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ADMINISTRATION OF SYSTEMIC PROPOLIS VERSUS MOXIFLOXACIN AS ADJUNCTIVE TREATMENT OF SEVERE GENERALIZED PERIODONTITIS

Mohamed M. Anees *, Tamer E. Aboshady ** *and* Hesham M. El-Sharkawy ***

ABSTRACT

To-date, emerging bacterial resistance to the commonly prescribed antibiotics in the management of periodontitis has become a challenging problem making scientists continually seek for new agents. In the present study, propolis (PRO) (natural bee glue) and moxifloxacin (MXF) (new quinolone antibiotic) were evaluated in the treatment of severe generalized periodontitis (gP) as adjuncts to scaling and root planing (SRP). Fifty four subjects with severe gP were randomly assigned into three groups (3 subjects did not complete the study after participation). Group I in which SRP alone was performed to gP patients (SRP group; n=17); group II in which SRP was done to gP patients combined with orally administered propolis 400 mg once daily for 7 days (PRO group; n=16); and group III in which SRP was performed to gP patients combined with oral moxifloxacin 400 mg once daily for 7 days (MXF group; n=18). Pocket depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), plaque (PI) and gingival (GI) indices were recorded at baseline, after 6 and 12 weeks of SRP. Salivary interleukin-1 beta (IL-1β) was evaluated at the same time points for all groups. All three procedures led to significant reductions in PD, CAL, BOP, PI and GI after 6 and 12 weeks. PD reduction and CAL gain were significantly greater in the PRO and MXF groups compared to SRP group at 6 and 12 weeks after therapy ($p < 0.01$). Importantly, there was no significant variation between PRO group and MXF group after treatment ($p > 0.05$). Likewise, in PRO and MXF groups, the salivary inflammatory marker (IL-1β) was significantly reduced in comparison to SRP group ($p < 0.01$). In all groups, salivary IL-1β levels were decreased at 6 and 12 weeks compared to baseline values. It was concluded that the adjunctive use of PRO and MXF to SRP had significantly improved the treatment outcomes in subjects with severe gP comparable to SRP alone. Thus, PRO and MXF showed promising results in the treatment of periodontal disease.

KEYWORDS Propolis, Moxifloxacin, Adjunctive treatment, Periodontitis.

* Assistant Professor of Oral Medicine & Periodontology, Faculty of Dentistry, Mansoura University.
** Lecturer of Oral Medicine & Periodontology, Faculty of Dentistry, Tanta University
*** Professor of Oral Medicine & Periodontology, Faculty of Dentistry, Mansoura University.

INTRODUCTION

Periodontitis is a chronic inflammatory disease which results from an imbalance between periodontopathogenic microorganisms and the host immune defense and is characterized by an irreversible progressive damage of the periodontal tissues.[1] It has been previously exhibited that most of the periodontal tissue destruction is caused by the host response to infection and not directly by the infectious agents.[2] In 2012, a newly established paradigm of periodontal disease etiopathogenesis stated that periodontitis is initiated by a synergistic and dysbiotic variety of microbial pathogens rather than by specific microorganisms, such as the 'red complex'. [3] In the polymicrobial synergy and dysbiotic model, certain microbes; known as keystone pathogens, have the potential to modulate the host response via the impairment of immune surveillance and tipping the ecological balance from homeostasis to dysbiosis. Moreover, keystone periopathogens enhance the virulence of the whole existing microorganisms via interspecies communication with other accessory pathogens.[3]

Efficient host response to the bacterial challenge is mainly mediated by polymorphonuclear leukocytes (PMNs) and characterized by an influx of PMNs into the gingival sulci. The PMNs recruitment and their influx in the vicinity of gingival crevices and periodontal pockets depend on a variety of chemotactic molecules, such as TNF-α, IL-1, IL-6 as well as matrix metalloproteinases (MMPs) which are synthesized and released into the area of inflammation.[4] The primary goal for periodontal therapy is to reduce infection, resolve inflammation, and prevent any further periodontal tissue destruction. The effectiveness of scaling and root planing (SRP) as an essential arm of non-surgical treatment of periodontitis patients has been evidenced through various investigations.[5-7] Thus, meticulous mechanical root debridement has been proved as a key determinant of periodontal therapy success. Nevertheless, various anatomical factors, such as narrow inaccessible furcation areas and deep pockets, have been suggested to reduce the efficacy of non-surgical periodontal therapy.[8] Furthermore, bacterial penetration deeper inside the periodontal tissues or yet in the dentinal tubules impairs the final outcomes of conventional periodontal therapy.[9] Therefore, adjunctive antibiotics can be used to improve the treatment outcomes in chronic periodontal disease.[10] Several antibiotics including amoxicillin, tetracyclines, clindamycin and metronidazole have been studied solely or in combination in periodontal therapy, however, problems of bacterial resistance suggest that alternatives for the currently used antibiotics may be needed.[11-13] Few years ago, moxifloxacin (MXF); which is a new fourth-generation fluoroquinolone antibiotic, has been found to exert excellent antibacterial activity against a wide variety of putative periopathogens, such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans* and Actinomyces species.[14] Its bactericidal activity against biofilm-embedded *P. gingivalis*, *A. actinomycetemcomitans*, and *Streptococcus constellatus* was found to be more potent than clindamycin, metronidazloe, and doxycycline.[15] MXF was found to have greater penetratability inside the soft tissues and potent bactericidal activity against intracellular periodontopathogens.[16] Hence, systemic MXF when used as adjuvant to scaling and root planing (SRP) in periodontitis therapy, yielded significant outcomes in comparison to doxycycline or SRP alone.[14]

