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Evaluation the Effects of Dentifrice Containing Propolis Extract in the Control of Plaque and Gingivitis with Measuring il-1β and il-6 Salivary Cytokines levels, (clinical and immunological trial)

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ABSTRACT

Background: Plants such as Propolis is a resinous substance obtained from the beehives that has antioxidant, anti-bacterial, anti-virus, antifungal, antitumor and anti-inflammatory activities. Gingivitis and periodontitis are diseases that involve the role of both the bacteria and the host immune response. Tooth brushing is effective in reducing levels of dental plaque. The aim of this study was to evaluate the effects of the toothpaste containing Propolis on control of plaque and gingivitis clinically and immunologically. Twenty patients diagnosed with generalized dental biofilm induced gingivitis were selected randomly to use propolis containing toothpaste (Ecodenta) and Colgate total toothpaste, for 7days for each type, and wash out period 7 days between them. Clinical periodontal evaluation is undertaken and saliva sample collection at baseline visit and after 7 days' tooth paste usage to estimate interleukin-1β and interleukin-6 levels. The study detected significant reduction in clinical periodontal parameters modified sulcus bleeding index and modified Quigley Hein index also in the levels of interleukins in group Ecodenta after 7days in comparison to the Colgate toothpaste. The interleukins-1β and 6 could be used as inflammatory biomarkers in patients with gingivitis. The propolis addition to the toothpaste leads to a more decrease in plaque accumulation and inflammatory response in patients with dental biofilm induced gingivitis and greater gingival health improvement than the sole use of a toothpaste.

Key words: Plaque, Gingivitis, Toothpaste, Interleukin-6, Antioxidant, Anti-bacterial, Anti-virus, Antifungal, Antitumor, Anti-inflammatory

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INTRODUCTION

Dental plaque is viewed as the key factor related to both inflammation of the gingiva and dental caries . Self-performed mechanical plaque removal such as using of tooth brush which is quite possibly the most acknowledged strategies for controlling plaque and gingivitis [1]. A wide types of toothpastes are present commercially and interest in natural items has expanded recently. Propolis herbal active agent one of these products. In the world markets propolis available in various structures as tincture, capsules, lozenges, creams and added to the mouth washes and toothpastes. Depending on published reports showing that propolis resin is a product with anti-inflammatory and bactericidal activities [2,3]. A clinical study regarding propolis-based herbal toothpaste revealed that Propolis was safe and efficient in decreasing accumulation of plaque when compared with Miswak and Colgate toothpaste [4]. Cytokines have been characterized as soluble components overlap or merge, building a complex immune-regulatory network in the immune system that is perturbed frequently in a disease. Many types of the cytokines such as interleukins (IL-6, IL-1), and tumor necrosis factor alpha (TNFα) are elevated in most, if not all, inflammatory states and have been documented as goals of therapeutic intermediation [5]. The IL-6 although mostly regarded as a pro-inflammatory cytokine, IL-6 also has many regenerative or anti-inflammatory activities while IL-1β is the first cytokine released after microbial stimulation and play a major role in the cell migration to the inflamed sites [6].

Saliva has been considered as a diagnostic liquid that could be utilized in the systemic and oral diseases diagnosis. The salivary markers levels, like cytokines, might actually be utilized to differentiate individuals with healthy periodontium from patients with periodontal disease [7,8]. Demonstrated that the IL-1β and IL-6 concentrations in patients with gingival disease were higher than in the control group, but with a non-significant difference. Studies that have been directed to find the

propolis effects on oral and periodontal health conditions were few. Thus, the current study has been conducted to evaluate and compare the Propolis containing tooth paste efficacy with Colgate total dentifrice in controlling plaque and inflammation of gingiva with measurement of salivary IL1β and IL-6 levels.

