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ACUTE ORAL TOXICITY STUDY OF DELTAMETHRIN IN COMBINATION WITH INSECT REPELLENTS, N,N - DIETHYL BENZAMIDE, N,N - DIETHYL PHENYLACETAMIDE AND N,N - DIETHYL METATOLUAMIDE IN RATS

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ABSTRACT

To control a variety of vector borne diseases insecticides and insect repellents are used. They share a common mode of toxicity, and are used together inside and outside the living areas. The present study was designed to evaluate the insect repellents N,N-diethyl benzamide (Deb), N,N-diethyl phenylacetamide (Depa) and N,N,-diethyl metatoluamide (Deet) in combination with insecticide deltamethrin (Del) in rats. The study was conducted in 2 phases. Phase I was determination of LD_{50} of Del by Gad and Weil moving average method and LD_{50} of Deb, Depa and Deet by Dixon Up and Down method. Phase II was determination of LD_{50} of insect repellents in combination with insecticide by Dixon Up and Down method in rats. Among the four, Del was more toxic than the insect repellents. Among the repellents, Deet was least toxic chemical. The estimated LD_{50} of Del, Deb, Depa and Deet were 129.7, 1035.1, 333.3 and 1894.1 mg/kg respectively. The extrapolated LD_{50} estimate of Del in presence of one LD_{50} of Deb, Depa and Deet was 65.9, 40.3 and 20.2 mg/kg respectively. The LD_{50} were additive and no potentiation was observed between the insecticide and the insect repellents showing they can be used together.

KEYWORDS: Deltamethrin, Deb, Depa, Deet, combined toxicity, LD_{50}, rats.

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INTRODUCTION

There are many methods to control mosquitoes viz., physical, biological, chemical and personal protection to prevent from vector borne diseases like malaria, dengue, chikungunya, yellow fever, filariasis and Japanese encephalitis[1]. The personal protection methods are insect repellents that are available in the form cream, spray, lotion and coil. When applied on body, the chemical which is present prevent the mosquitoes from biting for a certain period of time. The repellents are, plant derivatives like citronella, neem, and peppermint[2], and synthetic chemicals like N,N-diethyl benzamide (Deb), N,N-diethyl phenylacetamide (Depa) and N,N,-diethyl metatoluamide (Deet)[3,4]. Deltamethrin (Del) is an insecticide which is widely used against agricultural and household pests. It is a pyrethroid and an organic compound[5]. It is similar to natural pyrethrins extracted from pyrethrum flowers. The action of the insecticide is either by direct contact or by ingestion. Del action on insect is by affecting the ion channel in the nervous system due to the phosphorylation state and delays inhibition of sodium channel activation gate. Due to this there is a prolonged sodium in nerve that results in repeated nerve signals in sensory organ, nerves and muscles[6]. The toxicity of xenobiotics are of two types, acute toxicity i.e., single large dose which is fatal, and acute or chronic toxicity i.e., small or repeated doses which is fatal[7]. LD_{50} (lethal dose 50) means the administration of a chemical for a group of laboratory animals and when 50% of animals die than this dose is considered as LD_{50} [8]. If the LD_{50} value is small the toxicity is high and if the LD_{50} value is high than the toxicity is low. The LD_{50} of new chemicals and drugs are determined by various route of administration viz., oral, dermal, inhalation and parenteral routes[9]. The determination of the LD_{50} provides information of hazards from exposure to the chemicals and it also provides classifying and determining the dosage for both subacute and chronic toxicity studies[10]. In general the LD_{50} estimates of individual chemicals are reported, but in combination the information is not available[11]. If a chemical potentiates the toxic effect of another chemicals it will be more hazardous[12]. For controlling a variety of vector borne diseases insecticides and insect repellents are used. They share a common mode of toxicity, and are used together inside and outside the living areas. The present study was designed to evaluate the insect repellents, Deb, Depa and Deet in combination with insecticide, Del in rats.

MATERIALS AND METHODS

Chemicals

Deltamethrin (Del), N,N'–diethylbenzamide (Deb), and N.N'diethyl phenylacetamide (Depa) were obtained from M/s Tagros Chemicals India Ltd (Chennai, India) and N,N'- diethyl metatoluamide (Deet) from Alkyl Amine Chemicals Ltd. (Mumbai, India). All other chemicals used were of analytical grade.