Long time ago, propolis (PRO) has been used in folk medicine due to its recognized anti-inflammatory properties, particularly in ancient Egypt and Europe.[17] Propolis is a natural resinous mixture synthesized by honeybees from extracts collected from various plants.[18] Honeybees used propolis in order to protect their hives against

winds, dust and other foreign invaders. PRO has traditionally been used as a therapeutic in several diseases, including disorders of the gastrointestinal tract and mucocutaneous ailments of viral, bacterial and fungal etiologies.[19]

Noteworthy, there are several types of PRO which vary in composition depending on their plant sources based on the geographic zone. PRO contains more than 300 ingredients, such as caffeic acid phenethyl ester (CAPE), caffeic acid , flavonoids, cinnamic acids and their esters.[20] Furthermore, PRO revealed a bunch of different biological activities, such as: 1) antibacterial; 2) antiviral; 3) fungicidal; 4) anti-inflammatory; 5) antioxidant; 6) immunomodulatory; 7) antidiabetic activity and 8) hepatoprotective effects.[21] A recent review has exhibited that CAPE is a crucial bioactive compound present in PRO which is responsible for the majority of its therapeutic activities.[22]

Recently, an increased interest in whole mouth saliva as a diagnostic fluid has become evident. In periodontal disease, whole unstimulated saliva was reported to reflect the soluble mediator composition of the gingival and all mucosal tissues as well as gingival sulcular fluid of patients affected by periodontitis.[23] Potential biomarkers of periodontal disease were previously identified in saliva and shown to be specific for the unique physiologic and pathologic aspects of periodontitis. It was shown that salivary levels of IL-1β appear to serve as reliable biomarker of periodontitis.[24]

The purpose of the present study is to evaluate the impact of adjunctive use of systemic MXF versus systemic PRO in conjunction with scaling and root planing (SRP) compared to SRP alone in the treatment of severe generalized periodontitis. The current investigation focuses on the changes in clinical periodontal parameters and the salivary inflammatory biomarker IL-1β after 6 and 12 weeks of therapy.

MATERIALS AND METHODS

Patient Population

A total of fifty four systemically healthy individuals with severe generalized periodontitis selected from the outpatients coming to the Faculty of Dentistry , Mansoura University were enrolled in the study when they demonstrated interdental clinical attachment loss (CAL) ≥ 5 mm of more than 30% of sites and radiographic evidence of bone loss extending to or beyond the middle third of the root (stage III periodontitis) according to 2017 world workshop classification of periodontal diseases.[25]

Inclusion /Exclusion criteria

The eligible subjects were free from any systemic conditions which could influence the outcome of the treatment and had at least 20 treatable teeth in occlusion. Subjects were excluded if they had the following: 1) allergic to quinolones; 2) had received medication (e.g., antibiotics, corticosteroids, or non-steroidal anti-inflammatory drugs) in the previous 3 months; 3) had received any periodontal therapy within the last year; 4) had any systemic disease; 5) were smokers or 6) were pregnant/ lactating. The study proposal was approved by the Ethics Committee of the Faculty of Dentistry, Mansoura University. All participants signed a written informed consent before conduction of the study.

Study Design

The study was designed to assess the effectiveness of the antibiotic moxifloxacin (MXF) and the natural bee-product propolis (PRO) as adjuncts to mechanical periodontal treatment compared to SRP alone. The participants were randomly assigned into three groups. Group I which includes 18 patients performing SRP alone (SRP group) which represents the control group. Group II in which SRP was performed to 18 patients combined with orally administered MXF 400 mg (Moxacin

tablets; Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt) once daily for 7 days (MXF group). Group III in which SRP was done for 18 patients combined with systemically administered PRO 400 mg (BioPropolis capsule; Sigma Pharmaceutical Industries, Egypt) once daily for 7 days (PRO group). All subjects received instructions for good oral hygiene and given a medium dental brush and the same dentifrice (Colgate Total). Treatment started with chlorhexidine rinsing 0.12% (Hexitol, ADCO, Egypt) for 1 minute. The endpoint of SRP was a tactile smooth and clean root surface. The subjects began taking the antibiotics on the first day of the procedure. Individuals in the control group (SRP) received no drugs. Subjects in the MXF and PRO groups were extensively informed about the intake of the prescribed medication. Adverse effects were assessed and recorded throughout the duration of antibiotics given to the patients.

