

***In Vitro* Effectiveness of Neem Oil (*Azadirachta indica* A. Juss) Shampoo as Anti Head Lice (*Pediculus humanus capitis*)**

Norisca A. Putriana, Azizul Hakim, Patihul Husni, Taofik Rusdiana

Department of Pharmaceutical and Technology Pharmacy,
Faculty of Pharmacy, Universitas Padjadjaran, Bandung, West Java, Indonesia

Abstract

Head lice (*Pediculus humanus capitis*) is a parasite causing hair problem. The aim of this study was to evaluate *in vitro* effectiveness of the neem oil shampoo formulation as an anti head lice. We formulated four different concentration (5%, 10%, 15%, 20%) of neem oil and evaluated the shampoo, included skin irritation test, pediculicidal, and ovicidal activity of the shampoo. Skin and eye irritation tests were carried out by applying 0.5 ml shampoo to the skin and dripping 0.1 ml into the eyes of newzealand white rabbits, respectively. The data were analyzed by using Kruskal Wallis and Mann Whitney. The primary irritation index of 5%, 10%, 15%, and 20% neem oil shampoo respectively were 0.00; 0.0111; 0.222; and 0.222; which categorized as very mild irritation. Only the 20% neem oil shampoo showed a mild irritation to the eyes (the degree of corneal opacity ≥ 1) and recovered after 7 days of observation. All formulas of neem oil shampoo were effective as an anti lice, however, the 15% and 20% neem oil shampoo had similar effectiveness compared with 1% permethrin lotion. In conclusion, the 15% neem oil shampoo was the best anti head lice shampoo.

Keywords: Head lice, neem oil (*Azadirachta indica* A. Juss), shampoo

Introduction

Head lice or *Pediculus humanus capitis* is a parasite known as ectoparasites. Head lice are often found on hair and scalp, which are easily transmitted only through physical contact.¹

Head lice management is important to prevent secondary infections.² People usually treat head lice problems by using topical products, including Ivermectin (semi-synthetic macrocyclic lactone)³⁻⁶ and chemical compounds (organochlorides, natural and synthetic pyrethrins, carbamates), but these

treatments may induce resistance. However, neem oil is an alternative herbal shampoo to treat head lice that have pediculicidal activity.^{3,7}

The clinical trials of neem oil products are still limited to support therapeutic claims, but the use of neem plants has much developed.⁸ Neem plants contain several active substances, including azadiractin, nimbolides, nimbidin, nimbin, salanin, gedunin, meliacin, and valassin. Triterpene, an active metabolite of azadiractin, is effective (90%) against head lice.⁹ Mechanism of action remains

Corresponding author: Norisca A. Putriana. Department of Pharmaceutical and Technology Pharmacy, Faculty of Pharmacy Universitas Padjadjaran. email: norisca@unpad.ac.id

Received: 25 November 2019. **Revised:** 20 December 2019. **Published:** 28 December 2019.

unclear; however, neem oil works as appetite suppressant and cause death.^{10,11}

Previously, a mortality rate study of neem oil was performed. The result showed that neem oil concentration of 20% was the most effective against head lice (mortality rate 100% in 120 minutes), while the same concentration killed 50% of the nits in 9 days. *In vitro* evaluation needs to be conducted to ensure the safety and effectiveness of the shampoo before being clinically used in human. Therefore, this study was conducted to evaluate safety and effectiveness of neem oil as an anti head lice.

Methods

This study was approved by the Health Research Ethics Commission of the Faculty of Medicine, Padjadjaran University, Indonesia (No. 444/UN6.KEP/EC/2018).

Shampoo Formulation

Certificated neem oil was obtained from Happy Green Co, Jakarta, Indonesia. The shampoo contained: 5% neem oil, 13.6% sodium lauryl sulfate, 4% cocamidopropyl betaine, 5% coco-glycoside, 10% PEG-7 glyceryl cocoate, 7.6% PEG-40 hydrogenated castor oil, 0.2% guar hydroxypropyltrimonium chloride, 2% glycerin, 3% cocamide DEA, 4% acrylate co-polymer, 0.1% vitamin E, 0.18% methylparaben, 0.02% propylparaben, 2% perfume (jasmine) and deionized water. The oil phase (vitamin E, PEG-7 glyceryl cocoate and PEG-40 hydrogenated castor oil) was placed in a beaker and stirred until homogenous. Water phase (guar hydroxypropyltrimonium chloride, glycerin, sodium lauryl sulfate, cocamidopropyl betaine, coco-glycoside and deionized water) were mixed until homogenous. Then, the oil and water phases were mixed until shampoo was formed. Methylparaben and propylparaben were dissolved in deionized

water and poured into the shampoo base. Lastly, neem oil and perfume were added to the shampoo bases.

