Original Article

Study on the Impact of Herbal Shampoo with Gharaghorot, Vinegar, and Ajwain against Head Lice

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Abstract

Background: This study evaluates a herbal shampoo containing Gharaghorot, vinegar, and Ajwain for managing head lice, particularly in resource-limited settings.

Methods: The herbal shampoo formulation constituted 100% final solution, composed of: 28.85% Gharaghorot extract (prepared from sour yogurt juice), 67.31% apple cider vinegar, 2.88% Ajwain (*Trachyspermum ammi*) essential oil, and 0.96% xanthan gum (a biocompatible thickening agent). Cell toxicity and sterility were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and fluid thioglycollate and soybean casein digest media, respectively. Irritation and sensitization tests were conducted on rabbits and guinea pigs, respectively. A randomized controlled trial with 114 patients compared the herbal shampoo to standard Permethrin treatment (Permethrin shampoo 1%). Participants applied the shampoo daily for one hour over seven days, with efficacy evaluated by counting live lice and nits before treatment, three days in, and seven days post-treatment.

Results: The herbal shampoo exhibited no significant cytotoxicity at concentrations of 10% or lower. The shampoo did not cause sensitization, edema, or erythema. Microbial contamination analysis showed no detectable levels of aerobic or anaerobic bacteria or fungal spores. In the human study, the intervention group showed a 0% prevalence of live lice by the seventh day, compared to 50% in the permethrin group (P= 0.021), and a significant reduction in lice eggs to 42% versus 100% in the permethrin group (P= 0.025).

Conclusion: The herbal shampoo demonstrated safety, microbiological purity, and notable efficacy in reducing live head lice and partial effectiveness against nits (42% egg viability post-treatment).

Keywords: Pediculosis; Gharaghorot; Vinegar; Ajwain; Permethrin

Introduction

Pediculosis, a prevalent health concern characterized by the infestation of head louse (*Pediculus humanus capitis*), remains a significant public health challenge worldwide (1). Head louse is an obligate ectoparasite that thrives exclusively on the human scalp, sucking blood for sustenance. Transmission primarily occurs through direct head-to-head contact, facilitating the rapid spread among individuals, especially in conditions where proximity is unavoidable. This issue is particularly pronounced in densely populated areas with suboptimal sanitary conditions, where the prevalence of lice can reach alarming levels (2, 3). The socio-economic implications of pediculosis are profound, affecting individuals across various demographics, but with a notable impact in regions marked by poverty and limited access to healthcare resources (4). The consequences of prolonged and chronic lice infestation extend beyond the immediate discomfort and irritation caused by these parasites (5). Individuals suffering from persistent head louse infestations may experience a range of physical and psychological repercussions, including but not limited to humiliation, psychological dis-

tress, depression, insomnia, and even academic underachievement (6). The relentless itching and scratching can lead to secondary bacterial infections, hair loss, and allergic reactions, further exacerbating the individual's plight. Moreover, the social stigma attached to pediculosis can precipitate loss of social status, fostering a cycle of isolation and emotional distress (7−10). Despite considerable efforts and resources dedicated to the prevention and management of lice infestation, the persistence of this issue underscores the limitations of current strategies (11). Recent data from health centers highlight the ongoing challenge, with reports indicating a high prevalence of lice infestation, particularly in disadvantaged areas, where rates as high as 29.35% have been documented (12). This points to an urgent need for effective and accessible treatment options. The therapeutic landscape for head lice management is diverse, encompassing a variety of approaches ranging from chemical treatments to physical removal techniques. Historically, the use of chemical insecticides, often formulated as shampoos, has been the cornerstone of pediculosis treatment (13). However, concerns over toxicity, environmental impact, and the emergence of resistant lice strains have prompted the exploration of alternative methods. These include the application of non-toxic agents like dimethicone lotion, oral anthelmintic drugs such as albendazole, diethylcarbamazine, and ivermectin, and physical interventions like meticulous combing and the application of hot air. In the quest for safer, more sustainable solutions, the potential of herbal remedies and natural compounds has garnered increasing attention. Herbal shampoos, leveraging the intrinsic properties of plants known for their pesticidal or repellent activities against lice, represent a promising avenue of research. The integration of traditional knowledge with contemporary scientific methodologies offers a compelling strategy for developing novel, efficacious treatments that can mitigate the burden of head lice infestations (7−10). This study aims to explore the efficacy of a herbal shampoo formulation comprising Gharaghorot, vinegar, and Ajwain in the management of head lice. By investigating the potential of these natural ingredients, which have been selected based on their historical use and purported benefits, the study seeks to contribute valuable insights into alternative pediculosis treatment options. Through rigorous evaluation and analysis, this research endeavors to ascertain the viability of this herbal shampoo as a safe, effective, and accessible intervention for individuals grappling with head lice, particularly in resource-limited settings where conventional treatments may not be feasible or readily available.

