Original Article

Evaluation of the efficacy of lycopene gel compared with minocycline hydrochloride microspheres as an adjunct to nonsurgical periodontal treatment: A randomised clinical trial

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Abstract

Background/purpose: The prescription of antibiotics as an adjunct to mechanical periodontal therapy in patients with severe periodontitis is recommended; however, the side effects of antibiotics are a major concern. The aim of this study was to evaluate the efficacy of lycopene (Lyc) antioxidant gel versus minocycline hydrochloride microspheres (ARISTIN) as an adjunct to the nonsurgical treatment of periodontitis.

Materials and methods: Three identical periodontal pockets/patient received root surface debridement followed by the random application of either ARISTIN, Lyc, or placebo gel (control, Ctrl). Clinical parameters, plaque index, bleeding on probing, probing pocket depth, and clinical attachment loss, were recorded at the baseline and after 30 days. Additionally, the levels of interleukin-8 (IL-8), matrix metallopeptidase 9, and tissue inhibitor of metalloproteases 1 (TIMP1) in gingival crevicular fluid samples were assessed at the same time points.

Results: Twenty-three patients with periodontitis completed the study. Both ARISTIN and Lyc treatments showed significantly greater gains in attachment (1.94 ± 1.33 and 1.72 ± 0.88, respectively) than the Ctrl treatment (1.04 ± 0.96). Compared with those in the Ctrl, only ARISTIN showed a significant reduction in IL-8 level, whereas TIMP1 levels were significantly upregulated in the Lyc gel and ARISTIN sites. The effect size estimation indicated that Lyc gel exhibited considerably greater efficacy than the Ctrl gel.

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Introduction

The hallmark of periodontitis is the formation of periodontal pockets as collateral damage, which is mainly caused by an aberrant immune response to the dental biofilm.1 The presence of pathogenic bacteria and their virulence factors, such as lipopolysaccharide, upregulates the release of inflammatory cytokines, including interleukin (IL)-8.2 The persistent release of these cytokines, with increased oxidative stress (OS), potentially compromises the integrity of the epithelial-barrier lining the pocket.3 The loss of the epithelial-phenotype is attributed to an epithelial–mesenchymal transition (EMT) that is characterised by the downregulation of epithelial markers and acquisition of the mesenchymal-phenotype.4 The involvement of the EMT in the pathogenesis of periodontitis has been suggested by in vivo and in vitro studies.5,6 This process can be reversed by eliminating the triggering factors and modulated by certain drugs, including antioxidants.7

In a healthy individual, tissue remodelling by matrix metalloproteinases (MMP) is controlled by tissue inhibitor of metalloproteinases (TIMP) but, with an increased bacterial load in the periodontal pocket, this balance is disrupted, which consequently increases the susceptibility to periodontal tissue destruction.8 MMP9 is associated with periodontitis9 and the level of MMP9 increases with the severity of the disease.10

The basic principle of nonsurgical periodontal treatment is the removal of bacterial load, which allows the body to return to the healthy state.11 However, the effectiveness of this approach is not always guaranteed owing to several reasons, such as operator dexterity and the presence of certain bacteria, such as Aggregatibacter actinomyctemcomitans, that can re-colonise the root surface rapidly after treatment.12,13 Furthermore, some of these bacteria invade periodontal tissues, in which case they are difficult to remove by mechanical therapy.14 Therefore, several therapies have been proposed as adjuncts to scaling and root planing (SRP). Since the late 1970s, the interest in using locally delivered antimicrobials to treat periodontal pockets has increased.15 A recent systematic review and meta-analysis on the use of antimicrobials in different formulations as adjunctive agents to SRP showed that these agents enhance clinical outcomes with minimal side effects.16 Among these agents, minocycline hydrochloride microspheres (MM) have been reported to be effective in reducing probing pocket depth (PPD) and increasing attachment gain.17,18 The axial role of certain bacterial commensals in balancing a healthy subgingival flora has been reported,19 which can be changed non-specifically by antimicrobials.

