IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 19, Issue 11 Ser.4 (November. 2020), PP 34-39
www.iosrjournals.org

Efficacy of Hyaluronic Acid and Propolis as an Adjuvant Pocket Therapy: A Clinical and MicrobiologicalStudy.

Heba A. Shawky*

Associate Professor of Periodontics, Department of Preventive Dental Sciences, College of Dentistry, Princess Nourah Bint Abdulrahman University, Saudi Arabia.

Abstract:
Background: *Periodontitis is a complex inflammatory disease caused by the interaction between virulent bacteria and host immune response.Although mechanical debridement is considered the gold standard for periodontitis treatment, adjuvant therapy was introduced to inhibit the bacteria in deeper sites and to suppress the inflammatory mediators released in response to the periodontal pathogens. Local delivery drugsespecially those of natural origin were proved effective adjuvant therapy with minimal side effects.*
Aim of the study:*The present study was conducted to evaluate the efficacy of Hyaluronic acid and Propolis as adjuvant therapeutic agents in patients with periodontal pockets.*
Materials and Methods: *A total of 20 patients were enrolled in the present study. From each patient, threepockets were identified with a total of 60 pockets. The selected pockets received SRP and then were randomly allocated to three groups, each group comprises 20 pockets. The first treated group received Hyaluronic acid intra-pocket injections, the second treated group received Propolis, and the third group, Control received placebo.Clinical parameters included PBI, GI, PD and CAL, and were accessed at the baseline, after 1 and 3 months. Microbiological samples of GCF were obtained at baseline and after 3 months.*
Results: *Both treatment groups, Propolis and Hyaluronic acid revealed significant improvement concerning the clinical parameters as well as the bacterial load. These changes were significantly different from that of the control group. However, no significant difference existed between the two treatment groups.*
Conclusion:*Propolis and Hyaluronic acid are promising adjuvant therapeutic agents for periodontal pockets.*
Keywords:*Antimicrobial, clinical attachment loss, Hyaluronic acid, Propolis, pockets.*

Date of Submission: 31-10-2020 Date of Acceptance: 12-11-2020

I. Introduction

Periodontitis is a complex inflammatory disease caused by the interaction between virulent bacteria and host immune response. It destroys tooth-supporting structures manifested by periodontal pocket formation, gingival recession, and bone resorption that may lead to tooth loss.[1] Periodontitis therapy involves a variety of treatment intrusions such as oral hygiene measurements, dietary control, and mechanical debridement by scaling and root planing (SRP) in addition to appropriate plaque control methods. In advanced cases, surgical therapy is advocated.[2]

Although mechanical debridement is considered the gold standard for periodontitis treatment, bacterial recolonization is considered as a constraint for this approach. Adjuvant therapy has been introduced not only to inhibit the bacteria in deeper sites, which cannot be reached during SRP procedures but also to suppress the inflammatory mediators released in response to the periodontal pathogens.[3] Antibiotics such as tetracycline and doxycycline have been widely and efficiently used especially with cases of aggressive periodontitis. However, various side-effects as hypersensitivity, gastrointestinal problems, and bacterial resistance may occur associated with the systemic use of antibiotics.[4]To overcome these side-effects local delivery agents have been utilized. Local delivery drugs show higher concentration in the gingival crevicular fluid compared to systemic therapy. Examples of locally delivered antibiotics are tetracycline fibers, metronidazole gel, sustained-release doxycycline, and minocycline.[5]

In addition to antimicrobials, other drugs wereutilized to control the inflammatory reaction and to potentiate healing procedure. Hyaluronic acid was introduced as a local chemotherapeutic agent owing to its clinical therapeutic properties including control of inflammation and promotion of wound healing.[6] Hyaluronic acid (HA) is a naturally occurring linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, and other tissues. It retains numerous physiological and structural functions, which aid in keeping the structural and homeostatic integrity of the tissue.[7]

On the other hand, Natural medications were proved to be effective and safe alternatives to synthetic drugs.[8] These medications include different herbs as Turmeric (Curcuma longa),

