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ORIGINAL RESEARCH

Comparison of in vitro Killing Effect of *N, N*-Diethyl-Meta-Toluamide (DEET) versus Permethrin on *Demodex folliculorum*

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Background: There is no single effective treatment for demodicosis; successful treatment requires a multimodal approach. Relapse or recurrence of demodicosis is relatively high, making the therapy challenging. Several reports have documented the successful treatment of demodicosis with acaricidal agents, which aimed at reducing the excessive number of *Demodex* mites and improving the patients' symptoms. Reports of irritation and resistance to topical acaricidal agents have led to the search for effective alternative treatments.

Materials and Methods: A total of 100 standardized skin surface biopsy (SSSB) biopsy slides from 100 patients with demodicosis were randomly divided into five groups, each with 20 slides exposed to immersion oil, *N, N*-diethyl-meta-toluamide (DEET) 5%, 10%, 20%, and permethrin 1%, respectively. The microscopic evaluation started immediately after the test agents exposed the mites. The survival time (ST) was defined as the interval between the first exposure of *Demodex folliculorum* to the test agents to the time the movements ceased.

Results: The differences between the median ST of DEET 5% (44 min), 10% (22 min), and 20% (14 min) were significant when compared to the negative control group (240 min) with $p<0.001$, <0.001, <0.001, respectively. While the median ST of permethrin 1% (42 min) was not significantly different from the median ST of DEET 5% ($p=0.7395$).

Conclusion: This study demonstrated the dose-related acaricidal effect of DEET on *D. folliculorum*. The survival times of DEET 5%, 10%, and 20% were significantly shorter than the negative control (immersion oil). DEET 5% had a comparable in vitro killing effect as permethrin 1%. Further in vivo studies are necessary to determine the clinical efficacy in patients with demodicosis.

Keywords: *Demodex* mites, demodicosis, *N, N*-diethyl-*meta*-toluamide, DEET, permethrin, in vitro killing effect

Introduction

Demodex mite can cause a variety of skin disorders known as demodicosis. Clinical signs of demodicosis include dry, itchy, scaly skin, redness, increased skin sensitivity, burning, stinging sensation, rough skin like sandpaper, papulopustular lesions, and blepharitis. Demodicosis should be considered in differential diagnoses of unexplained eczema, seborrheic dermatitis, bacterial folliculitis, acne, perioral dermatitis, eosinophilic folliculitis, and blepharitis.[1] Furthermore, many studies revealed that rosacea patients also have a significantly higher density of *Demodex* mites than the average population.[2–4] The density of *Demodex* mites is an important factor in the appearance of clinical symptoms. Demodicosis can be diagnosed by clinical presentations and standardized skin surface biopsy (SSSB), which is abnormal when there are more than 5 mites/cm^2.[5–9]

Several reports have documented the successful treatment of demodicosis and rosacea with acaricidal agents, which aimed to reduce the excessive number of *Demodex* mites and improve the patients' symptoms.[10–16] Many topical acaricidal agents can cause mild to moderate irritation.[10,13] Moreover, resistance to these medications in *Pediculus humanus capitis* and *Sarcoptes scabiei* has been reported.[17–19]

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The limitations of topical acaricidal agents have urged a search for an alternative treatment.

N, N-diethyl-*meta*-toluamide (DEET) has been registered for commercial use as an insect repellent for more than six decades.[20,21] Recently, the in vitro *Sarcoptes scabiei* killing ability of DEET has been reported, but the in vitro *Demodex* mite killing effect has never been investigated.[22]

Permethrin is a commonly prescribed agent for demodicosis and scabies in Thailand. Studies have demonstrated that topical permethrin, crotamiton, benzyl benzoate, and ivermectin are effective in the treatment of demodicosis and all significantly decreased the *Demodex* count.[13–16] A review included randomized controlled trials demonstrating no difference in permethrin effectiveness compared to systemic or topical ivermectin for scabies.[23] Thus, the authors had permethrin as a positive control agent in the current study.

Although permethrin 1% is safer than 5%, the in vitro *Demodex* killing effect of permethrin 1% has not been explored. The authors included permethrin 1% as a positive control agent in this study. This study aims to investigate the in vitro killing effect of different concentrations of DEET versus permethrin 1% on *D. folliculorum* from SSSB slides of demodicosis patients.

Materials and Methods

Materials

Permethrin 1% solution is produced by HUNTER GROUP LIMITED PARTNERSHIP (Thailand). DEET 20% solution is a product from The British Dispensary (L.P.) Co., Ltd. (Thailand) and diluted with propylene glycol to the other study concentrations of 10% and 5%.

