

Can neem oil help eliminate lice? Randomised controlled trial with and without louse combing

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Abstract

Background: Neem oil and wet combing with conditioner are both claimed to facilitate elimination of head louse infestation. The aim of this pilot study was to identify whether a 1% neem oil lotion showed activity itself and/or enhanced the effectiveness of combing in treating infestation.

Methods: We treated 47 participants with 1% neem-based lotion on four occasions 3-4 days apart in a randomised, community based trial, analysed by intention to treat. The participants were randomly divided between two groups: One group used a grooming comb (placebo) and the other a head louse detection and removal comb (wet combing with conditioner method) to systematically comb the hair. Cure was defined as no lice on both Day 10 and Day 14.

Results: The cure rates of 6/24 (25.0%) for the placebo comb group and 8/23 (34.8%) for the louse comb group were not significantly different.

Conclusion: These results indicate that this formulation of neem oil was ineffective in the treatment of head louse infestations, even when accompanied by combing. Both combing methods were also ineffective, despite being implemented throughout by trained professionals.

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Introduction

Since the millennium, there has been a renewed and widespread interest, in both the developed and developing worlds, in using natural materials to treat head louse (*Pediculus humanus capitis*) infestation, known as pediculosis capitis. With the use of essential oils, there is a trade-off between efficacy and irritancy [1-3], and some materials approved for use against head lice have been implicated in potentially life threatening reactions in a small number of people [4, 5]. Alternative natural materials

with low irritancy, such as fixed oils, used in cosmetics, home remedies, and some commercial products, generally show low levels of efficacy against head lice [6, 7]. The most widely used fixed oils are derived from the neem tree *Azadirachta indica* A. Juss (Meliaceae). Neem oil contains variable low concentrations of a large number of putative pharmacologically active triterpenoids. However, the recognized insecticidal activity of neem is primarily an anti-feedant effect on phytophagous insects resulting from ingestion [8]. Neem is claimed to be active against head lice and several other

ectoparasites of animals and plants, none of which could ingest the neem limonoids because they feed only on host fluids [9-11]. The most prominent claims relate to a neem extract shampoo, for which there are *in vitro* and observational clinical reports of efficacy using a single application [9, 10]. In contrast, a 6% neem oil product required additional treatments over 1-2 weeks, used in combination with a comb, in order to achieve similar levels of cure [11].

In this study, we investigated a product that was originally developed as a wet-combing aid to test whether it could be effective without the use of a louse comb. The product, containing 1% neem seed oil and other vegetable extracts, was suspected of exhibiting pediculicidal activity independently of its use with a comb, and claimed to facilitate elimination of infestation. Prior to this investigation there was no clinical evidence to support the claims.

Materials and methods

Study site

This investigation was performed at the Health Protection Unit of the city of Leeds, UK, where the local recommended treatment for head lice was wet-combing with conditioner. This recommendation followed a policy audit in the year 2000 [12] because resistance to over the counter insecticide products had resulted in frequent treatment failures [13]. All the nurses were familiar with, and theoretically skilled at, wet-combing to eliminate lice.

Participants

We recruited participants through local advertising. Each contact received an information booklet at least 24 hours before a domiciliary visit. All household members were screened for lice using a plastic detection comb ("PDC", KSL Consulting, Helsingør, Denmark). Eligibility criteria were similar to previous studies [14] requiring the presence of at least one living louse for enrolment but excluding people who: had been treated for lice within 2 weeks; had other long term scalp conditions or known sensitivity to treatment components; had bleached, dyed, or permanently-waved their hair; or had used trimethoprim preparations within 4 weeks. We also

excluded pregnant or breast feeding females, recent participants in other clinical studies, and previous participants from this study. The minimum age for enrolment was 6 months, with no upper limit.

All volunteers stated that they understood the purpose and requirements of the investigation and gave written consent. Parents or guardians gave written consent for children younger than 16 years. Forty-seven participants, each from a different household, provided informed consent, demographic data, and agreed appointments for follow up treatments and visits. We provided any ineligible or non-participant household members who were also infested with lice with a standard of care treatment, 4% dimeticone lotion, to minimize the risk of reinfestation of study participants. No payment was offered for participation.