The use of antioxidants as adjuncts to periodontal treatment has been proposed recently as an alternative to antimicrobials.20 Lycopene (Lyc) is an antioxidant extracted from tomato that has a potential therapeutic effect for the treatment of periodontitis when applied locally or systemically.21 To the best of our knowledge, studies on the use of antioxidants as adjuncts to conventional periodontal therapy are limited. The rationale of this study is based on the potential use of Lyc gel as an adjunct to SRP in the treatment of periodontitis. The aim of this pilot trial was to evaluate the efficacy of Lyc gel as an adjunct to SRP based on clinical and biochemical markers of periodontitis. This was investigated by measuring the effects of Lyc on clinical parameters and selected inflammatory cytokines in gingival crevicular fluid (GCF) compared with the controls (SRP [control, Ctrl] and SRP + MM [ARISTIN]).

Materials and methods

Study design and population

A double-blind, split-mouth, randomised clinical trial with three arms was conducted at the Department of Periodontics, College of Dentistry, University of Baghdad from March 2019 to January 2020. Ethical approval was obtained from the ethics committee (Ref #013618, 08/01/2019) of the College of Dentistry, University of Baghdad in accordance with the Declaration of Helsinki and CONSORT 2010 checklist. The clinical registration number is NCT03964935 at www.clinicaltrials.gov/. The aims, design, and methods of the current study were explained to the potential candidates who were invited to participate and, when agreement was obtained, they were asked to sign a consent form before commencing any treatment.

Participants were recruited from patients referred for periodontal treatment and eligible individuals were selected according to the inclusion/exclusion criteria. The included participants were systemically healthy, non-smokers, and not pregnant or lactating, had not taken any drugs in the past 3 months, had no previous or unsuccessful periodontal treatment leaving a residual PPD >4 mm with bleeding, and were able to consent (Fig. 1). Additionally, only single-rooted teeth with infrabony pockets at interproximal sites were included. For pockets between adjoining surfaces, only one of these was selected to avoid possible overlap of the locally delivered agents.

According to the definition of periodontitis adopted by the American Academy of Periodontology and European Federation of Periodontology,24 the participants exhibited the following diagnostic features based on the clinical criteria and periapical radiography:
Generalised periodontitis (>30% involvement)
Stage II–III (bone loss extending to the coronal or middle third of the root)
Grade B (moderate rate of progression)
Currently unstable (PPD ≥ 4 mm with bleeding on probing [BOP]), any pocket without bleeding was excluded
No specific risk factors (smoking and diabetes mellitus)

Randomisation of the selected sites was performed using the lottery method (by A.A.A.). The number of teeth to be included in the treatment was written on pieces of paper, which were folded and placed in an opaque bag, and the same was done for the treatment modalities. A paper from each bag was drawn out to determine the tooth and corresponding therapy. Each site that received one of the treatment modalities was assigned a letter, 'A' for Ctrl, 'B' for ARISTIN, and 'C' for Lyc. Subsequently, all records for each site were sorted under the assigned letter to ensure the blindness of the statistician.

Clinical parameters and examiner calibration

Multiple clinical parameters were used to investigate the primary outcomes, including plaque index (PI), BOP, PPD, and clinical attachment loss (CAL). Clinical parameters were recorded following the Quadrant Tooth Site system; six sites per tooth were examined. CAL was determined by measuring the distance from the lower border of a pre-fabricated stent to the base of the pocket.

On the first visit, pre-treatment, initial clinical parameters were recorded. First, PI was recorded, followed by PPD and BOP, to reach a diagnosis, which was confirmed by periapical radiography for the suspected sites. All clinical parameters were recorded using William’s periodontal probe (MEDESY, Maniago, Italy). Impressions of the teeth were taken to fabricate a stent to measure CAL. This was followed by providing the patient with oral hygiene instructions, which included advice regarding toothbrushing and the use of interdental aids, while the use of mouthwash was prohibited during the study period. To ensure the blindness of the data collector, all treatments were performed by another clinician (A.H.A.). The treatment started with supragingival scaling using an ultrasonic scaler, DTE® D2 LED, (Guilin Woodpecker Medical Instruments Co Ltd, Guilin, China), followed by coronal polishing. Each patient was instructed to return after 7 days to complete the planned treatment.

