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ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

Evaluation of 4% Mangosteen (MG) Gel as a Local Drug Delivery in adjunct to Scaling and Root planing on Clinical Parameters and GCF MMP-9 levels in Chronic Periodontitis Patients – A Split Mouth Randomized Controlled Trial

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ABSTRACT

Objective: The current study was conducted to assess the efficacy of 4% Mangosteen (MG) gel in adjunct to scaling and root planing (SRP) on clinical parameters and gingival crevicular fluid (GCF) matrix metalloproteinase 9 (MMP-9) levels in the management of patients with chronic periodontitis.

Materials and Methods: This double blinded, randomized, placebo-controlled, split mouth, clinico-biochemical study was carried out on 13 patients (26 experimental sites) with Stage II and Grade B periodontitis. Following subgingival SRP, the 13 control sites were treated with LDD of placebo gel and the 13 test sites with 4% MG gel. Clinical parameters including plaque index (PI), gingival bleeding index (GBI), probing depth (PD) and relative attachment level (RAL) were recorded and GCF was collected from all the experimental sites for MMP-9 analysis, using Enzyme Linked Immunosorbent Assay (ELISA); at baseline and 3 months post treatment.

Results: Both, control and test sites, demonstrated improvement in the clinical parameters and reduction in GCF MMP-9 levels during the study period. Test sites exhibited a statistically significant reduction (P <0.05) in PD and RAL (gain in clinical attachment level; CAL) and GCF MMP-9 levels than the control sites. All the clinical parameters showed a positive correlation with the GCF MMP-9 levels.

Conclusions: Within the limitations of this study, 4% MG gel proves to be an efficacious adjunctive agent to SRP, for management of chronic periodontitis.

Keywords: *Garcinia mangostana*, GCF, Local drug delivery, MMP-9, Periodontitis, Scaling and root planing.

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INTRODUCTION

Periodontitis is a multifaceted inflammatory disease that results in the breakdown of tooth supporting apparatus. It occurs as a consequence of interplay between the host and pathogenic bacteria present in the biofilm and if left untreated, ultimately results in tooth loss (Singh et al., 2018). The host-immune interactions produce several inflammatory molecules locally such as cytokines, matrix metalloproteinases (MMPs), their inhibitors and regulators making them some of the important indicators of the disease (Checchi et al., 2020).

The main goal of periodontal therapy is to diminish the pathogenic microflora, thereby reducing the production of the inflammatory molecules. Scaling and root planing (SRP) are the foundation of periodontal therapy that aim at removal of the biofilm thus delaying the microbial repopulation (Singh et al., 2018; Wei et al., 2021) which reduces the inflammatory burden and provides a biocompatible root surface. The anatomical complexity of the tooth and the inadequate access to the pathogens deep within the pockets, limit the efficacy of SRP alone to entirely eradicate the infections. Combining SRP with various antimicrobial and anti-inflammatory agents, that improve tissue regeneration, has been shown to enhance its therapeutic effect (Goodson et al., 1985; Pradeep et al., 2013). These agents can be administered systemically as well as locally. Owing to several unintended effects of the systemic antimicrobials such as gastro-intestinal intolerance, development of resistance and requirement of large doses for administration, utilization of local drug delivery (LDD) has been amplified in periodontics (Drisko, 1996; Wei et al., 2021). LDD improves patient compliance, pharmacokinetics, & lowers the total drug dosage requirement. Herein, the drug is directly administered at the intended site with a sustained release,

and can be formulated as gels, chips, fibres, microparticles and nano systems (HR et al., 2019).

Even though numerous materials are being used as LDD, the necessity for effective, safe and affordable alternatives has continually driven researchers to explore natural products. *Garcinia mangostana* Linn., commonly known as Mangosteen (MG), is a popular fruit in the tropical Southeast Asia especially in Malaysia, Indonesia, and Thailand. MG rind contains multiple bioactive compounds that promote its therapeutic effects including xanthones, flavonoids, saponins, tannins, phenols, gartanines, and garcinones amongst several others (Aljuanid et al., 2022). Xanthones are polyphenols and are the major biomolecules that exhibit antioxidant, antiproliferative, antibacterial, and anti-inflammatory properties (Widyarman et al., 2019; Tangsuksan et al., 2022).

