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Clinical and anti-inflammatory efficacy of Berberine hydrochloride mouthwash versus chlorhexidine and saline in plaque-induced gingivitis: a randomized controlled trial

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Abstract

Background

Plaque-induced gingivitis is a reversible inflammatory condition resulting from microbial biofilm accumulation, and is managed through mechanical and chemical plaque control. Chlorhexidine remains the gold-standard chemical anti-plaque agent, but its long-term use is limited by adverse effects such as tooth staining and taste alteration. Berberine hydrochloride, an isoquinoline alkaloid derived from Berberis aristata, has demonstrated antimicrobial, and anti-inflammatory properties in preclinical studies, but clinical evidence for its use in periodontal therapy remains limited. This study evaluated the clinical and anti-inflammatory efficacy of a Berberine HCl-containing mouthwash compared with 0.2% chlorhexidine and saline in patients with plaque-induced gingivitis.

Methods

This randomized, double-blind, controlled clinical trial enrolled 93 patients aged 18–40 years with plaque-induced gingivitis, allocated equally into three groups (n = 31 each): 0.2% chlorhexidine, 0.9% saline, and Berberine HCl mouthwash, each used 10 mL twice daily for 15 days. Allocation concealment was achieved using identical amber plastic bottles. The primary outcomes, Full Mouth Plaque Score (FMPS) and Full Mouth Bleeding Score (FMBS), were recorded at baseline, day 15, and day 21. Secondary outcomes, salivary interleukin-4 (IL-4) and interleukin-6 (IL-6), were quantified by ELISA at baseline, day 15 and day 21. Intergroup comparisons were performed using one-way ANOVA with Tukey HSD post-hoc tests; intragroup comparisons over time were analysed using paired t-tests.

Results

At day 15 and day 21, all three interventions produced significant reductions in FMPS and FMBS from baseline ($p < 0.001$). Chlorhexidine showed the greater reduction in FMPS and FMBS at both timepoints, followed by Berberine, with Saline showing the least effect. Salivary IL-6 decreased significantly in all groups by day 15, with Chlorhexidine showing the greatest reduction, followed by Berberine. Salivary IL-4 declined significantly in the Chlorhexidine and Saline groups, whereas the Berberine group maintained comparatively higher IL-4 levels at day 15.

Conclusion

Berberine HCl mouthwash produced significant reductions in plaque accumulation, gingival bleeding, and salivary IL-6, with sustained clinical benefit through day 21. Although chlorhexidine demonstrated superior overall efficacy, Berberine HCl mouthwash may serve as a promising herbal adjunct in the management of plaque-induced gingivitis.

Trial registration

CTRI/2025/11/115219.

Introduction

Plaque-induced gingivitis is among the most prevalent inflammatory conditions affecting the oral cavity, arising from the accumulation of microbial biofilm at the gingival margin [1]. The disruption of homeostasis between the biofilm and the host immune response triggers the release of inflammatory mediators, including cytokines and chemokines, which drive the clinical signs of gingival inflammation [1]. Interleukin-6 (IL-6) is a key pro-inflammatory cytokine implicated in this

process, while interleukin-4 (IL-4) exerts a regulatory, anti-inflammatory role [1]. Since dental plaque biofilm cannot be permanently eliminated, management is directed at reducing microbial load through mechanical and chemical plaque control measures [1].

Chemical antiplaque agents, particularly mouthwashes, are widely used adjuncts to mechanical plaque control, acting by inhibiting bacterial adhesion, colonization, and metabolic activity [1]. Chlorhexidine gluconate mouthwash is considered as gold standard in plaque control, because to its broad-spectrum bactericidal and bacteriostatic activity and high oral substantivity [2]. However, prolonged chlorhexidine use is associated with adverse effects, including tooth staining, taste alteration, and mucosal irritation, which limit its suitability for long-term maintenance therapy.

This has prompted growing clinical interest in herbal alternatives that combine antimicrobial and anti-inflammatory efficacy reduced drawbacks. Berberine, an isoquinoline alkaloid originally isolated from Berberis vulgaris and abundant in the Himalayan medicinal plant Berberis aristata, has demonstrated antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties across a range of preclinical models [3–6]. Its anti-inflammatory action has been attributed to modulation of several signalling pathways, including nuclear factor kappa-B (NF-κB), activator protein-1 (AP-1), Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinases (MAPKs), and AMP-activated protein kinase (AMPK) [3]. Berberine hydrochloride (BBR-HCl) is the preferred salt form for mouthwash formulation owing to its water solubility and stability in aqueous media, which facilitate adequate contact with the oral mucosa during rinsing [5].

