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Evaluation of the efficacy of topical *Nigella sativa* L. with vinegar in the treatment of acne vulgaris: A randomized controlled trial

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ABSTRACT

Background and objectives: Acne vulgaris stands as the prevailing dermatological condition on a global scale, prompting exploration into diverse therapeutic modalities. Hence, this study aimed to assess the efficacy of a formulation comprising *Kalonji* (*Nigella sativa*) and *Sirka* (vinegar) in comparison to benzoyl peroxide 5 % for the treatment of acne vulgaris.

Methods: In this open-label randomized controlled trial, 40 participants with acne in the age bracket of 13–40 years completed the 28-day treatment period. The Unani group received a *Nigella sativa* seed powder and cane vinegar, while the control group received benzoyl peroxide 5 % gel. Both interventions were applied topically at night. Primary outcomes encompassed changes in the Global Acne Grading System (GAGS) and Cook's Acne Grading Scale using PSAG scores. The secondary outcome was the change in Quality of Life assessed through the Cardiff Acne Disability Index (CADI). Safety evaluations included baseline and post-treatment measurements of hemograms, serum creatinine, serum bilirubin, and random blood sugar levels.

Results: The GAGS scores showed a mean difference of −2.600 (95 % CI: −5.770 - 0.570, d = 0.57, $p = 0.105$), PSAG scores exhibited a mean difference of −0.600 (95 % CI: −1.793 - 0.593, d = 0.49, $p = 0.315$), and CADI scores displayed a mean difference of −1.500 (95 % CI: −3.470 - 0.470, d = 0.32, $p = 0.132$) at the trial conclusion.

Conclusions: This trial suggests that the formulation may have therapeutic potential as an alternative acne treatment. Further studies with larger sample sizes and extended follow-up periods are recommended.

Clinical trial registration: The clinical trial was registered with Clinical Trial Registry – India under the registration number CTRI/2022/05/042368 on May 4, 2022.

Introduction

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit, which includes the hair follicle, hair shaft, and sebaceous gland.[1] It is characterized by non-inflammatory open and closed comedones that can further develop into inflammatory papules, pustules, nodules, or even scars.[1,2] Primarily affecting the face, neck, upper trunk, upper back, chest, and upper arms,[3] it is among the most common dermatological conditions worldwide, affecting an estimated 650 million people. Additionally, it ranked as the 8th most prevalent disease globally in 2010[4]. It is more prevalent between the ages of 15 and 18 in both genders, with a potential increase in prevalence among women during adolescence, as indicated by recent reports.[5]

Acne is generally caused by genetic predisposition and hormonal factors, such as increased androgens during puberty, which leads to an elevated production of sebum by the sebaceous glands. This hormonal influence also extends to women before the start of their menstrual cycle.[1] Bacterial colonization with *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) also contributes to the development of acne.[6] Additionally, various other factors play a role in the onset of

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acne, including climate changes, certain drugs (e.g., anabolic steroids, progestin-only contraceptive pills, corticosteroids), consumption of dairy products or foods with a high glycemic load,[3,7] prolonged use of oil-based cosmetics, and psychological factors such as anger and anxiety.[8]

Conventionally, acne has been treated with topical or systemic agents like benzoyl peroxide, antibiotics, retinoids, and laser therapy. However, benzoyl peroxide often causes side effects, including mild dermatitis, skin bleaching, and facial hair growth. In recent years, oral isotretinoin (a retinoid derived from vitamin A) has transformed acne management, though it comes with notable mucocutaneous and systemic side effects, such as teratogenicity, hepatotoxicity, dryness of the lips and nasal mucosa, and conjunctivitis.[1,2,9–11] Consequently, there is a need to explore a safe and effective alternative treatment option.

