

Effectiveness test of neem oil cream (*Azadirachta indica* A. Juss) as antiscabies in New Zealand Rabbits

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ABSTRACT

Aim: This study aims to produce a cream formula made of neem oil (*Azadirachta indica* A.Juss) as anti-scabies with the best formula, optimal effectiveness and affordable price. **Method:** The method used is experimental method, variations of neem oil concentration in the cream preparations are made to determine which one is the most effective as anti-scabies. Physical stability of all cream preparations is determined by storing them for 3 months at 30±2 °C and 40±2 °C 75±5% RH and doing several physical evaluations every week including organoleptic, Homogeneity, pH, cream type and viscosity. Irritation test and effectiveness test of anti-scabies are done on rabbits, in which the use and the handling procedures of the laboratory animals must be approved by the committee of The Ethics of Animal Experimentation. **Result:** The effectiveness of neem oil 30% cream is better than that of 10% and 20%. However, compared to permethrin 5% cream, permethrin 5% cream is better in term of effectiveness. It can be seen from its speed in repairing or healing scabies. In average, neem oil 30% cream shows healing signs on Day-10 while the positive control (permethrin 5% cream) shows healing signs on Day-8. **Conclusion:** The effectiveness of permethrin cream 5% is better than the neem oil cream. Neem oil 30% cream is the best formula and the most effectiveness as anti scabies.

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

Introduction

Scabies is one of the infectious skin diseases caused *Sarcoptes scabiei var hominis* mites featured with oval and transparent shape, convex back, flat stomach, no eye and cannot be seen with naked eyes [1]. Scabies is also known as itch in Bahasa : *kudis, gudig, budukan and gatal agogo*.

According to World Health Organization (WHO) scabies is one of the most common dermatological conditions and mostly occurs in developing countries. Globally, scabies can affect more than 130 million people each year with occurrences ranging from 0, 3% to 46%. The highest level of scabies occurs in tropical countries with high levels of population density and relatively low socio-economic status [1].

Clinical symptoms of scabies are indicated itching on the skin. This itchy skin is characterized by red pimple-like rash and redness with allergies, that can also develop into small pus-filled blister.[2]

Scabies includes two main types of skin lesions, i.e. tunnel and rash. Tunnel is mainly found on the hands and feet (between fingers, wrists, and foot). In infants, tunnel often occurs in the palms of the hands, soles of the feet, and probably also on the body, head, and neck. The tunnel is a few-millimeter long, usually tortuous, and has a vesicle on one end adjacent to

the mites digging tunnels, and often surrounded by mild erythema. The tunnel can also be found in male genitalia, usually covered by inflamed papules, and the papules found in the penis and scrotum are pathogenic for scabies. The scabies rash is an inflamed small papular eruption. It is usually present around the axilla, umbilicus, and thighs. This rash is a body allergy to mites [3].

Based on Health Department of Republic of Indonesia, the occurrence of scabies is 5,6-12,95% according to 2008 data of health centers throughout Indonesia. In Indonesia, scabies ranks the third of 12 most common skin diseases [4]. Scabies can effect both men and women at all ages, race and social class. However, it becomes a major problem in densely populated areas with social disturbance, poor sanitation, and weak economy. Scabies can be transmitted through direct or indirect physical contact with the patient [5]. Topical medications are currently used for Scabies management such as permethrin 5% cream, sulfur precipitatum 5-10% cream, benzyl benzoate 10-25%, crotamiton 10% cream, and gamma benzene hexachloride 1% cream (Lindane lotion 1%) [6].

Resistance has been reported with drugs such as lindane, permethrin, and crotamitone. Resistance to lindane has been reported in El Salvador, Peru, Panama, New Zealand, Egypt, and from 18 different locations in the United States [7].

The use of medicinal plants as treatment alternatives are growing bigger. Neem (*Azadirachta indica* A.Juss) is a plant well known by the people of Indonesia and one of the potential medicinal plants because almost all parts of this plant can be used as a medicine (biopharmaca), cosmetics, and natural pesticides. Neem can be used for ulcer treatment, as antibacterial, insecticide, antifungal, antimalarial, anti-inflammatory, anti-pyretic, antihistamine, antiprotozoal, and many more [6].

Neem oil has been used in various countries for anti-parasitic and anti-scabies treatment. Moreover, the availability of neem oil as a raw material of medicine is quite plentiful and easy to obtain. Previous research result shows that the use of a mixture of neem oil and turmeric in scabies patients makes 97% improvement in 814 patients after receiving therapy for 3 - 15 days [7]. The study by Tabassam et al also proves the effectiveness of ointment methanol with 20% neem seed extract against *Sarcoptes scabiei* infestation on sheep.