Patients in the three groups were monitored at baseline, at 6 and 12 weeks after SRP completion. At these time points, the examiner recorded the periodontal parameters and saliva samples were collected as well. All subjects received supportive periodontal treatment and reinforcement of oral hygiene procedures at 2- week intervals for a period of 12 weeks.

Clinical Monitoring

Pocket Depth (PD), Clinical Attachment Loss (CAL), bleeding on Probing (POB), Gingival [26] and Plaque [27] Indices were recorded at baseline, 6 and 12 weeks for all groups. Measurements were taken with a calibrated UNC-15 periodontal probe at 6 standardized sites per each tooth excluding the wisdom teeth from recording. These were the mesiofacial, midfacial, distofacial, mesiolingual, midlingual and distolingual sites. All periodontal measurements were made by the same examiner (MA).

Saliva Sampling

Whole mouth unstimulated saliva samples were collected at baseline and 6 and 12 weeks after therapy for all subjects. Saliva samples (2 ml) were obtained by expectoration into graduated polypropylene tubes before periodontal measurements. Salivary samples were centrifuged to remove debris, and then immediately frozen at -80 ^{o}C till their evaluation time after study completion.

Biochemical Analysis

At the end of the study, the frozen saliva samples of all patients were left to thaw. Each saliva sample (2 mL) was centrifuged at 10,000 x g for 5 minutes; the supernatant was transferred to clean microcap tubes and used immediately for biochemical analysis. Detection of the levels of IL-1β in saliva was determined by enzyme-linked immunosorbent assay (ELISA) (Human IL-1 β Quantikine ELISA kit, R&D Systems, Minneapolis, MN, USA).

Data Analysis

All data were explored firstly by Kolomogrov-Smirnov test of normality. The parametric variables were presented as mean ± SD except salivary IL-1β levels that were expressed as mean ± SE (standard error of the mean). Chi-square and t-tests were used for comparing baseline data. The analysis of variance (ANOVA) of repeated measures with Bonferroni post hoc correction was used to determine significant differences among various time intervals. The significant level of difference was determined at 5%. All analyses were carried out by using SPSS (Statistical Package for the Social Sciences v. 19, Chicago, IL).

RESULTS

Clinical Outcomes

Fifty four patients with generalized severe periodontitis (stage III) were enrolled in the present study and subdivided randomly into three groups equally (n=18); SRP, PRO and MXF groups. After study completion, one patient and two patients dropped-out from SRP and PRO

groups; respectively, because these patients did not complete the study. Thus, the finally analyzed data belonging to each group were as follows: SRP group (n=17), PRO group (n=16) and MXF group (n=18). The demographic distribution and baseline periodontal parameters in the three groups revealed no statistical differences between groups regarding age, age range, male number to female number and number of present teeth per patient ($p > 0.05$) (Table1).

TABLE (1) Demographics of Patient Population and Baseline Periodontal Parameters (mean ± SD)

Parameter	SRP (n=17)	PRO (n= 16)	MXF (n=18)
Age (years)	47.3 ± 5.6	44.5 ± 7.3	45.8 ± 6.1
Age range (years) (min-max)	37-58	39-61	41-59
Male/Female (n)	11/6	9/7	13/5
Teeth (n)	22.4 ± 2.2	23.1 ± 1.9	21.8 ± 2.4
PI	2.2 ± 0.3	2.3 ± 0.2	2.4 ± 0.3
GI	1.7 ± 0.1	1.6 ± 0.3	1.8 ± 0.2
BOP (%)	62 ± 19	64 ± 21	61 ± 17
PD (mm)	4.8 ± 0.9	4.6 ± 0.8	4.7 ± 0.6
CAL (mm)	5.7 ± 0.7	5.8 ± 0.9	5.6 ± 0.8

There were no significant difference between groups (p >0.05).

Table 2 summarizes the full mouth mean (±SD) of Plaque Index (PI), Gingival Index (GI) and Bleeding on Probing (POB) index of all groups at baseline and after 6 and 12 weeks of therapy. The PI showed significant reduction in SRP, PRO and MXF groups at 6 and 12 weeks compared to baseline data. Notably, there was no statistically significant difference among groups at 6 and 12 weeks after treatment ($p > 0.05$).

TABLE (2) Full-Mouth Values (mean ± SD) of Periodontal Indices Before and After Treatment

Index	SRP (n=17)	PRO (n=16)	MXF (n=18)
PI			
baseline	2.2 ± 0.3	2.3 ± 0.2	2.4 ± 0.3
6 W	0.9 ± 0.3*	1.0 ± 0.2*	1.1 ± 0.4*
12 W	0.7 ± 0.2*	0.9 ± 0.3*	0.8 ± 0.3*
GI			
baseline	1.7 ± 0.1	1.6 ± 0.3	1.8 ± 0.2
6 W	0.8 ± 0.2*	0.6 ± 0.2*	0.7 ± 0.3*
12 W	0.7 ± 0.1*	0.5 ± 0.1*	0.6 ± 0.2*
BOP (%)			
baseline	62 ± 19	64 ± 21	60 ± 17
6 W	38 ± 15*	33 ± 14*	39 ± 16*
12 W	38 ± 14*	31 ± 11*	37 ± 13*

**** p < 0.05 compared to baseline (repeated ANOVA with the Holm-Sidak post hoc test between time points).***

The GI records tended to be lower in all groups at 6 and 12 weeks compared to baseline scores. There was also no significant variation among the three groups at 6 and 12 weeks after treatment ($p > 0.05$). BOP scores followed similar pattern with significant differences from baseline compared to 6 and 12- week scores. No statistically significant difference between groups at 6 and 12 weeks after therapy was noted ($p > 0.05$).