Ginseng,[9]Açaí,Ginger,[10] Miswak, Green tea,[11]Cinnamomum zeylanicum, Aloe vera, Azadirachta indica, Coptidis rhizome, and Propolis.[12] Propolis (bee glue) is an adhesive a resin-like material produced by bees from the buds of cone-bearing and poplar trees. Upon collection, Propolis encompasses abundant salivary and enzymatic secretions, which are used by the bees to protect their hives and are considered a potent chemical weapon to protect the bees against any attacking microorganisms.[13]Propolis as a natural product can be a suitable alternative to locally delivered antimicrobials. It has a complex chemical composition. Phenolic compounds, terpenes, alcohols, and aromatic acids were identified in Propolis.[14] Favorable relation existed between terpenes and phenolic compounds with antibacterial activity.[15]

The current study was performed to evaluate the efficacy of hyaluronic acid and Propolis as adjuvant therapeutic agents in cases with periodontal pockets.

II. Materials And Methods

Study Design

A randomized placebo-controlled clinical trial was conductedto compare the clinical and microbiologicaloutcomes of local application of hyaluronic acid and Propolis. The study was approved in 2018 by the Ethics Review Board of Facultyof Dentistry, Pharos University in Alexandria, Egypt.

Patient selection:

A total of 20 patients were enrolled in the present study. The patients were selected from the outpatient clinic of the Faculty of Dentistry, Pharos University.

Inclusion criteria:

1. Male patients of age ranging from 35 to 60 years old
2. Good general health
3. Presence of ≥25 teeth
4. Patients were diagnosed with mild to moderate chronic periodontitis (Stage I or II Periodontitis)
5. Patients have at least three non-adjacent periodontal pockets with 4 to 6 mm pocket depth and clinical attachment loss ranging from 1 to 4 mm.

Exclusion criteria:

1. Patients suffering from systemic diseases as diabetes, hyperthyroidism, or cardiovascular disorders.[16]
2. Smokers
3. Patients who were taking long term anti-inflammatory drugs or received antibiotics in the last 3 months.
4. Patients who were subjected to periodontal treatment or periodontal surgery during the previous 6 months.

The study outline was explained to the selected patients and written consent was obtained from each participant.

Clinical Parameters:

For each patient, three pockets were identified for the study with a total of 60 pockets. Acrylic stents were fabricated for each participant to standardize the measurements of probing depth and clinical attachment loss. The followingclinical parameters were accessed at the baseline, after 1 and 3 months: 1) Papillary Bleeding Index (PBI)[17] 2) Gingival Index (GI)[18] 3) Probing Depth (PD)[19] 4) Clinical attachment loss (CAL).[19]

Non-Surgical Treatment:

All participants received full-mouth SRP. The selected pockets were randomly allocated to three study groups, each group comprises 20 pockets. Hyaluronic acid group, Propolis group, and Control group.

- Hyaluronic group: A 23-gauge needle was usedto inject1 mL of 0.2% of hyaluronic gel[*]to the depth of the pocket once a week for foursuccessive weeks.[20]
- Propolis group: Treated by irrigation with3 ml of an aqueous solution of Propolis[†]once a week forfour successive weeks.[21]
- Control group: Placebo of 14% ethanol solution was prepared by mixing 14 ml ethyl alcohol in 100 of distilled water.[22]The pockets of the control group were irrigated with 3ml of placebo once a week for four weeks.

* HYALGAN® (Sodium Hyaluronate), Fidia Pharma, USA Inc.
† Bio-Propolis, Sigma Pharmaceutical Industries (S.P.I.)

Microbiological Sampling:

Microbiological samples of gingival crevicular fluid (GCF) were obtained by inserting sterile paper points into the pockets till resistance is felt and is left in situ for 30 seconds.[23] The paper points were then transmitted to capped test tubes, which contain brain heart infusion broth.‡ The test tubes were kept under anaerobic conditions for 4 hours at 37^0C, the tubes were then shaken by vortexing to mix the obtained GCF with the broth. Consequently, 50µl was placed on blood and MacConkey agar§ plates and were kept for 48 hours at 37^0C. The bacterial load was then determined.[24] Microbiological sampling was performed at baseline and by the end of the study period after 3 months.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. **(**Armonk, NY: IBM Corp**).**[25] The Kolmogorov-Smirnov test was used to verify the normality of distribution of variables; Comparisons between groups for categorical variables were assessed using the ***Chi-square test (Monte Carlo).*** ***Marginal Homogeneity Test***wasused to analyze the significance of the different stages**.** ***ANOVA*** was used for comparing the three studied groups and followed by the ***Post Hoc test (Tukey)*** for pairwise comparison. ***ANOVA with repeated measures***for normally distributed quantitative variables, to compare between the three periods, and Post Hoc test ***(Bonferroni adjusted)*** for pairwise comparisons. ***Kruskal Wallis test***was used to compare the three groups for not-normally distributed quantitative variables and followed by the ***Post Hoc test (Dunn's for multiple comparisons test)*** for pairwise comparison. ***Friedman test***to compare between the three periods for more than two categories, for pairwisecomparisons ***Wilcoxon signed ranks test*** was used between every two periods.The significance of the obtained results was judged at the 5% level.[26]