Animals

Adult female Wistar rats (*Rattus norvigicus*) weighing between 150 to 200 g from Biomedical Research Unit and Laboratory Animal Facility (BRULAC) were used. The animals were housed as per the guidelines of the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA, India). The rats were fed with standard pellet diet and given filtered water ad libitum. They were maintained at natural light and dark cycle. The study protocol was approved by the Institutional Animal Ethics Committee (SU/BRULAC/RD009/2014).

LD_{50} determination

The LD_{50} was determined in two phases, (i) in the phase I the LD_{50} of Del, Deb, Depa and Deet were estimated and (ii) in phase II the LD_{50} in combination was estimated.

LD_{50} determination of Del

The LD_{50} of Del was estimated by Gad and Weil moving average method[13]. Del was made into a suspension with olive oil and was administered orally using a 16 gauge oral feeding cannula (about 0.2 mL/100 g). Four logarithmic doses of 50, 100, 200 and 400 mg/kg of Del were given and for each dose 4 rats were used. Food and water were withheld for a period of 2 hr. The rats were weighed daily and mortality was observed for a period of 14 days.

LD_{50} determination of Deb, Depa and Deet

The LD_{50} of Deb, Depa and Deet were estimated by Dixon Up and Down method[14]. The insect repellents were dissolved in olive oil and were administered orally using a 16 gauge oral feeding cannula (about 0.2 mL/100 g). By this method, the animals were administered the dose on a logarithmic scale. If the dose produced mortality a lower logarithmic dose was given and if the animal survived a higher logarithmic dose was given. For each insect repellent 6 to 8 animals were used for estimating the LD_{50}. The rats were weighed daily, and survival or mortality for a period of 14 days was considered.

LD_{50} determination of combination of Del with Deb, Depa and Deet

The LD_{50} of Deb, Depa and Deet in combination with Del were estimated by Dixon Up and Down method[14]. For the combined LD_{50}, three combinations for each insect repellent was taken. Del+insect repellent = 1/4+3/4 LD_{50}, 1/2+1/2 LD_{50} and 3/4+1/4 LD_{50}. The required dose for 100 g body weight of the animal was made in 0.2 mL of olive oil and administered orally using a 16 gauge oral feeding cannula,about 0.2 mL/100 g as shown in Fig.If the dose produced a mortality a lower logarithmic dose (volume) was given and if the animal survived a higher logarithmic dose (volume) was given. The following logarithmic doses (volume) were used, 0.10, 0.14, 0.20, 0.28 and 0.40 mL. For each combination 6 to 8 animals were used for estimating the LD_{50}. The rats were weighed daily, and survival or mortality for a period of 14 days was considered.

Statistics

The survived animals body weight was calculated as percent change and expressed as mean ± SE. One way ANOVA with Dunnette's test was used for comparing with control. SigmaPlot 13 was used for statistical analysis and for plotting the graph (Systat Software, USA). The LD_{50} was estimated either by Gad and Weil method or by Dixon Up and Down method.

RESULTS

The animals did not show any sign and symptom immediately after the oral administration of Del or Deb, Depa and Deet. After 4 hr the animals showed slow activity and sometimes they were hyperactive. The animals showed gasping and death occurred within 24 hr. No mortality was observed after that. The individual oral LD_{50} estimate of Del, Deb, Depa and Deet are given in Table 1. Among the four the pyrethroid insecticide (Del) was more toxic than the insect repellents. Among the repellents Deet was the least toxic chemical. The estimated LD50 of Del, Deb, Depa and Deet was 129.7, 1035.1, 333.3 and 1864.1 mg/kg respectively. The dilutions used for the combined toxicity are shown in Table 2. The dose was calculated for 100 g rat for a oral feeding volume of 0.2 mL. The individual chemicals were weighed and made into a suspension in olive oil. This method of dilution was simple and easy to handle. Table 3 shows the combined oral LD_{50} of Del with Deb, Depa and Deet. In the combination the LD_{50} were dose dependent. The LD_{50} estimate of the combinations of Del with Deb, Depa and Deet is shown graphically in Figure 1. Good correlation was observed between the combinations of LD_{50}. The LD_{50} was additive in this experiment and no potentiation was observed between the insecticide and the insect repellents. The LD50 slope of Deb, Depa and Deet in presence of Del was 0.031, 0.403 and 0.043 respectively. The extrapolated LD50 estimate of Del in presence of one LD50 of Deb, Depa and Deet was 65.9, 40.3 and 20.2 mg/kg respectively. Table 4 shows the body weight changes of Del, Deb, Depa and Deet as independent chemical. Though, death occurred within 24 hr in all the chemicals recovery was observed after that. No change in body weight was observed among the survived animals except Depa showed a lower body weight compared to the control. Table 5 shows the body weight changes of Del in combination with Deb, Depa and Deet. No change in body weight was observed in any of the combinations, among the survived animals.