The study was conducted in conformity with Good Clinical Practices and the principles of the Declaration of Helsinki and of European Union Directive 2001/20/EC. Ethical approval was granted by Bradford Local Research Ethics Committee, Study reference 06/Q1202/19.

Treatments

The investigation was designed to evaluate which aspects of a treatment method showed efficacy, if any. Nice 'n Clear head lice lotion (A Nelson & Co Ltd, Wimbledon, UK) was developed for the "wet combing with conditioner" treatment method, used in combination with a head louse removal comb. However, the product manufacturer also mentioned it exhibited activity against lice if left in the hair to dry naturally. The product consisted of a cetearyl alcohol, glyceryl mono stearate, and liquid paraffin conditioner base with the addition of 1% neem seed oil, 0.25% tea tree oil, 0.25% lavender oil, and 1% nettle extract.

Prior to this study the nurses were understood to be experienced in combing methods for elimination of head louse infestation but to avoid any inconsistency, they were further instructed in the methods to be used at the study initiation meeting. They were required to apply conditioner systematically and evenly over the whole head after washing and towel drying the hair. They then combed systematically around the scalp,

drawing the comb from the roots of the hairs through to the tips, using the comb appropriate to the randomisation group and following the instructions applied to the “Bug-busting” treatment method [15]. On each occasion, each section of hair was combed at least three times or until no more lice were removed from that section.

To investigate whether the neem oil conditioner showed any activity independent of combing, the participants were divided into two study groups, each of which used a distinctive comb with different expected ability to remove lice.

Group 1 was treated using a regular grooming comb with teeth 1.3 mm apart, which was considered to be a placebo intervention because the teeth were wide enough for most lice to pass between. The comb was expected to have a minimal effect on the infestation because it was anticipated only a few lice would be removed; therefore, any efficacy of the overall treatment could be attributed to the effects of the neem oil conditioner.

Group 2 was treated using the “PDC” head louse detection and removal comb, which has teeth close enough (0.2-0.3 mm) apart to catch the smallest nymphs and some louse eggs in the same manner as the original “Bug-buster” comb developed for wet combing with conditioner [15].

On Day 0 we expected to remove some lice from all participants, although fewer using the placebo grooming comb than with the louse comb. Any lice combed out were collected and examined for their stage of development. After combing, any residual lotion was left to dry on the hair and rinsed out the following day using water. This part of the treatment regimen was intended to demonstrate whether the neem oil conditioner could exert any pediculicide effect to either enhance the effects of combing or to act independently of combing. Treatment was repeated on Days 3, 7, and 10. Nit combing, to remove lice or their eggs, and use of other treatment products by parents or carers was not permitted during the course of the study.

Post treatment assessment on Day 14 was performed by dry combing with a “PDC” comb drawn systematically through the hair following the same procedure used for wet combing, but without the

conditioner lubricant. Any participants found to have lice present at this examination were provided with the standard of care 4% dimeticone lotion treatment.

Outcome measures

The primary outcome measure was elimination of infestation, monitored by dry detection combing on Day 14. However, if lice were present on Day 10, they could have laid eggs that would hatch after Day 14. Consequently, for confirming a cure, no lice were permitted after the treatment combing on Day 7.

Sample size estimation

In this randomised, controlled, open-label, assessor blinded study of the neem-based lotion, an identifiable outcome was estimated as achievable using two groups of 20 participants (23 per group allowing for drop out), where one group used a grooming comb and the other a louse comb.

If the neem-based lotion had an independent effect on lice, it was necessary to demonstrate 50% of cases cured using the grooming comb because, if the lotion and grooming comb combination had no effect, we postulated only a 10% or 20% cure could be expected. For a true cure rate of 10%, the probability of observing 5 or more cures would be very low ($p = 0.002$). Even for a true cure rate as high as 20%, the probability would remain acceptably low ($p = 0.033$).

Similarly, to be confident that the observed cure rate would be 50% or more, if the true cure rate for the lotion was 70%, the probability of observing this rate using the grooming comb was high ($p = 0.953$) or, if the true cure rate was only 60%, the chance of observing the required rate was greater than 80% ($p = 0.834$).