Preclinical and in vitro studies have shown that Berberis aristata extracts exhibit antibacterial activity against both Gram-positive and Gram-negative oral pathogens, along with anti-adhesion, and anti-biofilm effects [6, 7]. Berberine has also been shown to downregulate pro-inflammatory mediators, including tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), IL-6, and monocyte chemoattractant protein-1 (MCP-1), and to inhibit cyclooxygenase-2 (COX-2) expression and matrix metalloproteinase activity relevant to periodontal tissue breakdown [8, 9]. Despite this substantial preclinical evidence, clinical studies evaluating berberine hydrochloride specifically as a therapeutic mouthwash in plaque-induced gingivitis remain limited [4]. Prior clinical work on herbal mouthwashes, including thymus extract, curcumin, and polyherbal formulations, has demonstrated comparable reductions in gingival inflammation and salivary cytokine levels relative to chlorhexidine [14–18], but berberine hydrochloride has not been comparably evaluated using both clinical indices and salivary inflammatory biomarkers within a single randomized trial.

This knowledge gap forms the basis of the present study. We hypothesized that a Berberine HCl-containing mouthwash would produce clinical and anti-inflammatory effects on plaque-induced gingivitis comparable to those achieved with 0.2% chlorhexidine, and superior to saline mouth rinse.

The aim of this randomized controlled trial was to evaluate and compare the effect of a Berberine HCl-containing mouthwash on the Full Mouth Plaque Score (FMPS) and Full Mouth Bleeding Score (FMBS), and on salivary levels of IL-4 and IL-6, against 0.2% chlorhexidine mouthwash and saline rinse in patients with plaque-induced gingivitis.

Materials and Methods

Study design and setting:

This study was designed as a prospective, randomized, double-blind, controlled clinical trial conducted in the Department of Periodontology, A.B. Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Deralakatte, Mangaluru, Karnataka, India. Salivary biomarker analysis was performed at the Speciality Central Research Laboratory, KSHEMA, Nitte (Deemed to be University). Berberine mouthwash was formulated at the NGSM Institute of Pharmaceutical Sciences.

Ethics approval

The study protocol was approved by the Institution's Central Ethics Committee, Nitte (Deemed to be University) (Reference No. ETHICS/ABSMIDS/451/2024/V2). Written informed consent was obtained from all participants prior to enrolment. The trial is registered with the Clinical Trials Registry – India (CTRI/2025/11/115219).

Study population and eligibility criteria

Ninety-three subjects aged 18–40 years, presenting to the Department of Periodontology with plaque-induced gingivitis, were recruited.

Inclusion criteria:

1. Age 18–40 years
2. Moderate to severe gingivitis, with a minimum of 20 permanent teeth exhibiting gingival inflammation
3. No history of periodontal surgery or use of antibiotics/anti-inflammatory medication in the preceding 6 months
4. Systemically healthy

Exclusion criteria:

1. Systemic disease with periodontal impact (e.g., diabetes mellitus, osteoporosis)
2. Uncooperative patients
3. Current use of antibiotics and/or anti-inflammatory drugs
4. History of drug allergy or hypersensitivity reaction
5. Smokers

Sample size calculation

The sample size was calculated using nMaster software (version 2), based on a pooled standard deviation of 0.0365, a mean difference of 0.3, an effect size of 0.8219, an alpha error of 5%, and 80% power. This yielded 31 participants per group, for a total sample size of 93.

Randomization, allocation concealment, and blinding

Ninety-three participants were allocated equally to three parallel groups (n = 31 each). Allocation concealment was achieved by dispensing all three mouthwash formulations in identical amber plastic bottles, maintaining double-blinding of both participants and outcome assessors throughout the study period (Fig. 1).

Interventions

- **Group A (Chlorhexidine)**: 0.2% chlorhexidine mouthwash, 10 mL rinsed twice daily for 15 days
- **Group B (Saline)**: 0.9% saline mouth rinse, 10 mL twice daily for 15 days
- **Group C (Berberine)**: Berberine HCl mouthwash, 10 mL twice daily for 15 days

All participants underwent non-surgical periodontal therapy (NSPT) and received standardized oral hygiene instructions, with follow-up scheduled at day 15 and day 21 post-baseline.

Preparation of Berberine mouthwash

Berberine HCl was procured from Herbal Creations (India); product quality and safety were confirmed through toxicity, heavy-metal, and microbiological testing in compliance with FSSAI standards, with manufacturing facilities certified under SSI and ISO guidelines. The formulation contained: Berberine HCl 25 mg, peppermint oil flavour 0.1 mL, saccharin sodium (sweetener) 0.1 mg, PEG surfactant 4 g, glycerol cosurfactant 8.5 mL, preservative 2 mL, lemon-yellow colouring agent 1–2 drops, and purified water to 100 mL. Preparation involved sequential addition of the salt to water, followed by surfactant, cosurfactant, and Berberine HCl (mixed until fully dissolved), and finally the sweetener, with the volume adjusted to 100 mL.