MATERIALS AND METHODS

The study design was approved by the Ethics Committee of the College of Dentistry, University of Baghdad and all patients were given detailed information relating to the study aim and an informed consent representing the patient's approval to participate in this study. Inclusion criteria were patients aged 20-35 years, apparently good general health, had generalized dental biofilm induced gingivitis with intact periodontium [9], 24 teeth as a minimum must be present and consented to stop for 24 h the measures of oral hygiene. Criteria for exclusion were persons with chronic disease, immunocompromised patients, pregnant, on contraceptive pills and lactating women, currently using any mouthwash, on antibiotic therapy and anti-inflammatory medications during the study and at the last 2 weeks before the study, having a background for hypersensitivity to any item utilized in the current study, with a new tooth extraction, having probing pocket depth (PPD) ≤ 4 or attachment loss, smoker or alcoholism, with a broad untreated dental caries, soft and hard palate lesions and wearing orthodontic apparatuses, removable dentures, implant, crown and bridge or presenting with abnormal salivary flow. Colgate total fresh mint strip tooth paste Manufactured by Colgate Palmolive Co with Active Ingredient: Stannous fluoride 0.454% (0.15% w/v fluoride ion) and Ecodenta triple force Manufactured by BIOK Laboratory UAB Vilnius with active ingredients White clay+Propolis+Teavigo™ were used in this study. All toothpaste tubes had a plain black covering to confirm appropriate hiding of the item from the examiner and the patients this done by subject not included in this study.

Clinical periodontal parameters examination (performed by the university of Michigan O probe with Williams markings) include: assessment of bleeding tendency by a modified sulcus bleeding index (mSBI) [10], and assessment of plaque accumulation using the modified Quigley Hein plaque index (mQHPI) and fluorescein disclosing tablets [11].

Study design

This study is a cross over double blinded randomized clinical trial had been conducted on 20 males and females. During the initial preparation visit, the patient received motivation and oral hygiene instructions (OHI), recording the bleeding on probing BOP, PPD and clinical attachment level CAL [12] then eliminate plaque, calculus, and stain by supra and sub gingival scaling and polishing. After prophylaxis, patients were gotten guidance about the clinical strides of the investigation. Next visit after one-week from initial visit: subjects must be with clinically healthy gingiva to continue in the study through clinical examination for (BOP, PPD and CAL) ,then the patient instructed to refrain from any oral hygiene measures for 24 hours. First visit (baseline) after 24 h of refrain from oral hygiene measures: Saliva sample collection was done. After that measure the (mSBI and mQHPI), the patients were given the Ecodenta or Colgate total toothpastes and instructed to use for 7 days twice daily at the morning after breakfast and at the evening before bedtime also take study tooth brush medium (Oral B) and use the modified bass technique tooth brushing method [13].

Second visit (after 7 days from first visit): Saliva sample collection then measuring (mSBI and mQHPI). After that scaling and polishing were done and the patient instructed about the washout period (7 days) instruction. After one week from second visit: Examination of (BOP, PPD and CAL) to ensure presence of clinically healthy gingiva, after that the subjects instructed to refrain from any oral hygiene measures for 24 h. First recall visit (baseline) after 24 h from refrain of oral hygiene measures: Saliva samples collection was done then measurement for (mSBI and mQHPI) after that the patient was given (Colgate) or (Ecodenta) toothpastes use it twice a day for 7 days. Second recall visit (after 7 days from first recall visit): Saliva sample collection then measurement for (mSBI and mQHPI).

Instruction for patients to use adequate amounts of tooth paste and brushing for 2 minutes.

Patients asked during test period to refrain from all other unassigned forms of oral hygiene measures, including non-study toothbrushes or toothpastes, interdental aids, chewing gum, or oral rinses. Patients motivated and instructed during 7 days' washout period to maintain oral hygiene measures by using whatever toothpaste, tooth brush and interdental aids. Patients instructed to brush their teeth before 2 hours prior to each visit.

Salivary samples collection

Unstipulated whole saliva had been collected prior to clinical periodontal evaluation according to a modification of the previous method by [14]. Subject expectorated at least 1 mL of saliva in a test tube.

After centrifuging 15 minutes 3000 RPM, aliquots had been prepared and samples had been frozen at -20°C in a freeze until analysis by enzyme-linked immunosorbent assay (ELIZA) kit for assessment of IL-6 and IL -1B levels [15,16].

Statistical analysis

Statistical Package for Social Sciences (SPSS) variant 25 was utilized to analyse the data which presented as mean, standard deviation (SD) and ranges with using of Independent t-test and Paired t test. A degree of probability of value p-value ≤ 0.5 represent the significant (S) also > 0.05 is non-significant (NS) while highly significant (HS) as $P < 0.001$.

RESULTS

Age and gender

Patients' age was from 21 to 33 years with a mean of 25.8 years. The higher percentage at age ≥ 25 years was 65% (13 patients). While the age <25 years was 35% (7 patients) revealed lower percentage. Concerning gender, percentage of males was higher than females (65% against 35%) respectively; hence the numbers were females 7 and males 13.

Baseline 1st visits before dentifrice usage

Non-significant differences were revealed between groups in means of mQHPI and mSBI, while significant differences were detected in means of IL-1β and IL-6, (Table 1).