Acclimatization of the animals

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

Acute dermal irritation test

The fur of the rabbit was shaved on the back area of approximately 10x15 cm or not less than 10% of the body surface for the exposure place of test preparation. Shaving started from the shoulder blades area (shoulder) to the groin bone (waist bone) and half down the body on each side. The irritation test was carried out by applying 0.5 ml of shampoo to the back of the rabbit and exposed to an area of 2x3 cm² of skin and covered with gauze and plaster.

The rabbits were divided into 6 groups; negative control (F0), positive control (1% permethrin lotion), 5% neem oil (F1), 10% neem oil (F2), 15% neem oil (F3), and 20% neem oil (F4). The degree of irritation was assessed at certain time intervals, respectively at 24, 48, and 72 hours after exposure to the test preparation. The degree of irritation was according to WHO testing guidelines and ISO 10998.¹⁰ The response category was assessed with a primary index BPOM RI.¹²

Eye irritation test

0.1 ml of F0, F1, F2, F3, F4, and permethrin were dripped in the conjunctival sack of the rabbit eyes.¹³ Observations were made at 1, 24, 48, and 72 hours after administration. The parameters observed were effects on the

Table 1. Primary Irritation Index

Shampoo	Primary Irritation Index (n-15)	Response Category
F1	0 (0 ± 0.43)	Very Light
F2	0.111 (0.37 ± 0.69)	Very Light
F3	0.222 (0.62 ± 0.48)	Very Light
F4	0.222 (0.62 ± 0.85)	Very Light

Table 2. Average Score of Eyes Observation Value

Shampoo	Cornea	Iris	Conjunctiva	Udema
F1	0	0	0.67	0
F2	0	0	0.33	0
F3	0.33	0	0.67	0.33
F4	2.33	0	1.67	1.67
Light Irritant				

cornea. If the test animal did not show eye injury, the test could be ended on the 3rd day after the administration of the test preparation.

Collection of Headlice and its nits

Head lice and nits' samples were taken from five pediatric patients in Hegarmanah Village, Jatinangor. Informed consent was signed by the parents. Head lice were taken using a specific comb. Inclusions criteria was patients without head lice treatment at least a month before sampling. Head lice and its nits were collected into petri dish.¹⁴

Pediculicidal and Ovicidal Activity Assay

Head lice were divided into 6 groups (n=10) and inserted into 6 petri dish containing filter paper that has been immersed with F0, F1, F2, F3, F4, and permethrin for 10 minutes. This assay was carried out by following by International Standard.¹⁴ Mortality rates were calculated by employing previous method.¹⁵

Results and Discussion

Physical Properties of Neem Oil Shampoo

The physical properties of neem oil shampoo was viscous (55.76 Ps), yellowish brown, distinctive odor, and pH 6.75.

Acute Dermal Irritation Test

Based on the results, all shampoo formulas were categorized as very light irritation. Thus, this shampoo formulas were safe to be used. The result is provided in Table 1.

Eyes Irritation Test

The eye irritation test indicated that neem oil shampoo did not cause any irritation, however the highest concentration showed mild irritant due to its high corneal opacity grade (2.33). The average results of score values is presented in Table 2.

Pediculicidal Activity of Neem Oil Shampoo

F3 and F4 showed 100% mortality to headlice in shorter time and had similar effect to 1% permethrin (Table 3).

Ovicidal Activity of Neem Oil Shampoo

Neem oil shampoo in all concentrations did not show 100% mortality rate, including 1% permethrin. F3 and F4 showed 37.5% mortality, eventhough 1% permethrin has the highest effectiveness (Table 4).