Clinical outcome measures

Primary outcomes

- Full Mouth Plaque Score (FMPS): Recorded as the percentage of tooth surfaces exhibiting plaque (four surfaces per tooth), scored as present (1) or absent (0), calculated as: FMPS (%) = (number of tooth surfaces with plaque / total tooth surfaces examined) × 100. Assessed at baseline, day 15, and day 21.
- Full Mouth Bleeding Score (FMBS): Recorded as the percentage of sites exhibiting bleeding on probing, scored as present (1) or absent (0), calculated as: FMBS (%) = (number of sites with bleeding on probing / total sites examined) × 100. Assessed at baseline, day 15, and day 21.

Instruments used: mouth mirror, periodontal probe, and two-tone disclosing agent.

Secondary outcomes

- Salivary interleukin-4 (IL-4) and interleukin-6 (IL-6): Assessed at baseline and day 15.

Saliva collection and biochemical analysis

Whole unstimulated saliva was collected by the passive drool method. Participants refrained from eating or drinking for 30 minutes to 1 hour before collection and were seated upright with the head tilted slightly forward to facilitate expectoration into a centrifuge tube. Samples were centrifuged at 3000 rpm for 10 minutes; the supernatant was aliquoted (0.5 mL) into microcentrifuge tubes and stored at − 80°C until analysis. Samples were transported to the Central Research Laboratory, KSHEMA, for estimation of IL-4 and IL-6.

Salivary IL-4 and IL-6 concentrations were quantified using commercially available sandwich ELISA kits per manufacturer instructions. Kit reagents were equilibrated to room temperature, and wash buffer was prepared from the supplied 20× concentrate. Recombinant human IL-4 and IL-6 standards were reconstituted and serially diluted to generate standard curves, with assay diluent as the zero standard. Standards, samples, and blanks were added in duplicate to antibody-coated microplate wells and incubated to allow cytokine binding. After washing, biotinylated detection antibody was added, followed by streptavidin–horseradish peroxidase (HRP) conjugate. Colour development was achieved with TMB substrate and terminated with acidic stop solution. Absorbance was measured at 450 nm using a microplate reader, and cytokine concentrations were derived from standard curves generated by four-parameter logistic regression.

Statistical analysis

Data were analysed using SPSS software (version 20.0). Descriptive statistics were expressed as mean ± standard deviation, with 95% confidence intervals. Intergroup comparisons were performed using one-way analysis of variance (ANOVA), with Tukey's honestly significant difference (HSD) post-hoc test applied for pairwise multiple comparisons.

Intragroup comparisons across time points were analysed using paired-samples t-tests. A p-value < 0.05 was considered statistically significant.

Results

Participant characteristics and flow

A total of 93 patients aged 18–40 years were recruited from the Department of Periodontology, A.B. Shetty Memorial Institute of Dental Sciences, Mangaluru, and equally allocated to three groups: Chlorhexidine (n = 31), Saline (n = 31), and Berberine (n = 31). All 93 participants completed the study protocol in full, with no dropouts or losses to follow-up (Fig. 2).

Baseline comparability

At baseline, no statistically significant intergroup differences were observed for salivary IL-4, IL-6, FMPS, or FMBS ($p > 0.05$ for all comparisons), confirming adequate baseline homogeneity across the three treatment groups (Table 1).

Table 1
Baseline characteristics of clinical and inflammatory parameters among the study groups

Variable	Group	n	Mean ± SD	95% CI	F-value	P-value
FMPS (%)	Chlorhexidine	31	64.90 ± 6.23	62.62–67.19		
	Saline	31	65.16 ± 5.90	63.00–67.33		
	Berberine	31	65.23 ± 5.76	63.11–67.34	**0.025**	**0.975**
FMBS (%)	Chlorhexidine	31	12.26 ± 2.95	11.17–13.34		
	Saline	31	12.23 ± 3.25	11.03–13.42		
	Berberine	31	12.32 ± 3.07	11.20–13.45	**0.008**	**0.992**
IL-4 (pg/mL)	Chlorhexidine	31	1.32 ± 0.13	1.28–1.37		
	Saline	31	1.33 ± 0.13	1.29–1.38		
	Berberine	31	1.33 ± 0.13	1.29–1.38	**0.054**	**0.948**
IL-6 (pg/mL)	Chlorhexidine	31	3.70 ± 1.14	3.28–4.12		
	Saline	31	3.70 ± 1.15	3.28–4.12		
	Berberine	31	3.68 ± 1.11	3.27–4.08	**0.005**	**0.995**

