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Topical Preparation of Newer and Safer Analogs of N,N-Diethyl-2-phenylacetamide (DEPA) against *Aedes aegypti* Mosquitoes

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ABSTRACT

Cosmetic acceptability and primary skin irritation are the two main parameters for assessing the suitability of any topical formulation meant for protection against the painful bites of mosquitoes. In the present study four newer analogs of N,N-diethyl-2-phenylacetamide (DEPA), were synthesized and formulated for topical application as insect repellent. They were assessed for their irritant behavior on rabbit's skin for erythema and edema. The topical formulations of the analogs were also assessed for their protection time at varying concentrations against *Aedes aegypti* mosquitoes.

Keywords: Insect Repellent; Mosquitoes; Protection

1. Introduction

Insect transmitted diseases remain a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million persons annually [1]. Mosquitoes belonging to three genera *Culex*, *Anopheles* and *Aedes* are known to transmit major mosquito borne diseases like malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya, dengue haemorrhagic fever and yellow fever [2]. Research shows that malaria kills about 3 million persons each year, including one child every 30 seconds [3]. Although dengue fever is known to exist in India for a long time, dengue haemorrhagic fever was reported in an outbreak which occurred in Calcutta in 1963 [4] and Delhi in 1996 [5]. Protection from arthropod bites can be best achieved by vector control aimed at mosquito eradication, disease prevention, prophylactic drug therapy, insecticides and insecticide-treated nets and repellents [6]. Personal protection is however one of the established methods to prevent mosquito bites [7].

In the past and before the discovery of synthetic organic insecticides and herbal products such as nicotine from tobacco leaves (*Nicotiana tabacum*), anasbasine and lupinine (alkaloids extracted from Russian weed *Anabasis aphylly*), rotenone from *Derris elliptica* and pyrethrums from *Chrysanthemum cinererifolium* flower have been playing an important role as natural insect repellent or insecticide in the interruption of the transmission of mosquito borne diseases both at the individual and community level [8-10]. Currently, many plants have been harnessed for their potential to act as larvicide, insecticides or repellents such as *Ocimum gratissimum* [6,11], lemongrass (*Cymbopogan citratus*) [12,13], *Solanum trilobatum* [14], *Catharanthus roseus*, *Lanata camara* [15], *Zanthoxylum piperitum* [16], *Syzygium aromaticum* [17] turmeric (*Curcuma longa*) [18], etc. against *Aedes*, *Anopheles* and *Culex* species of mosquitoes.

Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides. Pyrethrin and synthetic pyrethroids such as D-allethrin have been used in many mosquito coil formulations. Prolonged exposure to these chemicals may lead to local irritation, severe allergic dermatitis and other CNS disturbances [19]. Extensive use of synthetic organic insecticides during the last five decades have resulted in environmental pollution, residual effects, physiological resistance in major vector species [20].

Personal protection measures are apparently practical alternative to insecticides and economical way as compared to area repellents for preventing the transmission of vector-borne diseases to humans. Resistance against insect repellents are not reported so far. One important difference of the insect repellents from the insecticide is that insect repellents need to be used only when there is a requirement which is only on the exposed parts of the body. These personal protection measures include:

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1) Non-insecticidal repellent creams, lotion, sprays (for indoor as well as outdoor application);

2) Insecticide-impregnated bed nets (during night sleeps).

A number of mosquito repellents mainly in the form of mats, coils and liquid sprays are available in the market, which mostly contain synthetic pyrethroids like allethrin causing tremendous health hazards, have low effective period, produce knock-down effect and requires electricity.

Since none of the available materials to date were ideal repellents, research into new synthetic, non-insecticidal chemicals have been continued. In 1955, scientists synthesised N,N-diethyl toluamide (DEET), which is currently the most widely used active ingredient for mosquito repellents. DEET has broad-spectrum activity and effectively repels most mosquitoes, biting flies, chiggers, fleas and ticks [21]. It is the most effective insect repellent available for human use [22]. Currently, DEET is formulated in aerosols, pump sprays, lotions, creams, liquids, sticks, roll-ons and impregnated towelettes, with concentrations ranging from 5% to 100% [23]. Various formulations containing DMP (Dimethyl phthalate), picaridines, DEET, DEPA (N,N-diethyl 2-phenylacetamide) and DEB (N,N-diethyl benzamide) are commercially available.

Efforts to develop vaccines and new drugs have not yielded any major breakthrough. No new insecticide has been commercialized for more than two decades. The search for effective vaccines against these diseases is still in progress. The development of any insect repellent formulation requires the involvement of interdisciplinary research work; therefore it is generally left unattended. Keeping these aspects in view, the present study was carried out to develop a newer, safer, effective and broad-spectrum insect repellent formulation for topical application by using a non-insecticidal chemical which can be used by individual and communities in specific situation to minimize the transmission of vector-borne diseases. The work aims to develop a topical drug delivery formulations of newer derivatives of N,N-diethyl phenylacetamide (DEPA) and its safety evaluation.

