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Clinical Effects of Subgingival Application of *Salvadora persica* Gel in Generalized Chronic Periodontitis

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ABSTRACT

Background: *Salvadora persica* (SP), or miswak, possess anti-inflammatory, antioxidant, anti-coagulant and anti-bacterial properties that may provide therapeutic benefits in the treatment of chronic periodontitis (CP). In developing countries, prevalence of CP, bacterial resistance and adverse effects of antibacterial agents, has increased over the years. The current study aimed to explore the effectiveness of a locally delivered SP gel as an adjunct to scaling and root planing (SRP) in the treatment of CP.

Methods: Sixty-six subjects with chronic periodontitis were selected and full-mouth periodontal parameters (periodontal pocket depth, recession, clinical attachment loss, plaque score and bleeding) were measured. The subjects were divided into a control group [scaling and root planning (SRP)] and an SP group [subgingival placement of *Salvadora persica* gel in periodontal pockets]. On 6 weeks' follow-up, the periodontal parameters were recorded. The distributions of variables were analyzed by the Wilcoxon Signed Rank test and paired *t*-test. The $p \leq 0.05$ was considered statistically significant.

Results: A reduction in periodontal pocket depth (PPD), plaque score (PS) and bleeding on probing (BOP) and clinical attachment loss (CAL) was observed within both SP and control group on 6 weeks follow-up. Comparison of changes in periodontal parameters between the groups revealed a statistically significant difference in bleeding on probing score in SP group compared to control group ($p = 0.002$), while there was no statistically significant difference in changes in periodontal pocket depth, gingival recession, clinical attachment loss and plaque score between both groups.

Conclusion: The SP group demonstrated improved gingival health ($p=0.001$), reducing bleeding score compared to the control group. However, insignificant results were observed in periodontal pocket depth, recession, clinical attachment loss and plaque score.

Keywords: Miswak; Chronic Periodontitis; Scaling and Root Planing; Periodontal Pocket; Clinical Attachment Level.

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INTRODUCTION

Chronic periodontitis is a multifactorial, progressive, and destructive disease affecting the supporting structures of the teeth that are collectively referred to as the "periodontium", which if left untreated ultimately leads to tooth loss[1]. It is highly prevalent across the world, with mild periodontitis affecting as much as 50% of the global population[2, 3]. Mechanical debridement in the form of scaling and root planing (SRP) is the gold standard and often the sole treatment of chronic periodontitis, however, its efficacy is limited in areas with inadequate access to debridements, such as deep periodontal pockets and furcation areas[4]. To overcome the limitations of mechanical debridement, pharmaceutical adjunct therapies such as local antibiotics, statins, metformin and other treatment modalities such as laser-assisted photo-activated disinfection are currently being utilized[5,6]. While modern pharmaceutical agents have been proven efficacious as adjunctive therapy in periodontal disease, they carry undesirable side effects, including increased antibacterial resistance, taste disturbances, discoloration of the tongue and teeth, compliance issues and higher pricing.

Salvadora persica (SP) or miswak, is a root twig that was the predecessor to a toothbrush. It has remained a popular brushing tool in modern times amongst Muslim populations with its advantages being highlighted in Prophetic narratives[7,8]. The advantages of SP can be attributed to a combination of the mechanical action of brushing and its bioactive constituents. Among the various phytoconstituents of the SP extracts are the antioxidants Persicaline (a Sulphur-containing imidazoline alkaloid) [9], antivirals such as salvodourea, benzyl isothiocyanate and m-anisic acid[10], essential oils with antimicrobial activity[11], benzylamides which possess antibacterial activity and cause collagen-induced platelet aggregation[12], Trimethylamine and salvadorine with their antibacterial, antiphlogistic and gingival-stimulatory effects[13] and the antimicrobials benzaldehyde, benzyl nitrile and benzyl isothiocyanate[7]. With growing interest in the field of folk medicine which may be attributed to the minimal side effects and low cost of natural products, researchers have explored the advantages of SP as an adjunct therapy in the form of toothpaste and mouthwashes, however, whether it is effective in reducing bacterial loads in deep, inaccessible sites of CP needs to be assessed. Therefore, the present study aimed to investigate the clinical benefits of subgingival application *Salvadora persica* in subjects with CP, following scaling and root planing.

