Effectiveness of neem seed oil methanolic extract shampoo versus permethrin shampoo in the reduction of head lice infestation in children*

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Abstract

Introduction This study compared the effectiveness and safety of neem (*Azadirachta* indica) seed oil methanolic extract shampoo against permethrin shampoo in reducing head lice infestation among children.

Methods A single-blind, non-inferiority, randomized clinical trial was conducted on children aged 6-14 years with pediculosis. Using block randomization, the participants were assigned to receive either 10% neem seed oil methanolic shampoo, 1% permethrin shampoo, or pure shampoo for three treatment applications at 10-day intervals. The presence of head lice after each application was determined by standard quadrant counting and compared with the baseline count within and among treatment groups.

Results There was a statistically significant difference in lice count after treatment for both neem and permethrin, with mean reductions of 17.8 ± 23.97 (p = 0.043) and 22.5 ± 23.47 (p = 0.014), respectively. Repeated Measures ANOVA showed a summary p-value of 0.041 for neem, 0.013 for permethrin, and 0.193 for the shampoo alone with a linear trend indicating a significant decrease in the lice counts from the baseline to the third application of neem and permethrin shampoo, but not in the shampoo group. There was no significant difference in the mean decrease in lice count from baseline to the third application between the neem and permethrin shampoo groups.

Conclusion Neem seed oil methanolic extract shampoo is non-inferior and comparable to permethrin in the reduction of head lice count. There were no reported dermatologic adverse effects such as burning sensations, redness, skin irritation, and allergic reactions.

Keywords: Pediculosis, neem seed shampoo, *Azadirachta* indica methanolic seed extract shampoo

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Head lice infestation remains to be a worldwide health concern that affects all age groups, especially preschool and elementary-age children. Females are infested more often than males, probably due to more frequent head to head contact, length of hair and autonomy in their bath and body care, and common behaviors such as sharing of hair ornaments, combs and brushes.[1] In tropical countries like the Philippines, where head lice infestation is a common health problem, environmental factors such as air temperature and humidity influence the longevity and fecundity of *P. humanus capitis*,

particularly the propagation of lice populations and their dispersion.[2]

Pediculosis has been treated by methods that include the physical removal of lice, various domestic treatments, and conventional insecticides.[3] Commercially available medicated shampoos are often used as treatment of pediculosis for infestations not manageable by manual nit and lice removal because of their affordability and accessibility. Kwell Shampoo™ a pediculocidal shampoo considered as standard of treatment, contains the active ingredient permethrin. Permethrin is a synthetic pyrethroid proven to kill lice and nits quickly and exhibits extremely low mammalian toxicity. The potent insecticidal action of permethrin arises from repeated neuronal depolarization, leading to a reduction in neuronal excitability causing sensory hyperexcitability, incoordination, prostration, paralysis and eventual death.[4] However, evidence shows that the extensive use of commercial pediculicides has resulted in resistance and cross-resistance.[5] Non-toxic alternatives are hence needed for head lice treatment and prevention, and plant-derived natural products may offer safer alternatives with good pediculocidal activity.

Among these natural products is neem (*Azadirachta* indica) which contains azadirachtin, a complex tetranortiterpenoid limonoid found to cause toxicity to a wide spectrum of agricultural and household insects such as lice. Neem seeds with azadirachtin are comparable to synthetic chemicals in terms of efficacy and are deemed safe for human use according to previous studies. In addition, neem has added components such as azadirachtin B, nimbin, and salannin, which prevent the development of lice resistance against these products.[6] These natural products are useful sources of bioactive components in the development of new drugs and pharmaceutical targets due to their low toxicity to mammals and easy biodegradability.

The aim of this study was to determine the effectiveness of neem (*Azadirachta* indica) seed oil methanolic extract shampoo compared with permethrin in the reduction of head lice, and to determine the occurrence of adverse effects with its use.