Demodex Mites

The SSSB was performed during the consultation to determine the density of *Demodex* mites, by pressing two glass slides containing a drop of cyanoacrylate glue against the skin surface on both cheeks of the patients, one slide on each cheek, for 1 min before slowly and gently peeling them off. The first slide was exposed to immersion oil and covered with a coverslip to evaluate the density of *Demodex* mites, the second slide was randomly exposed to immersion oil or test agents and covered with a coverslip immediately for this trial under a microscope. The investigator selected only one *D. folliculorum*, the most active mite from each slide, as the study's subject for observation. The initiation of the microscopic evaluation occurred immediately after the mites were contacted with immersion oil or test agents.

Methods

A total of 100 SSSB slides from 100 patients with demodicosis were randomly divided into five groups, each with 20 slides. The positive control group was exposed directly to permethrin 1%, while the negative control group was exposed to immersion oil. The last three test groups were directly applied with DEET 5%, 10%, and 20%, respectively. The investigator was unaware of the test agent in each slide observed under a microscope. Viability was evaluated for 240 min by periodic observation of each slide for the movement of *D. folliculorum*'s head, body, and legs through the microscope. The observation period was 1 min in every 2 min of the first hour, and after that occurred every 10 min of the second hour and every 30 min of the third and the fourth hour of the study. Definition of the survival time (ST) is the interval between the first exposure of *D. folliculorum* to the test agents to the time the movements ceased. This cessation described the absence of any motion over two consecutive observation periods (2 min). The average ST from each study group was compared to evaluate the in vitro killing effect.

Statistical Analysis

Statistical analyses were performed using statistical software for data science, STATA for Windows, version 11.1. Numerical variables were shown as median, interquartile range, mean, and standard deviation (SD). The survival analysis using the Kaplan-Meier method demonstrated ST between the test agents. The comparison of ST between groups was evaluated by a Log rank test, p-value less than 0.05 was considered significant in all comparisons.

Results

The median ST of DEET 5%, 10%, 20%, and permethrin 1% were 44 min (IQR: 40–48 min), 22 min (IQR: 20–24 min), 14 min (IQR: 14–16 min) and 42 min (IQR: 40–46 min), respectively. All test agents were able to kill the mites within the first hour of exposure, except for two mites in permethrin 1% that had ST at 80 min. While all the mites survived in the negative control group (immersion oil) for up to 240 min of observation. The ST showed an inverse correlation with the DEET concentration, confirming the dose-related acaricidal effect among all DEET groups (Table 1).

The differences between the median ST of DEET 5%, 10%, 20%, and permethrin 1% were statistically significant compared to the negative control group ($p<0.001$). The median ST of DEET 10% and 20% were significantly shorter than permethrin 1% ($p<0.001$, $p<0.001$, respectively), while DEET 5% did not show significant difference from permethrin 1% ($p=0.7395$) (Table 2). Kaplan-Meier cumulative *D. folliculorum* survival curves of the five test agent groups are presented in Figure 1. Comparison of the differences in the median ST between test agents using the Log rank test are shown in Figure 2.

Discussion

This trial was performed in the adult form of *D. folliculorum* only because the SSSB collects mainly *D. folliculorum*, and previous studies have found that *D. folliculorum* is more tolerable to acaricidal agents than *D. brevis*.[24–26] Although permethrin 5% is widely used because it is more available in the market, many dermatologists prefer to use permethrin 1% as it has less skin irritation and side effects. A previous study showed the mean ST of *Demodex* mites in permethrin 5% was 12.5 ± 1.9 min, while the mean ST in permethrin 1% was 47.2 ±12.3 min (in this trial) which led to the conclusion that permethrin has a dose-related acaricidal effect.[27]

Table 1 The Survival Time of *D. folliculorum* from Standardized Skin Surface Biopsy Slides in Different Test Agents

Test Agents	n	Median	IQR	Mean	SD	Min	Max
Negative control (immersion oil)	20	240	240–240	240	0	240	240
DEET 5%	20	44	40–48	45.2	5.8	38	58
DEET 10%	20	22	20–24	22.4	5.1	16	34
DEET 20%	20	14	14–16	15.1	2.9	10	20
Positive control (Permethrin 1%)	20	42	40–46	47.2	12.3	38	80

Abbreviations: IQR, interquartile range; SD, standard deviation; DEET, *N,N*-diethyl-*meta*-toluamide.