Previous evidence indicated a cure rate for wet-combing alone of about 50–60% (12). Therefore, for a 50% true cure rate, an observed cure of 80% would occur with a low probability ($p = 0.055$), and an observed cure rate of 90% an even lower probability ($p = 0.011$). For a true cure rate of 60%, the probability of observing 80% or more successes would increase ($p = 0.167$) but the probability of observing a 90% cure would remain low ($p = 0.046$).

It was estimated that 20 participants per group could provide adequate security that:

- An observed cure of 50% or more using the grooming comb would not occur if the lotion was ineffective;
- An observed cure of <50% using the grooming comb group would not occur if the lotion was effective;
- An observed cure of 90% using the louse comb would not occur if the lotion was ineffective.

Randomisation and blinding

Treatment allocation used a computer-generated randomised sequence in balanced blocks of ten. Nurses received instruction sheets in opaque, sealed, sequentially numbered envelopes allocated in numerical order. Wherever possible, the same nurse applied all four treatments, but a different investigator performed the Day 14 assessment (blind of the comb allocation).

Statistical analysis

Analyses based on both "intention-to-treat" (ITT) and "per-protocol" (PP) populations measured differences in success rates by the 95% confidence interval (CI) calculated using a normal approximation to the binomial distribution. Fisher's exact test for yes/no variables and the Mann-Whitney U test for ranked variables were used to compare baseline characteristics, safety, and acceptability.

Trial registration

This trial was registered retrospectively on the U.S. National Institutes of Health website, <https://clinicaltrials.gov>, number NCT02974088.

Results

Participants

The ITT population analysed was 47, with 23 people treated using the louse comb and 24 using the

grooming comb between 26th May and 31st August 2006. Two participants and their data were lost to follow up when a nurse resigned from the Health Protection Unit and left the country without warning, leaving 37 children and 8 adults available for data analysis. One participant withdrew after two treatments following an adverse event and another refused treatment on Day 7 and was withdrawn (Figure 1).

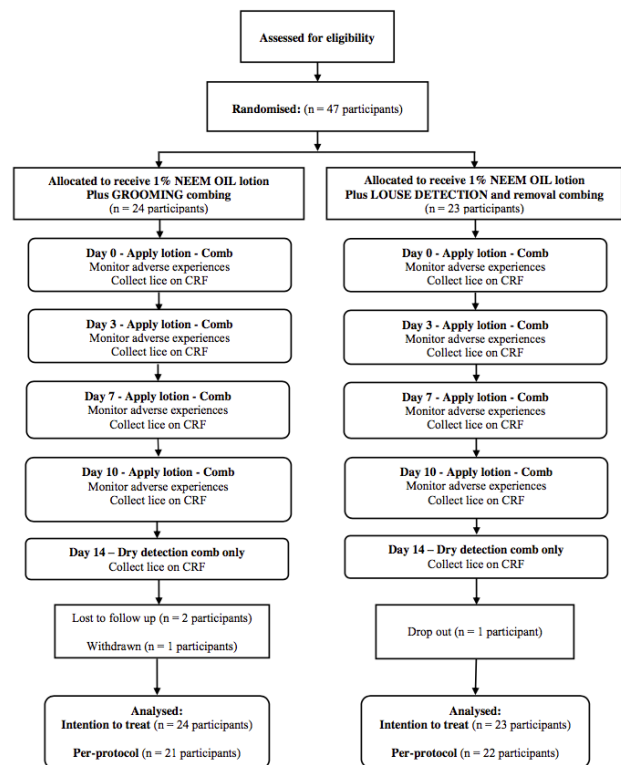


Figure 1. Flowchart of participants through the study. CRF: Case Record Form

Baseline characteristics recorded at Day 0 showed 39/47 (82.9%) female participants, and 28/47 (59.6%) had long hair; both characteristics were equally distributed between the groups with no significant differences in distribution of other baseline characteristics.