The effectiveness of neem oil for anti-scabies also has been tested by Zainal (2013) using clinical trial method before and after applying cream, comparing with permethrin 5% cream, and the test result shows that permethrin 5% cream is more effective than cream with 10% neem seed extract in terms of clinical recovery. Meanwhile, dermatologically, the effectiveness of 10% neem seed extract cream for scabies therapy has no difference from permethrin 5% cream. Therefore, to obtain clearer result of the effectiveness of neem seed extract cream, it should be tried in higher concentrations [8].

Methods

Materials

The materials used in this study are Neem oil, adeps lanae (Brataco), stearic acid (Brataco), glycerin (Brataco), sodium tetraborate (Brataco), triethanolamine (Brataco), methylparaben (Brataco) and aqua destillata.

The laboratory animals used are 6-to-8-week male albino New Zealand rabbits with weighed 1.5-2 kilograms obtained from Biofarma Animal laboratory. These laboratory animals are fed and given drink normally. They are kept in animal housing rooms with good lighting, temperature, humidity and air circulation. The use and handling procedures of the laboratory animals must be approved by the committee of The Ethics of Animal Experimentation, Ethics no. 881/UN6.C.10/PN/2017.

The Cream Formula

A formula in the form of vanishing cream is selected as the cream base through selection and then given concentration variations of neem oil as presented in Table 1.

Table 1 The Formula of Cream Preparations

Materials	F0	F1	F3	F4
Neem Oil (%)	-	10	20	30
Adeps lanae (g)	1	1	1	1
Stearic acid (g)	14.2	14.2	14.2	14.2
Glycerin (g)	10	10	10	10
Sodium tetraborate (g)	0.25	0.25	0.25	0.25
Triethanolamine (g)	1	1	1	1
Aqua destillata (g)	75	75	75	75
Methylparaben (%)	0.1	0.1	0.1	0.1

Note:

F0 = Formula does not contain neem oil

F1 = Formula contains 10 % neem oil

F2 = Formula contains 20 % neem oil

F3 = Formula contains 30 % neem oil

The Making of the Cream

Adeps lanae and stearic acid are melted in a vaporizer plate and heated over a water bath at 70°C (mass 1). Then, glycerin, sodium tetraborate, triethanolamine and distilled water are put in a vaporizer plate and heated over a water bath at 70°C (mass 2). The nipagin diluted in hot water is put in mass 2 and stirred homogeneously. At the same temperature, mass 1 and mass 2 are mixed in the super mixer and stirred constantly until homogeneous cream mass is formed. Furthermore, neem oil is added and stirred until homogeneous.

The Acclimatization of Laboratory Animals

Laboratory animals are acclimatized for 5 days. Their weight is observed daily. Those with weight loss more than 10% during the acclimatization period are not used for the study [9].

Acute Dermal Irritation Test

This test is conducted for all cream formulas with various neem oil concentration. The hair of the animals' back area is shaved. Test preparations are exposed on the skin area ± 6 (2 x 3) cm². The animals used in this experiment are animals with healthy skin. Irritation test is conducted by applying 0.5 g preparation cream on the rabbits' back and then covering the area with gauze and plaster. 3 laboratory animals are used for each formula (control (base), neem oil 10%, 20%, and 30% cream). The irritation degree is assessed at certain time intervals i.e. at hour- 1, 24, 48 and 72 after test preparations' exposure. The response category is assessed by Primary Irritation Index [9].

Sarcoptes scabiei Infestation

The rabbits' back is shaved, and then one group of *Sarcoptes scabiei* mites is infested from animals with scabies. The back area infested *Sarcoptes scabiei* is covered with gauze and plaster. Furthermore, they are left about 7-10 days in order that *Sarcoptes scabiei* mites can evenly spread out on the skin surface. After the scabies evenly spread out, a treatment therapy is done with the variations of neem oil concentration [10].