Table 3 shows that there was no statistical difference between groups for PD and CAL at baseline ($p > 0.05$). However, at 6 and 12 weeks, the three groups showed significant improvements in PD and CAL over baseline measurements. Importantly, there was no statistical difference between PRO and MXF groups at 6 and 12 weeks ($p > 0.05$). More interestingly, there were significant variations in MXF and PRO groups at 6 and 12 weeks compared to SRP group ($p < 0.01$).

TABLE (3) Full Mouth PD and CAL (mean ± SD) at different time points

	PD (mm)			CAL (mm)		
	baseline	**6 W**	**12 W**	**baseline**	**6 W**	**12 W**
SRP	4.8 ± 0.9	3.8 ± 0.6*	3.7 ± 0.6*	5.7 ± 0.7	4.8 ± 0.6*	4.7 ± 0.4*
PRO	4.6 ± 0.8	3.1 ± 0.5*	3.0 ± 0.6*	5.8 ± 0.9	4. 1 ± 0. 4*	4.0 ± 0.5*
MXF	4.7 ± 0.6	3.4 ± 0.3*	3. 3 ± 0. 4*	5.6 ± 0.8	4.0 ± 0.5*	4.0 ± 0.4*

**** Significant difference when compared to their baseline values; p <0.01.***

It was important to record any adverse effects of both propolis and moxifloxacin when given to the patients included in both PRO and MXF groups during the first week of the study. It was observed that there were no major deviations in using the drugs by the patients and there was no significant difference between the two groups regarding the recorded adverse events (Table 4).

TABLE (4) Adverse Events of the Drugs Used After 1st Week of the Study

Adverse Event	**PRO (n=16)**	**MXF (n=18)**
Headache	2	3
Dizziness	0	0
Nausea	2	1
Constipation	0	1
Diarrhea	1	2
Abdominal pain	0	1
Skin rash	0	0

There is no significant difference between the two groups; p> 0.05.

Figure 1 showed the mean values of pocket depth reduction (PDR) in the three groups at 6 and 12 weeks of therapy. The PDR value in the SRP, PRO and MXF groups were 1± 0.13 mm, 1.3 ± 0.14 mm and 1.5 ± 0.1 mm, respectively after 6 weeks of treatment. At 12 weeks, the PDR values for SRP, PRO and MXF were 1.1 ± 0.12 mm, 1.4 ± 0.12 mm and 1.6 ± 0.11 mm, respectively. There was a modest reduction in pocket depth of PRO group compared to MXF group at 6 and 12 weeks, however, it is not statistically significant. The PRO and MXF values of PDR at 6 and 12 weeks showed significant differences when compared to the corresponding values in SRP group ($p< 0.01$).

Figure 2 showed the mean values of clinical attachment (CAL) gain in the three groups at 6 and 12 weeks after therapy. The CAL gain value in the SRP, PRO and MXF groups were 0.09± 0.11 mm, 1.7 ± 0.15 mm and 1.6 ± 0.12 mm, respectively at 6 weeks of treatment. At 12 weeks, the CAL gain values for SRP, PRO and MXF were 1± 0.14 mm, 1.8 ± 0.14 mm and 1.6 ± 0.13 mm, respectively. There was also no statistically significant difference between PRO and MXF values at 6 and 12 weeks. Both PRO and MXF values of CAL gain at 6 and 12 weeks exhibited significant improvement compared to the corresponding values in SRP group ($p< 0.01$).

Biochemical Outcomes

The salivary IL-1β levels (picograms per milliliter) (mean ± standard error of the mean) of SRP, PRO and MXF groups at various time points are summarized in Figure 3. No significant difference of all groups at baseline was observed. It was statistically noted that significant reductions of salivary IL-1β are present in all groups at 6 and 12 weeks when compared to their baseline levels ($p< 0.01$). More importantly, there was significant decrease of salivary IL-1β levels of PRO and MXF groups at 6 and 12 weeks when compared to their corresponding values of SRP group ($p< 0.01$).