Table 1: Descriptive statistics and comparison for mQHPI, mSBI, IL 1β ng/L and IL-6 ng/L between study groups at baseline 1st visit.

Clinical periodontal and Immunological parameters	Study groups		Student t-test	P - Value	Sig.
	Colgate	Ecodenta			
	Mean ± SD	Mean ± SD			
mQHPI	1.9 ± 0.3	1.78 ± 0.5	0.858	0.396	NS
mSBI	2.96 ± 1.7	3.14 ± 1.9	0.316	0.754	NS
Interleukin-1β	566.84 ± 94.61	1099.4 ± 115.7	7.554	0.001	H S
Interleukin-6	38.31 ± 14.6	72.22 ± 16.67	2.802	0.012	H S

After dentifrice usage for one week (2nd visits)

Table 2 revealed decreases in means of all parameters at 2nd visits hence, there were significant differences detected between visits at both groups in means of mQHPI and mSBI , while non-significant differences were demonstrated at both groups regarding means of both interleukins except ,the significant difference at Ecodenta group for IL-1beta.

Table 2: Descriptive statistics and comparison of mQHPI, mSBI, IL1β and IL-6 between 1st and 2nd visits at each study group.

Study groups	mQHPI		Paired t-test	P- Value	
	1st visit(baseline) Mean ± SD	2nd visit Mean ± SD	Sig.		
Colgate	1.9 ± 0.34	0.89 ± 0.5	8.439	0.001	H S
Ecodenta	1.78 ± 0.5	0.69 ± 0.4	8.609	0.001	H S
mSBI					
Colgate	2.96 ± 1.7	1.02 ± 0.02	3.826	0.001	H S
Ecodenta	3.14 ± 1.9	0.3 ± 0.1	3.392	0.001	H S
Interleukin-1β					
Colgate	566.84 ± 94.61	550.42 ± 95.73	0.092	0.871	NS
Ecodenta	1099.4 ± 115.7	533.74 ± 87.32	8.243	0.001	H S
Interleukin-6					
Colgate	38.31 ± 14.6	34.82 ± 17.44	1.833	0.082	NS
Ecodenta	72.22 ± 16.67	57.55 ± 21.88	1.622	0.12	NS

There were significant differences between groups regarding mean percentage of change for clinical and immunological parameters at 2nd visit compared to 1st visit hence Ecodenta group registered higher values for mean percentage of change than Colgate group at all parameters, as shown in Table 3.

Table 3: Statistical analysis for mean percentage of change between study groups in mQHPI, mSBI, IL-1β and IL-6 at 2nd visit compared to that at baseline1st visit.

Study Groups	mQHPI	Student t-test	P - Value	Sig.

	Mean ± SD	Range			
Colgate	-52.67 ± 23.09	(- 92.7)–(-46.22)	2.415	0.016	HS
Ecodenta	-59.36 ± 23.67	(- 89.81)–(-8.75)			
mSBI					
Colgate	-97.77 ± 6.66	(-100.0)–(-80)	3.258	0.001	HS
Ecodenta	-100 ± 3.6	(-100.0)–(-84)			
Interleukin-1β					
Colgate	-7.89 ± 6.77	(-33.44)–(94.56)	3.677	0.001	H S
Ecodenta	- 31.42 ± 20.45	(- 51.41)-(84.66)			
Interleukin-6					
Colgate	- 14.63 ± 12.31	(- 62.4)–(-4.23)	6.124	0.001	H S
Ecodenta	- 19.2 ± 14.22	(- 59.42)–(89.3)			

DISCUSSION

The sample size used in this study was similar to other studies which have similar purposes like this study [4,17]. The use of crossover design in the current study reduced the variability among subjects, hence the comparison between dentifrices was made on a same patient consequently all patients represent as their own control also the required sample size was less. Obviously the short period of study which is 1week may considered a drawback and if the period prolonged more benefit have been obtained hence, [18] demonstrated that following 7 days of utilizing toothpaste containing ethanolic extract of propolis, plaque accumulation reduced significantly while after 1 month of utilizing it there was highly significant reduction.

Baseline 1st visits

Clinical periodontal parameters

There were non-significant differences between study groups regarding (mQHPI and mSBI) at baseline. These results similar to other study by [19], it means no influence on future outcomes as there were no significant differences at baseline.