Table 2 To Compare the Median Survival Time Difference Between the Pairs Using the Log Rank Test

The Difference Between the Pairs	P value
Negative control (immersion oil) vs DEET 5%, 10%, 20% and permethrin 1%	<0.001
DEET 5% vs DEET 10%	<0.001
DEET 5% vs DEET 20%	<0.001
DEET 10% vs DEET 20%	<0.001
DEET 5% vs permethrin 1%	0.7395
DEET 10% vs permethrin 1%	<0.001
DEET 20% vs permethrin 1%	<0.001

Abbreviation: DEET, *N,N*-diethyl-*meta*-toluamide.

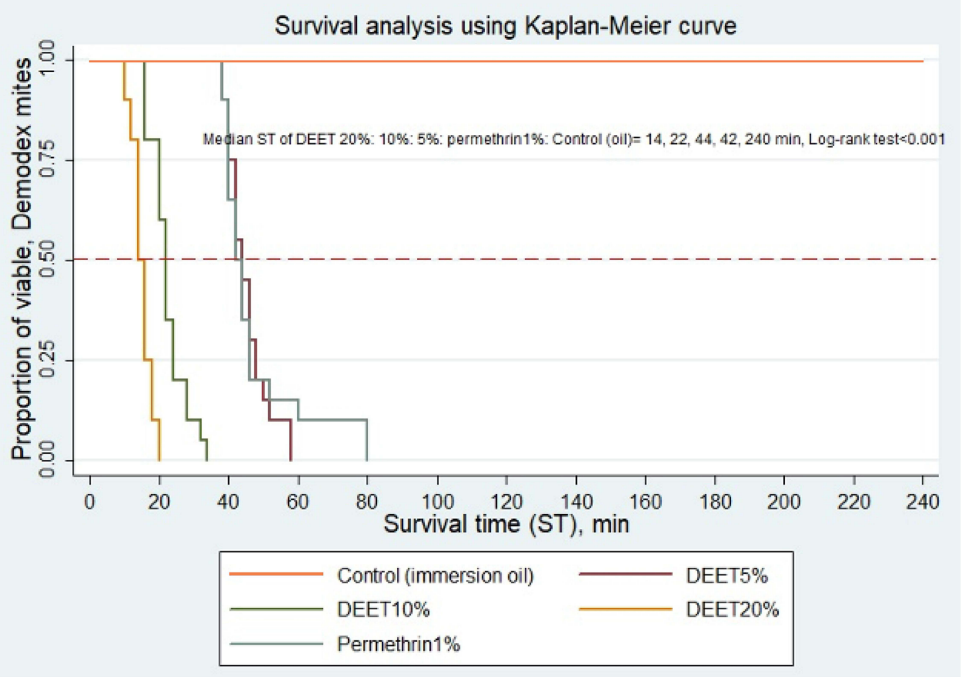

Figure 1 Kaplan-Meier, survival curve to demonstrate the survival time between the test agents.
Abbreviation: DEET, *N,N*-diethyl-*meta*-toluamide.

