers who come in direct contact and common man, who consumes them (through fruits and vegetables).[11-13] The combination of pesticides are gaining popularity in pest control programs as they exhibit a broad spectrum of activity coupled with better efficacy and economy.[14]

Synthetic pyrethroid and organochlorine pesticides are nowadays widely used in the agriculture industry to control pests on crops and ectoparasites on livestocks.[15]

Cypermethrin is a neurotoxic synthetic pyrethroid, primarily used as an insecticide and endosulfan is a neurotoxic organo chlorine compound of the cyclodine group, primarily used as an insecticide and secondarily as an acaricide. The oral LD_{50} of cypermethrin is 250 mg/kg (in corn oil).[16,17] The LD_{50} of endosulfan varied widely depending on the route of administration, species, vehicle, and sex of the animal.[18] The oral LD_{50} of endosulfan for rats is 80 mg/kg b.w.[19]

Several independent studies on cypermethrin and endosulfan toxicity have been carried out in different parts of the world. However, no attempts have been made to understand the combined toxic effect of these two pesticides, which is presently being used very commonly in India. Thus, keeping this in mind, the present study was designed to determine the oral median lethal dose of the combination of pesticide endosulfan, an organochlorine compound, and cypermethrin, a synthetic pyrethroid on Wistar rats, using dimethyl sulfoxide (DMSO) as vehicle.

MATERIALS AND METHODS

Test chemicals

Cypermethrin (92.21% pure, liquid) and endosulfan (95.80% pure, solid) were obtained from Hindustan Insecticides Limited (A government of India Enterprises).

Animals and experimental design

A total of 25 healthy adult male Wistar rats (weighing about 200 ± 20 gm) were obtained from Central Animal Facility (All India Institute of Medical Sciences, New Delhi). All animals were allowed to acclimatize to the experimental conditions for a period of 5 days. All the rats were housed in polyacrylic cages, not more than 3 animals per cage, and maintained under standard laboratory conditions (natural light/dark cycle, room temperature 22 ± 3°C). Animals were given standard dry rat pellet diet, and tap water was provided *ad libitum*. The institutional animal ethics committee approved the experimental protocol.

Dose preparation and administration

Rats were fasted for 18 h prior to dosing. The compound was administered once orally using 22-gauze oral feeding needle to the rats. The volume of the dose depends on the size of the animals. In rodents, it should not exceed 1 ml/100 g b.w.[20,21] In this study, the dose was mixed, i.e., combination of pesticide (endosulfan and cypermethrin, ratio 1:1) in 0.5 ml of DMSO.

Estimation of the dose range and percentage of mortalities

An approximate LD_{50} can be determined by a so-called "up and down" or the "staircase method" using two animals and increasing the doses of the combination of endosulfan and cypermethrin (ratio 1:1). Five doses were given orally to 5 groups of rats (5 rats in each group) for the determination of LD_{50} of the combination starting from 0% mortality to 100% mortality[22] [Table 1]. The animals were observed for 2 h and then at 4th, 6th, and 24th h for any toxic signs and symptoms. After 24th h, the numbers of deceased rats in each group were counted and the percentage of mortality

Table 1: Total dose of combination of endosulfan and cypermethrin (ratio 1:1) given orally to rats

Groups	No. of animals	Cypermethrin	Endosulfan	Total Dose of Combination in mg
(control)	5	--------	-------	----------
1	5	½ x LD 50 (125 mg)	½ x LD 50 (40 mg)	165 mg
2	5	LD 50 (250 mg)	LD 50 (80 mg)	330 mg
3	5	2x LD 50 (500 mg)	2x LD 50 (160 mg)	660 mg
4	5	4x LD50 (1000 mg)	4x LD50 (320 mg)	1320 mg

was calculated using the graphical method of Miller and Tainter.[5]

RESULTS

Signs recorded during experiment

Initially, the combination of endosulfan and cypermethrin did not produce any significant effect on central nervous system (CNS) at 165 mg/kg. However, when the doses of 330 mg/kg, 660 mg/kg, and 1320 mg/kg were administered, signs of CNS stimulation were observed for 16 to 24 h. The animals exhibited chewing, licking, salivation, tremors, arching and rolling, clonic convulsions, lacrimation, occasional pawing, or burrowing, and coarse whole-body tremors associated with movement of legs. Finally, choreoathetosis developed exhibiting slow writhing and twisting movement of the neck and tail of animals. The animals showed labored breathing, gasping, and death.