The next visit was the baseline for the study. First, compliance with the given oral hygiene instructions was evaluated using PI. For non-compliant patients, instructions were reinforced and the treatment visit was postponed for another week. For compliant participants, baseline data at selected sites (PI, PPD, BOP, and CAL) were recorded. This was followed by collecting the GCF samples. Each of the selected sites underwent thorough root surface debridement using a Gracey curette (MEDESY, Maniago, Italy), which was followed by the application of either placebo gel (Ctrl) or Lyc gel (100 μL), both of which were delivered by an insulin syringe with a blunt needle, or ARISTIN; the patients were blinded to the treatment. After 30 days, clinical parameters and GCF samples were collected again and coded data were sent to the statistician (H.S.R.) who blindly analysed the results.
Prior to the trial, one of the authors (S.S.J.) and one of the clinicians (A.H.A.) participated in calibration sessions for clinical parameters (PI, BOP, PPD, and CAL). The examiners met prior to these calibration sessions to discuss the criteria and methods of measurement. Calibration sessions were conducted using five volunteers not included in the study. Probing potential pressure increases the PPD,27 thus, a minimum of 15 min was allowed between repeated measurements by the two examiners to minimise any possibility of error.28 Alignment for BOP was conducted in a separate session. The weighted kappa (κ) was considered acceptable when it was ≥0.70 for all indices. In the case of disagreement, the methods were discussed and the calibration session was repeated on another date.

**GCF collection and enzyme-linked immunosorbent assay (ELISA)**

The site of the tooth was isolated with a cotton roll and debris and thin plaque film were removed manually with a gentle wiping movement using clean gauze.29 The site was then dried and the GCF was collected using the intracrevicular method. Tweezers were used to gently insert paper strips, PerioCol (Oraflow Inc., Hewlett, NY, USA), into the depth of the pocket until minimal resistance was felt; then, they were left in the site for 30 s, after which the PerioCol was removed from the pocket and inspected for any sign of contamination with saliva or blood. Contaminated papers were discarded and the procedure was repeated after 30 min; otherwise, the samples were transferred to a pre-weighed Eppendorf tube containing 200 μL of phosphate-buffered saline (PBS) (Euro Clone, Milan, Italy) and stored at −20 °C. The volume of the collected GCF was determined as previously described,30,31 which is essential for the proper calculation of the concentration of each marker. Briefly, the weight of the collected GCF was calculated according to the following formula:

\[ W_{\text{GCF}} = W_2 - W_1, \]

where \( W_{\text{GCF}} \) is the weight of GCF (μg), \( W_2 \) is the Weight of Eppendorf tube containing 200 μL of PBS + PerioCol after collecting GCF, and \( W_1 \) is the pre-weighed Eppendorf tube containing 200 μL of PBS. Then, the weight of GCF alone was calculated by subtracting the weight of the PerioCol paper from \( W_{\text{GCF}} \). Finally, the volume of GCF was converted from μg to μL, assuming that the density of GCF is (1 mg/mL). This was performed using the formula 'Volume = Mass/Density'.

The samples were thawed and then centrifuged at 400–500 g for 4–5 min and concentrations of IL-8, MMP9, and TIMP1 were determined using ELISA kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. The optical density (OD) was determined using a microplate reader (GloMax®, Promega, Madison, WI, USA) at 450 nm, which was converted into a corresponding concentration (ng/mL) using a specific equation for each cytokine obtained from plotting a standard curve. This value represented the concentration (in 1 mL) of the mediator eluted in 200 μL; therefore, the concentration of the cytokine in the original GCF samples was calculated according to the previously measured GCF volume as follows:

\[ \text{ELISA output} \times 0.2/\text{GCF volume (μL)}. \]32

**Study interventions and outcomes**

Three interventions were used: First, ARISTIN (MM), 1 mg (oraPHARMA, Bridgewater, NJ, USA). Second, Lyc gel, the study drug, prepared according to a previously reported method without any modifications.22 This procedure was used to convert pure Lyc powder (IBIS CHEMIÉ International, Mumbai, India) into gel. The concentration of Lyc in the final product was 2% at neutral pH (7.4). Third, the same steps were repeated to prepare a non-active control (placebo) gel without adding the active ingredient, i.e., Lyc powder.