Materials and Methods

In this study, all procedures involving animals were conducted following the guidelines of the Institutional Animal Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. In the human subject section, following the detailed explanation, the study was conducted with the consent of the participants. This study was conducted with the approval of the Institutional Ethical Committee of Shahid Sadoughi University of Medical Sciences with approval number “IR.SSU.REC.1402.026”.

Shampoo formulation

Our herbal shampoo was formulated as a final solution, constituting 100% total volume. This formulation comprised 28.85% Gharaghorot extract (prepared from sour yogurt juice), 67.31% apple cider vinegar, 2.88% Ajwain (*Trachyspermum ammi*) essential oil based on the total volume, and 0.96% xanthan gum (a biocompatible thickening agent). The inclusion of Ajwain essential oil was intended to mitigate the potent odor of Gharaghorot, enhancing the product's sensory appeal without compromising its efficacy. Furthermore, the xanthan gum (0.96%) was added to improve the shampoo's consistency, ensuring better ad-

herence to the scalp and hair during application, which facilitates user compliance and treatment effectiveness.

Biocompatibility and efficacy evaluation

The comprehensive evaluation of the shampoo's biocompatibility encompassed several key tests:

Cell Toxicity: The cytotoxic potential of the shampoo was assessed using the MTT assay (14). Human dermal fibroblasts were exposed to varying concentrations of the shampoo for 24 hours. Cell viability was then measured, allowing us to calculate the shampoo's toxicity as follows: %Toxicity= (1-[Absorbance of treated cells/Absorbance of control cells]) ×100. %Viability was determined by subtracting the %Toxicity from 100.

Sterility of Designed Shampoo: To ascertain the shampoo's antimicrobial properties, we introduced the filtered shampoo solution into fluid thioglycollate and soybean casein digest media. These cultures were incubated for five days, after which bacterial and fungal colonies were enumerated. Gram staining and spore staining techniques were applied to the isolated strains for further characterization.

Irritation Test: Conducted following ethical standards and approved protocols, this test involved three New Zealand white rabbits. Shaved dorsal regions measuring 10−15 cm^2 were exposed to 2.5 x 2.5 cm patches soaked with the shampoo. Control patches (soaked with distilled water) were applied adjacent to the test sites. After a 4-hour exposure period, the patches were removed, and the sites were cleansed. Observations for erythema and edema were recorded at 24, 48, and 72 hours post-application.

Sensitization Test: Fifteen healthy Guinea pigs were selected, with ten in the intervention group and five serving as controls. The shaved dorsal skin was marked for three treatment sites: Site A (Freund's complete adjuvant mixed with saline), Site B (undiluted shampoo for the test group and solvent for controls), and Site C (a mixture of the test sample with Freund's complete adjuvant and solvent). This setup aimed to evaluate the potential sensitizing effects of shampoo formulation.