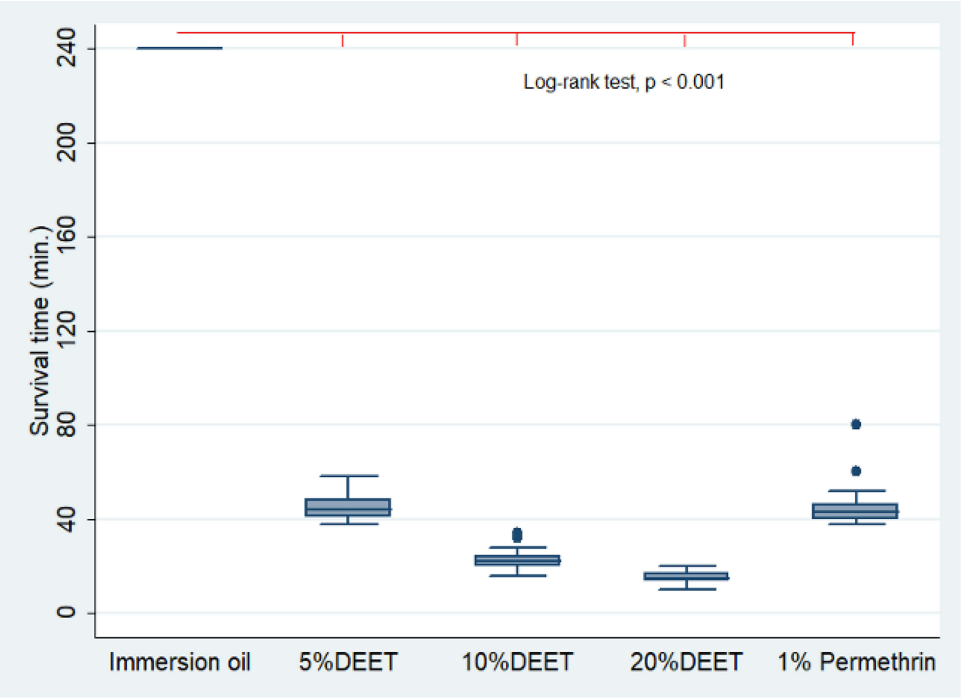

Figure 2 To compare the median survival time difference between the pairs using the Log rank test.
Abbreviation: DEET, *N,N*-diethyl-*meta*-toluamide.

Interestingly, two of the *Demodex* mites in permethrin 1% survived up to 80 min, while the remaining 18 mites in the same group had median ST at 42 min. It is possible that they were younger than the others, or the concentration of permethrin 1% may not be sufficient to kill all *Demodex* mites, or these mites may be less responsive or resistant to permethrin treatment, as had been reported in lice and scabies before.[17–19]

DEET has been used as an active ingredient in insect-repellent products for the US Army since 1946 and registered for public commercial use in 1957.[21] Products registered for human use can have a percentage of DEET from 4 to 100%. However, the most common concentration of products on the market is 30–40%.[28] DEET has become one of the most popular insect repellents, with 200 million applications annually worldwide for preventing bites from arthropods such as mosquitoes, black flies, ticks, mites, and fleas.[20] The mechanism of action of DEET is not fully understood, with several reports showing that odor, contact, and ingestion can prevent insect bites.[21] Insect repellents generally work by preventing insects from landing on human skin. However, many studies have shown that DEET also has insecticidal and acaricidal effects.[29,30] There is evidence showing that DEET works by interacting with insect's odorant and gustatory receptors.[31] DEET is likely targeting octopaminergic synapses and disrupting the calcium equilibrium in the nerve cells to induce neuroexcitation and toxicity in insects. The low potency of DEET for inhibiting human acetylcholinesterase makes it unlikely to cause toxicity in humans by this mechanism.[32]

The United States Environmental Protection Agency revealed minor side effects of DEET depending on the exposure route. DEET was classified to be as low toxicity (category III) based on the oral study, very low toxicity (category IV) based on an inhalation LC50 study, low toxicity for the eye, and very low toxicity for dermal irritation. Chronic toxicity studies did not show evidence of carcinogenicity.[33] Furthermore, there is a study about the effects of DEET exposure on human health using population-based data demonstrated that there was no significant correlation between DEET metabolite levels and biomarkers related to systemic inflammation (high sensitivity C-reactive protein), immune function (lymphocyte), liver function (aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transferase), and kidney function (estimated glomerular filtration rate).[21]

This study discovered that the ST of DEET 5% was equivalent to permethrin 1%, while the ST of DEET 10% and 20% were shorter in duration. DEET could be another option for demodicosis treatment. Since the most important thing in managing demodicosis is balancing the acaricidal effect and the safety of the treatment, the authors suggest that DEET 10% is probably the appropriate concentration for the further clinical trial.

The limitations of this study are the small number of *Demodex* mites, the age of the different mites at the beginning of the observation was not known, and comparison with propylene glycol alone (added to dilute) and other acaricidal agents (ivermectin, benzyl benzoate) was not performed.

Conclusion

The survival times of DEET 5%, 10%, and 20% were significantly shorter than the negative control (immersion oil). DEET 5% had a comparable in vitro killing effect as permethrin 1%. This is the first study that demonstrated the dose-dependent acaricidal effect of DEET. The findings of this study are limited to in vitro experiments and do not entirely reflect the efficacy of these agents in clinical practice; further in vivo investigations are necessary to determine the treatment efficacy of DEET in patients with demodicosis.

Data Sharing Statement

Unavailable data, but the reader can personally request to access the data via Dr. Anon Paichitrojjana; E-mail: anonpaic@gmail.com.

Statement of Ethics

The study was conducted in accordance with the World Medical Association Declaration of Helsinki. This study protocol was reviewed and approved by the Ethical Research Committee of Mae Fah Luang University, approval number COE 042/2022.

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Disclosure

The authors report no conflicts of interest in this work.

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