METHODS

A total of sixty-six participants with chronic periodontitis were recruited. The study included men and women aged 30 years old and above with a minimum of 20 teeth and a diagnosis of moderate to severe chronic periodontitis, defined minimal 4 sites with CAL ≥ 3 mm were included[14]. Out of these participants, those with systemic conditions modifying the progression of periodontitis (diabetes mellitus, metabolic syndrome, or immunologic disorders), pregnant or lactating females, or those who took antibiotics in the last 6 months were excluded from the trial. After stratification of the subjects based on their smoking status and social habits, thirty-three participants were randomly assigned to receive scaling and root planing (SRP) only (control group), while the other thirty-three were assigned to receive SP gel in addition to SRP (SP group).

At baseline (week 0) full-mouth periodontal charting of all subjects was performed including full-mouth 6-point clinical attachment level (CAL), periodontal pocket depth (PPD), gingival recession (GR), plaque score (PS) and bleeding on probing score (BOP). Following periodontal charting, the participants underwent SRP employing a combination of hand instruments and a piezoelectric ultrasonic handpiece with a slim scaler tip. The subjects of the SP group received SP gel in all periodontal pockets greater than 3 mm deep. All subjects were instructed to avoid eating and drinking for half hour after the procedure. They were provided thorough oral hygiene demonstrations and instructions. In the follow-up appointment at week 6, the five clinical parameter measurements were repeated. The study protocol has been explained in Figure 1.

The miswak gel was formulated and developed utilizing the methodology described by Al-Ayed et al. and Aslani et al. [15,16]. One hundred grams of *Salvadora persica* were washed and then cut into small pieces with a sharp knife. Once dried, the SP pieces were ground to powder using an electrical grinder. The powder produced was mixed with 95% ethanol (Merck, Germany) and filtered using a Whatman filter paper, forming an SP extract. To form the gel, potassium sorbate was dissolved in water and heated to 80°C. Hydroxypropyl methylcellulose (HPMC) was slowly added and stirred constantly. Distilled water was added and mixed until a homogenous transparent gel of the desired consistency was formed. The gel was stored in the refrigerator overnight. The SP extract was mixed with polyethylene glycol (PEG) 400 (Sigma Aldrich, Germany) and gradually added to the gel.

The data was expressed as Mean ± SD or Median (IQR) and SPSS was used for statistical analysis. The Mann-Whitney U test was used to find the mean difference among parameters. Distributions of PPD, GR, BI and CAL significance were found by independent paired *t*-test. The distributions of GR, PI and PG were non-normal within the group therefore, the Wilcoxon Signed Rank test was applied. The *p*-value ≤ 0.05 was considered statistically significant.

