The Impact of Natural Propolis on a Film Forming System of Chitosan in Non-Surgical Treatment of Periodontitis (Randomized Clinical and Biochemical Trial)

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Abstract

Aim: This study aimed to evaluate the effectiveness of natural propolis incorporated into a chitosan film-forming system as an adjunct to non-surgical periodontal therapy. **Patients and methods:** In this prospective, randomized controlled clinical trial, 45 patients with stage II periodontitis were allocated into three equal groups: Group I received SRP alone, Group II received SRP plus chitosan film-forming gel, and Group III received SRP plus propolis-loaded chitosan film-forming gel. **Results:** All treatment groups demonstrated significant improvements in clinical parameters and significant reductions in MPO levels throughout the study period ($p < 0.001$). At 3 months, Group III showed the greatest reductions in PI, GI, PPD, CAL, and MPO levels compared with Groups I and II. Group II also demonstrated superior outcomes to SRP alone, indicating the beneficial effect of chitosan as a local drug delivery system. **Conclusion:** Non-surgical periodontal therapy remains the cornerstone of stage II periodontitis management. However, adjunctive intra-pocket application of propolis incorporated into a chitosan film-forming system significantly enhanced clinical and biochemical outcomes compared with SRP alone or chitosan gel alone, suggesting a promising therapeutic approach for improving periodontal treatment success.

Keywords: Stage II periodontitis; Propolis; Chitosan film-forming gel; Myeloperoxidase (MPO).

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Introduction

Periodontitis is a chronic multifactorial inflammatory disease associated with plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus, loss of periodontal tissue support, clinical attachment loss (CAL), radiographically assessed alveolar bone loss, presence of periodontal pocketing and gingival bleeding. It may lead to tooth loss and disability, affect chewing function and aesthetics **[1]**.

According to 2017 new classification for periodontitis, Stage II represents periodontitis in which clinical periodontal examination identifies the characteristic damage that periodontitis has caused to tooth support. CAL is between 3-4 mm; the radiographic bone loss found in the coronal third 15% to 33% mostly horizontal bone loss with maximum 5 mm probing depth **[2]**.

The presence of inflammatory infiltrate and increase in the oxidative response of peripheral polymorphonuclear cells are characteristics of periodontitis, as the disease advances, there is greater damage because of the increase in the number of reactive oxygen substances (ROS). The deterioration caused by free radicals is regulated by an antioxidant defense system. Myeloperoxidase (MPO) is one of the body enzymes that produces ROS. It catalyzes the formation of hypochlorous acid and plays important roles in host defense mechanism **[3]**.

Chitosan is a hydrophilic biopolymer obtained by alkaline deacetylation of chitin, a major component of arthropod shells, has favorable properties such as nontoxicity, biocompatibility, and biodegradability.

Chitosan itself possesses antimicrobial activity and wound healing, hemostatic, and tissue regeneration properties. Chitosan is extensively used in biomedical applications as a carrier for drug encapsulation and controlled release as it stays for extended periods in the oral cavity and has adequate drug penetration **[4]**.
Propolis is a non-toxic resinous substance produced by bees that has anti-inflammatory, antibacterial, antifungal, antioxidant, and immunomodulating properties. Its main antioxidant components are flavonoids and phenolic esters. It's very useful to treat candidiasis, aphthous ulcers, gingivitis, and periodontitis. It also has an inhibitory effect on myeloperoxidase enzyme **[5]**.

Patients and methods

This prospective, randomized, and controlled clinical and biochemical study was conducted on 45 patients of both sexes, ranging in age from (25 to 44) years, diagnosed as stage II periodontitis. All patients were selected from those attending the outpatient clinic, Oral Medicine and Periodontology Department, Faculty of Dental Medicine, Al-Azhar University, Assiut Branch. **Patients were divided to: Group 1:** included 15 patients with Stage II periodontitis; patients received only scaling and root planing, **group 2:** included 15 patients with Stage II periodontitis, who received scaling and root planing followed by topical application of chitosan film-forming gel and **group 3:** included 15 patients with Stage II periodontitis, who received scaling and root planing followed by topical application of propolis on film-forming gel of chitosan.

Ethical consideration

The study protocol was approved by the Ethical Committee of the Faculty of Dental Medicine, Al-Azhar University. (Number: AUAREC202300007-02). All patients were fully informed about the nature and the possible risks of the study procedures; they signed the consent form before the work.