MMPs are protein molecules which breakdown the extracellular matrix (ECM) during the disease progression (Checchi et al., 2020). MMP-9 (Gelatinase B) is a 92 kilo Dalton (kDa) enzyme that primarily degrades type IV collagen of the basement membrane. Significantly higher level of gingival crevicular fluid (GCF) MMP-9 has been observed in periodontitis patients in contrast to healthy counterparts (Rai et al., 2010). The greater reduction of MMP-9 levels in GCF post-SRP in periodontal patients, deem it an important biomarker of periodontal disease (Luchian et al., 2022). MMP-9 is also expressed by osteoclasts, that are responsible for alveolar bone loss due to periodontal inflammation (Luchian et al., 2022). A xanthone of MG, α-mangostin, has demonstrated inhibition of the mRNA levels and promoter activity of MMP-9 in vitro on human renal carcinoma cells (Chen et al., 2017).

MG extract has been found to be bactericidal against periodontal pathogens including *P. gingivalis* and *A. actinomycetemcomitans* (Hendiani et al., 2016). Subgingival LDD of 4% MG gel, adjunctive to SRP, has demonstrated a significant reduction in pockets depth, gingival bleeding index values and improvement of clinical attachment levels along with antimicrobial and antioxidant effect in chronic periodontitis patients (Rassameemasmaung et al., 2008; Mahendra et al., 2017; Manjunatha et al., 2022).

To the best of our knowledge there is a paucity of trials that evaluated the efficacy of MG gel as a LDD agent and its effect on GCF MMP-9. Therefore, the present study intended to assess and compare the efficacy of 4% MG gel as a LDD, adjunctive to SRP on clinical parameters & GCF MMP-9 levels in the management of chronic periodontitis.

MATERIALS AND METHODS

The study was authorized by the Institutional Review Board (Ref. No. BDCH / Exam 574 / 2020 – 2021); Clinical Trials Registry – India Identifier: CTRI/2021/12/038758 and abided to the principles in the Helsinki Declaration (revised 2008).

Patients with chronic periodontitis, aged 30-65 years of either sex, who were systemically healthy, with minimum of 2 sites in different quadrants with probing depths ≥ 5 mm, loss of clinical attachment ≤ 3-4 mm i.e., Stage II and Grade B periodontitis, and who did not use chemotherapeutic mouth rinses or any other agents were included in the study. Patients with an identified or supposed allergy to MG, history of chronic smoking and alcohol consumption, having undergone systemic &/or topical antibiotic &/or anti-inflammatory therapy in last 3 months, who were treated for periodontitis in the past 6 months, pregnant and lactating mothers, those with systemic conditions affecting treatment outcomes, individuals who were unable to maintain the oral hygiene or come for the recall visits were excluded from the study.

This randomized, double blinded, placebo controlled, prospective, split mouth, clinico-biochemical study was conducted on 13 patients (26 experimental sites) fulfilling the aforementioned criteria. They were recruited from the Department of Periodontics, Bapuji Dental College and Hospital, Davangere, Karnataka, India. A thorough verbal and printed description regarding the study was given to all the patients and a signed consent to partake was obtained from them prior to the commencement. At screening, case history was taken followed by clinical examination and recording of the parameters. Full mouth supragingival scaling was done and oral hygiene instructions (OHIS) were given to them. Alginate impressions were made and casts poured for the preparation of acrylic stents. After 2 weeks, patients with full mouth PI and GBI scores ≤25% were chosen for the study. The selected sites were randomized (by computer generated random number sequence) equally into the following experimental groups:

Control Group – 13 Sites to be treated with subgingival SRP and placebo gel delivery.

Test Group – 13 Sites to be treated with subgingival SRP and 4% MG gel delivery.