Traditional systems of medicine encompass diverse methodologies for addressing various diseases, each characterized by its own nomenclature and classification.[12–14] Globally, multiple traditional systems of medicine have garnered recognition from the World Health Organization (WHO).[15] Unani, an ancient system of medicine that originated in Central Asia, evolved from the teachings and principles of the renowned Greek physician Hippocrates (460–370 BC). In the Unani system of medicine, acne vulgaris is clinically similar to "*Buthūr-i Labaniyya*," which is also referred to as *"Zīrwān"* due to its resemblance to *Zīra* (cumin).[16] *Buthūr-i Labaniyya* are small, white, pointed eruptions resembling drops of milk that typically develop on the forehead, cheeks, and nose.[14,17,18] Upon squeezing, these eruptions release a thick, oily, or cheesy material.[19] It is widely accepted among Unani scholars that *Ghalīz khilt* (morbid humor) and *"Mada-i Sadīdiya* (ichorous matter/pus-like substance) are responsible for the development of acne, and these conditions could be effectively managed with drugs possessing *Muḥallil* (resolvent), *Jālī* (detergent), *Jāzib* (absorbent), and *Dāfi-i Ta'affun* (antiseptic and antimicrobial) properties.[20–22] For this purpose, numerous single and compound drugs have been prescribed by Unani scholars. Among all the recommended options, the use of *Kalonji* (*Nigella sativa*) along with *Sirka* (vinegar) is also suggested for the effective treatment of *Buthūr-i Labaniyya.* This combination possesses *Jālī* (detergent), *"Mujaffif"* (desiccant), and *Muḥallil* (resolvent) properties.[22] In light of this, the current study has been meticulously designed to evaluate and compare the safety and efficacy of a formulation comprising *Kalonji* (*Nigella sativa*) and *Sirka* (vinegar) with 5 % benzoyl peroxide gel for the treatment of acne vulgaris.

2. Materials and methods

2.1. Trial design and setting

This prospective, open label randomized controlled clinical trial was conducted from September 14, 2022, to January 5, 2023, at the outpatient department of Moalajat, Luqman Unani Medical College, Hospital, and Research Centre, Vijayapura (Bijapur), Karnataka, India.

2.2. Participants

Male or female participants aged 13–40 years, clinically diagnosed with mild to moderate acne vulgaris characterized by the presence of comedones, papules, pustules, nodules, itching, and erythema, were included in the clinical trial. However, individuals with comorbid conditions such as acne rosacea, acne fulminans, acne necrotica, psoriasis, eczema, as well as those with cognitive impairments, individuals undergoing corticosteroid or anticonvulsant therapy, or taking oral contraceptive pills within the last month, pregnant and lactating women, and individuals with other severe systemic illnesses were excluded from participation due to non-compliance with the trial protocol.

2.3. Selection of the participants

To recruit trial participants, they underwent a thorough screening process that included a complete medical history, a general physical examination, and systemic examination. The trial enrolled participants who met the inclusion and exclusion criteria and conducted basic investigations. The demographic profile of the participants, a detailed history of the disease, and a physical and systemic examination were all recorded in the IEC-approved Case Report Form.

2.4. Interventions

Participants were randomly assigned to two groups. In the test group, a freshly prepared *Ṭīlā* (liniment) was used, made by mixing *Kalonji* (*Nigella sativa*) powder with *Sirka* (vinegar). This liniment was applied locally once daily for at least 20–30 minutes in adequate quantity. In the control group, Benzoyl peroxide 5 % gel was applied in the same manner locally, once daily for at least 20–30 minutes in sufficient quantity. Participants from both groups underwent treatment for 28 days and were followed up on the 14th and 28th days.

2.5. Herbal medicine formulation

The trial formulation of *Kalonji* (*Nigella sativa*) and *Sirka* (vinegar) was selected from authentic Unani textbooks: *Al-Qanoon fil Tibb*, *Al-Mukhtarat fil-Tibb*, and *Tibb-e-Akbar*.[17–19]

2.6. Dosage regimen and quantitative description

Participants in the test group were instructed to prepare a paste of *Kalonji* (*Nigella sativa*) powder and *Sirka* (vinegar) in a 1:6 ratio, sufficient to cover the affected area. They were shown the proper method for preparing the liniment to ensure consistency. This liniment was to be applied locally once daily for 20–30 minutes in an adequate amount, over a span of 28 days. Meanwhile, participants in the control group used benzoyl peroxide 5 % gel, applying it locally once daily for 20–30 minutes in a sufficient quantity, also for 28 days.

2.7. Qualitative testing of trial drugs

Dried *Kalonji* (*Nigella sativa*) seeds and sugarcane *Sirka* (vinegar) were procured from Dharwadkar Sidhlingappa Ayurvedic and Unani Medical, Vijayapura, Karnataka, India. These materials were subjected to taxonomical identification and authentication by Miss Pooja Desai, a botanist from SECAB's ARS Inamdar Arts, Science, and Commerce College for Women, Vijayapura, Karnataka, India. The authenticated drugs have been deposited at the Museum of Luqman Unani Medical College and Hospital and Research Centre in Vijayapura, Karnataka, India,bearing the herbarium file number 04/HERB/LUMC/21 and Voucher Specimen No. DBARSI-BJP2102. For additional verification, the botanical name of the plant was cross-referenced with the online database http://www.worldfloraonline.org.