Inclusion criteria

All patients were free from any systemic conditions that may affect periodontal status, as determined according to the Cornell Medical Index and its modified criteria **[6]**, Patients with stage II periodontitis; CAL 3–4 mm, with no tooth loss, and PD ≤ 5 mm **[2]**, Patients who have teeth with both mesial and distal neighbouring teeth **[7]**, Patient with more than 20 natural teeth, female patients were non-pregnant or lactating, patients with a history of traumatic occlusion were excluded and teeth with both endo-perio lesions were excluded.

Sample size calculation and power analysis

Based on previous study [10] and Using G power statistical power analysis program (version 3.1.9.4) for sample size determination, A total sample size (n=13) was sufficient for each group in the study to detect a large effect size (f)= 1.342 between two groups, with an actual power (1-β error) of 0.9 (90%) and a significance level (α error) 0.05 (5%) for two-tailed hypothesis test. The sample size was increased by 20% to compensate for possible patient drop-out, giving a total n=48 (16 for each group).

Preparation of propolis on chitosan film-forming gel

The film-forming propolis-loaded chitosan hydrogel was prepared in the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Al-Azhar University, Assiut Branch.

Intra-pocket application of chitosan film-forming gel and 1% propolis on film-forming gel of chitosan

The application site was isolated with cotton rolls, and the propolis-loaded gel was delivered into the periodontal pocket.

Clinical evaluation:

Periodontal status was assessed at baseline, 1 month, and 3 months after treatment using the following clinical parameters: Plaque Index (PI) **[8]**, Gingival Index (GI) **[9]**, Probing Depth (PD) **[10]**, and Clinical Attachment Level (CAL**) [11]**.

Biochemical Evaluation

Gingival crevicular fluid (GCF) samples were collected and subsequently analyzed for myeloperoxidase levels using enzyme-linked immunosorbent assay (ELISA) at baseline, 1 month, and 3 months in all three study groups.

Statistical analysis

The collected data were analyzed using a one-way ANOVA for parametric data and the Kruskal–Wallis test for non-parametric data, as appropriate. A p-value of 0.05 or less was considered statistically significant.

Results

Table (1): Intragroup comparison of PPD across the studied groups at different intervals.

Interval	Mean ± SD	Min	Max	P value	SIG
Group (1)					
Baseline	4.50 ± 0.20	4.06	4.70		
1m	3.58 ± 0.28	3.10	4.00	**<0.001****	**HS**
3m	3.31 ± 0.19	2.97	3.60	**<0.001****	**HS**
Group (2)					
Baseline	4.68 ± 0.17	4.40	5.00		

1m	3.23 ±0.45	2.68	4.10	**<0.001****	**HS**
3m	3.05 ±0.42	2.54	3.90	**<0.001****	**HS**
Group (3)					
Baseline	4.64 ±0.18	4.32	4.90		
1m	3.04 ±0.45	2.30	3.70	**<0.001****	**HS**
3m	2.36 ±0.26	1.90	2.80	**<0.001****	**HS**

Data are presented as mean ± SD (min–max). P values represent comparisons across follow-up intervals within each group. SIG indicates the level of statistical significance: NS = non-significant ($p \geq 0.05$), S = significant ($p < 0.05$), and HS = highly significant ($p < 0.01$).

Table (2): PPD across groups at baseline, 1 month, and 3 months (intergroup comparisons).

Interval	Groups (N=45)	Mean ± SD	P value	SIG
Probing pocket depth at baseline	Group (1)	4.50 ±0.20	0.059	**NS**
	Group (2)	4.68 ±0.17		
	Group (3)	4.64 ±0.18		
Probing pocket depth after 1 month	Group (1)	3.58 ±0.28	0.004*	**S**
	Group (2)	3.23 ±0.45		
	Group (3)	3.04 ±0.45		
Probing pocket depth after 3 months	Group (1)	3.31 ±0.19	<0.001**	**HS**
	Group (2)	3.05 ±0.42		
	Group (3)	**2.36 ±0.26**		

Note: n = 15/group. Group (1): SRP; Group (2): SRP + chitosan film-forming system; Group (3): SRP + propolis on chitosan film-forming system. P values represent between-group comparisons at each interval (baseline/1 month/3 months: Kruskal–Wallis). SIG: NS = non-significant ($p \geq 0.05$); S = significant ($p < 0.05$); HS = highly significant ($p < 0.01$).

Table (3): Intragroup comparison of CAL across the studied groups at different intervals.