Collection of GCF

At baseline, under adequate isolation, the supragingival plaque was debrided gently from the selected experimental sites to avoid contamination and blocking of the micro capillary pipette. A standardized volume of 3µl GCF was collected in 1-5µl calibrated and color-coded micropipettes (Sigma-Aldrich, USA) with extra-crevicular method (Fig. 1) and was transferred into sterile Eppendorf tubes (Flex-Tube® – Microtube, Eppendorf, North America) containing 50µl of 0.9% phosphate buffered saline (GibcoTM, Thermo Fisher Scientific, USA). All the collected samples were sent to the research laboratory, and were stored at – 80 °C until their analysis. Visibly contaminated samples were discarded. GCF samples were collected similarly at 3 months post-treatment from all the experimental sites.

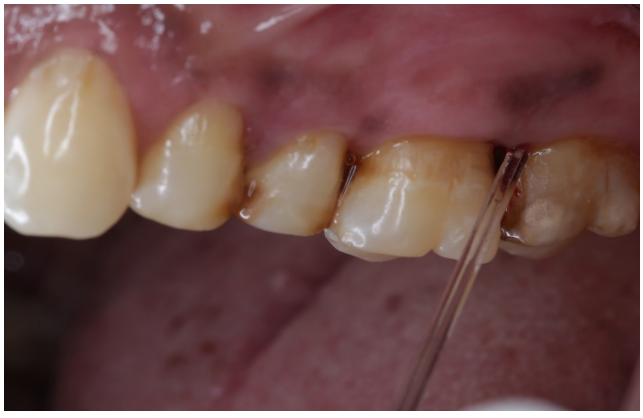

Figure 1: GCF collection by using a microcapillary pipette.

Clinical Measurements

A blinded periodontist (M.K.) examined and recorded the parameters, who was calibrated by a senior clinical investigator (G.G.V.) prior to the study. At baseline and 3 months post-treatment, the i) Plaque index (PI) (Silness and Loe,1964); ii) Gingival Bleeding Index (GBI) (Ainamo and Bay, 1975); iii) Probing Depth (PD) and iv) Relative Attachment Level (RAL) were recorded. The customized acrylic stents and UNC-12 Periodontal probe (HuFreidy®, USA) were used to record PD and RAL (in mm), with apical border of the stent as a fixed reference point.

Treatment protocol

All the selected patients were subjected to complete subgingival scaling with ultrasonic scalers (Woodpecker® India) and, root planing was carried out with Columbia universal 4R-4L, 2R-2L curettes (HuFreidy®, USA) at the selected experimental sites. The 4% MG gel was formulated according to Rassameemasmaung et al., (2008) and the placebo gel was similarly formulated, without the active ingredient i.e., MG. The prepared gels were packaged into similar tubes, and the demarcation for their identification was blinded from the administrator. The tubes were UV sterilized and stored at 4°C throughout the study period. In test group 4% MG gel (Fig. 2) while in control group placebo gel respectively were delivered in the designated sites with a 10cc syringe and a 21-gauge blunted tip needle (Unolok, Dispovan, HMD, Faridabad, Haryana, India), gently until it spilled over from the marginal gingiva and was allowed to settle inside the pocket. A periodontal pack (Coe-PakTM, GC America Inc., Illinois, USA) was placed up to a week, to prevent the delivered gel from flowing out, the ingress of oral fluids and the cross-over effect. OHIS were given.

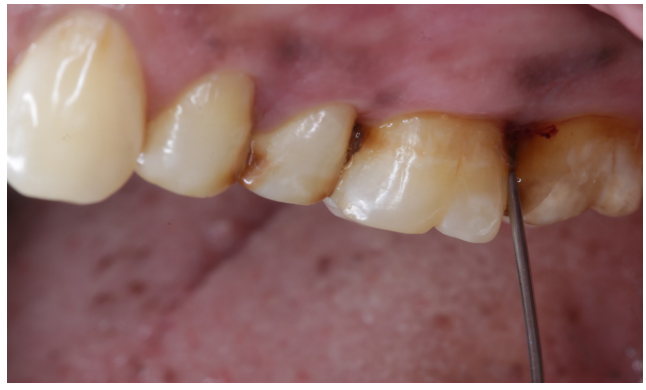

Figure 2: Subgingival LDD OF 4% MG gel.