Salivary interleukin-4 (IL-4)

Baseline salivary IL-4 levels were comparable among the Chlorhexidine, Saline, and Berberine groups ($F = 0.054$, $p = 0.948$) (Table 1). By day 15, significant intergroup differences emerged ($F = 320.99$, $p < 0.001$), with the Chlorhexidine group showing the lowest mean IL-4 concentration, followed by the Saline group, while the Berberine group retained the highest IL-4 level (Table 2). Tukey HSD post-hoc analysis confirmed statistically significant pairwise differences between all three groups at day 15 ($p < 0.001$ for all comparisons), whereas no significant pairwise differences were observed at baseline (Table 4).

Table 2
Comparison of clinical and inflammatory parameters among the study groups at Day 15

Variable	Group	n	Mean ± SD	95% CI	ANOVA (F)	P-value
FMPS (%)	Chlorhexidine	31	26.00 ± 2.56	25.06–26.94		
	Saline	31	48.90 ± 4.47	47.26–50.54		
	Berberine	31	31.61 ± 6.81	29.11–34.11	181.872	< 0.001
FMBS (%)	Chlorhexidine	31	4.94 ± 1.21	4.49–5.38		
	Saline	31	9.10 ± 2.31	8.25–9.95		
	Berberine	31	5.42 ± 2.32	4.57–6.27	39.465	< 0.001
IL-4 (pg/mL)	Chlorhexidine	31	0.73 ± 0.07	0.70–0.76		
	Saline	31	1.13 ± 0.11	1.09–1.17		
	Berberine	31	1.25 ± 0.06	1.22–1.27	320.99	< 0.001
IL-6 (pg/mL)	Chlorhexidine	31	1.85 ± 0.57	1.64–2.06		
	Saline	31	2.96 ± 0.92	2.63–3.30		
	Berberine	31	2.87 ± 0.78	2.58–3.15	19.912	< 0.001

Salivary interleukin-6 (IL-6)

Baseline salivary IL-6 levels did not differ significantly among groups (F = 0.005, p = 0.995) (Table 1). At day 15, a significant intergroup difference was observed (F = 19.912, p < 0.001), with the Chlorhexidine group showing the greatest reduction, followed by the Berberine group, and the Saline group showing the smallest reduction (Table 2). Post-hoc analysis showed that Chlorhexidine differed significantly from both Saline and Berberine at day 15 (p < 0.001 for both), whereas the difference between Saline and Berberine was not statistically significant (mean difference = 0.09 pg/mL; 95% CI: −0.37 to 0.56; p = 0.879) (Table 4).

Full Mouth Plaque Score (FMPS)

Baseline FMPS values were comparable among the three groups (F = 0.025, p = 0.975) (Table 1). Significant intergroup differences emerged by day 15 (F = 181.872, p < 0.001) and were sustained at day 21 (F = 255.513, p < 0.001), with Chlorhexidine demonstrating the greatest reduction, followed by Berberine, and Saline showing the least reduction at both time points (Table 2–3). Tukey HSD post-hoc comparisons confirmed statistically significant pairwise differences among all three groups at both day 15 and day 21 (p < 0.001 for all comparisons) (Table 4).

Table 3
Comparison of clinical parameters among the study groups at Day 21

Variable	Group	n	Mean ± SD	95% CI	ANOVA (F)	P-value
FMPS	Chlorhexidine	31	19.52 ± 1.82	18.85–20.18		
	Saline	31	42.48 ± 3.87	41.06–43.90		
	Berberine	31	29.32 ± 5.48	27.31–31.33	255.513	< 0.001
FMBS	Chlorhexidine	31	3.65 ± 0.84	3.34–3.95		
	Saline	31	7.90 ± 2.12	7.13–8.68		
	Berberine	31	4.58 ± 1.34	4.09–5.07	66.735	< 0.001

Table 4
Tukey HSD post hoc multiple comparisons between study groups for all outcome variables