Methods

The study utilized a single-blind, non-inferiority, randomized dual controlled clinical trial on a study population of males and females aged 6-14 years old from Barangay San Perfecto, San Juan City that was selected by purposive sampling. Mass screening was conducted through a house-to-house survey among school children belonging to the target age group in the identified barangay. The screening process included subjects who had eggs, nits, nymphs and adult lice. Excluded from this study were those who had 1) been previously treated with any pediculocidal solution within the last three months prior to the study, 2) erythema, scalp wounds and alopecia, and 3) hypersensitivity to the 1% permethrin treatment.

The presence of head lice was confirmed with a nit comb after thorough combing and detangling of dry hair with a wide-toothed comb. Having at least one head louse was considered positive for pediculosis infestation. A baseline lice count using a standard quadrant counting was performed prior to the administration of the treatment. Each subject's hair was partitioned into four major quadrants (Q): Q1 at the top of the head, Q2 and Q3 behind the ears and Q4 at the back of the head/above the nape. The hair was combed using a wide-toothed comb to detangle, followed by the use of the nit comb over the Q4.

The minimum required sample size of ten participants per treatment group was computed using the formula for the estimation of population mean using a 95% alpha error and 80% beta error and a mean and standard deviation of 1.96 with a margin of error of 10%, based on a previous study regarding cashew nut and its effect in reducing pediculosis.[7] A total of 30 participants was divided equally into three treatment groups.

The neem seeds utilized in this study were collected from the Bureau of Plant Industry in Manila. The leaves and seeds were collected, air dried, securely stored in resealable plastic bags and transported to the Division of Botany and Zoology of the National Museum of the Philippines for identification and authentication of plant species, as part of the requirements for herbal research. The seeds were then submitted to the Department of Science and Technology (DOST) Central Office in Taguig City for plant extraction and shampoo formulation. Under the protocols of the Chemicals and Energy Division-Pharmaceuticals Section of the DOST Central Office, the seeds were dried in an incubator at 60°C for 3 hours. The shells were removed to obtain the kernels inside which were then ground

and utilized for the extraction of the active component of neem seeds, azadirachtin. A suspension of 250g of ground neem seed kernel in one liter of hexane was stirred at 40°C for 2 hours and filtered. The hexane extract was concentrated in vacuo to give 106g of neem oil. The defatted marc was then extracted with one liter of methanol in the same manner as n-hexane extraction. The methanolic was dissolved in 100mL of 90% aqueous methanol and partitioned twice with 50mL hexane to remove remaining oil and other non-polar compounds. Mixtures were then filtered using Whatman No.2 filter paper. The solvent was removed from the oil using a rotary evaporator. The extracted oil was stored in aliquots of 10 mL vials and stored at 4 °C until use. A concentration of neem seed oil:shampoo at a dilution of 1:10 mL/mL was made as this was the concentration needed to exert its pediculocidal effect. This was assigned as Treatment A for this study.

The negative control, Treatment C, was the pure (pearlized) shampoo provided by the DOST, identical to the one used as the carrier shampoo for the neem seed methanolic extract. It contained a pearlizing agent known as glycol stearate which acts as an 1) opacifying agent, making it less transparent and giving the shampoo a pearly appearance, 2) skin conditioner to smooth and soften the tissue, 3) emulsion stabilizer which helps to hold oil particles in the water solution to prevent production separation, and 4) thickener. None of the said ingredients has any pediculocidal effect. The pediculocide shampoo Kwell™, with 1% permethrin (10 mg/g) as its main active ingredient, was used as Treatment B, the positive control.

Using block randomization technique, the participants were randomly assigned to their treatment groups through a computer software randomizer. The 10% neem seed oil methanolic shampoo treatment was given to Group A, permethrin shampoo was given to Group B as positive control, and pearlized shampoo was given to group C as negative control.