DISCUSSION

Pesticides and insect repellents are widely used but most of them show high degree of toxicity because they were designed to kill. Same time it can cause health hazards to human health which is of great concern[13]. In modern life, the use of pesticides and insect repellent are increased as people are very prone to mosquito bites. Normally the available mosquito repellents contain Deb, Depa or Deet. Therefore, in this study the various repellents were tested on rats to find out LD_{50} of the individual agents and in combination with the insecticide. There is a general tendency to use the insecticide, Del and the insect repellents like Deb, Depa or Deet together at home and at outside locations. Both the insecticide and the insect repellents are neurotoxic[14,15]. Acute toxicity experiments are very essential to assess the LD_{50}[16]. The three logarithmic concentrations of individual agent was used to study the LD_{50} and later their combination in three different ratio Del + insect repellent, 1/4+3/4 LD_{50}, 1/2+1/2 LD_{50} and 3/4+1/4 LD_{50}. Among the four, the insecticide Del was more toxic than the repellents and Deet was the least toxic. The acute inhalation toxicity studies on Deb, Depa and Deet showed that they have low mammalian toxicity[3]. The combination of Del with other repellents was not showing any potentiation. The extrapolated LD_{50} of Del in presence of one LD_{50} of Deb, Depa and Deet gave a positive value showing the effect is additive. A negative value would have shown potentiation. The pyrethroid poisoning effect is on sodium and chloride channels. It alters the sodium voltage channels by prolonging its closure and also decreasing the chloride in the chloride voltage channel[17]. This mechanism brings about the toxic action. The effect of Del is on the muscles and non-myelinated nerve fibres[18]. Studies conducted on Deet proved that it has low toxic effect. Unlike some insecticides it has no inhibitory action on acetylcholinesterase in insects and in mammals[19]. But, it may cause neuroexcitation. Information on Deb and Depa are not available. The present study shows that the toxic effect of insecticide, Del and the insect repellents, Deb, Depa or Deet were additive and no potentiation was observed. They can be used together within the permissible limits inside and outside the house. However, detailed sub-acute toxicological studies are required

Table 1
Oral LD_{50} of Del, Deb, Depa and Deet in rat

Abbre-viation	Dose (mg/kg)	Test Result Died out of 5 rats	Test out come; Lowest Dose (mg/kg)	Dose Difference (log)	f value	LD_{50} (mg/kg)	Confidence Limit (mg/kg)
Del	50	0	0,3,3,4;	0.301	0.375	129.7	66.4 -253.2
	100	3					
	200	3	50				
	400	4					
Abbre-viation	Dose (mg/kg)	Test Result O = survived X = Died	Test out come, Last Dose (mg/kg)	Dose Difference (log)	K value	LD_{50} (mg/kg)	Confidence Limit (mg/kg)
Deb	400	OO	OOXXOO	0.301	0.372	1035.1	425.6 - 2517.7
	800	XOO					
	1600	X	800				
Depa	100	OO	OOXOXO	0.301	0.737	333.3	137.1 – 811.0
	200	OOO					
	400	XX	200				
Deet	800	OO	OOOXXXOX	0.301	0.244	1894.1	899.5 – 3981.1
	1600	OXX					
	3200	OX	1600				
	6400	X					

LD_{50} of Del by Gad and Weil method and Deb, Depa and Deet by Dixon Up and Down method