The dried *Kalonji* (*Nigella sativa*) seeds were properly cleaned. These seeds were then pounded using a mortar and pestle and subsequently passed through a sieve (no. 80) to obtain a fine powder. This powder and vinegar were given to the participants, who were advised to mix them thoroughly to create a homogeneous paste. They were then instructed to apply the paste to the acne lesions.

2.8. Rationale for the type of control used

Topical benzoyl peroxide is one of the well-recognized and efficacious first-line treatments for acne vulgaris, whether it be inflammatory or noninflammatory acne.[9,23] Benzoyl peroxide is among the most widely used topical medication in dermatology.[24] In concentrations of 5 %, 10 %, and 20 %, benzoyl peroxide has been effectively employed to

suppress acne.[25] It possesses antibacterial, anti-inflammatory, keratolytic, and wound-healing properties.[9,24] Moreover, benzoyl peroxide suppresses the growth of *C. acnes*.[9]

2.9. Outcomes

2.9.1. Primary outcome measures

The primary outcome measures were changes in the Global Acne Grading System (GAGS) and Cook's Acne Grading based on Photographic Standards for Acne Grading (PSAG), assessed at baseline and the end of the trial. The GAGS scores range from 0 to 44 and assess acne severity across six regions (forehead, cheeks, nose, chin, chest/back) by multiplying lesion severity by regional weights. Higher GAGS scores indicate more severe acne, with improvement shown by a reduction in score over time.[26] The PSAG uses a photographic scale from 0 to 8, where each level corresponds to standard images representing varying acne severities. By comparing patients to these images, clinicians rate acne severity; a lower PSAG score at the end of the trial signifies improvement.[27]

2.9.2. Secondary outcome measure

Secondary outcome measures encompassed changes in the patients' quality of life, as quantified by the Cardiff Acne Disability Index (CADI), evaluated both at the baseline and at the conclusion of the trial. The CADI is a validated tool specifically designed to measure the impact of acne on a patient's quality of life. It consists of five questions addressing feelings of social, emotional, and psychological impact related to acne, each scored from 0 to 3. The total score ranges from 0 to 15, with higher scores indicating greater impairment in quality of life. Improvement in quality of life is reflected by a decrease in the CADI score from baseline to the conclusion of the trial.[28,29]

2.10. Safety and adverse event monitoring

The safety assessment was conducted through clinical evaluation and laboratory investigations, specifically focusing on the Complete Blood Counts (CBCs), serum creatinine, and serum bilirubin. In addition, any adverse events that occurred during therapy were meticulously documented in line with the appropriate guidelines.

2.11. Withdrawal criteria

Withdrawal criteria were clearly defined to include adverse effects requiring additional treatment, voluntary withdrawal by the participant, non-attendance or use of any concurrent therapy at the prescribed treatment sessions. The implications of withdrawal and the procedures to be followed were fully explained to the participants during the informed consent process.

2.12. Sample size

The initial sample size estimate for the study was 40 participants. However, accounting for a 10 % allowance for potential dropouts, we increased it to 44 participants, with 22 in each group. This calculation assumed a mean difference of 5 in GAGS scores between the test and control groups, a standard deviation of 5.1, a two-sided confidence level of 0.05, and 80 % power, as inferred from the previous study.[30] The sample size was derived using the following formulae: $n_1 = \kappa n_2 and n_2 = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2 (1 + 1/\kappa)}{(\mu_T - \mu_C)^2}$. where n_1 = sample size of Unani group, n_2 = sample size of control group, σ = expected population standard deviation, $\mu_T - \mu_C$ = difference in group means, κ = ratio of sample size, treat/control, $Z_{\alpha/2} = 1.96$ (at $\alpha = 0.05$ with two-tail), and $Z_{\beta} = 0.84$ (at 80 % power).[31]

2.13. Randomization and blinding

A block randomization method was employed, using four-person blocks constructed by the Medical Records Department (MRD) of the institute. These blocks were placed in sequentially numbered, sealed, opaque envelopes to maintain allocation concealment. Although the trial was open-label, the investigators who assessed the outcomes were blinded to the treatment allocation to minimize bias. Blinding was not employed in the study due to the different dosage forms and visible characteristics of the study drugs between the two groups.