A regular comb, a fine-toothed nit comb, and a shower cap were provided for each participant in this study. Hair washing, and administration of treatments were done by the investigators to ensure proper administration of the treatments. The treatment was kept on the hair for about 10-15 minutes during each application before it was thoroughly rinsed with water. The counting of head lice using the standard quadrant counting was done using a fine nit comb after every treatment application. Results in each quadrant were based on lice yield after stroking thrice using a fine-toothed nit comb. The number of live lice was then counted and recorded. Observation and documentation of any dermatologic side effects were also done by the investigators, participants, and their parents or guardians. Treatments were given at 10-day intervals for three applications. A 10mL solution of the respective shampoos were applied per treatment day, for a total of 30mL of shampoo applied throughout the study, in all treatment groups. At the end of the study, the Kwell™ permethrin shampoo was distributed and applied to groups A and C as standard treatment for pediculosis. Health education on pediculosis was also conducted to increase awareness and improve hygiene practices.

The study was approved by the Ethics Review Committee of the Medical Center. Written informed consent from the parents or guardian of the children, and assent from the children, as applicable, were obtained. Measures were taken to ensure confidentiality and to protect the privacy of the participants.

Results

Among 36 potential participants screened, 30 children were included in the study and randomly assigned to one of three treatment groups. The mean age of the participants was 10.2 years; more than 80% were girls and 80% were in Grades 1 to 6. All participants except two belonged to socioeconomic classes D and E. There was no statistically significant difference among the three treatment groups in terms of age, sex, grade level and socioeconomic status as seen in Table 1.

Compared with baseline, all groups showed a reduction in lice count following the first week of application, with the greatest reduction seen in the permethrin group. On Weeks 2 and 3, the permethrin and neem group continued to demonstrate a reduction in lice count. The group that received pearlized shampoo had an increase in lice yield on the third week. Repeated measures ANOVA showed that there was a significant decrease in the number of lice from baseline to Week 3 in the neem and

permethrin groups, with summary p-values of 0.041 and 0.013, respectively. There was no significant change in the pearlized shampoo group (Figure 1).

There was a decrease in the lice count from baseline to the third application in both the neem shampoo and the permethrin shampoo groups. The differences between the mean lice count in the two groups at Applications 1, 2 and 3 were not statistically significantly (Table 2). Upon completion of treatment, paired t-test showed a statistically significant difference of lice count from baseline to Application 3, in both the neem shampoo and permethrin shampoo groups (Table 3). Independent t-test showed no statistically significant difference between the

Table 1. Comparison of demographic characteristics of 30 participants in neem seed and permethrin shampoo groups.

Characteristics	Neem seed shampoo	Permethrin shampoo	Pearlized shampoo	p-value
Age (yr)				
Mean	9.4	10.4	10.8	0.309[a]
6-8	3	2	2	
9-11	6	6	4	
12-14	1	2	4	
Sex				0.383[b]
Male	1	1	3	
Female	9	9	7	
Grade level				0.506[a]
Preschool	2	0	0	
Grades 1-3	4	3	4	
Grades 4-6	3	5	5	
Grades 7-10	1	2	1	
Socioeconomic status				0.733[a]
AB	0	0	0	
C	1	0	1	
D	5	5	3	
E	4	5	6	