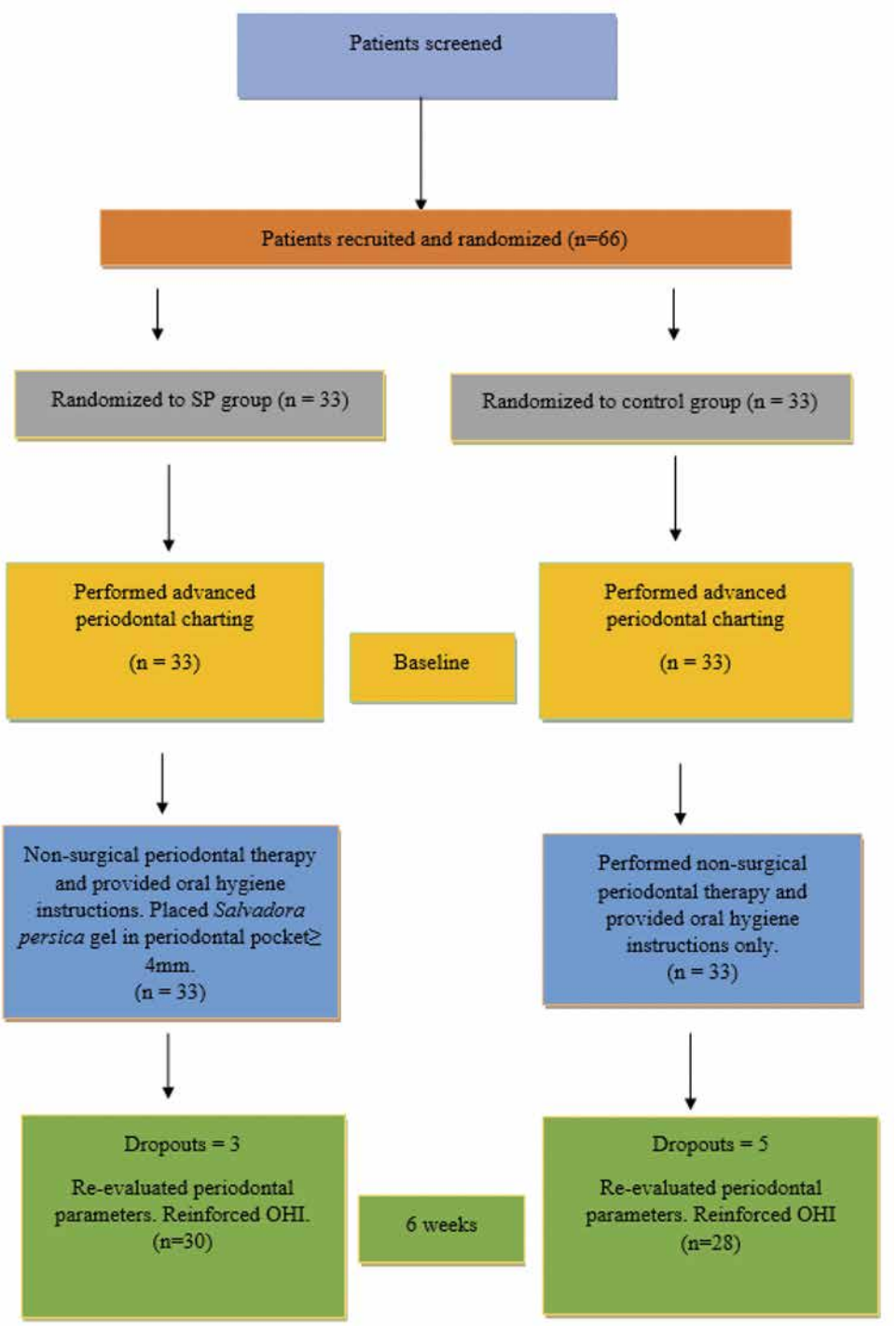

Figure 1: Study flow diagram.

RESULTS

A total of 66 participants were recruited for this study. The overall mean age of the patients was 48.68±9.12 years. Out of the 66 recruited participants, 38 were males (57.6%) and 28 were females (42.4%) (Figure 2) while Figure 3 represented the social habits of the study participants. Fifty-eight of sixty-six participants completed the study. In the SP group, three failed to follow up, while in the control group a total of five participants failed to follow up. None of the participants reported any adverse reaction or discomfort. Healing following SRP was uneventful.

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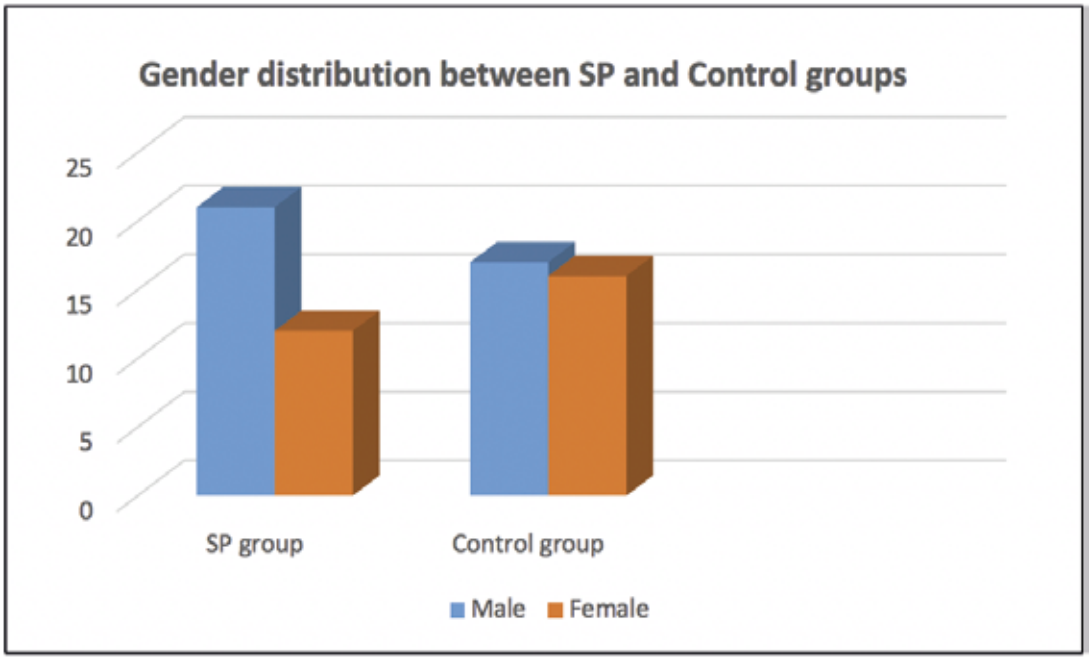