2. Materials and Methods

2.1. Mosquitoes

The laboratory colony of *Aedes aegypti* maintained for more than 25 years in the insectary of Entomology Division of Defence Research and Development Establishment (DRDE), Gwalior, India at 27˚C ± 2˚C and 75% ± 5% RH [24] has been utilized for the experiments. Five to seven days old female *Aedes aegypti* were taken from reared colony using an aspirator. The selection of female *Aedes aegypti* was based on the fact that it is a day biter, bites repeatedly and feeds on human beings in domestic and peridomestic situations as compared to *Culex* and *Anopheles* mosquitoes. All mosquitoes were starved of blood and sugar 24 hours before the tests. Laboratory tests were performed during daylight hours only.

2.2. Chemicals

A series of substituted aromatic amides that are analogs of DEPA were synthesized and characterized. All the compounds were initially tested for primary skin irritation test in rabbits. Laboratory studies were carried out to observe the behavioural responses and repellent activity of these compounds against *Aedes aegypti* mosquitoes. The compounds were compared with the well known insect repellents such as DEET, DEPA and DEB [25].

For selected compounds acute toxicity studies and haematological and biochemical changes were carried out in the Pharmacology and Toxicology Division of Defence Research and Development Establishment (DRDE), Gwalior [26]. A number of synthetic repellents were synthesized in the Synthetic Chemistry Division, Defence Research and Development Establishment (DRDE). Four effective compounds namely N,N-diethyl-2-(3-methylphenyl) acetamide (F1), N,N-diethyl-2-(4-methylphenyl)-acetamide (F2), N,N-diethyl-2-(3-methoxyphenyl)-acetamide (F3), and N,N-diethyl-2-(4-methoxyphenyl)-acetamide (F4) were used for the present study.

Stearic acid, stearyl alcohol, cetyl alcohol, potassium hydroxide, methyl and propyl paraben were purchased from Qualigens Fine Chemicals (Mumbai, India). The above mentioned repellent compounds were synthesized in the Synthetic Chemistry Division, Defence Research and Development Establishment (DRDE), Gwalior. All other reagents used were of analytical grade.

2.3. Preparation of Cream

The emulsification method was followed for the preparation of vanishing cream base. Stearic acid, stearyl alcohol, cetyl alcohol and propyl paraben (lipid phase) were heated together at about 70˚C. The active ingredients F1, F2, F3, F4 (in 10%, 15% and 20% v/v, respectively) were incorporated to it. Potassium hydroxides, glycerine (humectant), methyl paraben (preservative) were mixed together which comprised the aqueous phase. The aqueous phase was heated to the same temperature (70˚C) as that of the oil phase. The two phases were gradually mixed with continuous stirring. The cream was then allowed to cool at room temperature and used for further studies.

2.4. Primary Skin Irritation Study

Before conducting tests on humans, preliminary safety studies of the neat compounds were carried out in animal models as earlier reported [26]. The vanishing cream formulations were also subjected to the primary skin irritation test performed using the Draize method (1944) on male albino rabbits (New Zealand strain) [27]. The primary skin irritation index (PSII) gave an idea of the skin irritancy nature of the prepared cream formulations. The vanishing cream base without the active ingredient was used as control.

2.5. Bioefficacy Test on Human Volunteers

The protection time or repellent efficiency of the cream formulation was performed on human volunteers. The volunteers were informed about the test and consent was taken. The hand was washed thoroughly with tap water, dried with towel and then the cream was applied. For this, different concentration of repellent formulations (10%, 15% and 20% v/v) were applied on the external surface of the fist of human hand over an area of about 150 cm^2 at the rate of 1 mg/cm^2. The treated surface was exposed to 200 non blood fed female (5 - 7 days old) *Aedes aegypti* mosquitoes in 75 × 60 × 60 cm^3 test chamber for 5 min period at intervals of 30 min. Less than 5 bites in 5 min were considered to be indicative of repellency [28, 29]. The number of insects landing or biting was recorded for two (one male and one female) volunteers. Exposure of the human hand without the repellent (*i.e.* only cream base) to the mosquitoes served as the control. The repellent activity against *Aedes aegypti* was evaluated in the day time. The experiments were performed in triplicate (n = 3).