[a]One-way ANOVA
[b]Chi-square test

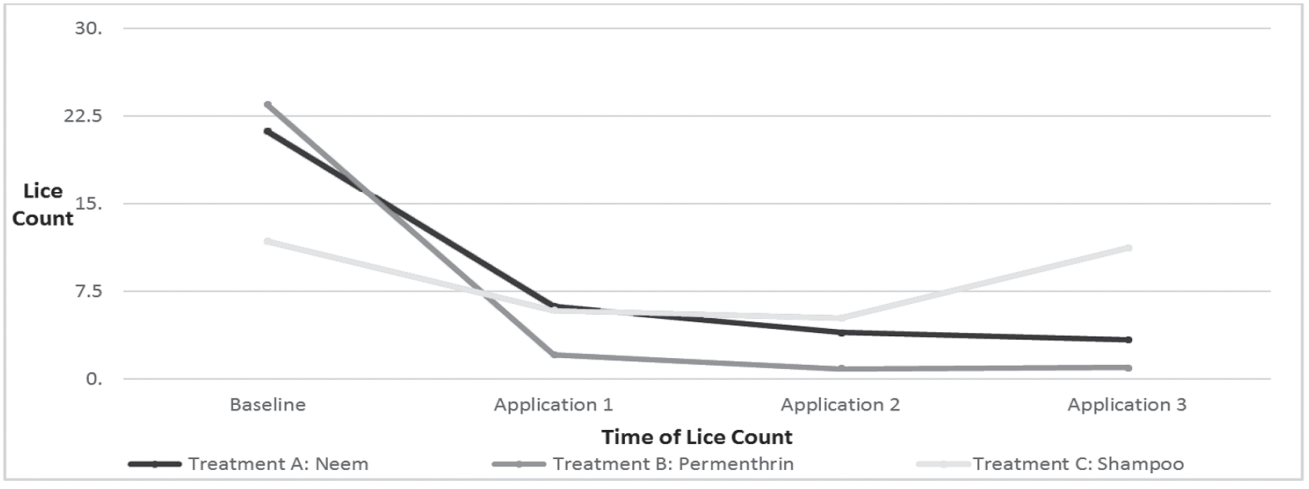

Figure 1. Mean count of head lice per treatment group at the baseline and after each treatment application.

reduction of head lice count after completion of treatment in the neem shampoo and permethrin shampoo groups as seen in Table 4.

No dermatologic adverse effects such as burning sensation, redness, skin irritation, allergic reactions or other adverse reactions were reported by the participants or observed by the investigators in any of the treatment groups.

Discussion

The larger percentage (83.3%) of female participants reflects the findings of Canyon and Meinking that pediculosis frequently affects girls more than boys.[8,9] Most of the subjects were familiar with each other as playmates and neighbors and were therefore prone to increased head-to-head contact. Having longer hair in females and sharing hair accessories (especially among the siblings in the current study participants), also increases the odds of transmission of head lice from one host to the other.[10] However, whether hair length indeed affects pediculosis capitis infestation is still debated upon in the literature. The participants had also recently resumed their classes during the study, which is consistent with the findings of Canyon that the highest rates of pediculosis occur from April to August where transmission is higher due to clustering of the children at a common site.[8] Abd El Raheem identified hygienic practices and overcrowding to be specific determinants influencing pediculosis prevalence.[11] However, the researchers were not able to obtain an average number of household members for the barangay, households of the study participants nor a baseline measure of the participants' hygienic practices, which could have supported this finding. Majority of the study participants belonged to the D and E socioeconomic strata, which reflects the findings of Willems that socioeconomic status is a significant factor in the increased incidence of pediculosis in children.[12]

The difference in effect of permethrin and neem shampoo on head lice reduction was not statistically significant, which may reflect that neem shampoo produces a comparable reduction of head lice to the standard treatment. These are consistent with the findings of Schmall that neem seed extract shampoo is pediculocidal and ovicidal.[13]

Table 2. Comparison of mean lice count in the neem seed and permethrin shampoo groups.

	Neem seed shampoo	Permethrin shampoo	p-value
Baseline	21.2 ± 8.41	23.5 ± 7.37	0.839
Application 1	6.2 ± 2.84	2.1 ± 0.78	0.497
Application 2	4.0 ± 1.5	0.9 ± 0.5	0.611
Application 3	3.4 ± 1.6	1.0 ± 0.7	0.663

Table 3. Comparison of reduction in lice count with from baseline to third application in the neem and permethrin shampoo groups.

	Baseline	Trial 3	Difference	p-value
Neem seed shampoo	21.2 ± 26.61	3.4 ± 5.28	17.8 ± 23.97	0.043
Permethrin shampoo	23.5 ± 23.30	1.0 ± 2.31	22.5 ± 23.47	0.014

Table 4. Comparison of mean differences from baseline to Application 3 between neem and permethrin shampoo.