Outcome	Time point	Groups (I)	Comparison(J)	Mean Difference (I–J)	95% CI	P-value	Significance
IL-4	**Baseline**	CHX	Saline	-0.01	-0.09 to 0.07	0.960	**NS**
		Berberine	CHX	-0.01	-0.09 to 0.07	0.954	**NS**
		Berberine	Berberine	0.00	-0.08 to 0.08	1.000	**NS**
	Day 15	CHX	Saline	-0.40	-0.46 to -0.35	< 0.001	**Yes**
		Berberine	CHX	-0.52	-0.57 to -0.47	< 0.001	**Yes**
		Berberine	Saline	-0.11	-0.16 to -0.06	< 0.001	**Yes**
IL-6	**Baseline**	CHX	Saline	-0.01	-0.69 to 0.68	1.000	**NS**
		Berberine	CHX	0.02	-0.67 to 0.71	0.997	**NS**
		Berberine	Saline	0.03	-0.66 to 0.71	0.995	**NS**
	Day 15	CHX	Saline	-1.11	-1.58 to -0.65	< 0.001	**Yes**
		Berberine	CHX	-1.02	-1.48 to -0.55	< 0.001	**Yes**
		Berberine	Saline	0.09	-0.56 to 0.37	0.879	**NS**
FMPS	**Baseline**	CHX	Saline	-0.26	-3.87 to 3.35	0.984	**NS**
		Berberine	CHX	-0.32	-3.93 to 3.29	0.975	**NS**
		Berberine	Saline	-0.06	-3.68 to 3.55	0.999	**NS**
	Day 15	CHX	Saline	-22.90	-25.89 to -19.92	< 0.001	**Yes**
		Berberine	CHX	-5.61	-8.60 to -2.63	< 0.001	**Yes**
		Berberine	Saline	17.29	14.31 to 20.27	< 0.001	**Yes**
	Day 21	CHX	Saline	-22.97	-25.40 to -20.54	< 0.001	**Yes**
		Berberine	CHX	-9.81	-12.24 to -7.38	< 0.001	**Yes**
		Berberine	Saline	13.16	10.73 to 15.59	< 0.001	**Yes**

Outcome	Time point	Groups (I)	Comparison(J)	Mean Difference (I–J)	95% CI	P-value	Significance
FMBS	**Baseline**	CHX	Saline	0.03	-1.84 to 1.91	0.999	**NS**
		Berberine	CHX	-0.06	-1.93 to 1.81	0.996	**NS**
		Berberine	Saline	-0.10	-1.97 to 1.78	0.992	**NS**
	Day 15	CHX	Saline	-4.16	-5.38 to -2.94	< 0.001	**Yes**
		Berberine	CHX	-0.48	-1.70 to 0.74	0.614	**NS**
		Berberine	Saline	3.68	2.46 to 4.90	< 0.001	**Yes**
	Day 21	CHX	Saline	-4.26	-5.18 to -3.33	< 0.001	**Yes**
		Berberine	CHX	-0.94	-1.86 to -0.01	0.046	**Yes**
		Berberine	Saline	3.32	2.40 to 4.25	< 0.001	**Yes**

Full Mouth Bleeding Score (FMBS)

Baseline FMBS values were comparable among groups ($F = 0.008$, $p = 0.992$) (Table 1). Significant intergroup differences were observed at day 15 ($F = 39.465$, $p < 0.001$) and day 21 ($F = 66.735$, $p < 0.001$), with the Chlorhexidine group showing the lowest bleeding scores at both time points, followed by Berberine, and Saline showing the highest (Table 2–3). At day 15, Chlorhexidine and Berberine did not differ significantly from each other (mean difference = − 0.48%; 95% CI: −1.70 to 0.74; $p = 0.614$), while both differed significantly from Saline ($p < 0.001$). By day 21, all pairwise comparisons reached statistical significance, including between Chlorhexidine and Berberine (mean difference = − 0.94%; 95% CI: −1.86 to − 0.01; $p = 0.046$) (Table 4).

Intragroup changes over time

Chlorhexidine group. Paired-samples analysis showed statistically significant reductions in IL-4 and IL-6 from baseline to day 15 ($p < 0.001$ for both), and in FMPS and FMBS from baseline to day 15, day 15 to day 21, and baseline to day 21 ($p < 0.001$ for all comparisons) (Table 5).

Table 5
Within-group comparison of clinical and inflammatory parameters at different time points