Table 2
Dilutions used for Del, Deb, Depa and Deet in olive oil for combined oral LD_{50} in rat

Chemical	Parameter	1/4 LD_{50}	1/2 LD_{50}	3/4 LD_{50}
		A	B	C
Del	Dose	32.4	64.9	97.3
	For 100 g rat	3.24	6.49	9.72
	Volume	0.2 mL	0.2 mL	0.2 mL
	Strength (mg/mL)	16.2	32.4	48.6
Chemical	Parameter	3/4 LD_{50}	1/2 LD_{50}	1/4 LD_{50}
		A	B	C
Deb	Dose	776.4	517.6	258.8
	For 100 g rat	77.63	51.76	25.88
	Volume	0.2 mL	0.2 mL	0.2 mL
	Strength (mg/mL)	388.2	258.8	129.4
Depa	Dose	250.0	166.7	83.3
	For 100 g rat	25.0	16.67	8.33
	Volume	0.2 mL	0.2 mL	0.2 mL
	Strength (mg/mL)	125.0	83.4	41.7
Deet	Dose	1420.6	947.1	473.5
	For 100 g rat	142.0	94.7	47.4
	Volume	0.2 mL	0.2 mL	0.2 mL
	Strength (mg/mL)	710.0	474.0	273.0

The volumes of the combination administered for the determination of LD_{50} are 0.10, 0.14, 0.28 and 0.40 mL.

Table 3
Combined oral LD_{50} of Del with Deb, Depa and Deet in rat

Combi-nation	Dose in volume (log value) 0.1 mL (- 1.0)	0.14 mL (-0.854)	0.20 mL (-0.699)	0.28 mL (-0.553)	0.40 mL (-0.398)	Test out come O = Survived X = Died	Last dose (log)	Dose Difference (log)	K Value
Del-Deb									
A	O	OO	OO	OOO	XX	OOOOXOXO	-0.553	0.150	0.741
B	O	OOO	XXO	X		OOOXXOXO	-0.854	0.150	0.952
C	OO	OO	XOO	XX		OOOXXOOX	-0.553	0.150	-0.753
Del-Depa									
A		OO	OX	OX	X	OOOXXXO	-0.854	0.150	1.544
B		O	OO	OX	X	OOOXXO	-0.699	0.150	1.139
C		O	OOO	XX	X	OOXOXO	-0.699	0.150	0.737
Del-Deet									
A		OO	OXO	X		OOXXOO	-0.699	0.150	0.372
B		OO	XX	OX		OXOX	-0.699	0.150	-0.500
C		OO	OXX	XX		OOXXOX	-0.699	0.150	-0.169

Combi-nation	LD_{50} Calculation log LD_{50} = log Last Dose + k value (log Dose Difference) = anti log (mL)			Del Dose (mg/kg) *		Insect Repellent Dose (mg/kg) *	
A	LD_{50}	-0.553 + 0.741 (0.150)	- 0.44185 = 0.3615 mL	Del	58.6	Deb	1403.2
B	LD_{50}	-0.854 + 0.952 (0.150)	- 0.7112 = 0.1944 mL	Del	63.1	Deb	503.1
C	LD_{50}	-0.553 + -0.753 (0.150)	- 0.66595 = 0.2157 mL	Del	104.9	Deb	279.1
A	LD_{50}	-0.854 + 1.544 (0.150)	- 0.6224 = 0.2386 mL	Del	38.7	Depa	298.3
B	LD_{50}	-0.699 + 1.139 (0.150)	- 0.52815 = 0.2964 mL	Del	96.2	Depa	247.0

C	LD_{50}	-0.699 + 0.737 (0.150)	- 0.5884 = 0.2580 mL	Del	125.5	Depa	107.5
A	LD_{50}	-0.699 + 0.372 (0.150)	- 0.6432 = 0.2274 mL	Del	36.8	Deet	1615.2
B	LD_{50}	-0.699 + -0.500 (0.150)	- 0.7740 = 0.1683 mL	Del	54.6	Deet	797.0
C	LD_{50}	-0.699 + -0.169 (0.150)	-. 0.72435 = 0.1886 mL	Del	91.8	Deet	446.5

Combinations (mg/kg)		Del (0.2 mL)	Deb (0.2 mL)	Depa (0.2 mL)	Deet (0.2 mL)
A Combination	1/4 Del + 3/4 Insect Repellent	32.4	776.3	250.0	1420.6
B Combination	1/2 Del + 1/2 Insect Repellent	64.9	517.6	166.7	947.1
C Combination	3/4 Del + 1/4 Insect Repellent	97.3	258.8	83.3	473.5

The Del, Deb, Depa and Deet doses were used for plotting the figure 1.