Conversion of percentage mortalities to probits and calculation of LD_{50}

The percentage of animals that died at each dose was then transformed to probit [Table 2] using Finney's method[23] [Table 3]. The percentage dead for 0 and 100 were corrected before the determination of probits. For 0% dead: 100 (0.25/n) and for 100% dead: 100 (n − 0.25/n), where n = 5 rats.[24]

The probit values thus obtained were plotted against log-dose and then the dose corresponding to probit 5, i.e., 50%, was found [Figure 1].

Table 2: Graphical method for combination of endosulfan and cypermethrin (ratio 1:1)

Groups	Dose (mg/kg)	Log dose	% Dead	Corrected %	Probits
1	0	0	0	0	0
2	165	2.21	0	5	3.36
3	330	2.51	1	20	4.16
4	660	2.82	2	40	4.75
5	1320	3.12	4	80	5.84

Table 3: Transformation of percentage mortalities to probit

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

In the present study, combination of endosulfan and cypermethrin (ratio 1:1), log LD_{50} is 2.84 and LD_{50} is 691.83 mg/kg. The SE of the LD_{50} was calculated using the following formula.[24]

$$\text{Approx. SE of } LD_{50} = \frac{(\text{Log } LD_{84} - \text{Log } LD_{16})}{\sqrt{2N}} \quad \ldots(a)$$

The probits of 84 and 16 from Table 1 are 5.99 and 4.01 (approximately 6 and 4), respectively. The log-LD values for the probits 6 and 4 are obtained from the line on the graph in Figure 1, which, in the present case, are 3.2 and 2.46, and their antilog are 1584.89 and 288.40, respectively. Using these values in formula (a), the SE of LD_{50} is 410.28.

Therefore, LD_{50} of combination of cypermethrin and endosulfan with DMSO, when given orally was observed as 691.83 ± 410.28, with 95% confidence interval of 281.55-1102.11.

DISCUSSION

Pesticides play an important role in modern agriculture by providing dependable, persistent, and relatively complete control against harmful pests with less labor and low cost. They have, no doubt, increased crop yields by killing different types of pests, which are known to cause substantial or total crop damage. At the same time, these chemicals are considered as potent pollutants of the environment with undesirable effects on non-target organisms.[25] Rampant use of pesticides in agricultural products continue to risk the life of the common man. Thus, it is very important to know the LD_{50} of the pesticide before using it in the fields. Data collected for the MAFF Pesticide Usage Survey of arable crops in England and Wales[26] in 1990 showed that over 25,000 different combinations of two active

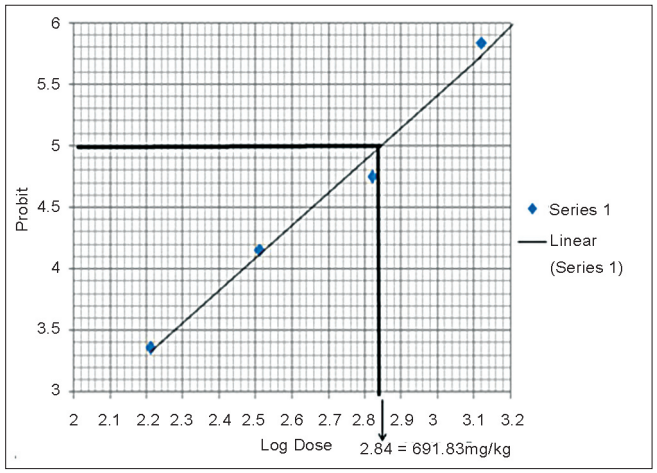

Figure 1: Plot of log-doses versus probits from Table 2 for calculation of oral LD_{50} of combination of endosulfan and cypermethrin (ratio 1:1)

ingredients were sprayed as tank mixes or co-formulations on the 761 farms surveyed, though only a small proportion were used on significant numbers of farms. A more detailed analysis of spray applications was carried out using data from the 1992 survey of arable crops in England, Scotland, and Wales.[27] This reported tank mixes and co-formulations were applied to 10.9 million ha, implying a mean of over two mixtures per crop. Fifty-nine different combinations of two or more active ingredients were applied to 30,000 ha or more.

