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The Effect of Calendula Extract Toothpaste on the Plaque Index and Bleeding in Gingivitis

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Abstract: The aim of this study is to investigate the efficacy of *Calendula officinal* extract toothpaste in reducing gingival inflammation and plaque formation. A total of 40 volunteers with established gingivitis (21 males and 19 females, with a mean age of 28.8±3.28) were included in this prospective, double blind clinical study. They were randomly assigned to 2 treatment groups: group 1 (n = 20), treated with a base dentifrice as the control toothpaste; group 2 (n = 20), treated with Calendula flower extract tooth paste. Subjects were instructed to brush their teeth three times daily using the Bass method and their assigned tooth paste for 4 weeks. Data was collected at baseline and at increment of 2, 3 and 4 weeks. This data includes an analysis of Plaque Index (PI), Gingival Index (GI) and Bleeding on Probing (BoP). The present study was carried out at Babol Dental School, Babol, Iran between December 2007 and October 2008. Over a period of 4 weeks, all indices were significantly decreased. A substantial reduction in PI was noted for group 2 (1.63 vs. 1.42) throughout the follow-up period, this reduction was seen in GI (1.23 vs. 0.66) and BoP (1.29 vs. 0.64) for group 2. Brushing with calendula toothpaste led to significant reductions in PI, GI and BoP. This treatment could therefore, be recommended as an adjunctive procedure effective for the improvement of gingival inflammation.

Key words: Calendula, toothpaste, plaque control, gingivitis

INTRODUCTION

The considerable prevalence of gingival inflammation within the general population suggests that most patients practice inadequate oral hygiene, particularly within certain regions of their mouths. In certain areas of their teeth. Elimination of microbial dental plaque biofilm prevents gingivitis, periodontitis and dental cavities (Lauten *et al.*, 2005). Although, brushing teeth twice a day and daily flossing is highly effective in plaque reduction, over 50% of adults have gingivitis on an average of 3 to 4 teeth (Oliver *et al.*, 1998). Although, self-performed mechanical plaque removal is an unquestionably effective method of controlling plaque and gingivitis (Axelsson *et al.*, 1991; Caton *et al.*, 1993; Loe *et al.*, 1965), teeth brushing and flossing are difficult tasks and most of the patients are not able to completely remove plaque on all teeth surfaces (Lindhe and Koch, 1967; Sowinski *et al.*, 2008). All mechanical procedures for plaque control, whether performed by patients or a

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dental professional, are time-consuming and some individuals may have no motivation for practicing these procedures. They may not achieve permanent complete plaque removal, therefore additional application of microbial agents (Owens *et al.*, 1997; Ozaki *et al.*, 2006) and medical mouth rinses is the subject of considerable scientific interest (Allen *et al.*, 1998; Charles *et al.*, 2004).

The most common and extensively used oral antiseptic and antiplaque is chlorhexidine gluconate (Aziz-Gandour and Newman, 1986; Brownstein *et al.*, 1990; Eaton *et al.*, 1997; Gehlen *et al.*, 2000). However, its long-term use is associated with a number of adverse side effects (Flotra *et al.*, 1971; Hiraishi *et al.*, 2009). On the other hand, a significant reduction of its anti plaque potential may be observed when it is used in a toothpaste preparation. Essential oils containing mouthrinse and triclosan toothpaste with a co-polymer are other effective anti-plaque, anti-gingivitis agents, less commonly used. Interest in alternative mouth rinses and toothpastes based on plant extracts has recently increased.

Numerous studies have shown that plant extracts can both improve gingivitis symptoms and inhibit the growth of pathogens in sub gingival plaque (Emling, 1988; Saxer *et al.*, 1994; Rassameemasuang *et al.*, 2007; Wan *et al.*, 2005; Lauten *et al.*, 2005; Tapsoba and Deschamps, 2006; Wołoszyn *et al.*, 2004).

Botanical extracts of calendula (*Calendula officinalis*) have become popular natural products believed to be anti-inflammatory and anti-microbial agents (Lauten *et al.*, 2005). Mouth rinses and dentifrices containing this ingredient purport to have anti-plaque and anti-gingivitis properties.

Calendula (marigold) extract has been used in Europe since before the 12th century primarily as a topical anti-inflammatory agent (Lauten *et al.*, 2005). *In vitro* studies have shown some evidence of calendula's anti inflammatory effects (Della *et al.*, 1994; Schmidgall *et al.*, 2000). At least one *in vivo* study using an herbal mouth rinse containing calendula has demonstrated its efficacy in reducing gingival bleeding (Scherer *et al.*, 1998). On the other hand, it is well documented that *Calendula officinalis* flowers have antimicrobial activity (De *et al.*, 2002; Koo *et al.*, 2000; Larrondo *et al.*, 1995).