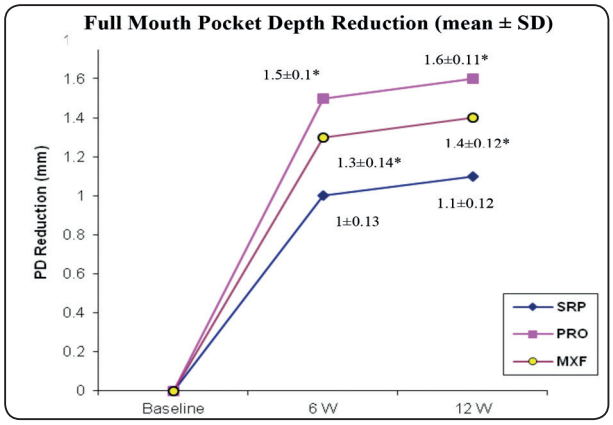

Fig. (1) Both Propolis and moxiflxacin groups showed a statistically significant reduction in PD compared to SRP group at 6 and 12 weeks ; (* $p< 0.01$).

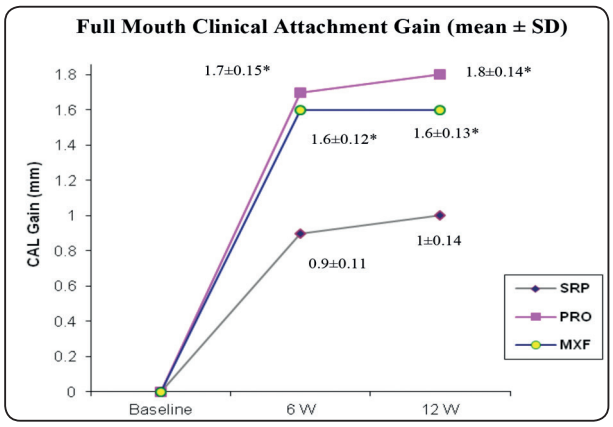

Fig. (2) There are significant differences in the CAL gain between both PRO and MXF groups compared to SRP group at 6 and 12 weeks after therapy; (* $p< 0.01$).

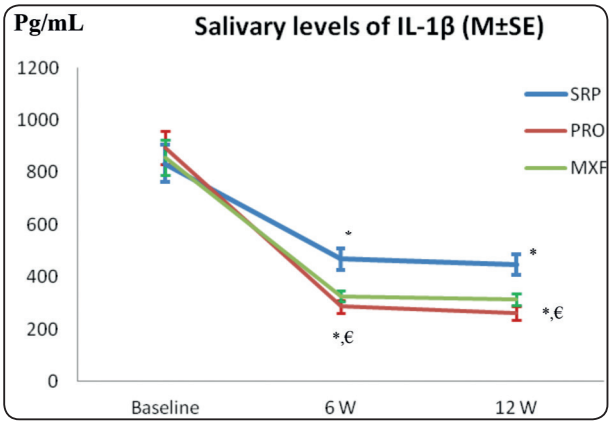

Fig. (3) The mean (± standard error) of IL-1β levels in saliva is significantly reduced in all groups after 6 and 12 weeks compared to baseline levels; (* $p< 0.01$). The same analyte in both PRO and MXF groups is significantly reduced after 6 and 12 weeks compared to SRP group at the same time points ; (€ $P< 0.01$).

DISSCUSION

The present comparative clinical study assessed the effectiveness of the use of moxifloxacin (MXF) and propolis (PRO) as new adjunctive therapeutics to the conventional non-surgical periodontal therapy represented by scaling and root planing (SRP) in generalized severe periodontitis. In general, antibiotics have been used as adjunctive therapy in chronic and aggressive periodontitis patients with inconsistent outcomes.[28,29] It was reported that increasing rates of bacterial resistance to some antibiotics became evident, hence, several warnings regarding their profligate prescription in all cases of periodontal disease were addressed.[30] For decades, the most extensively investigated periodontal antibiotic regimen is the combination of amoxicillin and metronidazole which is prescribed three times for one week, in conjunction with mechanical periodontal therapy.[31-33] However, another investigation demonstrated that mechanical debridement in addition to topical chlorhexidine exhibited the same efficacy as adjunctive amoxicillin/metronidazole after 6 months and also indicated that the early ameliorative effects were short lived.[34] Thus, pharmacologists are still searching

for new promising antimicrobials with least incidence of bacterial resistance and have potent bactericidal effect against most oral pathogens.

Recently, moxifloxacin (MXF) as one of the fourth generation of fluoroquinolones, showed better promising results in several *in vitro* and *in vivo* studies to be used as adjunctive antibiotic to periodontal infections.[35, 36] It was previously reported that MXF is more effective than doxycycline as adjunctive antibiotic in the treatment of severe cases of periodontitis.[14]

On the other hand, the promising properties of propolis, as a naturally produced substance by honeybees, especially the *in vitro* antimicrobial activity [37] have led us to evaluate its efficacy *in vivo* by assessing clinical and biochemical data. Therefore, the current study was tailored to explore and assess the adjunctive effects of systemically administered propolis with those of moxifloxacin, a recently potent well-established antibacterial agent.