Figure 2: Gender distribution of study subjects (n=66); *Salvadora persica* (SP).

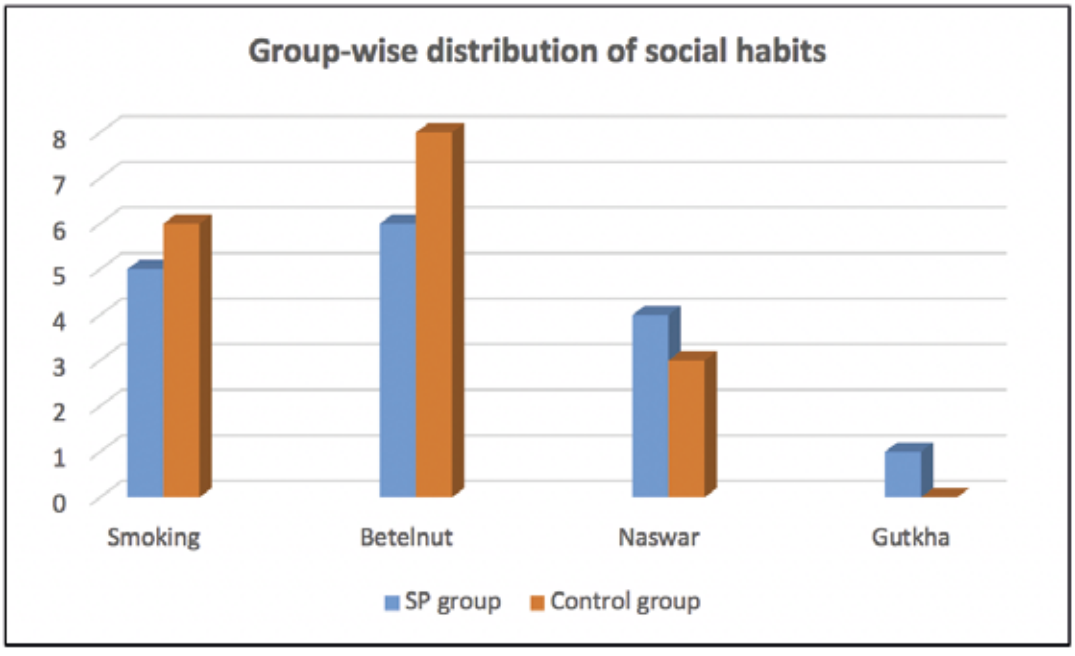

Figure 3: Social habits of study subjects (n=66); *Salvadora persica* (SP).

No statistically significant difference between the SP and control group was found between all clinical parameters at baseline. Both groups showed improvement in clinical attachment level (CAL), from 4.59 (3.86 -5.08) to 3.82 (3.38-4.25) in the SP group and 4.61 ± 0.77 to 4.03± 0.51 in the control group (Table 1), however a comparison of mean changes in CAL at 6 weeks showed no statistically significant differences between both groups (ΔCAL in SP 0.66 (0.25 to 1.05), in control group 0.75 (0.27 to 0.95), p= 0.901(Table 2).

Table 1: Comparison of clinical parameters from baseline to 6th week within groups.