III. Results

The clinical parameters (PBI, GI, PD, and CAL) at baseline, 1, and 3 months are presented in tables (1 – 3). No significant difference was found at baseline between the three groups for any parameter. Changes in PBI and GI were expressed in Table (1). Regarding the PBI, a significant decrease in PBI existed after 3 months in both Propolis and Hyaluronic groups. Concerning the GI, a significant decrease existed after only 1 month in bothtreated groups. While for the control group, the decrease in both parameters was not significant neither after 1 nor after 3 months.

Table (1):Comparison between the three studied groups according toPapillary Bleeding Index (PBI) and Gingival Index (GI)

		Propolis (n = 20)	Hyaluronic (n = 20)	Control (n = 20)	H	p
PBI	Baseline	1^{aA}± 0.6	1.1^{aA}± 0.7	1.1^{aA}± 0.6	0.236	0.889
	After 1 month	0.7^{aAB}± 0.7	0.7^{aAB}± 0.7	1^{aA}± 0.6	3.323	0.190
	After 3 months	0.6^{aB}± 0.5	0.6^{aB}± 0.5	1^{aA}± 0.7	4.865	0.088
GI	Baseline	0.5^{aA}± 0.5	0.6^{aA}± 0.5	0.6^{aA}± 0.5	0.922	0.631
	After 1 month	0.3^{aB}± 0.4	0.3^{aB}± 0.4	0.5^{aA}± 0.5	2.424	0.298
	After 3 months	0.2^{aB}± 0.4	0.2^{aB}± 0.4	0.5^{aA}± 0.5	4.036	0.133

Means in the same **raw** with **common small letters** are not significant
Means in the same **column** with **common capital letters** are not significant
H: H for **Kruskal Wallis test**
p: p value for comparing between the studied groups

Table (2) shows the change occurring in the PD for the 3 groups throughout the study. A significant decrease occurred between baseline and after 1 month and between 1 and 3 months in both Propolis and Hyaluronic groups. In the control group, significant decrease occurred only after 1 month, but no significance was found between 1 and 3 months. Comparing the three groups together, after one month the percentage of decrease in Propolis, Hyaluronic and Control groups was 28.3%. 28% and 20.8% respectively with no significant difference between them. The reduction in probing depth continued with a percentage of change between 1 and 3 months equal to 33.4%, 31.7% in both Propolis and Hyaluronic groups respectively, while it was only 7.9% in the control group with a significant difference with both treatment groups.

‡ Bd Bbl™, Becton, Dickinson And Company, USA.
§Oxoid Ltd, Waderoad,Basingstoke, Hampshire, Uk.

Table (2):Comparison between the three studied groups according to Probing Depth (PD)

PD	Propolis (n = 20)	Hyaluronic (n = 20)	Control (n = 20)	Test of Sig.	p
Baseline	5^{aA}± 0.7	4.8^{aA}± 0.4	4.8^{aA}± 0.4	F=0.777	0.465
After 1 month	3.6^{aB}± 0.7	3.5^{aB}± 0.5	3.8^{aB}± 0.6	F=1.354	0.266
After 3 months	2.4^{bC}± 0.6	2.3^{bC}± 0.7	3.5^{aB}± 0.8	F=18.74*	<0.001*
%decrease baseline - 1m	28.3^{a}± 9	28^{a}± 9.2	20.8^{a}± 11.4	H=5.280	0.071
%decrease 1m - 3m	33.4^{a}± 11.8	31.7^{a}± 24	7.9^{b}± 14.9	H=18.56*	<0.001*
%decrease after 3 month	52.5^{a}± 9	52^{a}± 14.5	27^{b}± 16.5	H=24.84*	<0.001*