Outcome	Time Comparison	Chlorhexidine Mean Difference (95% CI)	t-value	Saline Mean Difference (95% CI)	t-value	Berberine Mean Difference (95% CI)	t-value	P-value
IL-4 (pg/mL)	**Baseline vs Day 15**	0.60 (0.57–0.62)	58.40	0.20 (0.19–0.21)	55.22	0.09 (0.04–0.14)	3.80	< 0.001
IL-6 (pg/mL)	**Baseline vs Day 15**	1.85 (1.64–2.06)	17.97	0.74 (0.66–0.82)	17.94	0.81 (0.52–1.10)	5.73	< 0.001
FMPS (%)	**Baseline vs Day 15**	38.90 (37.55–40.26)	58.54	16.26 (15.71–16.80)	61.06	33.61 (31.43–35.80)	31.41	< 0.001
	Day 15 vs Day 21	6.48 (6.19–6.78)	44.50	6.42 (6.14–6.70)	46.73	2.29 (-0.47–5.05)	1.70	CHX, Saline: <0.001Berberine: 0.100 (NS)
	Baseline vs Day 21	45.39 (43.76–47.02)	56.92	22.68 (21.92–23.43)	61.42	35.90 (32.81–39.00)	23.71	< 0.001
FMBS (%)	**Baseline vs Day 15**	7.32 (6.66–7.99)	22.47	3.13 (2.77–3.49)	17.57	6.90 (6.16–7.65)	18.85	< 0.001
	Day 15 vs Day 21	1.29 (1.10–1.48)	13.59	1.19 (0.99–1.39)	12.34	0.84 (0.25–1.43)	2.89	CHX, Saline: <0.001Berberine: 0.007
	Baseline vs Day 21	8.61 (7.82–9.41)	22.09	4.32 (3.88–4.76)	20.16	7.74 (6.76–8.72)	16.14	< 0.001

Saline group. Statistically significant reductions were observed in IL-4 and IL-6 from baseline to day 15 ($p < 0.001$ for both), and in FMPS and FMBS across all time-point comparisons ($p < 0.001$ for all) (Tables 5).

Berberine group. IL-4 decreased significantly from baseline to day 15 (mean difference = 0.09 pg/mL; 95% CI: 0.04–0.14; $p = 0.001$), as did IL-6 (mean difference = 0.81 pg/mL; 95% CI: 0.52–1.10; $p < 0.001$). FMPS decreased significantly from baseline to day 15 ($p < 0.001$) and from baseline to day 21 ($p < 0.001$); however, the additional reduction from day 15 to day 21 was not statistically significant (mean difference = 2.29%; 95% CI: −0.47 to 5.05; $p = 0.100$). FMBS showed statistically significant reductions across all time-point comparisons, including from day 1.

Discussion

Main findings

This randomized controlled trial demonstrated that a Berberine HCl-containing mouthwash produced significant reductions in dental plaque accumulation, gingival bleeding, and salivary inflammatory cytokine levels in patients with plaque-induced gingivitis. While 0.2% chlorhexidine remained the most effective intervention across all outcome measures, Berberine produced clinically significant improvements that were evident on day 15 and were largely sustained through day 21, substantially exceeding the effect of saline rinse alone. Notably, the reduction in gingival bleeding (FMBS) achieved with Berberine did not differ significantly from that achieved with chlorhexidine at day 15, suggesting an early

anti-inflammatory effect comparable to the gold-standard agent, although chlorhexidine's advantage became statistically significant by day 21.

Comparison with previous studies

The baseline comparability observed across all three groups is consistent with the principles outlined by Altman for internal validity in controlled trials [30], and supports the attribution of observed changes to the interventions themselves rather than to baseline imbalance. The pattern of elevated pro-inflammatory (IL-6) and reduced anti-inflammatory (IL-4) cytokine activity in untreated or minimally treated gingival inflammation is consistent with the host-response model described by Kornman et al. and Genco and Van Dyke [31, 32].

The superior overall performance of chlorhexidine in this study is consistent with its established status as the gold-standard chemical plaque control agent, attributable to its broad-spectrum antibacterial activity and high oral substantivity. The clinical efficacy of Berberine observed here aligns with prior reports of herbal mouthwashes producing meaningful, if generally lesser, reductions in plaque and gingival inflammation relative to chlorhexidine. Bhat et al. similarly reported that a herbal mouthwash was comparably effective to chlorhexidine in reducing plaque and gingivitis [15]. The reduction in salivary IL-6 with Berberine parallels findings from other herbal mouthwash trials: Gupta et al. reported significant reductions in both IL-4 and IL-6 with a polyherbal mouthwash [17], and Kaur et al. observed a marked reduction in IL-6 with a curcumin-based formulation accompanied by improved clinical indices [18]. The early and sustained clinical response observed with Berberine in the present study is consistent with the pattern reported in these prior herbal mouthwash trials.