Several studies demonstrated that propolis is effective against *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* which are considered as keystone periopathogens for the etiology of periodontal diseases.[38, 39] In our study, we evaluated the systemic effect of MXF and PRO as adjunctive remedies in the treatment of generalized severe periodontitis and assess their effect clinically and biochemically via one reliable salivary biomarker; IL-1β. We have proved in the present study that PRO and MXF are superior to SRP alone in reducing pocket depth and increasing clinical attachment gain. This obtained improvement was supported biochemically by the obvious reduction of salivary IL-1β after 6 and 12 weeks of therapy. The appreciated effects of both PRO and MXF as adjuvant to SRP in the treatment of generalized severe periodontitis compared to SRP alone revealed in our findings are consistent with the results of other studies.[14, 40-42]

In our study, MXF showed significant changes in PD reduction and CAL gain when compared to SRP. There was an obvious reduction of salivary inflammatory biomarkers represented by IL-1β in MXF group compared to SRP group. Our findings come in agreement with a recent investigation.[14] This can be explained due to the greater activity of MXF against *P. gingivalis* and *A. actinomycetemcomitans* strains and unlikelihood of MXF to the development of resistant strains of these periodontopathogens.[43] In another Colombian study, superinfecting enteric were isolated in significant amounts from chronic periodontitis patients and it was found that these enteric rods and the putative periodontopathogens exhibited high susceptibility to MXF and showed variable susceptibility to amoxicillin/calvulanic acid.[15]

Previous studies reported that the antibacterial effect of propolis is attributed to a synergism of several compounds rather than a single component because propolis is a natural mixture of a myriad of different molecules which possess a plethora of biologic properties.[44] The antibacterial effect of propolis involves several mechanisms such as; inhibition of protein synthesis, partial bacteriolysis, and disorganization of the cytoplasm, the cytoplasmic membrane, and the cell wall.[45]

In our study, it was also noticed that MXF does not show any significant variation in comparison to PRO regarding the adverse events observed in the studied populations during the period of drug intake. This shows that MXF is similar to PRO in patient tolerability.

The adopted propolis dosage prescribed in the current study (400 mg/day) seems to be safe and effective adjunctive therapeutic modality to SRP. As anticipated, our findings revealed that SRP alone was effective in reducing POB, pocket depth (PD), and clinical attachment level (CAL) gain which are in accordance with previous investigations.[46-48] The PRO group showed greater PD reduction and more CAL gain when compared to the SRP group. The plausible biologic rationale for the expected anti-

inflammatory effects of propolis might result from the integration of the appreciated antibacterial, anti-inflammatory and antioxidant actions of propolis. Importantly, studies of topical propolis application as an adjunctive oral hygiene measure gave similar outcomes.[49-52]

The limitations of our study included the small sample size represented by the relatively small number of patients enrolled in the study. Thus, it is recommended to conduct future large scale studies of PRO and MXF in periodontitis patients. Second, the follow –up period for 12 weeks is short to assess the long-term effects of the used drugs. Moreover, the authors did not assess the microbiological aspects of periodontitis patients and correlating them with the clinical and biochemical findings. Another limitation of the present study is the lack of confirmation of its biochemical findings by collection of GCF samples as it reflects the condition of the local periodontal environment more accurately than saliva. In total, we concluded that propolis and moxifloxacin might be used as alternative adjunctive therapeutics and showed excellent improvements in severe generalized periodontitis patients over the use of SRP alone. It is also recommended to use them in other periodontal conditions like periodontal abscesses and adjuvant to surgical periodontal treatments.

REFERENCES

1. Novak MJ, Dawson DR, 3rd, Magnusson I, et al. Combining host modulation and topical antimicrobial therapy in the management of moderate to severe periodontitis: a randomized multicenter trial. Journal of periodontology 2008;79:33-41.
2. Garlet GP. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. Journal of dental research 2010;89:1349-1363.
3. Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Molecular oral microbiology 2012;27:409-419.
4. Kantarci A, Van Dyke TE. Lipoxin signaling in neutrophils and their role in periodontal disease. Prostaglandins, leukotrienes, and essential fatty acids 2005;73:289-299.
5. Alshehri M, Alshehri FA, Alshail F. Effect of scaling and root planing with and without adjunct use of an essential-oil-based mouthwash on whole salivary interleukin-1beta levels in patients with periodontal disease: A short-term follow-up study. Interventional medicine & applied science 2016;8:3-6.
6. Javed F, Salehpoor D, Al-Dhafeeri T, et al. Is adjunctive photodynamic therapy more effective than scaling and root planing alone in the treatment of periodontal disease in hyperglycemic patients? A systematic review. Photodiagnosis and photodynamic therapy 2018;22:1-6.
7. Soskolne WA. Re: Impact of local adjuncts to scaling and root planing in periodontal disease therapy: a systematic review. Bonito AJ, Lux L, Lohr KN (2005;76:1227-1236). Journal of periodontology 2006;77:323; author reply 323-324.
8. Matthews D. Conclusive support for mechanical nonsurgical pocket therapy in the treatment of periodontal disease. How effective is mechanical nonsurgical pocket therapy? Evidence-based dentistry 2005;6:68-69.
9. Pradeep AR, Raj S, Aruna G, Chowdhry S. Gingival crevicular fluid and plasma levels of neuropeptide Substance-P in periodontal health, disease and after nonsurgical therapy. Journal of periodontal research 2009; 44:232-237.
10. Bidault P, Chandad F, Grenier D. Systemic antibiotic therapy in the treatment of periodontitis. Journal 2007;73:515-520.
11. Veloo AC, Seme K, Raangs E, et al. Antibiotic susceptibility profiles of oral pathogens. Int J Antimicrob Agents;40:450-454.
12. Handal T, Caugant DA, Olsen I. Antibiotic resistance in bacteria isolated from subgingival plaque in a norwegian population with refractory marginal periodontitis. Antimicrobial agents and chemotherapy 2003; 47:1443-1446.
13. Veloo AC, Seme K, Raangs E, et al. Antibiotic susceptibility profiles of oral pathogens. International journal of antimicrobial agents 2012;40:450-454.
14. Guentsch A, Jentsch H, Pfister W, Hoffmann T, Eick S. Moxifloxacin as an adjunctive antibiotic in the treatment of severe chronic periodontitis. Journal of periodontology 2008;79:1894-1903.