Table 1. Numbers of lice recovered from each participant on each day of the study

Subject	Grooming comb group					Treatment outcome ITT	Subject	Louse detection and removal comb group					Treatment outcome ITT
	Assessment/treatment Day number							Assessment/treatment Day number					
	0	3	7	10	14			0	3	7	10	14	
3 L	0	0	0	0	0	Cure	1 M	4	3	7	9	16	Not cured
4 L	0	2	2	3	0	Not cured	2 M	2	6	4	4	0	Not cured
5 L	4	0	0	1	8	Not cured	6 M	6	2	0	5	12	Not cured
9 L	2	2	WD	WD	WD	Not cured	7 L	4	3	2	3	11	Not cured
10 L	0	0	0	0	13	Not cured	8 L	3	0	0	0	0	Cure
14 NR	3	3	3	0	28	Not cured	11 NR	5	0	0	0	0	Cure
15 CM	LTF	LTF	LTF	LTF	LTF	Not cured	12 L	3	4	DR	DR	DR	Not cured
16 CM	LTF	LTF	LTF	LTF	LTF	Not cured	13 L	1	0	0	0	0	Cure
18 L	0	0	0	2	3	Not cured	17 H	135	79	169	178	46	Not cured
19 L	2	11	0	4	0	Not cured	20 L	8	2	0	0	0	Cure
21 M	18	28	35	23	5	Not cured	22 L	1	1	0	0	0	Cure
24 L	13	0	0	3	0	Not cured	23 NR	0	0	0	2	0	Not cured
26 L	5	0	0	0	0	Cure	25 L	1	7	0	0	0	Cure
27 L	0	0	0	0	0	Cure	28 M	17	5	8	13	0	Not cured
29 L	2	1	0	4	1	Not cured	30 H	16	17	2	9	1	Not cured
31 M	7	9	24	30	38	Not cured	33 L	17	16	3	7	0	Not cured
32 M	3	0	0	1	0	Not cured	37 L	2	16	5	37	57	Not cured
34 L	1	3	6	1	1	Not cured	38 M	6	5	22	21	15	Not cured
35 L	5	0	3	2	2	Not cured	39 L	33	14	18	9	8	Not cured
36 L	1	2	5	0	6	Not cured	40 L	12	4	1	0	0	Cure
42 H	8	3	2	4	0	Not cured	41 L	24	14	1	1	0	Not cured
45 H	7	0	0	0	0	Cure	43 H	84	39	17	9	1	Not cured
46 L	1	0	0	0	0	Cure	44 L	2	1	0	0	0	Cure
47 L	0	0	0	0	0	Cure							

Key:

The Participant/Subject number is followed by the infestation level: L = Light, M = Moderate, H = Heavy, NR = No record, CM = Case Record Form missing

DR = Drop out; LTF = Lost to follow up; WD = Withdrawn by investigator

Outcomes

As expected, the grooming comb removed fewer lice than the louse comb (Table 1). Although all participants were confirmed as having at least one live louse present before treatment, lice were not found or recovered during the first or subsequent treatment combings of some participants.

Three people (participants 003, 027, 047) in the grooming comb group had no lice removed during treatment but also no lice were found during the final assessment so it was concluded that these could be

considered as cases of “Cured” because any immobilised insects may have been washed out from the hair when the conditioning lotion was rinsed out. Other participants from whom no lice were recovered at Day 0 (participants 004, 010, 018, and 023) all had at least one louse removed on other days (Table 1). Overall, lower mean numbers of lice were found and proportionately fewer participants demonstrated with lice using the grooming comb (Table 2). Unexpectedly, many lice were removed by the grooming comb because insects were trapped in the viscous fluid then combed from the hair.

Table 2. Comparison of groups for presence of lice and mean numbers of lice recovered

Study day	Grooming comb group		Louse comb group		Comparison of numbers of lice removed p value
	Presence of lice	Mean no. lice removed	Presence of lice	Mean no. lice removed	
Day 0	72.7%	3.4	95.6%	16.8	0.041
Day 3	45.5%	2.7	82.6%	9.9	0.0078
Day 7	38.1%	3.3	59.1%	10.8	0.099
Day 10	57.1%	3.3	63.6%	12.8	0.409
Day 14	47.6%	4.4	40.9%	7.0	0.926

However, no significant difference ($p = 0.573$) in the mean number of lice removed on Day 14 (4.86 versus 3.4 removed at Day 0) suggested no pediculicidal effect of the lotion. However, for the louse comb, the difference in the mean number of lice collected on Day 0 (16.8) and Day 14 (7.0) was significant ($p = 0.017$), indicating a cumulative impact of the whole intervention on louse numbers (Table 1).