Means in the same **raw** with **common small letters** are not significant.
Means in the same **column** with **common capital letters** are not significant.
F: **F for ANOVA test** H: H for **Kruskal Wallis test**
p: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$

Changes according to CAL are presented in Table (3). A significant decrease occurred between baseline and after 1 month and between 1 and 3 months for both Propolis and Hyaluronic groups. In the control group, significant decrease occurred only after 3 months. The percentage of decrease in Propolis, Hyaluronic and Control groups after 1 month was 11.2%, 18.3%, and 4.6% respectively. Whereas, the percentage of change between 1 and 3 months was 22.5% and 23.3 % for both Propolis and Hyaluronic groups, which was significantly higher than that of the control, group(1.7%).

Table (3):Comparison between the three studied groups according to Clinical Attachment Loss (CAL)

CAL	Propolis (n = 20)	Hyaluronic (n = 20)	Control (n = 20)	Test of Sig.	p
Baseline	3^{aA}± 0.7	2.9^{aA}± 0.6	3^{aA}± 0.6	F=0.160	0.853
After 1 month	2.6^{abB}± 0.5	2.3^{bB}± 0.6	2.9^{aAB}± 0.7	F=4.422*	0.016*
After 3 months	2^{bC}± 0.6	1.8^{bC}± 0.6	2.8^{aB}± 0.6	F=15.83*	<0.001*
%decrease baseline_1m	11.2^{a}± 14.4	18.3^{a}± 22.2	4.6^{a}± 11.3	H=5.649	0.059
%decrease 1m _ 3m	22.5^{a}± 19.7	23.3^{a}± 22.6	1.7^{b}± 15.9	H=12.82*	0.002*
%decrease after 3month	32.1^{a}± 18.4	37.9^{a}± 24.1	7.1^{b}± 12.8	H=21.57*	<0.001*

Means in the same **raw** with **common small letters** are not significant.
Means in the same **column** with **common capital letters** are not significant.
F: **F for ANOVA test** H: H for **Kruskal Wallis test**
p: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$

The bacterial load was assessed as Gram +ve, Gram –ve, or Free (Table 4). Significant change existed after 3 months in both Propolis and Hyaluronic groups with an increase in the number of free samples from zero to 70% and from zeroto 50% respectively. While in the control group though there was no significant change and the free samples increased only from 40 to 70%.

Table (4):Comparison between the three studied groups according to total bacterial load

Total bacterial load	Propolis (n = 20)	Hyaluronic (n = 20)	Control (n = 20)	χ^2	^{MC}p
Baseline					
Gram +ve	9 (45%)	9 (45%)	5 (25%)		
Gram –ve	11 (55%)	11 (55%)	7 (35%)	15.621*	0.002*
Free	0 (0%)	0 (0%)	8 (40%)		
After 3 months					
Gram +ve	1 (5%)	4 (20%)	4 (20%)		
Gram –ve	5 (25%)	6 (30%)	6 (30%)	3.187	0.561
Free	14 (70%)	10 (50%)	10 (50%)		
^{MH}p	**<0.001***	**0.003***	**0.577**		

χ^2: **Chi square test** **MC: Monte Carlo**
p: p value for comparing between the studied groups
^{MH}p: p value for **Marginal Homogeneity Test** for comparing between Baseline and after 3 months
*: Statistically significant at $p \leq 0.05$

IV. Discussion

Modern treatment strategies for periodontal pockets implies the use of locally delivered drugs as adjuvants to mechanical debridement. These drugs are not solely used to control bacterial infection but also to suppress the inflammatory response.[27] In the present study, Hyaluronic acid and Propolis were injected into periodontal pockets after SRP and were compared to control groups where patients were injected with placebo after SPR. Results showed that for both Propolis and Hyaluronic acid groups, GI and PBI were significantly