Effectiveness Test of Anti-scabies Cream

The number of samples is calculated by Federer's formula [11]:

$$(t-1)(n-1) \geq 15$$

Note:

t = the number of experimental groups

n = the number of repetitions or replication or number of samples per group

24 male New Zealand rabbits are divided into 6 test groups, and 3 replications for each formula. Group 1, 2, and 3 are given neem oil 10%, 20%, and 30% cream respectively, Group 4 functions as a positive control, and Group 6 as a negative control. Effectiveness test of neem oil cream is done to rabbits infected with scabies, namely by applying the cream on rabbits' part of body infected with scabies once a day and leaving it for 8 hours. Then, the skin is cleaned and the observation is done every day for a month. The effectiveness test is done to all cream formulas with permethrin 5% cream as the positive control and cream containing no neem oil as the negative control. The examination is conducted through observations on the wound healing. The wound healing is measured by parameter in the form of wound closure after neem oil cream applied with concentration variations. Then, it is scored from 1 to 5.

Statistical analysis

Statistical analysis of irritation test and antiscabies activity is performed using non parametric data analysis using Kruskal Wallis test. This test is used to learn the average difference of more than two groups. When H1 is accepted, a post hoc test will be performed using Mann Whitney U test. Mann Whitney test is performed to examine significant differences among categories.

Results

The Acclimatization of the Laboratory Animals

Before the test is performed, laboratory animals have been acclimatized first in test room for 5 days and put in individual cage (1 cage for 1 laboratory animal). Acclimatization is an adaptation process of an organism from its original environment to a new one with different physical and chemical condition in order to make the laboratory animals familiar with the laboratory atmosphere and to avoid stress that will affect the results of the study. The laboratory temperature is at room temperature with humidity between 40 - 60%.

The rabbits were fed according to standard, pellets and ad libitum. During the acclimatization, observation of behavior, ability to consume food, and weight weighing are done every day.

Based on Indonesian Pharmacopoeia 3rd edition, animals are said to be healthy if they do not undergo body weight change more than 10%, and show normal behavior during the maintenance. From the results of the acclimatization of laboratory animals for 5 days, all of the 24 laboratory animals are stated healthy since there is no one of them having weight loss more than 10%. This result indicates that the rabbits to be used for the study qualify Pharmacopoeia Indonesia Edition III and are healthy (Table 2).

Tabel 2. The Acclimatization of Laboratory Animals

No	Animals	Weight (kg)				
		Day-1	Day-2	Day-3	Day-4	Day-5
1	Rabbit -1	2.126	2.087	2.044	2.102	2.118
2	Rabbit -2	1.978	1.947	1.958	2.027	1.987
3	Rabbit -3	1.865	1.766	1.834	1.817	1.796
4	Rabbit -4	1.928	1.888	1.837	1.876	1.906
5	Rabbit -5	1.900	1.967	1.983	1.851	1.899
6	Rabbit -6	1.988	1.902	1.862	1.968	2.001
7	Rabbit -7	1.825	1.864	1.640	1.755	1.877
8	Rabbit -8	2.080	2.155	2.085	2.146	2.172
9	Rabbit -9	1.850	1.874	1.744	1.861	1.875
10	Rabbit -10	1.775	1.641	1.684	1.732	1.785
11	Rabbit -11	1.805	1.752	1.766	1.792	1.786
12	Rabbit -12	1.920	1.854	1.820	1.876	1.792
13	Rabbit -13	1.736	1.645	1.611	1.688	1.701
14	Rabbit -14	1.695	1.634	1.700	1.712	1.724
15	Rabbit -15	1.834	1.766	1.734	1.769	1.796
16	Rabbit -16	1.828	1.788	1.694	1.756	1.782
17	Rabbit -17	1.766	1.798	1.787	1.951	1.902
18	Rabbit -18	1.874	1.852	1.865	1.848	1.822
19	Rabbit -19	1.724	1.762	1.786	1.755	1.748
20	Rabbit -20	1.680	1.696	1.711	1.728	1.736
21	Rabbit -21	1.750	1.774	1.796	1.761	1.785
22	Rabbit -22	1.725	1.698	1.684	1.632	1.686
23	Rabbit -23	1.755	1.732	1.786	1.720	1.764
24	Rabbit -24	1.880	1.874	1.860	1.836	1.862

The irritation test is performed on 15 laboratory animals (3 for each test formula and control formula). The laboratory animals used are 6-to-8-week healthy male albino New Zealand rabbits (*Oryctolagus cuniculus*) with a weight between 1.5-2.5 kg. The albino rabbits have sensitive skin and their hair is more easily shaved than other rabbits. In addition, these rabbits has white skin so it will be easily observed in the case of irritation.

This irritation test is performed to examine the safety of the neem oil cream and to determine the level of erythema and oedema as well as its primary irritation index on the skin and to assess and evaluate the characteristics of a substance when exposed to the skin (Table 3).