2.14. Statistical methods

A per protocol analysis was employed to compare the efficacy of the test and control drugs. Data were presented as proportions (%), mean ± SD, mean difference with a 95 % confidence interval (CI), and effect size in terms of Cohen's d value, as appropriate. Categorical and continuous variables, both at baseline and post-treatment, were compared between the groups using the Chi-square/Fisher's exact test and Student's *t*-test, as appropriate.

2.15. Ethical statement

This research complied with all applicable national and institutional regulations and with the precepts of the Helsinki Declaration (as revised in 2013). It was approved by the Institutional Ethics Committee of Luqman Unani Medical College Hospital and Research Centre under the protocol number BJP/LUMC/IEC/2020–21/MOALIJAT/02. Additionally, the trial was registered on the Clinical Trial Registry of India (CTRI) with the CTRI number CTRI/2022/05/042368.

2.16. Informed consent

Written informed consent was obtained from the trial participants before administering the interventions. The subjects were given ample opportunity to discuss the aim and objectives of the study, its nature, the intervention to be administered, and the method of treatment, all in their native language. Participants were also informed of potential side effects, including skin irritation and dryness associated with both BPO and Unani medications.

Note: Assent was not required as all enrolled participants were 18 years or older, despite the inclusion criteria of 13-40 years.

3. Results

3.1. Participant flow

This study was conducted from September 14, 2022, to January 5, 2023. A total of 50 patients were assessed for eligibility in the study, of which 43 met the stipulated inclusion criteria and were consequently enrolled. The remaining 7 individuals were excluded due to non-compliance with the specified criteria. Of the 43 patients enrolled, 40 successfully completed the treatment course, while 3 participants (2 from the test group and 1 from the control group) were lost to follow-up and did not return for their final assessment without specifying the reason. The subsequent statistical analysis was performed on the 40 participants who adhered to and completed the prescribed therapy regimen (Fig. 1).

3.2. Clinico-demographic profile

The study involved 40 participants with mild to moderate acne vulgaris. The mean age ± SD of the participants was 21.60 ± 2.46 years. The majority (70.0 %) were in the 20–25 year age group, with 15.0 % each in the 18–20 year and 25–30 year groups. In the test group, there were 11 (50.0 %) males and 9 (45.0 %) females, while the control