	Mean	Standard deviation	Std. Error Mean	p-value
Neem seed shampoo	12.30	20.78	4.64	0.994
Permethrin shampoo	12.25	19.82	4.43	

The neem tree (*Azadirachta indica*), from the Meliaceae family, originally grown in East India and Burma, is considered as one of the most versatile plants for its therapeutic, ethnomedicinal, and cosmetic values, rendering it as the "wonder tree" of India.[14] Due to its wide range of uses, it has been included in the top 10 list of plants studied and used for development by the international scientific community. The WHO has also identified this plant as an environmentally powerful natural pesticide for its potential use in pest management, environment protection, and medicine.[14]

According to Asher, the neem tree has a wide spectrum of activity which includes anti-helminthic, anti-feedant, parasiticide, insecticide and pediculocide properties.[14] The plant's seed is enclosed by a sweet pulp containing 1-3 kernels, in which azadirachtin, a biologically active compound known to be toxic to insects, is contained. Azadirachtin is a complex tetranortiterpenoid limonoid, and this compound affects both physiologic and behavioral aspects in a wide variety of insects: anti-feedancy, severe growth reduction, increased mortality, and abnormally delayed molts. Neem seeds also contain other triterpenoid components such as 3-tigloylazadirachtin (azadirachtin B), nimbin, and salannin. These components have an additive effect when combined with azadirachtin and are useful in preventing resistance.[6]

According to Schmall, another important effect of neem is stunting in the development of immature insect stages.[15] Due to the reduced food intake by adults, postembryonic development of species is delayed. Neem also causes disruption of the endocrine system leading to disturbances of molting, pupation, and adult emergence. The reproduction of insects is also greatly affected, due to egg sterility and shortening of the longevity of male and female insects.

Deshmukh showed neem extract flowing into the opening of the aeropyles of the eggs, and slowly surrounding its surface.[16] It also penetrated the lateral tracheal openings in the motile stages of the lice. In the adult and larval stages, the tracheoles or the insect's respiratory tubes were also clogged. By covering the tracheoles and aeropyles, oxygen transfer between the water layer and the cell was essentially blocked resulting in the disruption of oxygen uptake by the insect's body muscles, heart, and other organs. Within just a short period, oxygen became depleted while the amount of lethal carbon dioxide increased. Sinha showed that 5 to 10 minutes of incubation with neem extract produced significant larvicidal and pediculocidal effects.[17] This protocol was implemented in the current study, and similar results were obtained among the 10 children utilizing neem shampoo.

The study of Schmall on the efficacy of neem extract showed that it was highly effective against all stages of head lice, with less side effects compared to the chemical based standard shampoo. The study tested a neem-based anti-louse shampoo as a single treatment vivo and in vitro. After a short incubation period of just 10 minutes, results show that none of the lice survived after a 22-hour observation and after 7 days of treatment. A second group of children was treated for 20 minutes with identical results. In vitro results of the said study show that only three minutes of submersion was needed to effectively kill both larval and adult stages of head louse. Both in vivo and in vitro results did not reveal freshly hatched larval stages of lice, suggesting the product's ovicidal activity.[15] Asher likewise demonstrated that a 1:10 dilution of the neem extract shampoo was sufficient to kill head lice in vivo within three minutes, or within 10-15 minutes within the wet hair of children.[14]

No side effects such as burning sensations, redness, skin irritation, allergic reactions or alopecia were observed and reported in any of the 10 children using neem seed extract shampoo, replicating the findings of Asher and Trob that neem seed extract shampoo has no associated toxicological risks and is suitable for human use.[14]

The overall results of the trials indicate that neem seed oil methanolic 1:10 mL/mL shampoo given at 10-day intervals for three applications is non-inferior and comparable in lice reduction to the standard treatment, permethrin 1% shampoo. There were no reported dermatologic adverse effects such as burning sensation, redness, skin irritation, allergic reactions based on the observation and feedback from subjects. The study offers support in the potential application of natural compounds for lice infestation reduction.

Factors such as physical removal of lice, difference in the hair length, reinfestation from other family members within the household and hygiene practices of each subject could have affected the results but were beyond the control of the researchers,

thus it is recommended that the future studies should consider and control such factors when feasible.

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