Biochemical analysis of GCF MMP-9 with ELISA

GCF MMP-9 levels were determined using EliKine™ Human MMP 9 ELISA Kit (Abbkine, Delhi, India), conferring to the instructions given by the manufacturer. The kit utilized the sandwich technique of double antibody type, to determine the Human MMP-9 concentrations in GCF samples. A microplate pre-coated with antibody specific to Human MMP-9 was employed. Standards and GCF samples were added to the microplate wells and the Human MMP-9 in the sample was bound by the immobilized antibody. Post removal of any unbound elements, an antibody specific for Human MMP-9 conjugated with biotin, was pipetted into the microplate wells. After washing, streptavidin-horseradish peroxidase (HRP) conjugates were added. Washing was repeated to eliminate any unbound streptavidin-enzyme reagent, which was followed by the addition of transparent HRP substrate (TMB). TMB turned blue by the breakdown of HRP, and further to yellow after the stop solution was added. The optical density (OD) value was determined

using an ELISA reader (iMark™ Microplate Absorbance Reader), measured at 450 nm wavelength (OD 450nm). The MMP-9 level was determined in picograms/microlitre (pg/µl), and was directly proportional to the OD 450nm value.

Statistical Analysis

The documented clinical and biochemical results were tabulated and statistically analysed by an expert. The data was presented as mean ± standard deviation. For the intragroup and intergroup comparisons, the Welch's paired-t test was applied to determine if significant changes occurred in the parameters at baseline and 3 months post treatment in both the groups. Pearson's correlation coefficient analysis was employed to check the linear correlation and strength of the relationship between two sets of data variables i.e., the clinical and biochemical (GCF MMP-9 levels) parameters. The *P* value < 0.001 was set as statistically highly significant (HS**); < 0.05 as significant (S*) and > 0.05 as non-significant (NS). GraphPad Software, Boston was used to carry out the statistical analysis.

RESULTS

Both control and test group sites demonstrated a significant reduction in the mean PI, GBI, PD (Figure 3A), RAL [gain in CAL] (Figure 3B) scores & in mean GCF MMP-9 levels (Figure 3C) from baseline to 3 months (Table 1). Comparison of mean PI, GBI, PD, RAL values and GCF MMP-9 levels in the test group sites demonstrated a significant decrease in contrast to the control sites at 3 months post treatment (Table 2). The clinical parameters and GCF MMP-9 levels demonstrated a positive correlation at 3 months post treatment, which was statistically not significant in both the groups (Table 3).

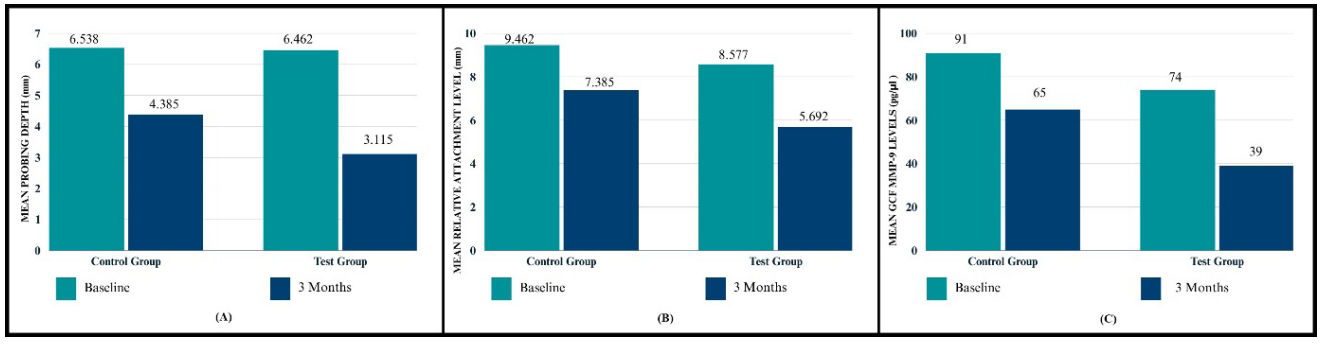

Figure 3: (a) Comparison of mean PD scores at baseline and 3 months post-treatment in control and test groups. (b) Comparison of mean RAL scores at baseline and 3 months post-treatment in control and test groups. (c) Comparison of mean GCF MMP-9 levels at baseline and 3 months post-treatment in control and test groups.