Biological explanation

The anti-inflammatory and antimicrobial actions of Berberine are supported by a substantial preclinical literature. Berberine has been shown to inhibit lipopolysaccharide-induced NF-κB signalling and suppress mitogen-activated protein kinase (MAPK) activation, thereby reducing downstream pro-inflammatory cytokine and chemokine production, as reported by Tillhon et al. and Imenshahidi and Hosseinzadeh [33, 34]. Sun et al. demonstrated a specific anti-inflammatory effect of berberine on lipopolysaccharide-induced periodontal inflammation [35], directly supporting the biological plausibility of the reductions in salivary IL-6 observed in the Berberine group of the present study. The relative maintenance of salivary IL-4 in the Berberine group, in contrast to the more pronounced decline seen with chlorhexidine and saline, may reflect an immunomodulatory action distinct from purely antimicrobial mechanisms, a pattern consistent with the alkaloid-mediated modulation of immune cell activity described by Chatterjee et al. [36]. Berberine's reported capacity to integrate into bacterial membranes, disrupt extracellular polysaccharide matrix formation, and interfere with quorum-sensing provides a plausible antimicrobial basis for the observed reductions in FMPS, complementing its anti-inflammatory action on FMBS and salivary cytokines.

Clinical implications

These findings suggest that Berberine HCl mouthwash may offer a favourable balance between antiplaque and anti-inflammatory efficacy and long-term tolerability. Chlorhexidine's well-documented adverse effects, including tooth staining, taste disturbance, and mucosal irritation, limit its suitability for extended use. In this context, Berberine's early and sustained reduction in gingival bleeding, together with meaningful plaque reduction, positions it as a potential adjunct or alternative for patients requiring longer-term chemical plaque control, or those unable to tolerate chlorhexidine.

Strengths

This study benefits from a randomized, double-blind design with adequate allocation concealment, achieved through the use of identical, indistinguishable mouthwash bottles across all three arms. The inclusion of both clinical (FMPS, FMBS) and biochemical (salivary IL-4, IL-6) outcome measures allowed the anti-inflammatory activity of Berberine to be

evaluated at both the clinical and molecular level. The relatively large, equally allocated sample size (n = 31 per group) and confirmed baseline comparability across groups strengthen confidence in the observed treatment effects.

Limitations

This study was limited by a relatively short follow-up period, precluding assessment of longer-term efficacy and safety of Berberine mouthwash. Microbiological analysis was not performed, limiting direct confirmation of the antimicrobial mechanisms proposed to underlie the observed plaque reduction. Patient-reported bitter taste and temporary staining were noted with the Berberine formulation, reflecting the inherent alkaloid nature of berberine and its interaction with oral mucosal surfaces, and highlighting a need for formulation refinement.

Future research

Future studies should incorporate longer follow-up periods to establish the durability of Berberine's clinical and anti-inflammatory effects, alongside microbiological and molecular analyses to directly characterize its antimicrobial and immunomodulatory mechanisms in vivo. Optimization of the mouthwash formulation to improve taste and reduce staining, while preserving substantivity, is warranted to enhance patient acceptability and compliance in future trials with larger, multicentric cohorts.

Conclusion

Within the limitations of this randomized controlled trial, Berberine HCl-containing mouthwash produced statistically significant reductions in dental plaque accumulation, gingival bleeding, and salivary pro-inflammatory cytokine levels in patients with plaque-induced gingivitis. Although 0.2% chlorhexidine demonstrated the greatest overall clinical and anti-inflammatory efficacy, Berberine achieved rapid early improvement that was largely sustained through day 21, with its effect on gingival bleeding approaching that of chlorhexidine at day 15. These findings support the potential of Berberine HCl mouthwash as a promising herbal adjunct to chlorhexidine, and a possible longer-term alternative, in the management of plaque-induced gingivitis, particularly for patients unable to tolerate the adverse effects associated with prolonged chlorhexidine use. Further studies with extended follow-up, microbiological analysis, and optimized formulations are warranted to validate and expand upon these findings.

Abbreviations

AMPK, AMP-activated protein kinase

ANOVA, analysis of variance;

AP-1, activator protein-1;

BBR, berberine; BBR-HCl, berberine hydrochloride;

CI, confidence interval;

COX-2, cyclooxygenase-2;

CTRI, Clinical Trials Registry – India;

DCs, dendritic cells;

ELISA, enzyme-linked immunosorbent assay;

FMBS, Full Mouth Bleeding Score;

FMPS, Full Mouth Plaque Score;

HSD, honestly significant difference;

IL-1β, interleukin-1 beta;

IL-4, interleukin-4;

IL-6, interleukin-6;

JAK/STAT, Janus kinase/signal transducer and activator of transcription;

MAPKs, mitogen-activated protein kinases;

MCP-1, monocyte chemoattractant protein-1;

MMP, matrix metalloproteinase;

NF-κB, nuclear factor kappa-B;

NSPT, non-surgical periodontal therapy;

SD, standard deviation;

TNF-α, tumour necrosis factor-alpha.

Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Institution's Central Ethics Committee, Nitte (Deemed to be University) (Reference No. ETHICS/ABSMIDS/451/2024/V2). Written informed consent was obtained from all participants prior to enrolment. The trial is registered with the Clinical Trials Registry – India (CTRI/2025/11/115219).

