Pharmacology and Clinical Pharmacy Research
Volume 4 No. 1 April 2019
ISSN:2527-7322 | e-ISSN: 2614-0020
doi: 10.15416/pcpr.v4i1.21388

In-Vivo Effectiveness of 5% *Azadirachta indica* Oil Cream as Anti-Scabies

Patihul Husni, Mayang Kusuma Dewi, Norisca Aliza Putriana, Rini Hendriani
Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy,
Padjadjaran University, Bandung, West Java, Indonesia

Abstract

Scabies is an infectious skin disease caused by mite *Sarcoptes scabiei.* Neem tree (*Azadirachta indica*) has the potential to be used as an anti-parasite due to the presence of azadirachtin compound that is commonly found in the seeds. The aim of this study was to evaluate *in-vivo* effectiveness of neem oil as an anti-scabies. This study used an experimental method. The effectiveness of the cream as an anti-scabies was tested on New Zealand white rabbits which were infected with scabies. Permethrin cream was used as a positive control and cream base was used as a negative control. Cream was applied once daily and left for 8 hours. The data were analyzed using Kruskal Wallis and Mann Whitney. Dermal acute irritation test was performed by applying 0.5 g cream on the rabbit dorsal. We found that 5% neem oil cream was effective as an anti-scabies with 20-21 days recovery time. The recovery time is longer than permethrin cream (7-8 days), but shorter compared to negative control with recovery time over 30 days. Primary irritation index for 5% neem oil creams was 0, indicating negligible irritation category. In conclusion, *A. indica* cream was effective for the treatment of scabies although its recovery time is shorter than permethrin cream.

Keywords: effectiveness test, irritation test, neem oil cream, scabies

Introduction

Scabies is a contagious skin disease caused by mites *Sarcoptes scabiei*. The infection cause erythema, papules, and vesicles which can lead to skin damage. Itching usually occurs at night. *S. scabiei* is an obligate parasite whose breeding requires a host. Scabies affects many children. Transmission can occur through direct contact with people with scabies or through indirect contact, such as the use of same clothing, towels, *etc.* Dense population with poor hygiene level can facilitate transmission of scabies. Indonesia is one of the five countries with the highest prevalence of scabies.[1,2] Scabies can be treated through systemic and topical therapy. However, systemic treatmet is only given for severe scabies. Thus, topical therapy is the main treatment choice for scabies.[3,4]

Neem tree (*A. indica*) is a medicinal plant originating from India. Neem tree can grow well in tropical and subtropical areas. Every part of this plant has its own function that can be used for empirical treatment. This

Corresponding author: Patihul Husni. Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Bandung, West Java, Indonesia. Email: patihul.husni@unpad.ac.id
Received: 14 December 2018. **Revised**: 25 March 2019. **Published:** 25 April 2019.

plant has many biological activities, *e.g.,* as anti-histamines, anti-dermatitis, anti-fungi, anti-inflammatory, anti-scabies, diuretics, insecticides, anti-protozoa, spermicidal, and other biological activities. Previous study found that there was no difference in the effectiveness of the cream containing 10% of neem oil and 5% of permethrin cream as positive control for scabies.[5,6] There is an urgent need to further evaluate the effectiveness of neem oil at different concentration with *in-vivo* method. *In-vivo* evaluation needs to be conducted to ensure the safety and effectiveness of the cream before being clinically tested in humans. Skin irritation potential also should be investigated to obtain complete safety profile of the cream.[7] Therefore, we conducted this study to evaluate *in-vivo* safety and effectiveness of neem oil as an antiscabies.

Methods

This study used an experimental method. The procedures in this study included cream formulation, acclimatization of test animals, acute dermal irritation test, and anti-scabies effectiveness test. The conduct of this study was approved by the Health Research Ethics Commission of the Faculty of Medicine, Padjadjaran University, Indonesia (No. 881/UN6.C.10/PN/2017).

Cream formulation

Certificated neem oil which was obtained from Happy Green Co, Jakarta, Indonesia. The cream was formulated: 5% neem oil, 1 g adeps lanae, 14.2 g stearic acid, 10 g glycerin, 0.25 g sodium tetraborate, 1 g triethanolamine, 75 g distilled water, and 0.1% nipagine. The oil phase (adeps lanae and stearic acid) was placed in a vaporizer dish and heated over a waterbath at 70 ^{0}C. The water phase (glycerin, sodium tetraborate, triethanololamine and distilled water) was placed in the evaporator cup and heated under the same conditions. The oil and water masses were mixed at the same temperature until cream was formed. Nipagin was dissolved in distilled water and then mixed into the cream. Neem oil was added to the base cream. The cream was then mixed until homogenous.[9,10]

Acclimatization of the animals

The experimental animals used were New Zealand albino male rabbits (6-8 weeks) with the weight of 1.5-2 kilograms. The animals were normally fed and maintained in a room with good air circulation, temperature, and lighting. The animals were acclimatized for 5 days. The body weight of the animals were observed every day. The animals that lost more than 10% of their body weight during acclimatization were excluded.[8]

Acute irritation test

A dermal acute irritation test was performed on healthy skin. Animal hair was shaved on the back area (2 x 3 cm). An irritation test was performed by applying 0.5 g of cream on the dorsal. It was then covered with gauze and plaster. The animals were divided into 2 group, *i.e.,* control formula (base) and 5% neem oil cream. Each group has 3 animals. The degree of irritation was assessed after 1 hour, 24, 48, and 72 hours after exposure.[11] Primary irritation index was calculated with the following formula:

$$\text{Primary Irritation Index} = \frac{(A-B)}{C}$$

Information:

A: The number of erythema and udema in tested observation area after 24, 48 and 72 hours divided by the number of observations.