Table 1. Intragroup comparison of the clinical and biochemical parameters in control and test groups at baseline and 3-months post treatment

Parameters	CONTROL GROUP (n=13)			TEST GROUP (n=13)		
	Baseline (C0)	3-months (C3)	P-Value #	Baseline (T0)	3-months (T3)	P-Value #
PI	1.423 ± 0.277	0.634 ± 0.299	<0.001**	1.308 ± 0.355	0.076 ± 0.120	<0.001**
GBI	1.084 ± 0.229	0.472 ± 0.128	<0.001**	1.042 ± 0.170	0.265 ± 0.274	<0.001**
PD (mm)	6.538 ± 0.720	4.385 ± 0.982	<0.001**	6.462 ± 0.557	3.115 ± 0.711	<0.001**
RAL (mm)	9.462 ± 1.898	7.385 ± 0.869	<0.05*	8.577 ± 0.909	5.692 ± 1.974	<0.001**
GCF MMP-9 (pg/µl)	91 ± 7.2	65 ± 24	<0.05*	74 ± 25	39 ± 25	<0.05*

Values are presented as mean ± standard deviation.
PI: Plaque Index; GBI: Gingival Bleeding Index; PD: Probing Depth; RAL: Relative Attachment Level; GCF MMP-9: Gingival Crevicular Fluid Matrix Metalloproteinase – 9
*# Welch's paired t – test. **HS: highly significant (P<0.001) *S: significant (P<0.05)*

Table 2. Intergroup comparison of the clinical and biochemical parameters between control and test groups at baseline and 3-months post treatment

Parameters	BASELINE			3 MONTHS			Mean difference ± SEM at 3 months (T3-C3)	P-Value #
	Control (n=13)	Test (n=13)	P-Value #	Control (n=13)	Test (n=13)	P-Value #		
PI	1.423 ± 0.277	1.308 ± 0.355	>0.05 NS	0.634 ± 0.299	0.076 ± 0.120	<0.001**	-0.557 ± 0.089	<0.001**
GBI	1.084 ± 0.229	1.042 ± 0.170	>0.05 NS	0.472 ± 0.128	0.265 ± 0.274	<0.05*	-0.206 ± 0.084	<0.05*
PD (mm)	6.538 ± 0.720	6.462 ± 0.557	>0.05 NS	4.385 ± 0.982	3.115 ± 0.711	<0.001**	-1.269 ± 0.336	<0.001**
RAL (mm)	9.462 ± 1.898	8.577 ± 0.909	>0.05 NS	7.385 ± 0.869	5.692 ± 1.974	<0.05*	-1.692 ± 0.598	<0.05*
GCF MMP-9 (pg/µl)	91 ± 7.2	74 ± 25	<0.05*	65 ± 24	39 ± 25	<0.05*	-25.66 ± 9.675	<0.05*

Values are presented as mean ± standard deviation and mean difference ± standard error of mean (SEM).
PI: Plaque Index; GBI: Gingival Bleeding Index; PD: Probing Depth; RAL: Relative Attachment Level; GCF MMP-9: Gingival Crevicular Fluid Matrix Metalloproteinase – 9
*# Welch's paired t – test. **HS: highly significant (P<0.001) *S: significant (P<0.05) NS: non-significant (P>0.05)*

Table 3. Correlation of GCF MMP-9 levels with clinical parameters at 3 months post-operatively

Parameters	GCF MMP-9 levels at 3 months			
	Control group		Test group	
	Correlation coefficient (r)	P-value #	Correlation coefficient (r)	P-value #
PI	0.475	> 0.05 NS	0.045	> 0.05 NS
GBI	0.209	> 0.05 NS	0.097	> 0.05 NS
PD	0.483	> 0.05 NS	0.132	> 0.05 NS
RAL	0.009	> 0.05 NS	0.056	> 0.05 NS