Parameters	SP Group (n=30)			Control Group (n=28)		*p*-Value
	Baseline	6th week	*p*-Value	Baseline	6th week	
PPD (mm)	4.21 (3.48-4.91)	3.54 (3.11-3.86)	0.001*	4.44±0.78	3.64±0.48	0.001^*
GR (mm)	0.22 (0.14-0.50)	0.22 (0.14-0.49)	0.063	0.33 (0.12-0.46)	0.34(0.12-0.46)	0.495+*
PS (%)	94.45 (88.4-95.7)	32.5 (23-45)	0.001*	92.4 (75.5-97.4)	29.6 (22.45-37.15)	0.001+*
BOP (%)	77.2 (66.8-88.60)	14.60 (12.55-16.60)	0.001*	74.28±12.94	22.81±6.81	0.001^*
CAL (mm)	4.59 (3.86-5.08)	3.82 (3.38-4.25)	0.001*	4.61±0.77	4.03±0.51	0.001^*

*^Distributions of PPD, GR, BI and CAL were normal within the group, therefore independent t-test was applied +Distributions of GR, PI and PG were non-normal within the group; therefore, Wilcoxon Signed Rank test was applied, *Significant at 0.05 level of significance. PPD= Periodontal pocket depth, GR=gingival recession, PS=plaque score, BOP=bleeding on probing, CAL=clinical attachment level.*

While both groups showed statistically significant differences in periodontal pocket depth (PPD) from week 0 to week 6 but there was no statistically significant difference in changes in PPD at follow-up (Δ PPD in SP group 0.79 (0.26 to 1.05), control group 1.02 (0.31 to 0.88), *p*=0.618 (Table 2), proving that while there was an improvement in PPD due to the treatment modality no additional benefit was achieved by placement of SP gel, compared to SRP only. In gingival recession (GR), no change in values was observed between week 0 and week 6 from 0.22 (0.14-0.50) to 0.22 (0.14-0.49) in the SP group and from 0.33(0.12-0.46) to 0.34 (0.12-0.46) in a control group, demonstrating that both treatment modalities did not affect GR.

Plaque score (PS) and bleeding score (BOP) are indicators of gingival health and measures of gingival inflammation. Both treatment modalities improved PS, from 94.45(88.4-95.7) to 32.5(23-45) in SP group and from to 92.4(75.5-97.4) to 29.6(22.45-37.15) in control group. However, there was no statistically significant difference in changes in PS between SP and control group at follow-up (SP: 56.7900±13.19431, control: 56.4821±12.82128, *p*=0.929, Table 2), While both groups demonstrated an improvement in bleeding score, a statistically significant improvement was observed in SP group compared to control group (*p*=0.006, Table 2), which attests that an additional benefit was achieved by the placement of SP gel.

Table 2: Comparison of mean changes in clinical parameters (from baseline to 6th week) between SP and control group.

Parameters	SP Group (n=30)	Control group (n=28)	*p*-Value
ΔPPD (mm)	0.79 (0.26 to 1.05)	1.02 (0.31 to 0.88)	0.618
ΔGR (mm)	0.000 (0.000 to 0.010)	0.000 (0.010 to 0.010)	0.832
ΔPS (%)	56.7900±13.19431	56.4821±12.82128	0.929
ΔBOP (%)	63.80 (53.60 to 75.00)	50.75 (40.40 to 61.85)	0.006*
ΔCAL (mm)	0.66 (0.25 to 1.05)	0.75 (0.27 to 0.95)	0.901

*Data expressed as Mean±SD or Median (IQR), Distribution of PPD, GR, PI, BI and CAL was non-parametric between groups; Mann-Whitney U test was applied, *Significant at 0.05 level of significance Δ denotes changes in values, calculated by the formula: Baseline value- 6th-week value PPD= Periodontal pocket depth, GR=gingival recession, PS=plaque score, BOP=bleeding on probing, CAL=clinical attachment level.*

DISCUSSION

The present study was carried out to assess the clinical benefits of a local drug-delivery system containing a plant extract *Salvadora persica* and found a significant improvement in bleeding score compared to SRP alone. Both treatments equally improved the mean CAL (primary outcome variable), PPD and PS compared to baseline, while no statistically significant improvement in GR was recorded in both groups compared to the respective values at baseline.

Scaling and root planing alone often serve as a definitive treatment of CP, causing cessation of the

disease process and restoring the periodontium's health and function[17]. The goal of SRP is to render the root surface completely free of plaque and calculus deposits, however, studies indicate that this goal is not always attainable[18]. Despite some residual plaque and deposits, however, SRP causes a significant reduction in the signs of inflammation (bleeding and plaque score), the PPD and the number of periodontopathogens and a gain in CAL[19-21].