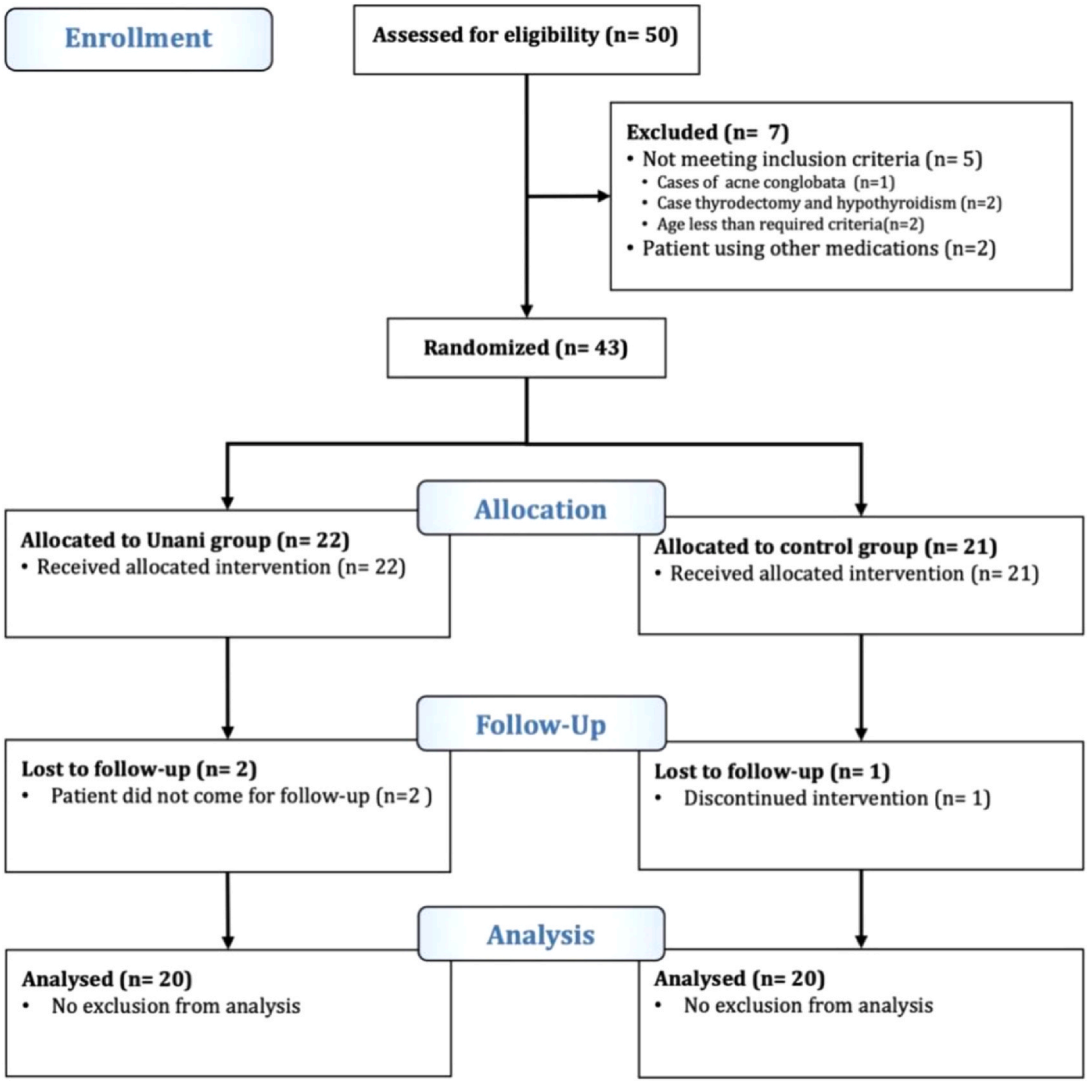

Fig. 1. CONSORT flow diagram of the participants studied in test and control groups.

group had 13 (65.0 %) males and 7 (35.0 %) females. The average BMI was 21.45 ± 2.85, with 47.5 % falling within the 20–25 kg/m^2 range, 35.0 % having a BMI < 20 kg/m^2, and 17.5 % in the 25–30 kg/m^2 range. Out of the 40 participants, the majority (95.0 %) were unmarried, with only 5.0 % married. Regarding occupation, 77.5 % were students, and 22.5 % had various occupations such as worker, housewife, mechanic, teacher, computer operator, and businessperson. Dietwise, 97.5 % followed a mixed diet pattern, while 2.5 % followed a vegetarian diet. In terms of socio-economic status, 37.5 % belonged to the Lower Middle class, 30.0 % to Upper Middle, 22.5 % to Upper Lower, and 5.0 % each to the Lower and Upper classes. Moreover, 50.0 % had oily skin, and 50.0 % had common skin. In terms of family history, 87.5 % had no family history of the condition, and 12.5 % had a positive family history. Habitat-wise, 92.5 % came from urban areas, and 7.5 % from rural areas. Regarding temperament, 70.0 % had *Damawī Mizāj*, 25.0 % *Ṣafrāwī Mizāj*, and 5.0 % *Balghamī Mizāj*. According to Unani concepts, human beings are classified into four temperaments that influence their body, mind, and pneuma. These types are based on dominant humors and include *Damawī* (sanguine, hot and wet), *Ṣafrāwī* (choleric, hot and dry), *Balghamī* (phlegmatic, cold and wet), and *Sawdāwī* (melancholic, cold and dry)[32]. In summary, the demographic and clinical characteristics at baseline were comparable between the Unani and control groups, with no statistically significant differences ($p > 0.05$) (Table 1).

3.3. Change in the GAGS score from baseline to end of the trial

In the test group, the mean ± SD of the GAGS score at baseline was 14.85 ± 4.41. Subsequently the score had significantly reduced at the end of trial to 5.55 ± 4.67 ($p < 0.0001$), indicating a considerable treatment effect. Similarly, in the control group, the mean ± SD of the GAGS score was initially 15.50 ± 4.39 at baseline and it significantly reduced to 8.15 ± 5.22 at the end of the trial ($p < 0.0001$) (Table 2). Furthermore, the end-of-trial observed mean difference between the groups was −2.600, with a 95 % confidence interval of −5.77–0.57, d = 0.52, and a p-value of 0.105 (Table 3).