Immunological markers

The present study demonstrated significant differences in mean concentrations of all tested cytokines at baseline visit. At 1st visit showed that group of Propolis containing dentifrice (Ecodenta) demonstrated significantly higher levels of both IL-1β and IL-6 than in group of Colgate dentifrice. The intra-subjects differences in every cytokines salivary levels most likely revealed the way that while a few subjects established prominent gingivitis, the other only presented mild degree of clinical signs of gingival inflammation during the period of study. Under quantitatively and-or qualitatively similar challenge of bacteria the registered significant differences in inflammatory response of gingiva assumed that the gingival reaction to accumulation of plaque might be a trait of an individual, probably hereditary in origin [20-22]. After refrain from oral hygiene measures a significant increase in microbial accumulation, gingival inflammation and level of salivary IL-1β were observed [23].

The baseline overall levels of the IL-6 in both groups were substantially lower than IL-1β, this study result revealed by other study done by [24]. Hence the major role of IL-6 is the terminal differentiation of B-lymphocytes to plasma cells, the predominant infiltrate cells in established and advanced periodontal disease [25].

Clinical periodontal parameters at 2nd visits

This study showed that means of both mQHPI and mSBI were significantly decreased in both Colgate and Ecodenta groups at 2nd visit compared to that at 1st visit, also means percentages of change for mQHPI and mSBI revealed significant differences between group. This result mean that the propolis containing tooth paste more efficient than Colgate tooth paste in controlling the plaque formation and gingival inflammation. Hence [4] studied the anti-plaque efficacy of propolis-based herbal toothpaste in a crossover clinical study and concluded that the Propolis was discovered to be safe and decreased accumulation of plaque effectively when contrasted with Miswak and Colgate total toothpaste. [26] searched about propolis mouth wash effect on dental plaque buildup and detected it to be efficient against plaque accumulation. [27,28] demonstrated that propolis was efficient against bacteria (Gram-positive and Gram-negative) consequently the decrease in the bacterial count could likewise be represented the explanation of decrease in the plaque score, as plaque contains bacterial colonies [4,29]. studied the Propolis-containing toothpaste biological activity on oral health status of subjects received implant-supported prosthodontic rehabilitation which revealed that the routine use of propolis-containing toothpaste seems to have a beneficial reduction of peri-implant tissues inflammation and plaque accumulation. The propolis anti-inflammatory activity was confirmed and by all accounts benefit for

prophylactic strategies for the patients with high susceptibility for inflammatory periodontal diseases, that is, gingivitis and chronic periodontitis [30,31]. The high levels of galangin, pinocembrin, chrysin, and caffeic acid esters in propolis could be related to its therapeutic effects on gingivitis [32]. The antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral properties of propolis related to the presence of flavonoids in its constituents, [33].

Immunological markers levels at the 2nd visits

In group Colgate there were no significant decrease in means of interleukin-1β and IL-6 at 2nd visit compared to that at 1st visit, whereas means percentages of change for interleukin-1β and IL-6 were significantly more decreased at 2nd visit in Ecodenta group than that in Colgate group compared to that at 1st visit. The IL-1β could be produced by gingival fibroblasts in response to bacterial stimulation [34], and the complete eradication of some oral pathogens provides a possible explanation of the registered decrease of its concentrations. Moreover, at the end of the study, the concentration of IL-1β in group Ecodenta was significantly lower than group Colgate and this result could be related to the absence of bacterial stimulation for its release. Given the complex composition of propolis, it was assumed that some of its components are responsible for this effect. [35] Reported that chrysin (a flavonoid) suppress the nuclear factor for IL-6. However, the role of other components could not be excluded. Propolis was confirmed to be aromatic acids rich source with their derivatives such as fatty acids, flavonoids, terpenes and cinnamic acids. Studies investigating the biological activities of propolis revealed that these types of compounds were involved in the antimicrobial, antioxidant, anticaries, anti-inflammatory activities of propolis [36,37].

CONCLUSIONS

Prosthetic stomatitis, when associated with angular cheilitis, may have a multifactorial etiology, despite recent scientific evidence suggesting that most cases are linked to fungal infections, namely by *Candida albicans*. Further studies and an increase in the sample of institutionalized elderly are needed to analyse other risk factors associated with the disease. To prevent the extension of lesions, prosthetic patients should be called regularly for dental appointments and a clinical examination of the oral cavity and removable prosthesis. Preventive measures should be developed to avoid the colonization of Candida albicans on the palatal mucosa and prosthesis, such as improvement of oral and prosthetic hygiene and prosthetic removable at night during sleep.

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