Based on the ITT population there was no significant difference between the groups in the rate of successful elimination of infestation ($Z = 0.733$, $p = 0.464$, 95% CI -0.164–0.359). However, significantly more lice were removed using the louse comb on Days 0 and 2 but not on Days 7, 10, or 14 (Table 2). Using the grooming comb, 6/24 (25.0%) participants were louse free after Day 0 and 11/24 (45.8%) on Day 14, of whom five had lice on Day 10 so they could not be considered cured. Similarly, 13/23 (56.5%) participants in the louse comb group had no lice on Day 14, but five of these also had lice on Day 10, leaving 8/23 (34.8%) who had been louse free throughout most of the treatment phase; odds ratio (OR) 0.625 (95% CI 0.177–2.206). Elimination of drop outs and lost participants gave a PP success of 6/21 (28.6%) for the grooming comb and 8/22 (36.4%) for the louse comb (OR 0.70, 95% CI 0.194–2.53).

The study nurses delivering the treatment varied considerably in their ability to eliminate infestation and in the quantities of neem-based lotion used, overall and according to hair length of the participants (Table 3). There was some correlation between lotion use and success rate, with more cures in both groups when total lotion use exceeded 276 g.

In the cohort of 276–858 g lotion used there were 25 participants (12 grooming comb, 13 louse comb). On Day 14, this cohort comprised 6/12 (50%) cured and 3/12 (25%) louse free using the grooming comb and 5/13 (38.5%) cured and 4/13 (30.8%) louse free using the louse comb. This comparison showed no statistical difference between the two comb groups for cure ($\chi^2 = 0.34$, $p = 0.56$; relative risk 1.3, 95% CI 0.53–3.17) and for being louse free even less difference ($\chi^2 = 0.1$, $p = 0.75$). Neither increased lotion use nor investigator experience were reliable predictors of outcome, e.g. nurse ‘B’, the most successful treatment provider, applied some of the highest aggregate quantities of lotion, 585 g and 605 g, to participants 029 and 043, respectively. Both were treatment failures.

Adverse events

No serious adverse events were recorded. One participant from each group reported an adverse event considered possibly or probably related to treatment. The first, a 4-year-old male who had not previously been treated for louse infestation and had no prior history of sensitisation, reported itchiness and rash around the eyes, probably related to treatment, developed following the second treatment and prompting withdrawal. The irritation was still present 4 days after withdrawal but had resolved without intervention when examined after 2 weeks. The other, a 33-year-old female, reported spots on the face and neck at Day 14, which may have been present earlier but not previously reported by the participant. The condition resolved within 2 weeks, although it is possible the rash was present before starting treatment and association with the product was coincidental.

Table 3. Comparison of treatment nurses by cure rate achieved and quantity of lotion used

Nurse	Number treated	Treatment outcome		Mean weight of lotion used per application according to hair length		
		Cure	Failure	Long	Medium	Short
A	16	4 (25%)	12 (75%)	86.66g	55.75g	35.24g
B	16	7 (44%)	9 (56%)	102.35g	86.28g	79.46g
C	5	1 (20%)	4 (86%)	41.42g	62.90g	-
D	5	0	5 (100%)	98.23g	97.68g	31.10g
E	2	1 (50%)	1 (50%)	41.13g	45.58g	-
F	1	1 (100%)	0	Data missing	-	-

Discussion

This study evaluated two approaches to head louse treatment: the activity of a neem-based lotion, and combing with a louse detection and removal comb using the lotion as a lubricant (wet-combing with conditioner). Wet-combing with conditioners has the potential to cure head louse infestation, with varying reported outcomes of up to about 57% success [15-17]. In this study, if it is assumed the neem-based product had no effect, the best case outcome for wet-combing was 56.5% (i.e. no lice on Day 14), but a 34.8% success is more likely, for participants also louse free on Day 10 or earlier (Table 1).