Interval	Mean ± SD	Min	Max	P value	SIG
Group (1)					
Baseline	3.54 ±0.23	3.20	4.00		
1m	2.95 ±0.13	2.70	3.20	**<0.001****	**HS**
3m	2.57 ±0.28	2.10	3.07	**<0.001****	**HS**
Group (2)					
Baseline	3.68 ±0.20	3.30	4.00		
1m	2.86 ±0.33	2.30	3.30	**<0.001****	**HS**
3m	2.45 ±0.33	1.90	2.90	**<0.001****	**HS**
Group (3)					
Baseline	3.69 ±0.18	3.37	4.00		
1m	2.41 ±0.15	2.17	2.70	**<0.001****	**HS**
3m	2.21 ±0.21	1.90	2.65	**<0.001****	**HS**

Data are presented as mean ± SD (min–max). P values represent comparisons across follow-up intervals within each group. SIG indicates the level of statistical significance: NS = non-significant ($p \geq 0.05$), S = significant ($p < 0.05$), and HS = highly significant ($p < 0.01$).

Table (4): Intragroup comparison of MPO across the studied groups at different intervals.

Interval	Mean ± SD	Min	Max	P value	SIG
Group (1)					
Baseline	81.69 ±5.99	71.96	90.46		
1m	73.00 ±8.72	60.37	85.33	**<0.001****	**HS**
3m	69.97 ±7.39	59.90	81.56	**<0.001****	**HS**
Group (2)					
Baseline	79.55 ±8.35	66.24	90.85		
1m	69.42 ±8.87	55.43	79.47	**<0.001****	**HS**
3m	62.52 ±9.70	44.43	71.20	**<0.001****	**HS**
Group (3)					
Baseline	83.47 ±4.76	76.01	90.95		
1m	69.77 ±3.57	63.02	74.96	**<0.001****	**HS**

3m	56.42 ±4.54	50.74	63.73	**<0.001****	**HS**

Data are presented as mean ± SD (min–max). P values represent comparisons across follow-up intervals within each group. SIG indicates the level of statistical significance: NS = non-significant ($p \geq 0.05$), S = significant ($p < 0.05$), and HS = highly significant ($p < 0.01$). (Table 8)

Discussion

In the present research, stage II periodontitis patients were selected to accommodate the criteria of this stage, which can be treated by non-surgical approach according to the study of Ambili et al **[12]**.

With reference to the age of patients participated in the present study, it ranged from (25-44) years old, with a mean age of (36) because the prevalence of periodontitis in adults (73%), and adolescents (59%), as reported by Lijan et al **[13]**.

In the present study, all patients were free from any systemic disease to ensure that systemic risk factors were excluded from the results of the research because of the interrelationship between periodontal disease and systemic health **[14]**.

Chitosan was used in the present research for its distinctive physicochemical properties, biodegradability, biocompatibility, and antimicrobial activity, which made it an effective carrier for the drug (propolis) by enabling sustained drug release, as mentioned by Olivia et al **[15]**.

In the present study, patients were instructed to restrict eating, spitting, and drinking for 1 hour after application of film-forming gel. Teeth brushing and flossing were restricted for 4 hours after application to ensure drug delivery of the ingredients of the gel to all cells of the sulcus, following the instructions given by Hakan et al **[16]**.

Regarding both the plaque index and the gingival index, in all studied groups, the study showed a significant reduction at both 1 and 3 months compared with baseline. This reduction reflects the oral hygiene measures in the study groups and indicates effective plaque control, which leads to improved clinical parameters and reduces the signs of inflammation **[17]**.

The utilization of local drug delivery as an adjunct to scaling and root planing enables the sustained release of the drug in short doses over an extended period, eliminating the need for repetitive dosing and decreasing the probing depth as well as subgingival microflora and clinical signs of inflammation, as reported by McFall et al **[18]**.

Intra-pocket application of chitosan gel is helpful in unresponsive patients to conventional periodontal treatment. In addition, chitosan has the ability to hinder bacterial colonization on the tooth surface, as recorded by the study of Schwach-Abdellaoui et al **[19]**.

The results of probing pocket depth measurements in the current research demonstrated a significant reduction of pocket depth in group III, followed by group II, as compared to group I. These outcomes were in agreement with the study of Chun et al **[20].** This discrepancy between the examined groups could be attributed to the application of propolis on the film-forming system of chitosan, which appeared better in group III as compared to other groups.

The results of the present study showed a highly significant reduction in MPO in all examined groups, especially at the period of 3 months compared to the baseline, while group III showed the lowest level of this enzyme (56.42) compared to group II (62.52) and in group I the level was (69.97). Some investigators attributed the increased level of MPO in periodontitis patients before treatment could be due to neutrophil degranulation, which indicates the hyperactive state of these cells due to antigenic stimulation **[21]**.

Yamulik et al **[22]** reported that MPO activity was lower in healthy versus diseased periodontal sites; similar results were obtained in this study.

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