These results underscore the efficacy of both therapeutic strategies in mitigating acne vulgaris symptoms, as reflected by the GAGS score. Even though both treatments notably reduced GAGS scores throughout the trial, the statistically insignificant difference between the groups attests to the comparable efficacy of the two interventions.

3.4. Change in the Cook's Acne Grading based on PSAG score

In the test group, the mean ± SD of the PSAG score at the baseline was 3.70 ± 1.62. By the end of the trial therapy, this score had decreased significantly to 1.60 ± 1.90 ($p < 0.0001$). Similarly, at baseline, the control group had a mean ± SD PSAG score of 3.90 ± 1.78. This too saw a significant decrease by the end of the trial,

Table 1
Baseline demographic and clinical profiles of the participants.

Characteristics		Unani group (n = 20)	Control group (n = 20)
Age	mean ± SD, years	22.10 ± 2.85	21.10 ± 1.94
BMI	mean ± SD, kg/m^2	22.11 ± 2.86	20.98 ± 2.80
Gender	Male, n, %	11 (55.0 %)	13 (65.0 %)
	Female, n, %	9 (45.0 %)	7 (35.0 %)
Marital status	Married, n, %	2 (10.0 %)	0 (0.00 %)
	Unmarried, n, %	18 (90.0 %)	20 (100.0 %)
Occupation	Student, n, %	16 (80.0 %)	15 (75.0 %)
	(Worker, housewife, mechanic, businessman, teacher, computer operator), n, %	4 (20.0 %)	5 (25.0 %)
Religion	Muslim, n, %	20 (100.0 %)	18 (90.0 %)
	Hindu, n, %	0 (0.00 %)	2 (10.0 %)
Diet	Vegetarian, n, %	0 (0.00 %)	1 (5.0 %)
	Mixed, n, %	20 (100.0 %)	19 (95.0 %)
Socio Economic Status	Upper, n, %	0 (0.00 %)	2 (10.0 %)
	Upper Middle, n, %	7 (35.0 %)	5 (25.0 %)
	Lower Middle, n, %	9 (45.0 %)	6 (30.0 %)
	Upper lower, n, %	4 (20.0 %)	5 (25.0 %)
	Lower, n, %	0 (0.00 %)	2 (10.0 %)
Mizaj (Temperament)	Damawī, n, %	17 (85.0 %)	11 (55.0 %)
	Ṣafrāwī, n, %	2 (10.0 %)	8 (40.0 %)
	Balghamī, n, %	1 (5.0 %)	1 (5.0 %)
Skin Type	Oily, n, %	10 (50.0 %)	10 (50.0 %)
	Common, n, %	10 (50.0 %)	10 (50.0 %)
Family History	Present, n, %	1 (5.0 %)	4 (20.0 %)
	Absent, n, %	19 (95.0 %)	16 (80.0 %)
Habitat	Urban, n, %	20 (100.0 %)	17 (85.0 %)
	Rural, n, %	0 (0.00 %)	3 (15.0 %)
GAGS Score	mean ± SD	14.85 ± 4.41	15.50 ± 4.39
PSAG	mean ± SD	4.60 ± 2.62	5.85 ± 2.72
CADI	mean ± SD	3.70 ± 1.62	3.90 ± 1.78

*Independent samples *t*-test, #Chi-square test
Between-group difference was not significant ($p > 0.05$) in all the characteristics

registering at 2.20 ± 1.82 ($p < 0.001$) (Table 2). Furthermore, at the end of the trial, the observed mean difference between the groups was −0.60 (95 % CI: −1.79 - 0.59, d = 0.32, $p = 0.315$) (Table 3).

These findings reveal consistent reductions in the PSAG scores for both the test and control groups. However, the between-group difference was not statistically significant, emphasizing the comparable effectiveness of both drugs.

3.5. Change in the CADI score

In the test group, the mean ± SD of the CADI score at baseline was 4.60 ± 2.62. After completion of the trial therapy, this score significantly reduced to 3.05 ± 2.78 ($p < 0.0001$), representing a substantial improvement in the quality of life as related to the dermatological condition. Similarly, in the control group, the mean ± SD of the CADI score was initially 5.85 ± 2.72 at baseline. By the end of the trial, this score had significantly reduced to 4.55 ± 3.35 ($p < 0.001$) (Table 2). However, the observed mean difference between the groups at the end of the trial was −1.500 (95 % CI: −3.470 - 0.470, d = 0.49, $p = 0.132$) (Table 3).

Table 3
Between-group comparison of the scores and effect estimates.