Conclusion

Based on the study, it can be concluded that neem oil shampoo in all concentrations were

Table 3. Neem Oil Shampoo Corrected Mortality

%Neem Oil Shampoo	Corrected mortality (%) n=10										
	Time (minute)										
	5	10	20	30	60	120	360	480	720	1440	2160
F1	0	13.33	10.34	31.03	37.93	60.71	70.37	85.19	100	100	0
F2	6.67	13.33	24.14	31.03	52	60.71	74.07	100	100	100	0
F3	13.33	23.33	27.58	51.72	69	75	100	100	100	100	0
F4	13.33	23.33	31.03	58.62	86.2	96.43	100	100	100	100	0
Permethrin	16.67	23.33	41.38	65.52	82.76	92.86	100	100	100	100	0

Table 4. Neem Oil Shampoo Corrected Mortality

Neem Oil	% Corrected Mortality
F1	25
F2	37.5
F3	37.5
F4	37.5
Permethrin	63

effective as an anti lice. However, the 15% neem oil shampoo was chosen as the best shampoo because of its high anti lice effect and light irritation response.

Acknowledgements

None declared.

Conflict of Interest

None declared.

References

1. Sembel DT. Entomologi kedokteran edisi I. Yogyakarta: Percetakan Andi; 2009.
2. Handoko RP. Pedikulusis dalam ilmu penyakit kulit dan kelamin edisi III. Jakarta: Balai Penerbit FK UI; 2002.
3. Burkhart CG, Burkhart CN, Burkhart KM. An assessment of topical and oral prescription and over the counter treatments for head lice. *Journal of the American Academy of Dermatology*. 1998;38(6):979–983.
4. Jones KN, English JC. Review of common therapeutic options in the United States for the treatment of Pediculosis capitis. *Clinical Infectious Diseases*. 2003;36(11):1355–1361.
5. Develoux M. Ivermectin. *Annales de dermatologie et de vénéréologie*. 2004;131(6–7 Pt 1):561–570.
6. Hotson IK. The avermectins: a new family of antiparasitic agents. *Journal of South African Veterinary Association*. 1982;53(2):87–90.
7. Mac-Mary S, Messikh R, Jeudy A, Lihoreau T, Sainthillier J, Gabard B, Schneider C, Auderset P, Humbert P. Assessment of the efficacy and safety of a new treatment for head lice. *International Scholarly Research Network (ISRN) Dermatology*. 2012;1-6.
8. Burgess IF. Human lice and their control. *Annual Review of Entomology*. 2004;49:457–481.
9. Girish K, Shankara BS. Neem – a green treasure. *Electronic Journal Biology*. 2008;4(3):102-111.
10. Nicoletti M, Petitto V, Gallo FR, Multari G, Federici E, Palazzino G. The modern

- analytical determination of botanicals and similar novel natural products by the HPTLC fingerprint approach. *Studies in Natural Product Chemistry*. 2012;37:217–258.
11. Brahmachari G. Neem – an omnipotent planta retrospection. *Chembiochem*. 2004;5:408–421.
 12. Cox C. Pyrethrins/pyrethrum insecticide factsheet. *Journal of Pesticide Reform*. 2002;22:14–20.
 13. BPOM RI. Peraturan kepala badan pengawas obat dan makanan republik Indonesia nomor 7 tahun 2014 tentang pedoman uji toksisitas nonklinik secara In Vivo. Jakarta: BPOM RI; 2014.
 14. International Standard ISO 10993-10. Biological evaluation of medical devices, part 10-tests for irritation and delayed-type hypersensitivity, second Edition. 2002.
 15. Kosalge SB, Fursule RA. Investigation of licicidal activity of some plants from Satpuda hills. *International Journal of PharmTech Research*. 2009;1(3):564–567.
 16. Kerry AG, Barnes TM. The efficacy of Australian essential oils for the treatment of head lice infestation in children. *Australasian Journal of Dermatology*. 2017;59:99–105.
 17. Abdel-Ghaffar F. Efficacy of a single treatment of head lice with a neem seed extract: an in vivo and in vitro study on nits and motile stages. *Parasitology Research*. 2012;110:277–280.
 18. Making cosmetics.com.. Shampoo Formulas. [online] Available at: https://www.makingcosmetics.com/Shampoo-Formulas_ep_90.html [accessed on November 2018].