15. Ardila CM, Fernandez N, Guzman IC. Antimicrobial susceptibility of moxifloxacin against gram-negative enteric rods from colombian patients with chronic periodontitis. Journal of periodontology 2010;81:292-299.
16. Ardila CM, Guzman IC. Clinical Factors Influencing the Efficacy of Systemic Moxifloxacin in the Therapy of Patients With Generalized Aggressive Periodontitis: A Multilevel Analysis From a Clinical Trial. Global journal of health science 2015;8:80-88.
17. Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 1998;36:347-363.
18. Bankova V, Popova M, Trusheva B. Propolis volatile compounds: chemical diversity and biological activity: a review. Chemistry Central journal 2014;8:28.
19. Sung SH, Choi GH, Lee NW, Shin BC. External Use of Propolis for Oral, Skin, and Genital Diseases: A Systematic Review and Meta-Analysis. Evidence-based complementary and alternative medicine : eCAM 2017;2017:8025752.
20. Velikova M, Bankova V, Sorkun K, Houcine S, Tsvetkova I, Kujumgiev A. Propolis from the Mediterranean region: chemical composition and antimicrobial activity. Zeitschrift fur Naturforschung C, Journal of biosciences 2000;55:790-793.
21. de Groot AC. Propolis: a review of properties, applications, chemical composition, contact allergy, and other adverse effects. Dermatitis : contact, atopic, occupational, drug 2013;24:263-282.
22. Tolba MF, Azab SS, Khalifa AE, Abdel-Rahman SZ, Abdel-Naim AB. Caffeic acid phenethyl ester, a promising component of propolis with a plethora of biological activities: a review on its anti-inflammatory, neuroprotective, hepatoprotective, and cardioprotective effects. IUBMB life 2013;65:699-709.
23. Wu Y, Shu R, Liu H. [Comparison of proteomic profiles of whole unstimulated saliva obtained from generalized aggressive periodontitis patients and healthy controls]. Hua xi kou qiang yi xue za zhi = Huaxi kouqiang yixue zazhi = West China journal of stomatology 2011;29:519-521, 525.
24. Miller CS, King CP, Jr., Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: a cross-sectional study. Journal of the American Dental Association 2006;137:322-329.
25. Chapple ILC, Mealey BL, Van Dyke TE, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of periodontology 2018;89 Suppl 1:S74-S84.
26. Loe H, Silness J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. Acta odontologica Scandinavica 1963;21:533-551.
27. Silness J, Loe H. Periodontal Disease in Pregnancy. Ii. Correlation between Oral Hygiene and Periodontal Condtion. Acta odontologica Scandinavica 1964;22:121-135.
28. Smiley CJ, Tracy SL, Abt E, et al. Systematic review and meta-analysis on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. Journal of the American Dental Association 2015;146:508-524 e505.
29. Herrera D, Sanz M, Jepsen S, Needleman I, Roldan S. A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients. Journal of clinical periodontology 2002;29 Suppl 3:136-159; discussion 160-132.
30. Bonine NG, Berger A, Altincatal A, et al. Impact of Delayed Appropriate Antibiotic Therapy on Patient Outcomes by Antibiotic Resistance Status From Serious Gram-negative Bacterial Infections. The American journal of the medical sciences 2019;357:103-110.
31. Winkel EG, Van Winkelhoff AJ, Timmerman MF, Van der Velden U, Van der Weijden GA. Amoxicillin plus metronidazole in the treatment of adult periodontitis patients. A double-blind placebo-controlled study. Journal of clinical periodontology 2001;28:296-305.
32. Cionca N, Giannopoulou C, Ugolotti G, Mombelli A. Amoxicillin and metronidazole as an adjunct to full-mouth scaling and root planing of chronic periodontitis. Journal of periodontology 2009;80:364-371.
33. Duarte PM, Feres M, Yassine LLS, et al. Clinical and microbiological effects of scaling and root planing, metronidazole and amoxicillin in the treatment of diabetic and non-diabetic subjects with periodontitis: A cohort study. Journal of clinical periodontology 2018;45:1326-1335.
34. Kaner D, Bernimoulin JP, Hopfenmuller W, Kleber BM, Friedmann A. Controlled-delivery chlorhexidine chip versus amoxicillin/metronidazole as adjunctive

antimicrobial therapy for generalized aggressive periodontitis: a randomized controlled clinical trial. Journal of clinical periodontology 2007;34:880-891.