The ability to scale and root planing to cause a reduction in gingival inflammation, as demonstrated by a decrease in plaque and bleeding scores, is a well-established fact[22]. The significant reduction in the supra-gingival plaque scores seen in both the SP and control groups in our study could be attributed to an effective SRP regimen and the implementation of a comprehensive oral hygiene maintenance protocol and the instructions provided to all the patients recruited in the study. The observation that there was no additional improvement in supra-gingival plaque score compared to that seen in the control group, is indicative of the fact that the improvement in plaque score, when compared to baseline, could be attributed to the SRP and oral hygiene instructions rather than the constituents of the SP gel. No statistically significant difference in plaque scores was found between the SP and control group at both timeframes, which indicates that both groups followed equally effective levels of oral hygiene maintenance through the study period.

Subgingival or local drug-delivery (LDD) systems offer the advantages of achieving high intra-sulcular drug concentrations, without being affected by patient compliance and with minimal systemic side effects[23]. The most extensive research has been performed on LDDs delivering antimicrobials, which provide as much as a 100-fold greater concentration compared to systemic regimens[24]. In this clinical trial, we used the LDD system for offering a high concentration of SP in the gingival sulcus of subjects with chronic periodontitis.

An important and interesting observation was that while both SP and control groups showed significant improvement in bleeding scores, the SP group showed a statistically significant difference in bleeding scores when compared to the control group. This point to the fact that SP gel exerts a strong anti-inflammatory and anti-coagulant effect in the gingiva, resulting in decreased gingival bleeding. Additionally, the biostatic nature of SP suggests that it can reduce the number of periodontopathogens, and the subsequent destruction caused by them[25].

When used as an adjunct in previous studies, SP has reported improvement in plaque and bleeding scores. SP is a combination of a series of organic and inorganic products including tannins, vitamin C, calcium, silica, calcium, and sodium bicarbonate. The role of silica in the maintenance of normal pH, plaque inhibition and caries prevention is well established and documented[26]. The bacteriostatic nature of SP reduces periodontopathogenic bacteria including *Porphyromonas gingivalis*, *Streptococcus mutans*, *Enterococcus faecalis* and *Aggregatibacter actinomycetemcomitans*, thereby secondarily reducing sulcular inflammation and the bleeding levels. Azaripour et al. stated that SP as an active ingredient in toothpaste caused a reduction in plaque scores[27]. Similarly, it has been reported that SP as a mouthwash formulation also led to significant plaque reduction[28,29]. These formulations, however, had a supra-gingival or mechanical plaque-control-related action, while in our study the gel was placed in the gingival sulcus. A study comparing the efficacy of SP gel and photodynamic therapy (PDT) as adjuncts to SRP yielded similar results to our study with only a statistically greater improvement in bleeding score in the SP group compared to SRP only[30].

The present trial carries several limitations. Firstly, it is a short clinical trial. Longer clinical trials with an assessment of radiographic findings would provide a more robust clinical picture. Secondly, standardization and quantification of the SP gel, along with an assessment of the release of pharmacological constituents and an analysis and comparison of different vehicles of SP should be performed in *in-vivo* studies. Third, the assessment of biomarkers or periodontopathogenic levels from local sites could further help identify the therapeutic benefits and efficacy provided by the adjunct and validate the clinical results. Hence, the results presented in this *in-vivo* trial lay a guide that may design larger and long-term clinical trials aimed specifically to assess the additional benefits of adjunctive use of SP in subjects with varying degrees of periodontal destruction.

CONCLUSION

The adjunctive use of SP gel has a positive effect on gingival health, as evident from a reduced bleeding score in the SP group compared to the control group. However, multicenter, long-term, randomized, controlled clinical trials should be conducted to assess its clinical, histological, and radiographical effects in bone regeneration. These can help us better understand the therapeutic efficacy of SP gel in CP.

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CONFLICT OF INTEREST
All authors confirm that there is no conflict of interest.

ETHICS APPROVAL
The institutional approval of the research study was attained by the Ethical Review Committee at Ziauddin University (Reference code: 0860319THOM).

PATIENT CONSENT
Written consent was taken from all participants.

FUNDING
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AUTHORS' CONTRIBUTION
TH was the principal investigator who performed periodontal charting of all subjects. She also prepared the manuscript. SQ was the study coordinator who confirmed that SRP was performed successfully. AJ contributed to the manuscript. ZN prepared the SP gel.

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