The clinical parameters were set as the primary outcomes of the current study. The outcomes were measured by calculating the differences in these parameters between the baseline and endpoint of the study (after 30 days). In brief, gain of attachment (ΔCAL), reduction in PPD (ΔPPD), and reduction in the percentage of BOP (Δ%BOP) were used to investigate the effect of each treatment modality. Concentrations of inflammatory cytokines in GCF samples were considered as secondary outcomes and were calculated similarly to the primary outcomes.

**Sample size**

Calculation of the required sample was based on the changes in CAL measurements per site. A preliminary study was initially conducted on six patients to determine the mean and standard deviation of CAL for the Lyc and Ctrl groups at the baseline and endpoint. The CAL value of the Ctrl group was 4.325 ± 1.166, whereas that of the Lyc group was 3.34 ± 1.079. The calculated sample size was 22 sites per group at power 0.8 and 0.05 α-error probability. The total number of required sites to reject the null hypothesis was 66 but 24 sites were included per group to avoid dropout in the sample. The sample size calculation was performed using G*Power (version 3.1.9.2).

**Statistical analysis**

Descriptive statistics were used to analyse the data in terms of mean, standard deviation, frequency, and percentages. Before inferential analysis, a normality test showed that the data were not normally distributed; thus, nonparametric statistics were selected for analyses. For continuous variables, intragroup differences were compared using the Wilcoxon signed-rank test, whereas intergroup differences were compared using the Kruskal–Wallis test followed by Dunn-Bonferroni post hoc test. Changes in PI scores over multiple visits within each group were determined using the Friedman and Dunn-Bonferroni post hoc tests. Fisher’s exact test was used to analyse the dichotomous variable (BOP). The effect size of the difference in means was calculated using Cohen’s d and the Koopman asymptotic score was used to calculate the effect size of changes in BOP%. Differences were considered significant when the

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**Equations and Formulas**

\[ W_{\text{GCF}} = W_2 - W_1, \]

\[ \text{ELISA output} \times 0.2/\text{GCF volume (μL)}. \]
Results

Recruitment of patients and demographic characteristics of the study groups

A total of 109 patients were assessed for eligibility and 24 patients were recruited after excluding 85 patients (Fig. 1). The final analysis of the study included 23 patients and 69 sites, which were equally distributed among the three groups. One patient did not comply with appointments and was thus excluded from the final analysis (Fig. 1). The demographic variables of the participants are summarised in Table 1. No adverse events were reported as a consequence of this study.

Intragroup comparisons of clinical and biochemical parameters

PI scores were significantly lower \((P < 0.05)\) at the baseline visit when treatment was provided. All groups showed an increase in PI at the end of the study; however, these increases were not significant compared with baseline scores (Fig. 2A). All treatments resulted in a significant reduction \((P < 0.05)\) in the percentage of BOP at the end of the trial compared with their baseline score. For CAL and PPD, all groups showed significant differences \((P < 0.05)\) at the endpoint compared with their baseline scores (Table 2). The concentrations of cytokines in GCF at the baseline and endpoint of the trial showed that patients in the Ctrl group only exhibited a significant difference in the level of MMP9. For combined SRP with ARISTIN or Lyc, both adjunctive drugs showed significantly reduced \((P < 0.05)\) levels of IL-8 and MMP9 and increased levels of TIMP1 at the endpoint compared with the baseline (Table 2).

Intergroup comparisons of clinical and biochemical parameters

At the baseline, there were no significant differences in two clinical parameters, PPD and CAL, among all groups (Fig. 2B). ARISTIN and Lyc showed a significantly greater gain in attachment \((\Delta \text{CAL} = \text{CAL}_{\text{baseline}} - \text{CAL}_{\text{endpoint}})\) than the Ctrl. Additionally, both drugs had a high effect size \((0.774–0.792)\) compared with the Ctrl (Table 3). Although no significant differences in \(\Delta \text{PPD}\) were apparent when the Ctrl was compared with ARISTIN and Lyc, a moderate effect size was observed between ARISTIN \((0.655)\) or Lyc \((0.461)\) and the Ctrl (Table 3). The same pattern was observed in association with \(\Delta \%\text{BOP}\), in which ARISTIN and Lyc were more effective than the Ctrl (Table 3).