35. Tomas I, Alvarez M, Limeres J, et al. In vitro activity of moxifloxacin compared to other antimicrobials against streptococci isolated from iatrogenic oral bacteremia in Spain. Oral microbiology and immunology 2004;19:331-335.
36. Huelves L, Sevillano D, Martinez-Marin C, et al. Correlation between in vitro and in vivo activity of levofloxacin and moxifloxacin against pneumococcal strains with different susceptibilities to fluoroquinolones. International journal of antimicrobial agents 2006;27:294-299.
37. Stepanovic S, Antic N, Dakic I, Svabic-Vlahovic M. In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbiological research 2003;158:353-357.
38. Santos FA, Bastos EM, Rodrigues PH, et al. Susceptibility of Prevotella intermedia/Prevotella nigrescens (and Porphyromonas gingivalis) to propolis (bee glue) and other antimicrobial agents. Anaerobe 2002;8:9-15.
39. Agarwal G, Vemanaradhya GG, Mehta DS. Evaluation of chemical composition and efficacy of Chinese propolis extract on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An in vitro study. Contemporary clinical dentistry 2012;3:256-261.
40. El-Sharkawy HM, Anees MM, Van Dyke TE. Propolis Improves Periodontal Status and Glycemic Control in Patients With Type 2 Diabetes Mellitus and Chronic Periodontitis: A Randomized Clinical Trial. Journal of periodontology 2016;87:1418-1426.
41. Sanghani NN, Bm S, S S. Health from the hive: propolis as an adjuvant in the treatment of chronic periodontitis - a clinicomicrobiologic study. Journal of clinical and diagnostic research : JCDR 2014;8:ZC41-44.
42. Flemmig TF, Petersilka G, Volp A, et al. Efficacy and safety of adjunctive local moxifloxacin delivery in the treatment of periodontitis. Journal of periodontology 2011;82:96-105.
43. Tsaousoglou P, Nietzsche S, Cachovan G, Sculean A, Eick S. Antibacterial activity of moxifloxacin on bacteria associated with periodontitis within a biofilm. Journal of medical microbiology 2014;63:284-292.
44. Nina N, Quispe C, Jimenez-Aspee F, et al. Antibacterial Activity, Antioxidant Effect and Chemical Composition of Propolis from the Region del Maule, Central Chile. Molecules 2015;20:18144-18167.
45. Inui S, Hatano A, Yoshino M, et al. Identification of the phenolic compounds contributing to antibacterial activity in ethanol extracts of Brazilian red propolis. Natural product research 2014;28:1293-1296.
46. Griffiths GS, Smart GJ, Bulman JS, Weiss G, Shrowder J, Newman HN. Comparison of clinical outcomes following treatment of chronic adult periodontitis with subgingival scaling or subgingival scaling plus metronidazole gel. Journal of clinical periodontology 2000;27:910-917.
47. Acharya AB, Thakur S, Muddapur MV. Effect of scaling and root planing on serum interleukin-10 levels and glycemic control in chronic periodontitis and type 2 diabetes mellitus. Journal of Indian Society of Periodontology 2015;19:188-193.
48. Brignardello-Petersen R. No benefits important to patients from the use of chlorhexidine rinse as an adjunct to scaling and root planing in patients with chronic periodontitis. Journal of the American Dental Association 2017; 148:e172.
49. Pereira EM, da Silva JL, Silva FF, et al. Clinical Evidence of the Efficacy of a Mouthwash Containing Propolis for the Control of Plaque and Gingivitis: A Phase II Study. Evidence-based complementary and alternative medicine: eCAM 2011;2011:750249.
50. Anauate-Netto C, Anido-Anido A, Leegoy HR, et al. Randomized, double-blind, placebo-controlled clinical trial on the effects of propolis and chlorhexidine mouthrinses on gingivitis. Brazilian dental science 2014;17:11-15.
51. Aral CA, Kesim S, Greenwell H, Kara M, Cetin A, Yakan B. Alveolar bone protective and hypoglycemic effects of systemic propolis treatment in experimental periodontitis and diabetes mellitus. Journal of medicinal food 2015;18:195-201.
52. Toker H, Ozan F, Ozer H, Ozdemir H, Eren K, Yeler H. A morphometric and histopathologic evaluation of the effects of propolis on alveolar bone loss in experimental periodontitis in rats. Journal of periodontology 2008;79:1089-1094.