PI: Plaque Index; GBI: Gingival Bleeding Index; PD: Probing Depth; RAL: Relative Attachment Level; GCF MMP-9: Gingival Crevicular Fluid Matrix Metalloproteinase – 9
 # Pearson's Correlation Coefficient Analysis r : correlation coefficient NS: non-significant (P>0.05)

DISCUSSION

Herbal agents have been employed in the field of medicine for treatment of several diseases (Aljuanid et al., 2022). MG is one such source of herbal medicine, originating from the tropical Southeast Asia. The significant biomolecules in MG pericarp are the xanthones like α – and γ-mangostin that have antioxidant (Manjunatha et al., 2022), anti-inflammatory (Hendiani et al., 2017; Putri et al., 2017; Lim et al., 2019; Ridwan et al., 2021), antibacterial (Rassameemasmaung et al., 2008; Mahendra et al., 2017; Widyarman et al., 2019; Tangsuksan et al., 2022) and antitumorigenic (Chen et al., 2017; Xu et al., 2017) properties.

Xanthones of MG can substantially regulate and minimalize oxidative damage due to the reactive oxygen species, thus inhibiting the degeneration of cells (Manjunatha et al., 2022). They also inhibit the inflammation by suppressing the lipoxygenase (LOX) and cyclooxygenase (COX) production, which are responsible for the release of prostaglandins (PGs) that further induce MMPs and result in osteoclastic bone resorption. Kresnoadi et al., (2017) in their study demonstrated a substantial reduction in the expression of nuclear factor kappa B (NF-kB) and receptor activator of NF-kB ligand (RANKL), which are essential for osteoclast development and progression of periodontitis, when MG was used along with a xenograft for preservation of extraction sockets. α-mangostin has demonstrated inhibitory effect on interleukin 1β (IL-1β) induced expression of COX-2, PGs, inducible nitric oxide synthase (iNOS), nitric oxide (NO), in

chondrocytes, indicative of its anti-inflammatory and anti-oxidant effects on the chief inflammatory mediators (Pan et al., 2017).

The xanthones present in MG pericarp, also destroy bacterial adhesins to inhibit the attachment of bacteria to the tissue, result in coagulation of bacterial cells, eliminate bacterial virulence factors and interfere with bacterial metabolism thus demonstrating its anti-bacterial properties on periodontal pathogens (Widjaja et al., 2019; Ridwan et al., 2021). MG pericarp extract has shown bactericidal activity against *P.gingivalis, T.denticola, S.aureus* at a minimum inhibitory concentration (MIC) range of 3.12 – 4 μg/ml (Phongpaichit et al., 1994; Rassameemasmaung et al., 2008; Mahendra et al., 2017).

LDD in the form of gels have been widely used as an adjunct to SRP and have demonstrated their potential role in improving the periodontal clinical parameters (Pradeep et al., 2013). Gels are easy to prepare, administer and they offer a sustained release of the drug with minimum dose frequency and toxicity (Bural et al., 2018). Subgingival LDD of 4% MG gel following SRP has shown a significant reduction in PD, PI, BI values and improvement in CAL gain in chronic periodontitis patients (Rassameemasmaung et al., 2008; Hendiani et al., 2017; Mahendra et al., 2017; Manjunatha et al., 2022). Hence in the present study a 4% concentration i.e., 4 μg/ml of the MG gel was considered as LDD in adjunct to SRP.

MMP-9 is a catabolic enzyme mainly involved in the degradation of collagen type IV, the fundamental constituent of the basement membrane (Luchian et al., 2022). It also degrades collagen type V & XI, proteoglycans, and elastin present in the connective tissue (Bildt et al., 2008). Neutrophils are the chief source of MMP-9, with increased concentrations being produced by the inflamed junctional epithelial cells in advanced periodontal disease (Yabluchanskiy et al., 2013). Other cells such as macrophages, keratinocytes, fibroblasts, osteoclasts and eosinophils in periodontitis are also responsible for expression of the MMP-9 gene (Franco et al., 2017). MMP-9 has been found to regulate IL-1, – 6, – 8, and PGs during various stages of inflammation in periodontitis (Franco et al., 2017). Hence, it can be assumed that increased MMP-9 concentrations may precisely indicate the inflammatory status of periodontitis in patients, and be a valuable biomarker for diagnosis and prognosis following periodontal therapy.