Regarding changes in the concentration of cytokines, ARISTIN and Lyc failed to cause significant changes in the level of MMP9 compared with the Ctrl; however, they showed a moderate effect size \((0.532–0.482)\) compared with the Ctrl. For \(\Delta \text{IL-8}\), only ARISTIN resulted in significant changes \((P = 0.016)\) in the level of this cytokine at the endpoint, with a high effect size \((0.971)\) compared with the Ctrl (Table 3). A significant alteration in the level of TIMP1, with a high effect size, was observed between ARISTIN \((P = 0.041, \text{effect size} = 0.786)\) or Lyc \((P = 0.038, \text{effect size} = 0.897)\) and the Ctrl (Table 3).

Discussion

The main results of this phase I clinical trial showed that Lyc gel and ARISTIN were equally effective in improving clinical CAL measurements and levels of biomarkers involved in periodontal tissue destruction compared with the Ctrl group. The exact etiopathological mechanism of periodontitis has not been fully elucidated; however, it is well-recognised that the destruction of periodontal tissues is a collateral event to an intense immune response to pathogenic bacteria or their virulence factors.15 Tissue integrity is maintained by homeostasis, which involves an antioxidant system that counteracts the adverse effects of reactive oxygen species. Increased OS has been proposed to be associated with periodontal diseases.16 Data regarding the effectiveness of locally delivered antioxidant agents as an adjunct to nonsurgical periodontal treatment are limited. The aim of this study was to test the hypothesis that the local application of Lyc as an antioxidant in periodontal pockets potentially improves periodontal status. The selection of Lyc was based on reported results for using this drug as supportive therapy for SRP, both systemically20 and locally.21

Despite satisfactory results using subgingival antimicrobials to treat periodontal diseases, an in vitro study has shown that subgingival bacteria in steady-state biofilms require a 250-fold greater concentration of antimicrobials to inhibit their growth than bacteria outside biofilm, and approximately half of these bacteria remain viable.35 MM is an antimicrobial that has been shown to be effective as an adjunct therapy to SRP in many studies;17,18,36 therefore, it was selected as a positive control for this study. The main mechanism of action of this antimicrobial is the elimination of Gram-negative anaerobes.37 A reduction in the bacterial load in the subgingival domain is of paramount importance in periodontal therapy as this provides the immune system enough time to overcome the remaining bacteria and decrease the level of the cytokines, thereby restoring the healthy state.11 It is important to acknowledge that, in clinical trials, besides the \(P\) value, the effect size needs to be taken into consideration, especially when the sample

| Table 1 | Demographic data of the patients. |
|---------|----------------------------------|
| Sex     | N      | %     |
| Male    | 15     | 66    |
| Female  | 8      | 34    |
| Age groups (years) | Mean ± SD | Range |
| 30-39   | 7      | 30.4  | 33.1 ± 2.4 | 33–36 |
| 40-49   | 6      | 26.1  | 42.5 ± 1.8 | 40–45 |
| 50-59   | 7      | 30.4  | 52.3 ± 2.3 | 50–55 |
| >60     | 3      | 13.0  | 66.3 ± 5.1 | 62–72 |
| Total   | 23     | 100   | 45.7 ± 11.5| 30–72 |

N, Number.
size is small. Therefore, the effect size has been reported in the current study.

The results of this study showed that MM significantly reduced PPD by the end of the trial but there was no significant difference compared with Ctrl and Lyc. This is in agreement with a previous study that indicated no additional benefit of using MM as an adjunct to nonsurgical treatment over SRP alone. However, this disagrees with results from other studies that have shown a significant reduction in PPD in the MM group compared with the SRP group, which is an inconsistency that could be owing to differences in the sample size and follow-up period. Furthermore, with Lyc, the gain of clinical attachment at the endpoint was almost half the amount of the reduction.
in PPD, which is similar to previous results from a systematic review of the effectiveness of SRP. Combined Lyc and SRP showed a significant gain in attachment compared with the placebo group. These results are consistent with other trials that also utilised Lyc as an adjunct to the nonsurgical treatment of periodontitis. Additionally, both MM and Lyc were more effective in the reduction of BOP compared with SRP alone, but not significantly so.

