JJBS

Jordan Journal of Biological Sciences

Volume 10, Number 3,September 2017
ISSN 1995-6673
Pages 193 - 197

Estimation of Median Lethal Dose of Commercial Formulations of Some Type II Pyrethroids

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Received February 14, 2017 *Revised May 3, 2017* *Accepted June 4, 2017*

Abstract

The present study aims to investigate the median lethal dose (LD_{50}) of three commercially formulated type II pyrethroids, namely Fenvalerate (Hafen), Deltamethrin (Decis) and Cypermethrin (Cybil) against female albino rats. Acclimatised experimental animals were divided into three sets and respective doses of pyrethroids under investigation were administrated orally to them. Survival and mortality number was observed following 96 hours of intoxication. LD_{50} was calculated statistically by log-dose/probit regression line method from the numerical data obtained and came out to be 1532.5, 656.2 and 618.2 mg/kg b.wt. for Hafen, Decis and Cybil respectively. Structural changes in type II pyrethroid *vide supra* have been considered responsible for the differences in LD_{50}.

Key words: LD_{50}, Hafen, Decis, Cybil, Female albino rat.

1. Introduction

Synthetic pyrethroids have emerged as a group of multipurpose pesticides, considered comparatively safer than other available counterparts, such as Organochlorines and Organophosphates. These pesticides presently account for about 30% of the total insecticide market worldwide by virtue of comparatively higher LD_{50}, easy biodegrability and less non-target toxicity. These pesticides are synthetically modified analogues of basic pyrethrins contained in the flowers of genus *Chrysanthemums.* On routine basis, many new pyrethroids are synthesised and added up in the market to meet enhanced global food demands, vector borne diseases and genetically modified pesticide resistant pest species. On the same line, Fenvalerate, Deltamethrin and Cypermethrin in the formulated state have been added up in the market and broadly used throughout the world (Saxena and Tomar, 2003; Saxena and Doneriya, 2004; Bhushan *et al.*, 2010; Pande *et al*., 2014; Moustafa and Hussein. 2016.

A consequence of their indiscriminate and excessive use can also be in the form of toxicity to various non-target species (Doneriya and Saxena, 2004; Bhushan *et al.*, 2013; Bhushan *et al.*, 2013; Rajawat *et al*., 2015). Considering the same concept, comparative median lethal dose (LD_{50}) evaluation has been undertaken in the present study, so as to assess the effect of structural modifications on non-target toxicity.

2. Materials and Methods

2.1. Experimental Animal

Ninety female albino rats, *Rattus norvegicus*, eight weeks old, selected from an inbred colony weighing 110 ± 20 gm were used in the present study. These experimental animals were well acclimatized for two weeks to laboratory conditions and provided standard rat pellet feed and water *ad libitum.* The rats were then divided into three main sets each of which was further sub-divided in five sub-sets comprised of seven rats corresponding to different doses of Hafen (Fenvalerate), Decis (Deltamethrin) and Cybil (Cypermethrin), respectively. The experimentation was approved by the Ethical Committee of Dr. B. R. Ambedkar University, Agra, India.

2.2. Experimental Compounds

Commercial formulations of Fenvalerate, Deltamethrin and Cypermethrin were obtained from Bayer India Ltd., Mumbai. Concentrations of experimental pyrethroids in these commercial formulations was 92, 92.6 and 91%, respectively. The LD_{50} was calculated (Finney, 1971).

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2.3. Dose Administration and Determination of LD50

2.3.1. Hafen (Fenvalerate)

Experimental albino rats were divided into five groups, each consisting of seven individuals. Standard solution of experimental test compound Hafen (Fenvalerate) was prepared by dissolving in distilled water. Different doses 1200, 1600, 2000, 2400 and 2800 mg/kg b.wt. were administrated orally. The mortality and survival numbers of the rats were recorded for each dose after 96 hours. The data were analyzed statistically by log dose/probit regression line method (Finey, 1971). Regression line was drawn on the basis of two variables, log dose and empirical probit on a simple graph paper and used to determine the expected probit necessary for LD_{50} determination (Table 1).

2.3.2. Decis (Deltamethrin)

Experimental albino rats were divided into five groups, each consisting of seven individuals. Standard solution of experimental test compound Decis was prepared by dissolving in distilled water. Different doses 640, 800, 960, 1200 and 1280 mg/kg b.wt. were administrated orally. The mortality and survival numbers of rats were recorded for each dose after 96 hours. The data were analyzed statistically by log dose / probit regression line method (Finey, 1971). Regression line was drawn on the basis of two variables, log dose and empirical probit on a simple graph paper and used to determine the expected probit necessary for LD_{50} determination (Table 1).