Tabel 3. Acute Dermal Irritation Test

Treatment		1 hour		Σ	24 hour		Σ	48 hour		Σ	72 hour		Σ	Σ
		^o erythe ma	^o oed ema		^o erythe ma	^o oed ema		^o erythe ma	^o oede ma		^o erythe ma	^o oed ema		
Control	Rabbit 1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rabbit 2	0	0	0	0	0	0	0	0	0	0	0	0	
	Rabbit 3	0	0	0	0	0	0	0	0	0	0	0	0	
	Amount			0			0			0			0	
F 1	Rabbit 1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rabbit 2	0	0	0	0	0	0	0	0	0	0	0	0	

	Rabbit 3	0	0	0	0	0	0	0	0	0	0	0	0	0
	Amount			0				0					0	0
F 2	Rabbit 1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rabbit 2	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rabbit 3	0	0	0	0	0	0	0	0	0	0	0	0	0
	Amount			0				0					0	0
F 3	Rabbit 1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rabbit 2	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rabbit 3	0	0	0	1	0	1	0	0	0	0	0	0	0
	Amount			0			1			0			0	1
F 4	Rabbit 1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rabbit 2	0	0	0	1	0	1	0	1	1	1	0	1	1
	Rabbit 3	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total			0			1			1			1	3

Sarcoptes scabiei Infestation

The infestation is done on the shaved back of the rabbits. As for the *Sarcoptes scabiei* mites used, they are obtained from other rabbits infected with scabies. The transmission is done by swabbing the scabies affected parts to the gauze, and then attached it to the back of the laboratory animals using non-irritant plasters. Furthermore, since the incubation period of *Sarcoptes scabiei* mites takes 14 days, after the infestation process, the rabbits are left for 2-3 weeks until the *Sarcoptes scabiei* mites evenly spread out on the surface of their skin in accordance with the life cycle of *Sarcoptes scabiei* which takes about 10-14 days for the eggs to become adult mites.

The Effectiveness Test of Anti-scabies Cream

All rabbits are divided into 6 test groups, 4 replications for each formula. Effectiveness test of neem oil cream is done to all formulas of neem oil cream with a positive control using cream available at the market, namely permethrin 5% cream as a positive control and vanishing cream as negative control. Every formula contains active neem oil varying from one to another

Table 4. Effectiveness Test of Anti-scabies Cream

Day	Cream 10%				Cream 20%				Cream 30%				Positive control				Negative control			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	5	5	5	5
3	5	5	5	5	4	4	4	4	4	4	4	4	4	3	4	4	5	5	5	5
4	4	4	4	5	4	4	4	4	4	4	4	4	3	3	4	3	5	5	5	5
5	4	4	4	4	3	4	3	4	4	3	3	3	3	2	3	2	5	5	5	5
6	4	4	4	4	3	3	3	3	3	3	3	3	2	2	3	2	5	5	5	5
7	4	4	4	4	3	2	3	3	3	2	3	3	2	1	2	1	5	5	5	5
8	4	4	4	4	2	2	2	3	2	1	2	2	1	1	1	1	5	5	5	5
9	4	3	4	4	2	2	2	2	2	1	2	2	1	1	1	1	5	5	5	5
10	4	3	4	4	2	1	2	2	1	1	1	1	1	1	1	1	5	5	5	5
11	4	3	4	4	2	1	2	2	1	1	1	1	1	1	1	1	5	5	5	5
12	3	3	3	3	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5

13	3	3	3	3	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
14	3	2	3	3	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
15	3	2	3	3	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
16	3	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
17	3	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
18	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
19	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
20	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
21	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
22	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5

Data Analysis

Table 5. Comparison of Recovered Times in five Dose Groups

Dose Groups	Average	p-value	Note
Cream 10%	14.00	0.000	There is a difference
Cream 20%	11.50		
Cream 30%	9.50		
Negative control	7.50		
Positive control	31.00		

Table 6. The Difference of Recovered Time

Dose Groups	Day Heal
Cream 10%	14.00 ± 0.82^{ab}
Cream 20%	11.50 ± 1.00^{ab}
Cream 30%	9.50 ± 1.41^{ab}
Negative control	7.50 ± 0.58^b
Positive control	31.00 ± 0.00^a

Discussion

The principle of dermal acute irritation test is the exposure of the test dosage in a single dose to the previously shaved rabbit's back. The degree of irritation was assessed at the hour interval of 1, 24, 48 and 72 hours after the exposure of the test preparation. Animals that show signs of agonizing pain or suffering should be sacrificed in accordance with the procedures of animal reduction. The dosage of the neem oil cream used is 0.5 g.