Scale	Mean Difference	p-Value*	95 % CI	Cohen's d
GAGS	−2.60	0.105	−5.770, 0.570	0.52
CADI	−1.50	0.132	−3.470, 0.470	0.49
PSAG	−0.60	0.315	−1.793, 0.593	0.32

Effect sizes are indicated by Cohen's d: d = 0.2 for small effect, d = 0.5 for medium effect, and d ≥ 0.8 for large effect.

These findings highlight the therapeutic benefits of both treatments on the quality of life of the acne vulgaris patients. Both interventions resulted in significant improvements in the quality of life, with no

Table 2
Changes in the GAGS, CADI and PSAG scores.

Scale	Visit	Test group (mean ± SD)	Control group	*p*-Value[a]
GAGS	**Baseline**	14.85 ± 4.41	15.50 ± 4.39	0.105
	After treatment	5.55 ± 4.67	8.15 ± 5.22	
	***p*-Value**[b]	$p < 0.0001$	$p < 0.0001$	-
CADI	**Baseline**	4.60 ± 2.62	5.85 ± 2.72	0.132
	After treatment	3.05 ± 2.78	4.55 ± 3.35	
	***p*-Value**[b]	$p < 0.0001$	$p < 0.0001$	-
PSAG	**Baseline**	3.70 ± 1.62	3.90 ± 1.78	0.315
	After treatment	1.60 ± 1.90	2.20 ± 1.82	
	***p*-Value**[b]	$p < 0.0001$	$p < 0.0001$	-

[a] Independent samples *t*-test. The differences were not statistically significant ($p > 0.05$) for all outcomes, including GAGS, CADI and PSAG scores.
[b] Paired samples *t*-test (the within-group differences were statistically significant with $p < 0.0001$)

significant difference between the two groups, reinforcing their comparable effectiveness.

3.6. Safety parameters

Throughout the duration of the trial, the participants remained largely unaffected by adverse side effects, as no such events were reported. This suggests that both interventions were well-tolerated by the participants. Furthermore, while the trial did register statistically significant changes in certain laboratory parameters, it is crucial to note that these changes remained within the clinically accepted normal ranges. In other words, despite the statistical variations, the actual health implications of these changes were negligible, reinforcing the safety of the interventions tested.

4. Discussion

The study was conducted to compare the efficacy of topically applied *Kalonji* (*Nigella sativa*) seed powder mixed with *Sirka* (vinegar) versus Benzoyl peroxide 5 % in the management of *Buthūr-i Labaniyya* (acne vulgaris). Based on the preliminary findings, it may be inferred that the efficacy and safety of the test and control drugs were comparable in ameliorating the overall disease severity and clinical features.

The specific mechanism by which *Nigella sativa* and vinegar treat acne remains elusive, but it may be postulated that its anti-microbial action against *P. acnes*, combined with anti-inflammatory, antioxidant, and anti-keratinization properties, is crucial. Its efficacy can be linked to its pharmacological characteristics outlined in Unani medicine, substantiated by modern scientific studies. These include its properties like *Jālī* (skin cleansing), *Qātil-i Jarāsīm* (anti-microbial), *Mujaffif* (desiccative), *Muḥallil-i Awrām* (anti-inflammatory), and *Musakkin-i Alam* (analgesic) properties.[33–36]

Recent studies have indicated that inflammation and oxidative stress may play an early role in initiating the pathogenesis of acne. The activities of antioxidant defense enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in leukocytes are decreased in acne patients. Thus, the drugs with anti-inflammatory and antioxidant effects may be valuable in acne treatment.[37] *Nigella sativa* seed extract, especially thymoquinone, has proven anti-inflammatory effects in various conditions like pancreatic cancer, allergic airway inflammation, mix glial cells, asthma, and rheumatoid arthritis. It reduces pro-inflammatory cytokines, inhibits NF-kB, and exhibits analgesic and antipyretic properties in animal models.[38–42] Moreover, vinegar contains bioactive compounds, including polyphenols and vitamins. These compounds are renowned for their strong antioxidant properties, offering protection against oxidative stress.[43]