Competing interests

The authors declare no competing interests.

Author contributions

B.B. — Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft.
R.B. — Supervision, Conceptualization, Methodology, Writing – review & editing.

A.N.V. — Formulation development, Methodology.
S.K.N. — Supervision, Methodology, Writing – review & editing.
R.R. — Investigation, Data curation
N.H. — Investigation, Data curation

All authors read and approved the final manuscript.

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Figures



FIGURE 1: IDENTICAL AMBER PLASTIC BOTTLES

Figure 1

See image above for figure legend.

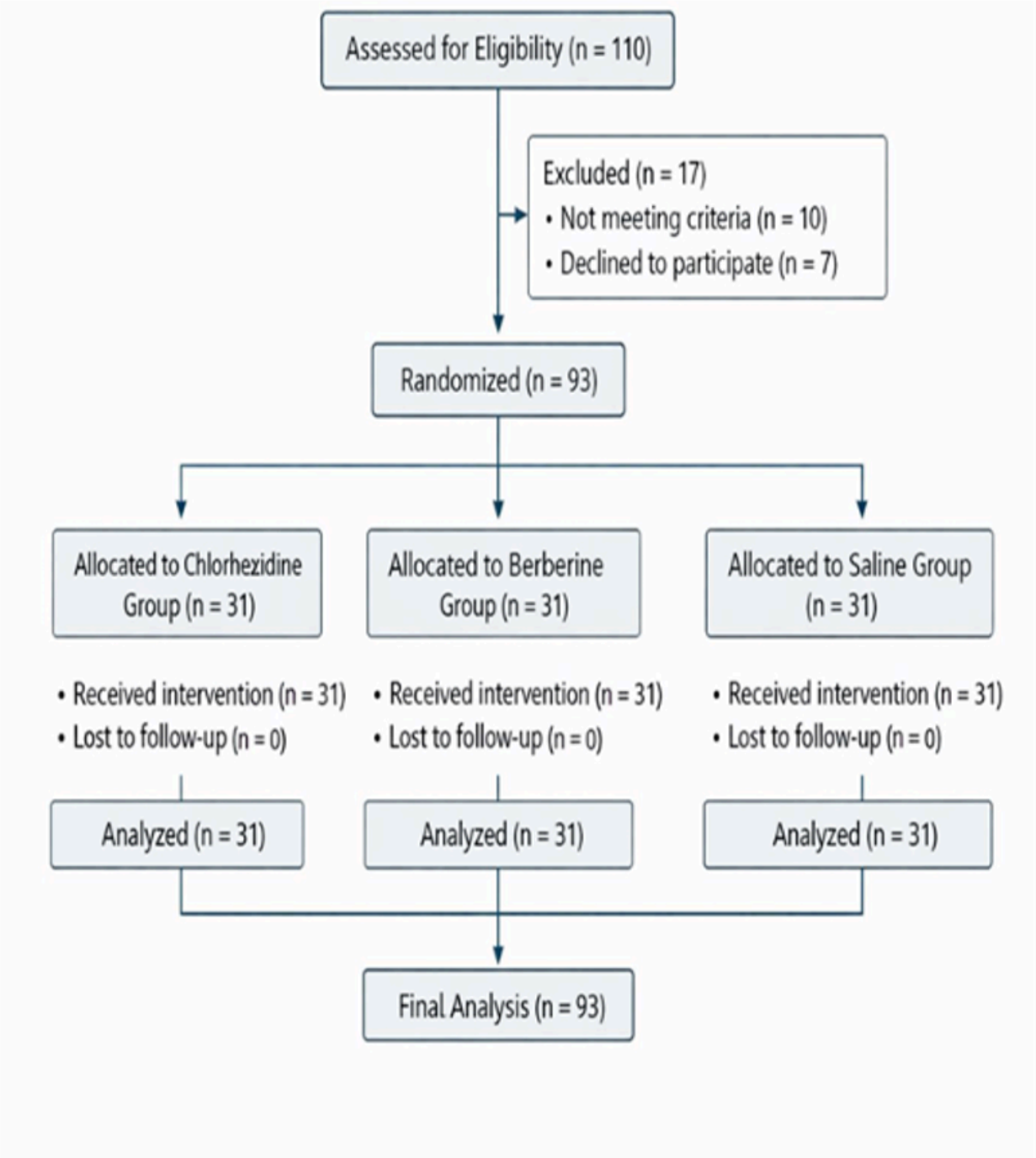


FIGURE 2: CONSORT DIAGRAM

Figure 2

See image above for figure legend.