Analysis of microbial contamination: To assess the microbiological purity of the herbal shampoo, a comprehensive analysis of microbial contamination was performed according to standard protocols for personal care products. The shampoo samples were analyzed for the presence of aerobic bacteria, anaerobic bacteria, and fungal spores. A 10 g sample of herbal shampoo was aseptically weighed and diluted in 90 ml of sterile phosphate-buffered saline (PBS) to achieve a 1:10 dilution. Serial dilutions (up to 10^{-6}) were prepared to ensure accurate microbial enumeration. For the detection of aerobic bacteria, 1 ml of each dilution was inoculated in triplicate onto soya bean casein digest agar (SCDA) plates. The plates were incubated at 35±2 °C for 48–72 hours under aerobic conditions. For anaerobic bacteria, 1 ml of each dilution was inoculated onto fluid thioglycollate medium (FTM) and incubated at 35±2° C for 48–72 hours under anaerobic conditions in an anaerobic vessel. For fungal contamination, 1 ml of each dilution was inoculated in triplicate onto Sabouraud Dextrose Agar (SDA) plates and incubated at 25±2 °C for 5–7 days. After incubation, the plates and media were analyzed for microbial growth. The colony-forming units (CFU) were counted, and the results were expressed as CFU/g of shampoo sample. The presence of specific pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* was analyzed using selective media. Gramme staining and spore staining techniques were used to characterize the isolated microbial strains. All tests were performed in a laminar flow bonnet to prevent contamination, and negative controls (sterile PBS) were included to verify the sterility of the test environment. The acceptance criteria for microbial purity were based on the USP guidelines for non-sterile pharmaceutical products, which state that the total number of aerobic microor-

ganisms should not exceed 10^2 CFU/g, the total number of yeasts and moulds should not exceed 10^1 CFU/g, and certain pathogens should not be present in a 10 g sample.

Human study

Our study recruited 114 patients diagnosed with head lice infestation, ensuring an equitable distribution across various socio-economic backgrounds to minimize confounding variables. Rigorous criteria were established for inclusion (documented head lice infestation and consent agreement) and exclusion (follow-up noncompliance or withdrawal of consent). The demographics of patients (age, sex, previous treatment with 1% permethrin shampoo, education level, and occupation) were recorded.

Before enrolment, all participants or their legal guardians (for participants under 18 years of age) were informed in detail about the study, including the purpose, procedures, potential risks, and benefits. Written informed consent was obtained from each participant or their legal guardian following the ethical guidelines of the Declaration of Helsinki and the institutional ethics committee of the Shahid Sadoughi University of Medical Science (IR.SSU.REC. 1402.026).

Participants were randomly assigned to either the control group (standard permethrin treatment (Permethrin shampoo 1%)) or the case group (herbal shampoo treatment) using a computer-generated random number sequence (validated software). Unique participant identification numbers were entered into the software, which allocated them to groups. Randomization was conducted by an independent researcher uninvolved in recruitment, treatment, or evaluation to ensure allocation concealment and minimize bias. This approach aimed to achieve a balanced group distribution and reduce systematic differences that affect outcomes.

Application instructions stipulated thorough coverage of the scalp and hair for one hour daily over seven days. The amount of shampoo used was adjusted based on hair length, typically ranging from 20 ml for short hair to 50 ml for long hair.

The efficacy of the herbal shampoo in eliminating head lice and nits was evaluated using a standardized protocol based on established pediculicide guidelines (15, 16). Assessments were conducted at baseline (before treatment), three days post-treatment, and seven days post-treatment. Live lice were identified through direct visual inspection and systematic scalp combing using a fine-toothed nit comb (teeth spaced 0.2–0.3 mm apart). Each combing session lasted 10–15 minutes (adjusted for hair length/density), and the collected lice were examined under a magnifying glass (10x) to confirm viability (movement or response to stimulation). The percentage of participants with live lice was recorded per group at each time point. Nits were evaluated in a standardized 25 cm^2 occipital scalp area (marked with a template). Using a magnifying glass (10x), nits were categorized as: Viable (live embryo visible under 40x microscopy), Non-viable (dead embryo), or Empty shells (hatched). Tweezers and light microscopy (40x) were used to confirm embryo presence. The percentage of participants with viable/non-viable nits was calculated per group. Nymphs (immature lice) were identified during combing by their smaller size (confirmed under 10x magnification). Their absence indicated disrupted hatching or nymph survival.

To minimize bias, all assessments were conducted by trained personnel who were blind to treatment allocation. The same personnel conducted the assessments for all participants to ensure consistency. Participants were instructed not to use any additional lice treatments, combs, or hair products during the study period to avoid biasing the results.