2.3.3. Cybil (Cypermethrin)

Experimental albino rats were divided into five groups, each consisting of seven individuals. Standard solution of experimental test compound Cybil was prepared by dissolving in distilled water. Different doses 315, 650, 1260 and 2520 and 5040 mg/kg b.wt. were administrated orally. The mortality and survival numbers of rats were recorded for each dose after 96 hours. The data were analyzed statistically by log dose/probit regression line method (Finey, 1971). Regression line was drawn on the basis of two variables, log dose and empirical probit on a simple graph paper and used to determine the expected probit necessary for LD50 determination (Table 1).

2.4. Statistical Analysis

Results obtained were statistically analysed using log-dose/probit regression analysis (Finey 1971).

3. Results

In the different experimental sets, experimental rats were treated with different doses of different concentrations of Hafen, Decis and Cybil for estimation of LD_{50}. Survival number and percentage for each dose have been noted after 96 hours which decreased with increasing dose of Hafen, Decis and Cybil (Table 1). LD_{50} has been calculated (Tables-2-4, Figure 1-3) by log dose/probit regression line method (Finney, 1971). The test doses were then converted to their logarithm. The empirical probit values, equivalent to percentage mortality were then obtained from the table (Finney, 1971) and plotted against log dose on graph paper. The provisional lines, drawn fitting to the expected values, were read for the values of log dose (X).

Working probits

$$Y = Y0 + kp$$

where, p = percentage mortality

Y0 and k are two factors.

The weighting coefficients for each point were also obtained from the table. The weight has been calculated by multiplying each coefficient by number of rats used (Table 2-3). The value of 'b' is obtained by the formula:

$$b = \left(\sum wxy - \overline{X}\sum wy\right) / \left(\sum wx^2 - \overline{X}\sum wx\right)$$

The values of Y can be obtained by regression equation (Figures1-3)

$$Y = \overline{Y} + b(X - \overline{X})$$

From the equation values of X, equivalent to Y and $\overline{Y}$ were estimated, and the calculated values of LD_{50} thus been obtained (Tables-2-4, Figures 1-3). Variance V was calculated followed by 95% confidence fiducial limits as below:

$$\text{Variance (V)} = \frac{1}{b^2}\left(\frac{1}{\sum w} + \frac{(X - \overline{X})^2}{\Sigma wx^2 - \frac{(\sum wx)^2}{\sum w}}\right)$$

By the following formula, 95% confidence fiducial limits have been obtained (Table 5):

m1 = m + 196 V

m2 = m-1.696 V

Applying all these calculation, LD_{50} for Hafen, Decis and Cybil came out to be 1532.5, 656.2 and 618.2 mg/Kg b.wt., respectively.

Table 1: Mortality index and percentage of albino rat on treatment with experimental compounds

Sr. No.	Dose (mg/Kg b.wt.)			No. Of individuals treated	Exposure time (in hours)	Mortality Number	Mortality percentage
	Hafen	Decis	Cybil				
1	1200	640	315	6	96	2	33.33
2	1600	800	630	6	96	3	50.00
3	2000	960	1260	6	96	4	66.66
4	2400	1120	2520	6	96	5	83.33
5	2800	1280	5040	6	96	6	100.00

Table- 2

Determination of LD_{50} by log-dose/probit Regression analysis after oral intoxication of different doses of Hafen (Fenvalertae) to *Rattus norvegicus*

S.No.	Dose in mg/kg b.wt.	No. of rats	Mortality Number	Mortality (%)	Log dose 'X'	Empirical Probit	Expected Probit 'Y'	Working Probit 'y'	Weighing coefficient 'N'	Weight W=n×N	WX	WY	WX^2	WY^2	WXY
1.	1200	6	2	33.33	3.079	4.56	4.56	4.561	0.781	4.686	14.428	21.373	44.424	97.482	65.807
2.	1600	6	3	50.00	3.204	5.00	5.06	5.00	0.627	3.762	12.053	18.810	38.619	94.050	60.267
3.	2000	6	4	66.66	3.301	5.44	5.44	5.440	0.601	3.606	11.903	19.617	39.293	106.715	64.755
4.	2400	6	5	83.33	3.380	5.95	5.78	5.944	0.503	3.018	10.201	17.939	34.479	106.629	60.634
5.	2800	6	6	100.00	3.447	-	6.01	6.656	0.439	2.634	9.079	17.532	31.306	116.692	60.432
										ΣW= 17.706	ΣWX= 57.664	ΣWY= 95.271	ΣWY^2= 188.115	ΣWX^2= 521.568	ΣWy^2= 311.895