Microulceration of the epithelial pocket lining exposes the underlying highly vascularised connective tissue, which increases the tendency for bleeding. Additionally, discontinuity in the epithelial layer allows bacteria to enter deeper tissues and cause more damage to tooth-supporting structures. The presence of Gram-negative bacteria, OS, and high levels of inflammatory cytokines negatively affect the epithelial-barrier function of the pocket lining. Exposure of epithelial cells to gingipains, virulence factors of Porphyromonas gingivalis, results in the cleavage of epithelial adhesion-molecules and increases the expression of MMP, which enhances the dispersion of the epithelial layer. Furthermore, results from a previous study show the significant downregulation in the expression of major epithelial markers, E-cadherin and β-catenin, associated with the acquisition of the fibroblast-like phenotype following exposure of epithelial cultures to OS. The authors suggested that OS could act alone or synergistically with other inflammatory cytokines to alter the epithelial-phenotype. The mechanism by which Lyc minimised bleeding could be attributed to decreased OS in the pocket microenvironment, which has been previously demonstrated by another study that showed that 8-oxo-2′-deoxyguanosine is restored to almost normal levels after 1 week following the local application of Lyc in periodontal pockets.

The reduction of OS has a positive impact on the tissue that enhances healing and restores the architecture of the tissue. Tissue plasticity is an elusive process that cannot be tracked clinically in humans owing to several technical and ethical issues. However, the events of this process can be predicted indirectly by measuring the level of inflammatory cytokines, which are considered the main driver for the initiation and sustainability of the EMT. Therefore, inflammatory cytokines that are differentially expressed during periodontitis and the EMT were used in this study. Lyc treatment resulted in the significant downregulation of IL-8 and MMP9 levels and significant upregulation of TIMP1 levels, which are known to balance the destructive effect of collagenases; this could provide additional support for the healing effect of Lyc. The change in the level of these cytokines was associated with a significant gain of attachment and reduced bleeding in sites treated with Lyc. Previous studies have demonstrated that SRP significantly upregulates the level of TIMP1 in GCF samples from patients with periodontal disease, which is accompanied by a significant gain of attachment and reduction in PPD. Similar results were obtained in the current study in association with SRP and further upregulation of TIMP1 was observed when SRP was combined with Lyc.

This study lacked the measurement of OS in GCF samples, which could better reflect the response of the periodontal tissue to the treatments. A comparison of alterations in the subgingival microflora before and after each treatment modality requires consideration in future investigations. Additionally, this study only monitored the effect of a single application of Lyc over a short period of time. Thus, studies over a longer period and utilising a larger population are recommended.

Despite the limitations of this study, combining SRP with Lyc gel in the treatment of mild to moderate periodontitis showed promising results. This was reflected by significant differences in clinical and biochemical parameters compared with the controls. Additionally, Lyc was almost as effective as MM in improving outcomes of nonsurgical periodontal therapy. These results suggest that Lyc significantly modulated the expression of inflammatory cytokines in GCF, which positively influenced clinical outcomes. Although antioxidants represent an alternative to antimicrobials in the treatment of periodontitis, the current results should be interpreted with caution, and further

| Table 2 | Intragroup comparison of clinical parameters and cytokines concentrations at baseline and endpoint of the study. |
| Parameters | Clinical | Mean ± SD |
| --- | --- | --- |
| BOP baseline (N, %) | Ctrl | ARISTIN | Lyc |
|  | 23, 100 | 23, 100 | 23, 100 |
| BOP endpoint (N, %) | 13, 57 | 6, 26 | 7, 30 |
| p value<sup>a</sup> | <0.001 | <0.001 | <0.001 |
| CAL baseline (mm) | 7.33 ± 1.93 | 7.69 ± 2.16 | 7.83 ± 2.04 |
| CAL endpoint (mm) | 6.28 ± 1.82 | 5.76 ± 2.21 | 6.11 ± 1.98 |
| p value<sup>b</sup> | <0.001 | <0.001 | <0.001 |
| PPD baseline (mm) | 5.82 ± 0.88 | 6.04 ± 0.82 | 5.91 ± 0.95 |
| PPD endpoint (mm) | 4.00 ± 1.41 | 3.44 ± 1.12 | 3.57 ± 1.08 |
| p value<sup>b</sup> | <0.001 | <0.001 | <0.001 |
| Biochemical<sup>c</sup> | IL-8 Baseline | 46.75 ± 28.75 | 56.87 ± 23.94 | 59.30 ± 22.50 |
| IL-8 endpoint | 41.63 ± 24.47 | 31.97 ± 10.97 | 43.84 ± 23.73 |
| p value<sup>b</sup> | NS | 0.002 | 0.005 |
| MMP-9 Baseline | 85.76 ± 23.98 | 79.79 ± 29.86 | 82.88 ± 32.98 |
| MMP-9 endpoint | 70.14 ± 35.95 | 51.58 ± 26.20 | 52.13 ± 35.95 |
| p value<sup>b</sup> | 0.025 | 0.001 | 0.001 |
| TIMP-1 Baseline | 3.31 ± 1.87 | 3.31 ± 1.37 | 3.18 ± 1.29 |
| TIMP-1 endpoint | 3.86 ± 1.84 | 5.19 ± 1.59 | 5.31 ± 1.94 |
| p value<sup>b</sup> | NS | 0.004 | <0.001 |