decreased after 1 and 3 months respectively. These observations highlight the anti-inflammatory effect of both drugs. The results observed in this study concerning the efficacy of Hyaluronic acid as an adjuvant to mechanical debridement confirms those of Pilloni *et al*. who conducted a randomized controlled clinical study and concluded that the group treated with hyaluronic acid showed reduced inflammation manifested by a reduction in plaque index, bleeding on probing and gingival index.[28]The anti-inflammatory effect of Hyaluronic acid can be attributed to the scavenging activity of exogenous Hyaluronan for metalloproteinases and prostaglandins, potent inflammatory mediators implicated in connective tissue destruction and bone resorption. Besides, Hyaluronic acid was proved to have anti-edematous property due to its osmotic activity.[29]On the other hand, the results of the present study proved the positive effect of Propolis in the suppression of inflammation manifested by the significant decrease of PBI and GI. Previous studies demonstrated that Propolis can modulate key inflammatory mediators causing upregulation of anti-inflammatory cytokines, downregulation of pro-inflammatory cytokines, and inhibition of nuclear factor-(NF) κB.[30,31]Moreover, Campos *et al*. observed that Propolis inhibitshyaluronidase enzyme in a concentration-dependent manner.[32]Hyaluronidase enzyme degrades hyaluronic acid causing bone resorption and inflammation.[33]

Significant reduction in both PD and CAL was detected in the group treated with Hyaluronic acid after 1 month and after 3 months with a total percentage of change 52% for PD and 37.9% for CAL. These results were following Johannsen *et al*. who demonstrated a significant reduction in PD in the group treated with Hyaluronic acid compared to the control group.[34]These findings confirm the regenerative effect of Hyaluronic acid due to its ability to store and deploy growth factors, which enable it to stimulate cellular adhesion, migration, proliferation,and activation. Thus, it promotes extracellular matrix formation, tissue organization, and attachment of gingival epithelium to basal lamina.[35]Likewise, PD and CAL decreased significantly in the Propolis group after 1 and 3 months. These results agree with El-Sharkawy *et al*. who concluded that the group treated with Propolis showed a significant reduction in PD and CAL compared to the control group.[36]Comparing both Hyaluronic acid and Propolis, the total decrease after 3 months in PD was nearly equal in both Propolis and Hyaluronic group (52.5% and 52% respectively), but the decrease of the control was only 27%. While regarding CAL, the maximum decrease existed in the Hyaluronic group (37.9%) followed by Propolis (32.1%). However, the difference between Propolis and Hyaluronic acid was insignificant. Both treatment groups showed significantly higher attachment gain compared to Control group (7.1%). These findings demonstrate that both Hyaluronic and Propolis are nearly equally efficient in reducing PD and CAL.

The efficacy of Antimicrobial drugs is lowered by time due to the development of drug-resistant microorganisms. Thus, finding other alternatives that can overcome bacterial resistance became an essential concern.

Microbiological results of the present study showed that Hyaluronic acid significantly changed the bacterial load by decreasing both Gram +ve and Gram –ve samples and increasing the number of free samples fromzero to 50%. Pirnazar *et al*. reported that hyaluronic acid exhibited a patent bacteriostatic effect in various concentrations on periodontal pathogens as *Staphylococcus aureus, Actinobacillus actinomycetemcomitans*, and *Porphyromonas gingivalis.*[37] On the other hand, Propolis offered the best results as it decreased significantly the bacterial load and increased the free samples from zero to 70%. Propolis was demonstrated to inhibit the growth and proliferation of bacteria.[38]The phenolic compounds of the Propolis show antimicrobial activity by potentiating cell membrane destruction and by preventing nucleic acid synthesis, thus hindering the bacterial action.[39]In the current study, the sensitivity of Gram +ve samples to Propolis was more than that of Gram –ve. The percentage of decrease in the bacterial load was 40% in Gram +ve compared to 30% in Gram –ve. These findingsconfirm that of Choudhari*et al*. who reported that gram-positive bacteria are more sensitive to the action of Propolis than gram-negative bacteria, which may be due to structural differences of the cell wall.[40]

V. Conclusion

Hyaluronic acid and Propolis demonstrated a positive impact in cases of periodontal pockets manifested by significant improvement in clinical parameters and bacterial load. Although Propolis showed better antimicrobial action, yet no significant difference was observed between both treatments. Thus, both Hyaluronic acid and Propolis are considered effective local agents that can be used as adjuvant therapy for periodontal pockets.