GCF originates from and is specific to the periodontium, constituting numerous reaction products associated with periodontal inflammation, analysis of which can indicate the changes in the tissues with progression of the disease. A thorough literature search revealed that the elevation in GCF MMP-9 levels is associated with chronic periodontitis (Rai et al., 2010; Han et al., 2012; Yakob et al., 2013). Non-surgical management of localized aggressive periodontitis, involving SRP and systemic antimicrobial therapy, has demonstrated a reduction in the levels of MMPs locally and a positive correlation with the clinical periodontal parameters (Gonçalves et al., 2013). But there is a paucity of evidence on the effect of LDD, particularly of MG, on GCF MMP-9 levels and the clinical parameters when used as an adjunct to SRP. Hence, we carried out this study to assess and compare the effect of 4% MG gel as an LDD, on clinical parameters and GCF MMP-9 levels in chronic periodontitis, as an adjunct to SRP.

There were no dropouts during the entire study period. All the patients tolerated the MG gel without any adverse reactions. All the clinical parameters demonstrated a non-significant difference in the mean baseline values of both groups, indicative of uniformity in the selection of experimental sites (Table 2). At 3 months post treatment, the mean PI scores of the test sites were reduced significantly ($P < 0.001$) than the control sites, which can be attributed to the antibacterial effect of the MG gel against the plaque microorganisms (Mahendra et al., 2017). These findings were similar to the trials carried out by Mahendra et al., (2017) and Manjunatha et al., (2022). Removal of the biofilm, reinforcement of oral hygiene instructions at screening and baseline visits, in addition to the "Hawthorne effect" could have improved the hygiene maintenance and treatment outcomes in the patients.

Similarly, the mean GBI scores showed a significant reduction ($P < 0.05$) in test sites than the control sites at 3 months post treatment. Our results were in congruence with the findings of Rassameemasmaung et al., (2008), Mahendra et al., (2017) and Manjunatha et al., (2022). The local vasoconstrictive and strong anti-inflammatory effect of MG mediated via inhibition of PGs, ILs and MMPs (Pan et al., 2017; Lim et al., 2019; Aljuanid et al., 2022; Luchian et al., 2022) could be responsible for the reduced ulceration of the gingiva and thus its tendency of bleeding on probing.

Increase in the probing depth and loss of clinical attachment are salient features of periodontitis. We observed a highly significant mean PD reduction ($P < 0.001$) in the test sites than the control sites at 3 months post operatively. Although shrinkage of gingival tissues naturally occurs

after SRP (Goodson et al., 1985) resulting in the PD reduction, the additional reduction in inflammation by 4% MG gel application, could have enabled the reconstruction of connective tissue fibres thereby improving the resistance to penetration by the probe. Recording of RAL is a clinical method to determine disease severity and progression of disease activity. RAL denotes the extent of the periodontal support that is present around a tooth. Post treatment, at the end of 3 months the test group sites showed a significant decline ($P < 0.05$) in the mean RAL scores i.e., betterment in the attachment levels, than the control sites which may be substantiated by synergistic effects of mechanical debridement and LDD of the MG gel. SRP reduces gingival inflammation, which results in tissue contraction & better gingival tissue adaptation. Root planing also aids in increasing the clinical attachment either via generation of new periodontal ligament fibres (new attachment) or via establishment of long junctional epithelium (repair) (Greenstein, 2000). In adjunct to SRP, the anti-inflammatory (Hendiani et al., 2017; Putri et al., 2017; Lim et al., 2019; Ridwan et al., 2021), antioxidant (Manjunatha et al., 2022), anti-bacterial properties (Rassameemasmaung et al., 2008; Mahendra et al., 2017; Widyarman et al., 2019; Tangsuksan et al., 2022) of the 4% MG gel might have brought out better results in the test group. Studies have also revealed an analogous reduction in PD and increase in the clinical attachment levels (CAL) with the sub gingival delivery of MG gel (Rassameemasmaung et al., 2008; Mahendra et al., 2017; Hendiani et al., 2017; Manjunatha et al., 2022).

