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

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**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)³⁶ 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.³⁷

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, salalin, gedunin, meliacin, and valassin. Triterpene, an active metabolite of azadiractin, is effective (90%) against head lice.⁹ Mechanism of action remains
unclear; however, neem oil works as appetite suppressant and cause death.\textsuperscript{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. \textit{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.

\textbf{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).

\textit{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.

\textit{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.

\textit{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 cm\textsuperscript{2} 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.\textsuperscript{10} The response category was assessed with a primary index BPOM RI.\textsuperscript{12}

\textit{Eye irritation test}

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

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. Mortality rates were calculated by employing previous method.

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

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**Table 1. Primary Irritation Index**

<table>
<thead>
<tr>
<th>Shampoo</th>
<th>Primary Irritation Index (n-15)</th>
<th>Response Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0 (0 ± 0.43)</td>
<td>Very Light</td>
</tr>
<tr>
<td>F2</td>
<td>0.111 (0.37 ± 0.69)</td>
<td>Very Light</td>
</tr>
<tr>
<td>F3</td>
<td>0.222 (0.62 ± 0.48)</td>
<td>Very Light</td>
</tr>
<tr>
<td>F4</td>
<td>0.222 (0.62 ± 0.85)</td>
<td>Very Light</td>
</tr>
</tbody>
</table>

**Table 2. Average Score of Eyes Observation Value**

<table>
<thead>
<tr>
<th>Shampoo</th>
<th>Cornea</th>
<th>Iris</th>
<th>Conjuctiva</th>
<th>Udema</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0</td>
<td>0</td>
<td>0.67</td>
<td>0</td>
</tr>
<tr>
<td>F2</td>
<td>0</td>
<td>0</td>
<td>0.33</td>
<td>0</td>
</tr>
<tr>
<td>F3</td>
<td>0.33</td>
<td>0</td>
<td>0.67</td>
<td>0.33</td>
</tr>
<tr>
<td>F4</td>
<td>2.33</td>
<td>0</td>
<td>1.67</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Light Irritant
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


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