Acknowledgment

The author would like to thank Dr. Walid A. Lotfy, (Microbiology Department, Faculty of Dentistry, Pharos University in Alexandria) for his valuable assistance throughout this study.

REFERENCES

[1]. Könönen E, Gursoy M, Gursoy UK. Periodontitis: A Multifaceted Disease of Tooth-Supporting Tissues. J Clin Med. 2019; 8(8):1135.
[2]. Apatzidou D, Kinane D. Nonsurgical Mechanical Treatment Strategies for Periodontal Disease. Dental clinics of North America. 2010; 54: 1-12.
[3]. Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: how many options for one disease?.Periodontol 2000. 2017; 75: 152-188.
[4]. Sulijaya B, Takahashi N, Yamazaki K, Yamazaki K. Nutrition as Adjunct Therapy in Periodontal Disease Management. Curr Oral Health Rep. 2019; 6: 61–69.
[5]. Matesanz-Pérez P, García-Gargallo M, Figuero E, Bascones-Martínez A, Sanz M, Herrera D. A systematic review on the effects of local antimicrobials as adjuncts to subgingival debridement, compared with subgingival debridement alone, in the treatment of chronic periodontitis. J ClinPeriodontol 2013; 40: 227–241.
[6]. Engström PE, Shi XQ, Tronje G, Larsson A, Welander U, Frithiof L, Engstrom GN. The effect of hyaluronan on bone and soft tissue and immune response in wound healing. J Periodontol. 2001; 72(9):1192-1200.
[7]. Fakhari A, Berkland C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. ActaBiomaterialia. 2013; 9 (7):7081-7092.
[8]. EidAbdelmagyd HA, Ram Shetty DS, Musa Musleh Al-Ahmari DM. Herbal medicine as adjunct in periodontal therapies- A review of clinical trials in past decade. J Oral BiolCraniofac Res. 2019;9(3):212-217.
[9]. Koura A, Shawky H, Ahmed N. Histological and Biochemical Evaluation of Curcumin and Panax ginseng in Rats with Ligature Induced Periodontitis. E.D.J. 2016; 62 (3): 3393 – 3403.
[10]. Koura A, Shawky H, Ahmed N. Effects of Açaí and Ginger in Senile Rats with Experimental Periodontitis. Histological and Biochemical Study. Aust. J. Basic & Appl. Sci. 2016; 10 (14): 10-19
[11]. Shawky H, Khalil D. Comparative Evaluation of Natural Mouthwashes Miswak and Green tea with Synthetic Mouthwash Chlorohexidine: A Clinical and Microbiological study. E.D.J. 2015; 61(1): 415-425
[12]. Shama N.S., Prasanna K.R., Joshna A., Lakshmi Srinivas T. Effect of herbs on periodontitis – a serious gum infection. Int J Pharmacol Res. 2014;4(1):17–22.
[13]. Daleprane JB, Abdalla DS: Emerging roles of propolis: antioxidant, cardioprotective, and antiangiogenic actions. Evid Based Complement Alternat Med. 2013, 2013:175135.
[14]. Bankova V, Popova M. Propolis of stingless bees: a promising source of biologically active compounds. Pharmacognosy Reviews. 2007;1(1):97–101.
[15]. Salomão K, Pereira PR, Campos LC, Borba CM, Cabello PH, Marcucci MC, de Castro SLEvid. Brazilian propolis: correlation between chemical composition and antimicrobial activity.Based Complement Alternat Med. 2008; 5(3):317-324.
[16]. Abiodun O. Arigbede, B. OsagbemiroBabatope, M. KoludeBamidele. Periodontitis and systemic diseases: A literature review. J Indian SocPeriodontol. 2012;16:487–491.
[17]. Muhlemann, H. R. Psychological and chemical mediators of gingival health. J of Prev Dent. 1977; 4:6–16.
[18]. Loe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. ActaOdontol Scand. 1963; 21: 533 – 551.
[19]. Ramfjord SP. The periodontal disease index (PDI). J Periodontol. 1967; 6: 602 – 10.
[20]. Gontiya G, Galgali SR. Effect of hyaluronan on periodontitis: A clinical and histological study. J Indian SocPeriodontol. 2012;16(2):184-192.