Table 4
Body weight (percent) of rats following p.o. administration of Del, Depa and Deet.

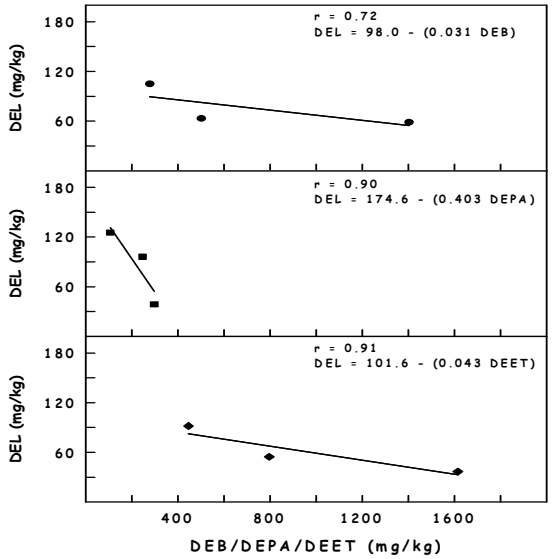
Groups	3 days		7 days		14 days	
	Mean	SE	Mean	SE	Mean	SE
Control	102.9	1.7	99.1	2.9	105.4	3.6
Del	99.2	0.8	101.5	1.0	103.6	1.3
Deb	96.7	3.2	94.7	2.5	100.2	3.5
Depa	99.3	0.7	101.3	2.3	90.3*	4.3
Deet	98.6	1.1	99.6	1.7	99.7	3.4
F		1.73		1.97		3.22
P		NS		NS		< 0.05

Values are mean ± SE (n = 4 to 6) of survived animals.
Statistically significant from control by one way ANOVA with Dunnett's test.

Table 5
Body weight (percent) of rats following p.o. administration of Del in combination with Deb, Depa and Deet.

Groups	Combina-tion LD_{50}	3 days		7 days		14 days	
		Mean	SE	Mean	SE	Mean	SE
Control	-	102.9	1.7	99.1	2.9	105.4	3.6
Del-Deb	1/4 Del + 3/4 Deb	96.6	2.0	98.5	2.9	105.2	6.3
	1/2 Del + 1/2 Deb	96.9	1.2	96.2	0.9	101.4	1.0
	3/4 Del + 1/4 Deb	100.6	1.8	100.2	1.8	99.8	0.8
Del-Depa	1/4 Del + 3/4 Depa	104.6	1.1	105.1	4.8	110.5	5.9
	1/2 Del + 1/2 Depa	98.4	1.6	103.2	4.5	104.6	3.5
	3/4 Del + 1/4 Depa	98.5	0.7	97.4	0.8	104.9	1.6
Del-Deet	1/4 Del + 3/4 Deet	101.5	3.6	101.8	3.5	100.0	2.5
	1/2 Del + 1/2 Deet	96.3	3.2	96.3	3.7	95.1	5.9
	3/4 Del + 1/4 Deet	100.8	2.1	98.2	1.5	94.5	3.7
F		1.69		1.06		1.73	
P		NS		NS		NS	

Values are mean ± SE (n = 4 to 6) of survived animals.
Statistically significant from control by one way ANOVA with Dunnett's test.



CONCLUSION

The present study shows the combined LD_{50} effect of various insect repellents (Deb, Depa and Deet) with pyrethroid insecticide (Del) by oral route in Wistar rats. The study shows that the toxicity of pyrethroid insecticide and the insect repellent are additive and there is no potentiating of toxicity. Hence, they can be used together either indoor or outdoor.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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