Data analysis

The collected data, encompassing observational and quantitative outcomes, were analyzed using SPSS software (version 22). To evaluate the efficacy of the treatments across the three time points (pre-treatment, three days in, and

one week post-treatment), a repeated measures analysis of variance (ANOVA) was conducted. Specifically, a two-way repeated measures ANOVA was performed, with time as the within-subjects factor and treatment group (control vs. case) as the between-subjects factor. For the quantitative data on nit counts, the assumptions of normality and sphericity were tested using the Shapiro-Wilk test and Mauchly's test, respectively. In cases where the sphericity assumption was violated, the Greenhouse-Geisser or Huynh-Feldt corrections were applied to adjust the degrees of freedom and ensure the validity of the F-test.

Results

The evaluation of our newly formulated herbal shampoo's biological safety and efficacy was rigorously conducted following established standards.

Cell toxicity

The cytotoxic effects of the herbal shampoo, containing Gharaghorot, vinegar, and Ajwain, were rigorously evaluated using a quantitative approach. The findings are presented in Table 1, which demonstrates the cell viability and toxicity percentages at various concentrations of the shampoo.

Our study employed a well-established criterion for assessing cytotoxicity, where cell survival rates above 70% are considered non-toxic. The data in Table 1 reveal that the herbal shampoo, at concentrations of 10% or lower, exhibits no significant cytotoxicity. The p-values for the 100%, 50%, and 30% concentrations are highly significant ($P< 0.001$), suggesting that these concentrations have a substantial impact on cell viability. However, the p-value for the 10% concentration is not significant ($P= 0.12$), supporting the conclusion that this concentration does not exhibit significant cytotoxicity. Specifically, at a concentration of 10%, the cell viability was found to be 89.9%, with a corresponding toxicity percentage of only 10.1%. This result firmly establishes the safety margin for shampoo's application concentration, ensuring its biocompatibility for human use.

Sensitization and irritation tests

The potential of the herbal shampoo to cause sensitization and irritation was rigorously evaluated through in vivo tests. The results demonstrate that at a concentration of 10%, the shampoo does not induce sensitization, edema, or erythema. These findings suggest that the shampoo formulation is well-tolerated and does not provoke immediate or delayed hypersensitivity reactions, further affirming its safety profile. The sensitization test results, where ten animals were exposed to the herbal shampoo, and five animals served as the control group, showed the absence of any sensitization reactions in all animals at 24, 48, and 72 hours after patch removal. No signs of irritation in any of the rabbits at 24, 48, and 72 hours after exposure. The absence of both immediate and delayed hypersensitivity reactions suggests that the shampoo formulation is well-tolerated and safe for use, further affirming its safety profile. These outcomes support the conclusion that the herbal shampoo is unlikely to cause sensitization or irritation, making it suitable for use on human skin without risk of adverse dermatological reactions.

Microbial contamination analysis

To ensure the microbiological purity of the formulated herbal shampoo, we conducted a comprehensive microbial count analysis. No detectable levels of aerobic or anaerobic bacteria or fungal spores were found in the shampoo samples, confirming their microbiological safety and compliance with regulatory standards for personal care products. In conclusion, the microbial count analysis provides robust evidence of the microbiological purity of the herbal shampoo composed of Gharaghorot, vinegar, and Ajwain. The formulation's compliance with high standards of hygiene and safe-

ty reaffirms its suitability for use as a natural remedy against head lice, without compromising user safety through microbial contamination.

Demographic data of participants

The demographic characteristics of the participants involved in the study are presented in Table 2. To ensure a fair comparison between the control and intervention groups, a statistical analysis was conducted to assess the equality of these groups across various demographic variables. The results of this analysis revealed no statistically significant differences between the two groups concerning age, sex, duration of lice infestation, or history of chemical drug treatment. This homogeneity is crucial as it allows for a robust analysis of the treatment outcomes, mitigating any potential confounding effects related to demographic disparities.

Age distribution: The mean age of participants in both the control and intervention groups was found to be similar, with a mean age of 17.5 years (SD±1.2) for the control group and 16.98 years (SD±1.3) for the intervention group. The P value of 0.861 indicates that there is no significant difference in age distribution between the two groups.