Cutibacterium acnes, formerly known as *Propionibacterium acnes*, is a principal microbial contributor to the onset of acne vulgaris, as highlighted in numerous studies.[6,44] In relation to this, *Nigella sativa* has emerged as a potent antimicrobial agent. Extracts from *N. sativa* seed have exhibited remarkable antibacterial efficacy against a range of bacterial strains, such as *MDR E. coli, Klebsiella pneumoniae, MRSA, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and certain oral bacteria. Both the essential oils and seed extracts of *Nigella sativa* have displayed profound inhibitory actions against these microbes, underscoring their potential for antimicrobial applications.[45–53] Complementing the antimicrobial properties of *Nigella sativa*, vinegar brings its own set of antibacterial properties to the table. The inherently low pH of vinegar fortifies its antimicrobial prowess. Notably, the organic acids found in vinegar, with acetic acid being the chief constituent, have the ability to breach the cell membranes of bacteria, subsequently leading to bacterial cell apoptosis.[43] Thus, the combination of *Nigella sativa* and vinegar offers a promising dual approach in targeting the bacterial elements associated with acne vulgaris (Fig. 2).

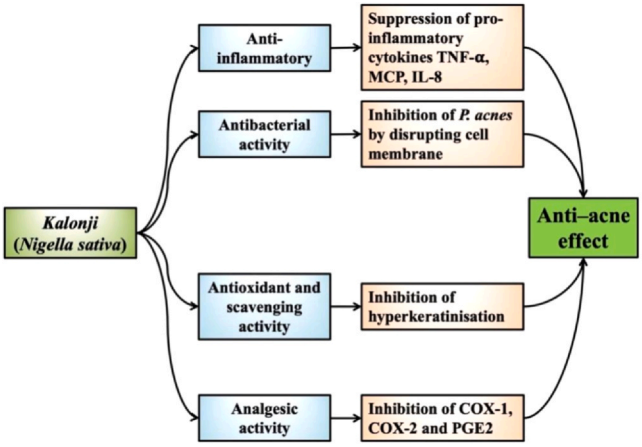

Fig. 2. Postulated mechanism of action by which *Nigella sativa* influences acne pathogenesis.

This alignment in pharmacological results reinforces that the tested drugs might be comparable with benzyl peroxide in terms of efficacy for acne treatment. Such findings advocate for further research into these medications, positioning them as promising alternatives against acne vulgaris.

4.1. Strength and limitations of the study

This study was meticulously designed and executed to minimize the potential for bias and confounding variables, and reported in line with the guidelines established by the CONSORT extension for herbal medicine interventions. To enhance the robustness of our findings, we used two validated primary outcome measures, the Global Acne Grading System (GAGS) and Cook's Acne Grading, providing a comprehensive assessment of acne severity. A rigorous randomization process was implemented to ensure balanced distribution across groups, thereby minimizing the impact of known and unknown confounding variables.

However, several limitations should be noted. The small sample size may reduce the statistical power, potentially limiting the ability to detect significant differences or associations. The study duration was brief, and without a post-treatment follow-up period, we were unable to assess the long-term efficacy or recurrence of acne vulgaris. Additionally, as an open-label trial, the lack of blinding may introduce bias, as participants and researchers were aware of treatment assignments. Differential treatment experiences may also have influenced participant-reported outcomes, as the Unani group had to prepare a liniment before application, while the control group used a pre-prepared gel. Finally, conducting the study at a single centre limits the generalizability of these findings to broader populations.

4.2. Future recommendations

Future studies should be designed to address the limitations identified in this study. Furthermore, considerable research has been conducted to better understand the biological systems using computational approaches such as genomics, transcriptomics, proteomics, metabolomics, and metagenomics, which can be combined with further experimental work in this area. In addition, the cream formulation of the test formulation may improve patient compliance and efficacy.

5. Conclusion

The study results suggest that the trial drug, *Kalonji* (*Nigella sativa*) combined with *Sirka* (vinegar), may offer comparable efficacy to the standard treatment of benzoyl peroxide for acne vulgaris. Given its favorable safety profile and observed improvements, this therapy shows

promise as a potential alternative treatment. However, further studies with larger sample sizes and extended follow-up periods are recommended to fully assess its long-term efficacy and safety.

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CRediT authorship contribution statement

Shaikh Nisar Ahmed Abbas: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Arshan Horti:** Writing – review & editing, Visualization, Validation, Resources, Formal analysis. **Muhammed Rashid Allapat:** Writing – review & editing, Visualization, Validation, Software. **Mamadapur Saba Abdul Razzaq:** Visualization, Validation, Resources, Conceptualization. **Farooqui Shazia Parveen:** Writing – review & editing, Visualization, Validation, Formal analysis. **Nazim Husain:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization..

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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