[21]. Escobar E, Pustiglioni A, Lima L, Mayer M. Propolis extract as an adjuvant to periodontal treatment. Oral health & preventive dentistry. 2003; 1: 29-35.
[22]. Coutinho A. Honeybee propolis extract in periodontal treatment: A clinical and microbiological study of propolis in periodontal treatment. Indian J Dent Res 2012;23:294.
[23]. Guentsch A, Kramesberger M, Sroka A, Pfisters W. Comparison of Gingival Crevicular Fluid Sampling Methods in Patients with Severe Chronic Periodontitis. J Periodontol. 2011;82: 1051–1060.
[24]. Gamboa F, Garcia DB, Acosta A, Mizrahi D, Paz A, Martinez D, Arévalo A, Aristizabal F, Abba M. Presence and antimicrobial profile of gram-negative Facultative anaerobe rods in patients with chronic Periodontitis and gingivitis. ActaOdontolLatinoam. 2013; 26: 24-30.
[25]. Kirkpatrick LA, Feeney BC. A simple guide to IBM SPSS statistics for version 20.0. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning; 2013.
[26]. Kotz S, Balakrishnan N, Read CB, Vidakovic B. Encyclopedia of statistical sciences. 2nd ed. Hoboken, N.J.: Wiley-Interscience; 2006.
[27]. Levine WZ, Samuels N, Bar Sheshet ME, Grbic JT. A novel treatment of gingival recession using a botanical topical gingival patch and mouthrinse. J Contemp Dent Pract. 2013;14(5):948-953.
[28]. Pilloni A, Annibali S, Dominici F, Di Paolo C, Papa M, Cassini MA, et al. Evaluation of the efficacy of an hyaluronic acid-based biogel on periodontal clinical parameters. A randomized-controlled clinical pilot study. Ann Stomatol (Roma). 2011;2:3–9.
[29]. Dahiya P, Kamal R. Hyaluronic Acid: a boon in periodontal therapy. N Am J Med Sci. 2013;5(5):309-315.
[30]. Wang L.-C., Lin Y.-L., Liang Y.-C., et al. The effect of caffeic acid phenethyl ester on the functions of human monocyte-derived dendritic cells. BMC Immunology. 2009;10, article 39.
[31]. MacHado J. L., Assunção A. K. M., da Silva M. C. P., et al. Brazilian green propolis: anti-inflammatory property by an immunomodulatory activity. Evidence-Based Complementary and Alternative Medicine. 2012;2012:10.
[32]. Campos JF, Dos Santos UP, da Rocha Pdos S, et al. Antimicrobial, Antioxidant, Anti-Inflammatory, and Cytotoxic Activities of Propolis from the Stingless Bee Tetragoniscafiebrigi (Jataí). Evid Based Complement Alternat Med. 2015;2015:296186.
[33]. Pascoal A., Rodrigues S., Teixeira A., Feás X., Estevinho L. M. Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food and Chemical Toxicology. 2014;63:233–239.
[34]. Johannsen A, Tellefsen M, Wikesjö U, Johannsen G. Local delivery of hyaluronan as an adjunct to scaling and root planing in the treatment of chronic periodontitis. J Periodontol. 2009;80:1493–1497.
[35]. Dicker KT, Gurski LA, Pradhan-Bhatt S, Witt RL, Farach-Carson MC, Jia X. Hyaluronan: A simple polysaccharide with diverse biological functions. ActaBiomater 2014;10:1558-1570
[36]. 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. J Periodontol. 2016;87(12):1418-1426.
[37]. Pirnazar P, Wolinsky L, Nachnani S, Haake S, Pilloni A, Bernard GW. Bacteriostatic effects of hyaluronic acid. J Periodontol. 1999;70:370–374.

[38]. Wojtyczka R. D., Dziedzic A., Idzik D., et al. Susceptibility of Staphylococcus aureus clinical isolates to propolis extract alone or in combination with antimicrobial drugs. Molecules. 2013;18(8):9623–9640.
[39]. Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids.Int J Antimicrob Agents. 2011; 38(2):99-107.
[40]. Choudhari M. K., Punekar S. A., Ranade R. V., Paknikar K. M. Antimicrobial activity of stingless bee (Trigona sp.) propolis used in the folk medicine of Western Maharashtra, India. Journal of Ethnopharmacology. 2012;141(1):363–367.