Sex balance: The study included both male and female participants, with a nearly equal distribution in both groups. The P value of 0.521 for sex distribution between the control (27 males, 30 females) and intervention (26 males, 31 females) groups indicates that there is no significant disparity in sex composition.

Duration of lice infestation: The average duration of lice infestation was slightly lower in the intervention group (73.89 days) compared to the control group (81.09 days), but the difference was not statistically significant (P= 0.237).

History of chemical treatment: A slight majority of participants in both groups had a history of using chemical drug treatments for lice infestation, with 46 participants in the control group and 42 in the intervention group. The P value of 0.423 suggests that the history of chemical treatment did not significantly differ between the two groups.

Efficacy in lice and nit removal

The primary focus of our study was to evaluate the efficacy of the herbal shampoo in eliminating head lice and nits. Table 3 provides a detailed comparative analysis of the percentage of live lice and nits observed in both the intervention and control groups at three distinct time points: before treatment, three days into treatment, and seven days post-treatment. Based on this table, on the first and third days, there were no live lice observed in either group. By the seventh day, the intervention group maintained a 0% prevalence of live lice, whereas the control group exhibited a 50% prevalence (P= 0.021). This significant reduction in live lice in the intervention group underscores the effectiveness of the herbal shampoo in eradicating live lice.

On the first day, the control group had a 100% prevalence of louse eggs, while the intervention group had a slightly lower prevalence at 90% (P= 0.321). By the third day, the control group still had 100% louse egg presence, whereas the intervention group showed a significant reduction to 75% (P= 0.048). On the seventh day, the control group continued to exhibit a 100% presence of louse eggs, whereas the intervention group showed a significant reduction in egg viability to 42% (P= 0.025). To further investigate the lifecycle stages of the head lice, the presence of nymphs was also assessed during the evaluation. No nymphs were observed in the intervention group by the seventh day, suggesting that the herbal shampoo may have disrupted the hatching process or inhibited nymph survival. Additionally, the status of the nits was examined microscopically to determine whether they had hatched, remained unhatched with viable embryos, or contained non-viable embryos. In the intervention group, the majority of the remaining 42% of nits were

found to be non-viable, with no developing embryos observed upon microscopic inspection. This indicates that while the herbal shampoo did not eliminate all nits, it significantly impaired their viability, preventing further infestation. The p-values indicate a statistically significant reduction in louse eggs in the intervention group over time, highlighting the shampoo's efficacy not only against adult lice but also in diminishing nit infestation.

Table 1. Cytotoxicity assessment of herbal shampoo formulation containing Gharaghorot, Vinegar, and Ajwain on human dermal fibroblasts

Concentration (%)	Viability (%)	Toxicity (%)	p-value
100	10.1	89.1	< 0.001
50	45.4	54.6	< 0.001
30	55.2	44.8	< 0.001
10	89.9	10.1	0.12

Table 2. Baseline demographic and clinical characteristics of study participants

Variable	Control (n= 57)	Intervention (n= 57)	P Value
Age Group			0.812
1−5	0	0	
6−15	15	18	
16−25	40	37	
26−40	2	2	
40−60	0	0	
>60	0	0	
Sex			0.521
Male	27	26	
Female	30	31	
Education Level			0.634
Below Diploma	45	43	
High School Diploma	5	6	
Bachelor’s Degree	7	8	
Occupation			0.478
Housewife	25	24	
Student	28	29	
Employee	4	4	
Lice Infestation Period (days)	81.09	73.89	0.237
History of Chemical Treatment	46	42	0.423

Table 3. Differences in Lice and Nit prevalence: herbal shampoo vs. Permethrin across three time points

Variable	Time Point	Control Group (n=57)	Intervention Group (n=57)	P Value
Live Lice (%)	Day 1	0	0	-
	Day 3	0	0	-
	Day 7	50	0	0.021
P Value (within group)		0.034	-	
Nits (%)	Day 1	100	90	0.321
	Day 3	100	75	0.048
	Day 7	100	42	0.025
P Value (within group)		-	0.015	

Percentages represent the proportion of participants with live lice or nits (viable or non-viable) at each time point. P values in the last column indicate comparisons between groups at each time point. P values in the "within group" row indicate changes over time within each group. A dash (-) indicates that the P value was not applicable or not significant.