The analysis of GCF is amongst the least invasive methods which can provide information about the current and future disease outcomes after treatment, in contrast to the measurement of clinical parameters which are comparatively invasive and only indicative of the periodontal destruction that has occurred in the past (Qasim et al., 2020). It helps in improving the accuracy of diagnosis for better management of the periodontal disease. Pre-treatment mean GCF MMP-9 levels were greater in the control group (91±7.2 pg/µl) than test group (75±25 pg/µl) with a significant difference ($P < 0.05$), though the mean values of all four clinical parameters had a non-significant difference (Table 2). This highlights the importance and precision of biochemical analysis in determining the disease severity at specific diseased sites when compared to clinical parameters alone. At 3 months, a statistically significant reduction ($P < 0.05$) was observed in the mean GCF MMP-9 levels of the test group sites than those of control group. Study results of Marcaccini et al., (2010) revealed elevated GCF MMP-9 concentrations in patients with stage II grade B periodontitis compared to the healthy patients, which were lowered when re-evaluated at 3 months after SRP, similar to our results. Our results are also analogous to those of Rai et al., (2010) who reported increased levels of GCF MMP-9 concentrations in periodontal patients compared to healthy patients.

Xanthones of MG have shown downregulation of MMP-9 and upregulation of tissue inhibitors of metalloproteinase (TIMP-1 and 2) in ovarian cancer cells (Xu et al., 2017). α-mangostin has also exhibited a reversal in the expression MMP-3, – 9 and – 13 levels (Pan et al., 2017). It is effective in increasing IL-10 levels, an anti-inflammatory cytokine, which can downregulate the production of NO, collagenase, and gelatinase (MMP-9) thereby regulating inflammation and maintaining homeostasis (Ridwan et al., 2021). Thus, the adjunctive use of MG in our study might have reversed and/or indirectly decreased the MMP-9 concentrations via the aforementioned mechanisms. Hence the potent anti-inflammatory effect of MG against MMP-9 can be credited for reduction in the GCF MMP-9 levels, which was also evident after 3 months of LDD.

In our study, the clinical parameters along with GCF MMP-9 levels presented a non-significant positive correlation in both the groups signifying a possible role of MMP-9 in the periodontal inflammatory process. Our results are comparable to those of Preshaw (Preshaw et al., 2020) wherein GCF MMP-9 levels post non-surgical periodontal therapy, demonstrated a reduction along with an improvement in the clinical status in Type 2 diabetics with moderate periodontitis. In contrast, study by Peniche (Peniche et al., 2019) demonstrated highest levels of GCF MMP-9 levels in healthy subjects compared to subjects having periodontitis with Type 2 diabetes. They attributed this disparity to limitations of the ELISA test kit and its variations, which cannot quantify active forms. The difference observed in our results compared to Peniche et al. can be credited to the changes in the study design, groups employed for comparison, duration of follow-up, methods of GCF sampling and statistical analysis.

CONCLUSIONS

From the present study we can conclude that following SRP, the sub gingival LDD of 4% MG gel effectively controlled the periodontal disease progression as observed by the improved clinical parameters and decreased GCF MMP-9 levels in the test group. This substantiates the

synergistic anti-inflammatory activity of MG gel and that it can be used adjunctively with SRP for better clinical results in the treatment of chronic periodontitis patients. The GCF concentrations of MMP-9 were correlated positively with the four clinical parameters, though they were not statistically significant. Thus, it can be postulated that the presence of MMP-9 locally influences the periodontal status and can be considered as a promising biomarker for the disease.

Conducting the study with a bigger sample size, an extended follow up period with a positive control and multiple applications of the MG gel are needed for further validation of its efficacy as a LDD agent. Evaluation and comparison among variants of MG LDD in the form of chip, micro & nanoparticle systems, electro spun fibres etc. can shed more light on Mangosteen's potential as a targeted therapeutic modality against MMP-9, in the management of chronic periodontitis.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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