Based on expected outcomes, the data do not suggest that the neem-based lotion was effective because, in the grooming comb group, although the most optimistic Day 14 outcome was 45.8% success, only 25.0% were also louse free on Day 10. However, the most surprising outcome was the low cure rate achieved by nurses whose normal duties included advising how to wet-comb with conditioner as a treatment for pediculosis. This finding is not new [16, 17] but, if professionals cannot perform the method reliably, is it surprising that the public experience difficulty using it successfully?

The evidence for the insecticidal activity of neem originates from agricultural pest and mosquito larval control, where the large complex molecules in neem are ingested [8, 18, 19]. As head lice feed exclusively on host blood, it is difficult to conceive how these compounds, too large to pass across an insect's cuticle, could impact upon their physiology. Evidence

for neem activity in non-phytophagous insects is sparse and any reported effects are possibly more attributable to commercial formulation excipients than pharmacologically active limonoid compounds. In the cases of any reported effects, prolonged or repeated exposures were required [20-22].

In our laboratory, we have investigated neem oils, extracted limonoids, and fortified azadirachtin mixtures (IF Burgess, unpublished data), but failed to achieve results similar to those reported by others [9]. Marketed products, field tested in rural areas of developing countries where even the formulation base could adversely affect louse viability [9-11], do not confirm activity for neem against head lice. Formulation plays a significant role in the activity of other pediculicides [23] and we found components of the Nice 'n Clear head lice lotion conditioner base, tested in isolation, were more active against lice than neem oil [24], which may explain why larger quantities of the lotion were more effective.

This clinical study suggests that in practice 1% neem seed oil contributes no detectable activity to kill or inhibit head lice or to prevent the eggs from hatching, and the same can be concluded for the other plant extracts and oils in the product, being too diluted in the conditioner base to exert any effect on the insects. Figure 2 shows newly emerged nymphs and adult lice capable of laying eggs were present at every stage of the treatment regimen.

Lice were removed by the grooming comb because the conditioner had a limited but unsustainable "immobilization" effect from which they later recovered.

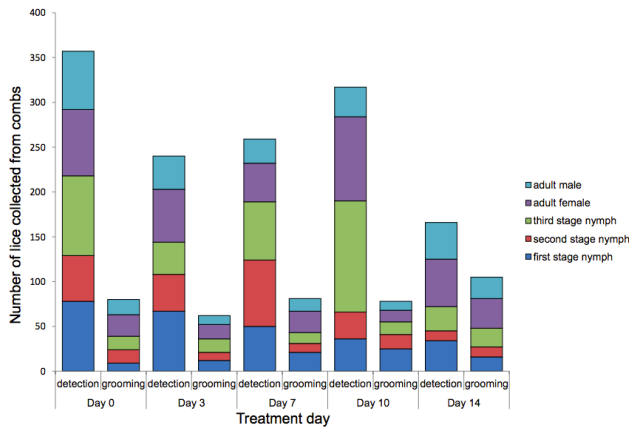


Figure 2. Numbers and stages of development of lice from each study group at each combing

Consequently, the outcomes of this study not only indicate that this neem oil formulation should not be regarded as offering efficacy as a treatment for head louse infestations but also show that four sessions of wet combing with conditioner by experienced practitioners are inadequate to eliminate lice from a majority of infestations.

Despite the claim on the pack that the product was “dermatologically tested”, the occurrence of two adverse events suggesting skin sensitization-like effects indicate that plant extract components in this type of formulation should be more rigorously tested for sensitization risk.

Conclusions

This randomized, controlled, clinical study has shown that four overnight applications of a 1% neem oil-based lotion in combination with combing were not effective to eliminate head louse infestation in most participants. The study also showed that using louse combing in conjunction with the lotion (wet combing with conditioner method) did not contribute significantly towards a successful outcome for pediculosis treatment compared with using a grooming comb to spread the lotion

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