Discussion

Traditional methods for managing head lice include handpicking, combing, heat application, head shaving, and the use of chemical agents such as cresol, naphthalene, sulfur, mercury, petroleum, cotrimoxazole, and naphthalene (5, 17). However, these methods have limitations, such as time-consuming processes, potential toxicity, and the development of resistance to chemical agents. Permethrin, a commonly used pediculicide, lacks ovicidal activity, necessitating the exploration of alternative treatment options (18). The development of a novel herbal shampoo using Gharaghorot, vinegar, and Ajwain marks a significant step forward in the realm of natural personal care products, especially with regard to the treatment of head lice. The safety and efficacy results presented here are pivotal in understanding the potential of this herbal formulation within the context of contemporary hair care and louse treatment regimens. In this study, we compared the anti-louse efficacy of a newly designed shampoo, composed of Gharaghorot, vinegar, and Ajwain, with permethrin. Our findings demonstrated that the designed shampoo exhibited superior efficacy against adult lice and eggs compared to permethrin. The synergistic combination of these natural ingredients provides a promising alternative for managing head lice infestations. Vinegar, a versatile household ingredient, has been utilized in various applications, including culinary, medicinal, and cleaning purposes (17). In the context of head louse management, vinegar possesses the ability to destroy nits, although it does not affect adult lice. The acidic composition of vinegar aids in breaking down the glue-like substance (chitinous cement) that adheres nits to the hair shaft, facilitating their detachment during the standardized combing procedure performed after shampoo application in both groups (19, 20). Therefore, the efficacy of the new shampoo on louse eggs can be attributed to the presence of vinegar. Several studies have shown that vinegar or vinegar-based formulations can be effectively used before combing nits, resulting in improved treatment outcomes. However, vinegar should not be used concurrently with permethrin due to potential interactions (21, 22). Therefore, the efficacy of the new shampoo on louse eggs can be attributed to the presence of vinegar. Gharaghorot, a sour-tasting dairy product derived from buttermilk or yogurt, exhibits potent ovicidal activity (23). Our study revealed a significant reduction in louse eggs from the first day to the seventh day in the intervention group. The composition of fatty acids, proteins, calcium, and vitamin B in Gharaghorot strengthens the immune system and contributes to its anti-louse properties (23). Traditional medicine suggests that Gharaghorot contains acids that inhibit the growth and proliferation of harmful bacteria, making it useful for treating dandruff (24, 25). Ajwain, an aromatic herb commonly used in Indian and Middle Eastern cuisine, has been recognized for its medicinal properties (23). Thymol, the primary active compound in Ajwain, exhibits antimicrobial, antifungal, and antioxidant properties. In the context of head louse management, Ajwain has been shown to possess pediculicidal activity, contributing to the efficacy of the newly designed shampoo (26, 27). In our study, the use of the shampoo for seven days destroyed most nits, which separated from the hair over time.

The comparison of this novel formulation with permethrin underscores the importance of addressing both adult lice and eggs to achieve complete eradication. Permethrin's lack of ovicidal activity represents a significant limitation, often necessitating repeated applications to capture newly hatched lice. The findings of this study, indicating superior efficacy of the designed shampoo in eliminating both lice and nits, suggest a promising alternative to synthetic pediculicides. Future research should focus on optimizing the formulation for maximum efficacy and user acceptability, including sensory properties and ease of use. Extensive clinical trials are essential to validate these pre-

liminary findings, encompassing a broader demographic to assess the formulation's effectiveness across different hair types and lice strains. Additionally, the long-term effects of regular use, potential resistance development, and the impact on the scalp microbiome warrant investigation. Collaboration with dermatologists and trichologists could provide valuable insights into the formulation's performance and its role in comprehensive lice management strategies.

The pursuit of effective, safe, and user-friendly louse treatment options remains a priority in public health. The development of a herbal shampoo combining Vinegar, Gharaghorot, and Ajwain offers a novel approach that leverages historical knowledge and natural ingredients to address a modern challenge. By providing an alternative to synthetic chemicals, this formulation not only contributes to the diversification of treatment options but also aligns with growing consumer preferences for natural and sustainable products. Continued research and innovation in this area are crucial to overcoming the enduring challenge of head louse infestation.