Combination of two or more pesticides may have additive, synergistic, or antagonistic effect. Therefore, the present study was conducted to find out the LD_{50} of the combination of endosulfan and cypermethrin (ratio 1:1). This combination did not show any gross visible changes, and no toxic signs at 165 mg/kg b.w and considered to be the No Observed Adverse Effect Level (NOAEL). However, animals administered with a combination of endosulfan and cypermethrin at the doses of 330 mg/kg b.w, 660 mg/kg b.w, and 1320 mg/kg b.w dose showed a sequence of the signs of toxicity, viz., acute cholinergic symptoms, chewing, licking, salivation, writhing, tremors, arching and rolling, clonic convulsions, lacrimation, occasional pawing, or burrowing, coarse whole-body tremors associated with movement of legs, hyperactivity to sound/touch, abnormal gait pattern, incoordinaton, imbalance, difficulty in breathing, and convulsions immediately after dosing and these symptoms were persisted for 6 h. The pattern of the signs after the administration of combination of endosulfan and cypermethrin is strongly suggestive of CNS toxicity. Similar signs were observed by Nagarjuna *et al*.,[17] and Manna *et al*.,[28] while using only cypermethrin and chlorinated hydrocarbons insecticides.[29]

The geographic mean of two doses i.e.: The lowest dose that killed one animal and the highest dose that did not kill any animal determines LD_{50} of a particular substance. The graphical representation of probit versus log dose showed a typical straight line, which was in agreement with the principle of probit analysis [Figure 1]. According to Miller and Tainter probit analysis method, at 24 h, the acute oral LD_{50} value of combination of endosulfan and cypermethrin (ratio 1:1) in DMSO was calculated as 691.83 mg/kg b.w (with 95% confidence interval) in the present study, which is 8.64-folds higher (in terms of LD_{50}) than the LD_{50} values of endosulfan alone and 2.76-folds higher (in terms of LD_{50}) than the LD_{50} of cypermethrin alone determined using other vehicles like corn oil.[16-17,19] Actual decrease in LD_{50} or increases in mortality are used to assess the scale of the increase in toxicity following combined exposure and vice versa. Various studies suggests that combination of pesticides always have an additive effect and when two or more pesticides are used together, their toxicity is always more than the toxicity of single pesticide;[30] but, in the present study, increase in LD_{50} value of combination is seen, indicating that when endosulfan and cypermethrin are used in combination with DMSO, the toxicity of the combination is reduced in Wistar rats. Manna *et al*.,[28] reported that DMSO also reduces toxicity.

However, published experimental work on cypermethrin and endosulfan toxicity in rat is limited and there is no experimental work reported on the combination of endosulfan and cypermethrin toxicity with DMSO as a vehicle. However, it is concluded that, the overall result of the present study clearly demonstrates that oral LD_{50} of the combination of endosulfan and cypermethrin (ratio 1:1) with vehicle DMSO is as 691.83 mg/kg b.w (with 95% confidence interval) and NOAEL of the combination of the above mentioned pesticide is 165 mg/kg b.w in Wistar rats.

ACKNOWLEDGMENT

University Grant Commission (UGC) is hereby acknowledged for awarding financial support to conduct this study (SRF-Ms. Jaya Raj).