3. Results and Discussion

Cosmetic acceptability is the most important criterion in the wide-scale use of an insect repellent in vanishing cream base [28]. PSII was computed as the average sum of erythema and edema on all sites of rabbit's skin. Scoring scale for the PSII values are 0.0 (not-irritant), > 0.0 - 0.5 (negligible irritant), >0.5 - 2.0 (mild irritant), > 2.0 - 5.0 (moderate irritant) and >5.0 - 8.0 (severe irritant), respectively. The PSII value for neat F1 was 0.125 and that of neat F2, F3 and F4 was found to be 0.00 whereas the PSII values of the neat compounds DEB, DEET and DEPA were 1.875, 0.875 and 0.75, respectively [26]. In the present study the PSII values of all the formulations were 0.0 (**Table 1**) showing that they are safer to be used as a vanishing cream for topical application. Vanishing cream without the active ingredient served as control and was also non-irritating to the skin (PSII value was zero) (**Table 1**).

The bioefficacy test in the protection time against *A. aegypti* with 20% concentration of the compounds in isopropanol was reported earlier. The compounds N,N-diethyl-2-(3-methylphenyl)-acetamide was found to protect for 4.5 h, N,N-diethyl-2-(4-methylphenyl) acetamide for 5.0 h, N,N-diethyl-2-(3-methoxyphenyl) acetamide for

Table 1. Protection time and primary skin irritation index (PSII) values of the cream formulations.

S.No.	Code	Concentration (%)	PSII*	Average protection time (in hours)#
1	Control (cream without active compound)	-	0.00	<0.5
2	F1	10	0.00	3.5
3	F1	15	0.00	4.0
4	F1	20	0.00	5.0
5	F2	10	0.00	4.0
6	F2	15	0.00	5.0
7	F2	20	0.00	5.5
8	F3	10	0.00	4.0
9	F3	15	0.00	4.5
10	F3	20	0.00	5.5
11	F4	10	0.00	2.0
12	F4	15	0.00	2.5
13	F4	20	0.00	3.0

*First scoring was done after 4 hours. #(n = 3). F1 = N,N-diethyl-2-(3-methylphenyl)-acetamide, F2 = N,N-diethyl-2-(4-methylphenyl)-acetamide, F3 = N,N-diethyl-2-(3-methoxyphenyl)-acetamide, and F4 = N,N-diethyl-2-(4-methoxyphenyl)-acetamide.

5.0 h and N,N-diethyl-2-(4-methoxy- phenyl)-acetamide for 3.0 h. The known compounds DEB, DEET and DEPA gave a protection of 1.5 h, 6.0 h and 5.0 h, respectively [25].

Table 1 shows the average protection time for the control and the cream formulations on human volunteers. The vanishing cream containing 20% of the compounds provided maximum repellent behaviour and thus suitable for protection against mosquito bites. For the initial 5 minutes, no landing of mosquitoes was observed for the tested preparations. The mosquitoes started landing only after 5 minutes on the hand of volunteers. However, in case of the control, the mosquitoes started landing immediately after exposure of hand in the cage. **Figure 1** shows the volunteer's hand with 20% of the vanishing cream exposed to female *Aedes aegypti* mosquitoes in the cage. The average protection time for the control was found to be less than 30 minutes showing that the formulation has good insect repellency and cosmetic compatibility. **Figure 2** shows the volunteer's hand after the exposure of the vanishing cream (20%) containing N,N-diethyl-2-(3-methoxyphenyl)-acetamide (F3) to female *Aedes aegypti* mosquitoes. It can be seen that there was no observable toxicity in terms of edema and erythema post exposure of the cream for 5.5 h duration. The repellent activity of the formulations indicated that the active principle present in it was solely responsible for the repellent response against *Aedes aegypti* mosquitoes. Among the formulations F2 and F3 were capable of protecting the human from bites of *Aedes aegypti* up to 5.5 h duration.

Figure 1. Volunteer's hand with 20% of the vanishing cream containing N,N-diethyl-2-(3-methoxyphenyl)-acetamide (F3).

Figure 2. Volunteer's hand after the exposure of the vanishing cream (20%) containing N,N-diethyl-2-(3-methoxyphenyl)-acetamide (F3) to female *Aedes aegypti* mosquitoes.

At present a number of mosquito repellents mainly in the form of mats, coils and liquid sprays are available in the market, but all of them contain synthetic compound e.g. allethrin and cause tremendous health hazards. Several personal protection devices require electricity for their operation and therefore may not be useful in remote rural and forest areas [30]. Sprays mostly contain synthetic compounds and their effective period is short and also causes health hazards. Coils are harmful to health, cause irritation on skin and eyes. Natural plant repellents suffer from drawbacks such as limited availability, high cost of extraction and usually short protection time (<2 h). In such circumstances, topical repellents can give immediate protection to individuals exposed in areas where suppression of arthropod vectors is not feasible [31].

4. Conclusion

The significant repellency exhibited by the formulation containing the compound against *Aedes aegypti* mosquitoes suggests that they can be further studied to develop on a commercial repellent. The bioefficacy of repellent formulations against different species of mosquitoes can be studied.

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