Table- 3

Determination of LD_{50} by log-dose/probit Regression analysis after oral intoxication of different doses of Decis (Deltamethrin) to *Rattus norvegicus*

S.No.	Dose in mg/kg b.wt.	No. of rats	Mortality Number	Mortality (%)	Log dose 'X'	Empirical Probit	Expected Probit 'Y'	Working Probit 'y'	Weighing coefficient 'N'	Weight W=n×N	WX	W X^2	WY	W Y^2	WXY
1.	640	6	2	33.33	2.806	4.56	4.56	4.561	0.781	4.686	13.149	36.896	21.373	97.482	59.972
2.	800	6	3	50.00	2.903	5.00	5.02	5.00	0.627	3.762	10.921	31.704	18.810	94.050	54.605
3.	960	6	4	66.66	2.983	5.44	5.44	5.440	0.601	3.606	10.757	32.087	19.617	106.715	58.516
4.	1200	6	5	83.33	3.049	5.95	5.48	5.844	0.581	3.486	10.629	32.407	20.546	121.101	62.646
5.	1280	6	6	100.00	3.107	--	5.96	6.656	0.439	2.634	8.184	25.427	17.532	116.692	54.472
										ΣW= 18.174	ΣWX= 53.64	ΣWX^2= 158.565	ΣWY= 97.878	ΣWY^2= 536.04	ΣWXY= 290.211

Table- 4

Determination of LD_{50} by log-dose/probit Regression analysis after oral intoxication of different doses of Cybil (Cypermethrin) to *Rattus norvegicus*

S.No.	Dose in mg/kg b.wt.	No. of rats	Mortality Number	Mortality (%)	Log dose 'X'	Empirical Probit	Expected Probit 'Y'	Working Probit 'y'	Weighing coefficient 'N'	Weight W=n×N	WX	WY	WY^2	WX^2	WXY
1.	315	6	2	33.33	2.498	4.56	4.560	4.561	0.781	4.686	11.706	21.373	97.482	29.241	53.389
2.	650	6	3	50.00	2.799	5.00	5.06	5.00	0.627	3.762	10.530	18.810	94.050	29.473	52.649
3.	1260	6	4	66.66	3.100	5.44	5.44	5.440	0.621	3.606	11.179	19.617	106.715	34.654	60.812
4.	2520	6	5	83.33	3.401	5.95	5.84	5.949	0.503	3.018	10.264	17.939	106.629	34.909	61.011
5.	5040	6	6	100.00	3.702	-	6.22	6.793	0.370	2.22	8.218	15.080	102.442	30.425	55.826
										ΣW= 17.292	ΣWX= 51.897	ΣWY= 93.819	ΣWY^2= 507.318	ΣWX^2= 158.702	ΣWXY= 283.687

Table 5: Toxicity evaluation of Hafen, Decis and Cybil in albino rats specifying Fiducial limits

Experimental animal	Experimental compound	Regression equation	Median lethal dose(LD_{50})	Variance	Fiducial limit
Albino rat	Hafen	5=5.381+5.271(X-3.257)	1532.5	0.008	m_1=665.3 m_2=641.1
Albino rat	Decis	5=5.699+5.029(X-2.951)	656.2	0.005	m_1=665.3 m_2=641.1
Albino rat	Cybil	5=5.368+1.736(X-3.001)	618.2	0.047	m_1=665.3 m_2=641.1

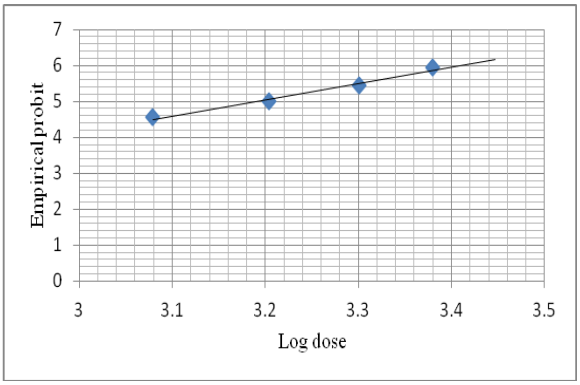

Figure 1.Graph between probit mortality and log-dose showing the regression line for the calculation of mortality index (LD_{50}) of Hafen(Fenvalerate)