N, number; BOP, bleeding on probing; CAL, clinical attachment loss; PPD, probing pocket depth; IL, interleukin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; Lyc, Lycopene.

<sup>a</sup> Significance at p < 0.05 by Fisher’s exact test at 95% confidence interval.

<sup>b</sup> Concentrations of cytokine (ng/ml).

<sup>c</sup> Concentrations of cytokine (ng/ml).
studies are needed to better clarify the feasibility of the antioxidant.

Declaration of Competing Interest

The authors have no funding and no conflicts of interest relevant to this article.

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Table 3 Intergroup comparison of the changes in clinical parameters (primary outcomes) and inflammatory mediators’ concentration (secondary outcomes) at baseline and at the endpoint of the trial.

| Δ Primary outcomes Non-bleeding sites at endpoint | Comparisons | p valuea | Effect sizec |
|-------------------------------------------------|-------------|----------|-------------|
| Ctrl | ARISTIN | Lyc |
| **BOP (N, %)** | | | |
| 10 (44) | 17 (74) | 16 (70) | Ctrl vs ARISTIN NS 1.212 |
| Ctrl vs Lyc 1.200 | ARISTIN vs Lyc 1.026 |
| **Mean difference± SD** | | | |
| Ctrl | ARISTIN | Lyc |
| **CAL (mm, gain)** | 1.04 ± 0.96 | 1.94 ± 1.33 | 1.72 ± 0.88 | Ctrl vs ARISTIN 0.029 | 0.792 |
| Ctrl vs Lyc 0.036 | ARISTIN vs Lyc NS 0.150 |
| **PPD (mm, reduction)** | 1.83 ± 1.27 | 2.61 ± 1.12 | 2.35 ± 0.98 | Ctrl vs ARISTIN NS 0.461 |
| Ctrl vs Lyc NS 0.655 | ARISTIN vs Lyc NS 0.118 |
| **Δ Secondary outcomes** | | | |
| **IL-8 ↓** | 5.119 ± 14.14 | 24.90 ± 25.10 | 15.46 ± 16.63 | Ctrl vs ARISTIN 0.016 | 0.971 |
| Ctrl vs Lyc NS 0.667 | ARISTIN vs Lyc NS 0.376 |
| **MMP9 ↓** | 15.62 ± 24.10 | 28.21 ± 27.96 | 30.75 ± 32.11 | Ctrl vs ARISTIN NS 0.482 |
| Ctrl vs Lyc NS 0.532 | ARISTIN vs Lyc NS 0.084 |
| **TIMP1 ↑** | 0.55 ± 0.92 | 1.83 ± 2.08 | 2.13 ± 1.61 | Ctrl vs ARISTIN 0.038 | 0.897 |
| Ctrl vs Lyc NS 0.135 | ARISTIN vs Lyc NS 0.088 |

N, number; BOP, bleeding on probing; CAL, clinical attachment loss; PPD, probing pocket depth; IL, interleukin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; Lyc, Lycopene.

a Significance at p < 0.05 by Fisher’s exact test at 95% confidence interval.
b Significance at p < 0.05 by Kruskal-Wallis test.
c Effect size by Koopman asymptotic score at 95% confidence interval.
d Effect size by Cohen’s d.

N, number; BOP, bleeding on probing; CAL, clinical attachment loss; PPD, probing pocket depth; IL, interleukin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; Lyc, Lycopene.
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