B: The number of erythema and udema in control observation area after 24, 48 and 72 hours divided by the number of observations.

C: Number of animals

Table 1. Irritation Response Category on Rabbit [9]

Average	Category
0.0-0.4	Negligible
0.5-1.9	Slight
2.0-4.9	Moderate
5.0-8.0	Severe

Anti-scabies effectiveness test

The *S. scabei* were injected to the back skin of animal. The transmission was performed indirectly, *i.e.,* by swabing the rabbit dorsal infected with scabies into gauze and then affixed to the back of the test animal using plester. The incubation period of *S. scabiei* is 14 days from eggs to adult mites. Thus, the rabbit is left 2-3 weeks until the parasites spreads evenly to the surface of the skin.

The effectiveness of anti-scabies cream was calculated using the Federer formula.[11,12] New Zealand male rabbits were divided into 3 groups, *i.e,* the test group (5% neem oil cream), the positive control (5% permetherin cream), and the negative control group (cream base). The effectiveness of the cream was performed on rabbits infected with scabies by applying the cream to the infected skin once a day and left for 8 hours before it was cleaned. Observations were conducted every day for 1 month. Examination of wound healing was conducted by observing the wound closure. Score of 1-5 was given in each observation (Table 3). Data analysis was performed using the Kruskal Wallis test and the Mann Whitney test. The Kruskal Wallis test was used to examine value differences in more than two groups. The Mann Whitney test was used to evaluate significant differences between categories.

Results and Discussion

Cream formulation

5% neem oil cream had thick consistency, faded yellow white co, distinctive odor, pH 8, and viscosity around 4195 Cps. Cream was selected as a dosage form since it has good ability to spread on the skin surface and is more convenient for the animals. The pH value was within the recommended

Table 2. Assessment of Skin Reactions[10]

Erythema	Score
There is no erythema	0
Very small erythema (almost indistinguishable)	1
Erythema is clearly visible	2
Moderate to severe erythema	3
Severe erythema (flesh red) to eschar formation which inhibits erythematous assessment	4

Edema	Score
There is no edema	0
Edema is very small (almost indistinguishable)	1
Small edema (visible area boundary)	2
Mid-level edema (increase in area by about 1 mm)	3
Severe edema (the area increases more than 1 mm and widens beyond the exposure area)	4

Table 3. Assessment of the Effectiveness of the Cream1[1]

No	Assesment	Score
1	The wound closes 100% (the wound closes completely and has no scab)	1
2	The wound closes 75% (the wound closes and has few scabs)	2
3	The wound closes 50% (the wound partially closes and has few scabs)	3
4	The wound closes 25% (the wound slightly closes and has many scabs)	4
5	The wound completely opens and has many scabs	5

range based on the Regulation of the Head National Agency of Drug and Food Control No.7/2014. Cream should not be administered to the skin if it has extreme pH (pH ≤ 2 or ≥ 11.5).[8]

Dermal acute irritation test

The irritation test aimed to evaluate the safety of the neem oil cream by observing the the level of eryhtma and edema. Albino rabbit was selected as it has sensitive skin. Beside, the color of skin is brighter than the other type of rabbits. These characteristics allowed straightforward observation on the skin irritation. The primary irritation index for 5% neem oil cream is 0, indicating very negligible irritation category (a range of 0.0-0.4). Thus, it was safe to be applied to the skin.

Anti-scabies effectiveness

We found that 5% neem oil was effective as an anti-scabies with 20-21 days recovery time (Table 4 and 5). The recovery time is longer than permethrin cream (7-8 days), but shorter compared to negative control with recovery time over 30 days. Statistical analysis showed that there was significant difference between three dose groups. Azadirachtin compound in neem oil acted as an anti-parasites. This compound affects the hormonal cycle of parasites, prevents the growth and development of mites, makes the eggs sterile, and gives an antifeedant effect.[9-14] Neem oil does not terminate scabies mites directly, but interfere with the proliferation and reproduction of mites, thus there is a reduction in the population of parasites over time. Therefore, the application of the cream should be conducted daily. Besides, the cream can also relieve itching and moisturize cracked skin.[15,16]

The 5% permethrin cream was used as the positive control because it is the first line of anti-scabies drugs. Permethrin cream has very low toxicity to mammals but the results of *in vivo* studies showed children are more sensitive to permethrin.[1,16] Besides, it has very little absorption and was metabolized quickly. It was excreted through sweat, sebum, and urine.[17-19] Nevertheless, its recovery time is shorther than neem oil.

This study has limitation, particularly in the transmission process of *S. scabiei.* The administration of parasites on rabbit dorsal

Table 4. Comparison of Recovery Times

Group	Average (day)	p-value	
5% neem oil cream	20.75 ± 1.71		
5% permethrin cream	7.50 ± 0.58	0.000	Significant difference
cream base	31.00 ± 0.00		

parts is relatively difficult. Besides, we did not perform the assessment to evaluate the spread of scabies in the dorsal skin. Microscopic testing with the use of 10% KOH as keratolytic could be conducted.[20]

Conclusion

A. indica oil cream was effective for the treatment of scabies although its recovery time is shorter than permethrin cream.

Acknowledgements

None declared.

Funding

This research was funded by the 2017 Padjadjaran University Internal Grant.

Conflict of Interest

None declared.

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