REFERENCES

1. Anwar WA. Biomarkers of human exposure to pesticide. Environ Health Perspect 1997;105:801-6.
2. Ahmad L, Khan A, Khan MZ, Hussain I. Cypermethrin induced anemia in male rabbits. Pakistan Vet J 2009;29:191.
3. Environmental Information System-National Institute of Occupational Heal. News Letter 2007;2.
4. Grewal KK, Sandhu GS, Kaur RR, Brar RS, Sandhu HS. Toxic impact of cypermethrin on behavior and histology of certain tissues of albino rats. Toxicol Int 2010;17:94-8.
5. Miller LC, Tainter ML. Estimation of LD50 and its error by means of log-probit graph paper. Proc Soc Exp Biol Med 1944;57:261.
6. Bliss CI. The method of probits. Science 1934;79:38-9.
7. Litchfield JT Jr, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949;96:99-113.
8. Finney DJ. Probit Analysis. 3rd ed. Cambridge: Cambridge University Press; 1971.
9. Weil CS. Tables for convenient calculation of median effective dose (LD 50 or ED 50) and instructions in their use. Biometrics 1952;8:249.
10. Thompson WR. Use of moving averages and interpolation to estimate median effective 4 dose. Bacteriol Rev 1947;11:115.
11. Report of National Institute of Environmental Health Sciences (2009). Available from: http://www.niehs.nih.gov/health/topics/agents//pesticide/index.cfm. [Last accessed on 24 oct 2011]
12. United States Environmental Protection Agency. Recognition and Management of Pesticide Poisoning (2009). Available from: http://www.Epa.gov/opp00001/safety/healthcare/handbook/handbook.htm. [Last accessed on 24 oct 2011]
13. World Health Organization. Issue Brief Series: Pesticides. (2009). Available from: http//www.who.in/heca/infomaterials/pesticides.pdf. [Last accessed on 24 oct 2011]
14. Sekar BH, Uma Devi, Ch Susma, Venkateswara RJ, Vijaya

Kumar TM, Thirumurugan G. Effect of polytrin C (combination pesticide) on the ach ease inhibition in plasma and brain of wistar rats. Am J Biochem Mol Biol 2011;1:101.

15. Nolan MP, Roberson EL. Veterinary Pharmacology and Therapeutics. New Delhi: Oxford and IBH Publication, Co.; 1979. p. 1107.
16. Ray DE. Pesticides derived from plants and organisms. In: Hayes WJ, Laws ER Jr, editors. Handbook of Pesticides Toxicology. New York: Academic Press; 1991. p. 2-3.
17. Nagarjuna A, Doss JP. Acute oral toxicity and histopathological studies of cypermethrin in rats. Indian J Anim Res 2009;43:235.
18. Uboh FE, Asuquo EN, Eteng MU, Akpanyung EO. Endosulfan-induces renal toxicity independent of the route of exposure in rats. Am J Biochem Mol Biol 2011;1:359.
19. International Programme on Chemical Safety. The WHO Recommended Classification of Pesticides by Hazards and Guidelines to Classification 1998-1999. (WHO/PCS/98.21 Rev. 1 World Health Organisation, Geneva) 1998b.
20. Ghosh MN. Toxicity studies. Fundamentals of Experimental Pharmacology. Calcutta: Scientific Book Agency; 1984. p. 153.
21. Turner R. Quantal response. The determination of ED 50. Screening Methods in Pharmacology New York: Academic Press; 1965. p. 61.
22. Randhawa MA. Calculation of LD50 values from the method of Miller and Tainter, 1944. J Ayub Med Coll Abbottabad 2009;21:184-5.
23. Finney DJ. Probit Analysis. 2nd ed. Cambridge: Cambridge University Press; 1952.
24. Ghosh MN. Statistical Analysis, Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta: Scientific Book Agency; 1984. p. 187.
25. Shafiq-ur-Rehman. Endosulfan toxicity and its reduction by selenium: A behavioral, hematological and peroxidative stress evaluation. Internet J Toxicol 2006;3.
26. Davis RP, Garthwaite DG, Thomas MR. Pesticide Usage Survey Report 85, Arable Farm Crops in England and Wales 1990. London: MAFE; 1991.
27. Davis RR, Thomas MR, Garthwaite DG, Bowen HM. Pesticide Usage Survey Report 108, Arable Farm Crops in Great Britain 1992. London: MAFE; 1993.
28. Manna S, Bhattacharya D, Basak DK, Mandal TK. Single oral dose toxicity study of α-cypermethrin in rats. J Pharmacol 2004;36:25.
29. Smith AG. Chlorinated hydrocarbons insecticide. In: Hayes WJ Jr, Laws ER Jr, editors. Handbook of Pesticide Toxicology. New York: Academic Press Inc.; 1991. p. 63.
30. Thompson HM. Interactions between pesticides: A review of reported effects and their implications for wildlife risk assessment. Ecotoxicology 1996;5:59.

How to cite this article: Raj J, Chandra M, Dogra TD, Pahuja M, Raina A. Determination of median lethal dose of combination of endosulfan and cypermethrin in wistar rat. Toxicol Int 2013;20:1-5.

Source of Support: UGC (University Grant Commission). **Conflict of Interest:** None declared.