Skin irritation of a preparation is an important element of safety [12]. Positive skin irritation is indicated by erythema and oedema in the treated skin area. Erythema is a redness

of the skin produced by capillary vessel congestion. While oedema is an abnormal accumulation of fluid under the skin or in one or more body cavities.

Skin irritation score (Primary Irritation Index) is assessed along with the severity of the wound. Individual scores of skin reactions as shown in Table 3 do not represent a definite standard for irritant properties of the test preparation. The test irritation scores are the total number of erythema and oedema scores of all observation points of the samples at hour- 24, 48 and 72 divided by the total number of observations minus the number of erythema and oedema score of all observation point of the control at hour- 24, 48 and 72 divided by the number of observations, and then divided by the total number of the laboratory animals. The results of the assessment of primary irritation are presented in the form of irritant response category as in Table 3. The observation results show the erythema and Oedema score at hour- 24, 48 and 72. The Primary Irritation Index of neem oil 10%, 20%, and 30% cream is 0; 0.111; and 0.333 respectively. Accordingly, the four formulas of neem oil cream include in the category of negligible irritation response with range from 0.0 to 0.4, therefore they are safe to use.

Neem oil is obtained from neem seeds containing a high azadirachtin compound that acts as an anti-parasite. Azadirachtin affects the parasitic hormonal cycle, prevents the growth and the development of mites, makes the eggs sterile, and acts as an antifeedant (making them stop eating, preventing them from eating or allowing them to eat but in limited frequency).

Neem oil does not directly kill the scabies mites, but it interferes with their reproduction and reproduction thereby reducing their population over time. Therefore, scabies treatment using neem oil cream is done every day not only to treat the scabies, but also to calm down the itching and to soften and moisturize the dry and cracked skin.

Permethrin 5% cream is used as a positive control because this cream is the first option in the scabies treatment. It has a very low toxicity to mammals and its poisoning tendency due to errors is also very low. In addition, it also has a very little absorption power in the skin and can be rapidly metabolized and then released back through sweat and sebum as well as through the urine.

Effectiveness of the test results, 30% neem oil cream is more effective when compared with neem oil cream 10% and 20% (Table 4). However, when compared with permethrin cream 5%, permethrin cream 5% more effective, this is indicated by faster healing time compared with 30% neem oil cream. For the negative control, there is no improvement, but rather worsen the situation of scabies. The higher the concentration of neem oil used, the faster the rate of improvement or the healing rate of scabies. During the therapy, the cream base, neem oil 10%, 20%, and 30% cream, also permethrin 5% cream as well, are well tolerated since there is no irritation or allergic reaction from the treatment to all laboratory animals from the five groups.

Table 5 shows the result of comparison test using Kruskal Wallis. Obtained p-value of $0.000 < 0.05$, then H_0 rejected. This suggests that there is a significant (significant) difference between the healing time in the five dose groups. Furthermore, to find out which dose groups had different healing time, a further test was performed using Mann Whitney. Table 6 shows that each cream concentration has a difference of time healed with positive controls as well as negative controls. In addition it can be seen also that; 10% cream takes 14 days to heal; 20% cream takes 11-12 days to heal; 30% cream takes 9-10 days to heal; Positive control takes 7-8 days to heal; and Negative Control takes more than 30 days to recover. Can be seen also the order of dosage with the fastest healing time until the longest is Positive Control, Cream 30%, Cream 20%, 10% Cream, and Negative Control.

This study is in line with a research by Nasriyani et al 2013 in a journal entitled Effectiveness test of 10 % neem oil cream (*Azadirachta indica* A. Juss) on scabies patien using a research method through clinical trials of before and after the use of cream compared with

permethrin 5% cream. In terms of clinical cure, permethrin 5% cream is more effective than neem seed extract 10% [13]. However, dermoscopic effectiveness of neem seed extract 10% cream for scabies therapy is not different from permethrin 5% cream. In addition, there is another study on the effectiveness of neem oil as an antiscabies conducted by Charles V and Charles SX using a mixture of neem and turmeric oils in patients with scabies, and the results show 97% [13].

Conclusion

The effectiveness of neem oil 30% cream is better than that of 10%, and 20%. However, compared to permethrin 5% cream, permethrin 5% cream is better in term of effectiveness. It can be seen from its speed in repairing or healing scabies. In average, neem oil 30% cream shows healing signs on day-10 while the positive control (permethrin 5% cream) shows healing signs on day-8.

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