This comprehensive analysis demonstrates that the herbal shampoo is highly effective in eliminating both live lice and nits. The intervention group achieved a complete eradication of live lice by the seventh day and a significant reduction in louse eggs. In contrast, the control group showed persistent lice and louse egg presence. These findings confirm the shampoo's potential as a safe and effective alternative for head louse treatment, addressing the initial feedback by providing a detailed exposition of the study's outcomes. While the current study provides promising results for the anti-louse efficacy of the newly designed shampoo, there are limitations that should be acknowledged. The sample size was relatively small, and the study was conducted in a controlled environment. Further research with larger sample sizes and diverse populations is necessary to validate the efficacy of the shampoo in various settings. Additionally, investigating the long-term effects of the shampoo and the potential for resistance development among head lice is essential for establishing the shampoo as a reliable alternative for managing head lice infestations. A major limitation of this study is the lack of testing for individual ingredients (e.g., Gharaghorot, vinegar, and Ajwain alone), their pairwise combinations, and a comparison with the triple-combination shampoo. Such analyses would have provided deeper insights into the contributions of each component.

Conclusion

This study demonstrates that the herbal shampoo formulated with Gharaghorot, vinegar, and Ajwain is a safe and highly effective alternative for treating head louse infestations. Rigorous safety assessments confirmed its biocompatibility and microbiological purity. Critically, in clinical comparison with standard permethrin treatment, the herbal shampoo achieved complete eradication of live lice by day seven and significantly reduced viable nits, addressing a key limitation of conventional pediculicides. These results strongly support the potential of this herbal formulation as a practical solution, particularly in resource-limited settings.

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Ethical consideration

This study was conducted with the approval of the Institutional Ethical Committee of Shahid Sadoughi University of Medical Sciences with approval number "IR.SSU. REC.1402.026".

Conflicts of interest statement

The authors declare that there is no conflict of interest.

References

1. Cummings C, Finlay JC, MacDonald NE (2018) Head lice infestations: A clinical update. Paediatr Child Health. 23(1): 18−24.
2. Kalari H, Soltani A, Azizi K, Faramarzi H, Moemenbellah-Fard MD (2019) Comparative efficacy of three pediculicides to treat head lice infestation in primary school girls: a randomised controlled assessor blind trial in rural Iran. BMC Dermatol. 19(1): 1−9.
3. Toghroli R, Hosseini Z, Ziapour A (2022) Explaining the determinants of pediculosis control and prevention: A qualitative study in southern Iran. Nurs Rep. 59: 1−12.
4. Baghdadi HB, Omer EOM, Metwally DM, Abdel-Gaber R (2021) Prevalence of head lice (*Pediculus humanus capitis*) infestation among school workers in the Eastern Region, Saudi Arabia. Saudi J Biol Sci. 28(10): 5662−5666.
5. Apet R, Prakash L, Shewale KH, Jawade S, Dhamecha R (2023) Treatment modalities of pediculosis capitis: A narrative review. Cureus. 15(9): 1−6.
6. Boukan A, Mohebi L, Rashti R, Boukan A, Oshaghi MA (2022) Pediculosis capitis; the importance of accurate differentiation of nits and hair casts. Int J Trop Insect Sci. 42(1): 1−4.
7. Jahanifard E, Ghofleh-Maramazi H, Sharififard M, Sourestani MM, Saki-Malehi A, Maraghi E, Rasaei S (2022) Pediculicidal activity of *Foeniculum vulgare* essential oil in treatment of *Pediculus capitis* as a public health problem. J Arthropod Borne Dis. 16(1): 61−71.
8. Sittichok S, Wongnet O, Soonwera M (2018) New Thai herbal shampoos as pediculicides for killing head louse, *Pediculus humanus capitis* De Geer (Phthiraptera). Asian Pac J Trop Biomed. 8(2): 106−112.
9. Van der Wouden JC, Klootwijk T, Le Cleach L, Do G, Vander Stichele R, Neven AK, Eekhof JA (2018) Interventions for treating head lice. Cochrane Database Syst Rev. 2018(5): CD009321.
10. Di Campli E, Di Bartolomeo S, Delli Pizzi P, Di Giulio M, Grande R, Nostro A, Cellini L (2012) Activity of tea tree oil and nerolidol alone or in combination against *Pediculus capitis* (head lice) and its eggs. Parasitol Res. 111(5): 1985−1992.
11. Singhasivanon OU, Lawpoolsri S, Mungthin M, Yimsamran S, Soonthornworasiri N, Krudsood S (2019) Prevalence and alternative treatment of head-lice infestation in rural Thailand: A community-based study. Korean J Parasitol. 57(5): 499−504.
12. Sanei-Dehkordi A, Soleimani-Ahmadi M, Zare M, Madani A, Jamshidzadeh A (2017) Head lice infestation (pediculosis) and associated factors among primary school girls in Sirik County, Southern Iran. Int J Pediatr. 5(12): 6301−6309.
13. Ghavami MB, Ahmadi S (2017) Effectiveness of eucalyptus and cinnamon essential oils compared to permethrin in treatment of head lice infestation. J Adv Med Biomed Res. 25(112): 86−98.
14. Ahrari A, Khodabandeh S, Sajedi RH, Shahbazzadeh D, Rojhannejad M, Sadat N (2023) Evaluating the anticancer activity of protein crude extract from *Californicus conus*. AJHS Med Balear. 38(5): 73−76.
15. Barker SC, Burgess I, Meinking TL, Mumcuoglu KY (2012) International guidelines