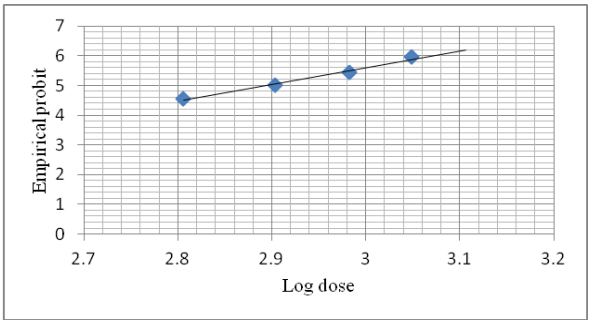

Figure 2. Graph between probit mortality and log-dose showing the regression line for the calculation of mortality index (LD_{50}) of Decis(Deltamethrin)

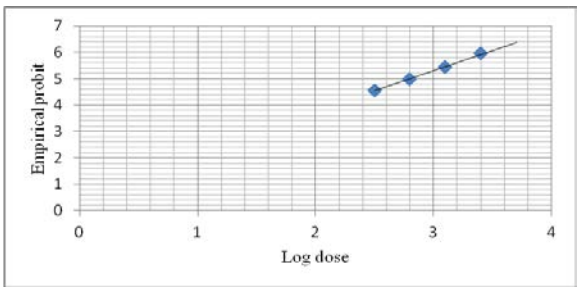

Figure 3. Graph between probit mortality and log-dose showing the regression line for the calculation of mortality index (LD_{50}) of Cybil(Cypermethrin)

4. Discussion

Hafen, Decis and Cybil, are all type II pyrethroids, which are commonly and extensively used all over the world, therefore reflect risk to non-target species (Tomar and Saxena, 2003; Saxena and Doneriya, 2004; Doneriya and Saxena, 2004; Bhushan *et al.*, 2013; Bhushan *et al.*, 2013; Amir *et al.*, 2015; Abdul-Hamid *et al.*, 2017).

Median lethal dose (LD_{50}) determination provides an overview of the toxic potential of a compound and is helpful in minimizing the accidental acute exposures such as in case of domestic intoxications. In the present era of competition, research and development of new more effective pesticides and further their indiscriminate use makes it necessary to compare the toxicity of new compounds with formerly present counterparts. However, limited guidelines for toxicological analysis in pyrethroid poisoning cases have been cited; evaluation of LD_{50} becomes essential for better evaluation of toxic characteristic of pyrethroids (Saxena and Yadav, 2011; Raj *et al.*, 2013; Chandra *et al.*, 2014; Deasi *et al.*, 2015; Elblehi *et al.*, 2015; Rajawat *et al.*, 2015).

In the present investigation, the order of toxicity in increasing order based on LD_{50} values has been observed as Cybil followed by Decis and lastly Hafen. Structural differences may be kept the sole factor responsible for this difference in the toxicity. Fenvalerate contains two halogenated groups, which in turn are chlorine, where as two bromine atoms attached to a tri cyclic structure in case of deltamethrin, where as in case of Cypermethrin, a chlorine atom attached to a benzene ring (Figures. 4-6). Chlorine is more electronegative than bromine, but bromine atoms attached in case of Deltamethrin are attached to a cyclic group which provides the compound an additional stability, which in turn increases many folds in case of Cypermethrin where chlorine atom is attached to a benzene ring, providing an additional stability and therefore the present order of toxicity (Key *et al.*, 1997; Finar, 1998; Bhushan *et al.*, 2013; Bhushan *et al.*, 2013; Yadav *et al.*, 2014).

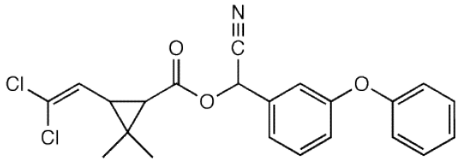

Figure 4. Structure of Fenvalerate

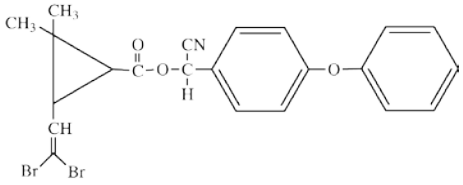

Figure 5. Structure of Deltamethrin

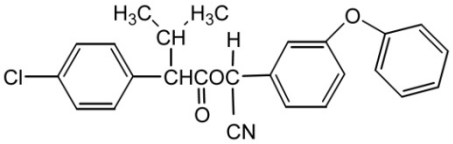

Figure 6. Structure of Cypermethrin

5. Conclusion

A preliminary idea of the comparative toxicity evaluations of newer pyrethroids with the former representatives has been provided in the present study, which revealed enhanced toxicity with advancement, thus also is a suggestive of proper precautions and appropriate use so as to minimize risk to non-target organisms.

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