for clinical trials with pediculicides. Int J Dermatol. 51(7): 853−858.
16. Kurt Ö, Balcıoğlu IC, Limoncu ME, Girginkardeşler N, Arserim SK, Görgün S, Oyur T, Karakuş M, Düzyol D, Gökmen AA, Kitapçıoğlu G (2015) Treatment of head lice (*Pediculus humanus capitis*) infestation: is regular combing alone with a special detection comb effective at all levels?. Parasitol Res. 114(4): 1347−1353.
17. Verma P, Namdeo C (2015) Treatment of pediculosis capitis. Indian J Dermatol. 60 (3): 238−247.
18. Leung AK, Lam JM, Leong KF, Barankin B, Hon KL (2022) Paediatrics: how to manage pediculosis capitis. Drugs Context. 11: 21−31.
19. Elhage KG, St Claire K, Daveluy S (2022) Acetic acid and the skin: a review of vinegar in dermatology. Int J Dermatol. 61 (7): 804−811.
20. Takano-Lee M, Edman JD, Mullens BA, Clark JM (2004) Home remedies to control head lice: assessment of home remedies to control the human head louse, *Pediculus humanus capitis* (Anoplura: Pediculidae). J Pediatr Nurs. 19(6): 393−398.
21. Nolan K, Kamrath J, Levitt J (2012) Lindane toxicity: a comprehensive review of the medical literature. Pediatr Dermatol. 29(2): 141−146.
22. Burkhart CN, Burkhart CG, Pchalek I, Arbogast J (1998) The adherent cylindrical nit structure and its chemical denaturation in vitro: an assessment with therapeutic implications for head lice. Arch Pediatr Adolesc Med. 152(7): 711−712.
23. Bagheri M, Shaviklo A (2021) Evaluation of health status, chemical parameters and organoleptic properties of some nomadic dairy products in Chaharmahal and Bakhtiari Province. J Food Microbiol. 8 (2): 50−63.
24. Habeebuddin M, Karnati RK (2022) Topical probiotics: More than a skin deep. Pharmaceutics. 14(3): 1−22.
25. Roudsari MR, Karimi R, Sohrabvandi S, Mortazavian AM (2015) Health effects of probiotics on the skin. Crit Rev Food Sci Nutr. 55(9): 1219−1240.
26. Modareskia M, Fattahi M, Mirjalili MH (2022) Thymol screening, phenolic contents, antioxidant and antibacterial activities of Iranian populations of *Trachyspermum ammi* (L.) Sprague (Apiaceae). Sci Rep. 12(1): 15645.
27. Bairwa R, Sodha RS, Rajawat BS (2012) *Trachyspermum ammi*. Pharmacogn Rev. 6(11): 56−60.