Calendula used as a healing agent in the treatment of gingivitis and its clinical relevance in dentistry has not been thoroughly documented. For this reason, this study set out to investigate the efficacy of toothpaste containing calendula flower extract in the reduction of plaque and gingivitis in subjects with established gingivitis.

MATERIALS AND METHODS

The present study was carried out at Babol Dental School, Babol, Iran between December 2007 and October 2008. Volunteers for this study were recruited from the Department of Periodontology, Babol University of Medical Sciences, Babol, Iran. Initially 40 patients (21 males, 19 females with a mean age of, 28.8±3.28 years and range 17-37 years) were enrolled in this study. They were admitted to the study after meeting the following eligibility criteria:

Age ≥16 years, a minimum of 15 teeth, good general health, a baseline Plaque Index (PI) mean >1.5 (Turesky *et al.*, 1970) and presence of established gingivitis. Established gingivitis was defined as a baseline (Loe and Silness, 1963), Gingival Index (GI) mean was based on inclusion criteria used by Binney *et al.* (1996) and Owens *et al.* (1997). Exclusion criteria were: presence of systemic disease, using drugs affecting periodontal disease, antibiotic therapy for periodontitis or gingivitis during the last three months, pregnancy, breast-feeding, smoking, the use of orthodontic appliances, continuous use of mouth rinses containing chemical agents in the previous 3 months and any history of allergies to toothpaste or herbal medicine.

All subjects were given verbal and written information concerning the study and after entering the study, signed a written consent form regarding all information received. The study protocol was approved by the Ethics Committee of Babol Medical University. All procedures in this experiment were performed according to the ethical principles established by the Declaration of Helsinki.

A standard toothpaste formulation as a base for the preparations (Pooneh, Tehran, Iran) was used in this study. *Calendula officinal* flowers were prepared from a registered herbal medicine shop (Rezaeian Herbal Shop, Tehran, Iran) and a specimen was deposited in the Department of Pharmacology, Babol University of Medical Sciences. The extracts were isolated by maceration of *Calendula officinal* flowers with ethanol within a 48 h period. After filtering and drying the extract, toothpastes containing 2% calendula flower extract were prepared. Two series of toothpastes were prepared including toothpastes without the extract as placebo and toothpastes containing 2% of calendula extract. All toothpastes were prepared in the lab section of the pharmacology department and were separately packaged in the label-free tubes. Patients were supplied with the assigned oral hygiene kit, toothpaste and a soft- bristled toothbrush.

All patients were instructed in the use of the oral hygiene items. They were asked to brush their teeth thrice daily using the Bass technique for approximately 2 min.

The study was designed as a randomized double-blind, parallel arm controlled trial. The subjects were randomly assigned to either the control or calendula extract toothpaste (drug) group. Blinding and allocation concealment were controlled by the independent technician in the pharmacology department, who distributed the toothpastes in A and B tubes. All investigators and subjects were unaware of the contents of each tube. Subjects in the drug group received a toothpaste tube containing 2% calendula extract dentifrice and the control group received a toothpaste tube containing the base formulation without calendula extract. All participants used dental floss during the study based on Ozaki *et al.* (2006). The baseline plaque, gingival and bleeding indices were measured on all teeth at the buccal, mesial, distal and lingual aspects, with the exception of third molars. All which had been previously calibrated measurements were conducted by the main investigator. For calibration, two measurements were performed with a 1 h interval. Intra-examiner calibration was performed in 5 patients until an 80% agreement was obtained between measurements. This study was conducted over a 4 week duration. Clinical re-examination was performed at the end of weeks 2, 3 and 4 after initial use of toothpastes. Throughout the study all measurements and instrumentations were obtained by the main investigator. On the other hand, the clinical data was collected by one investigator who was blinded to the respective group allocation.

Statistical Analysis

The protocol-defined primary outcome variable shows the reduction of PI from baseline. At the 2, 3 and 4 week visits, a change from the baseline for PI, GI and BoP for each site was calculated. A mean value was calculated for each treatment and an overall mean of the differences from baseline was calculated for each treatment. Summary of statistics were determined and unpaired student t-test was used to identify statistically significant differences between the treatments in each time section. Repeated measure test was used for intra-groups variations at different points of time. For distribution-free scores at the 1 month visit non-parametric Mann-Whitney U-test was applied to compare data across the treatment. Any differences with $p < 0.05$ was considered significant.

RESULTS

All subjects completed this study. During the trial only 2 subjects reported mild unpleasant tastes from one type of the toothpastes. The ratio of male to female subjects was 1.1. Baseline data for placebo and drug treatment groups were analyzed on a subject wise basis which has been shown in Table 1. This data confirms the target sites in the drug and placebo groups were very similar without any significant differences with respect to PI, GI and BoP at the baseline.

Plaque Index (PI)

Plaque-covered surfaces ratios on all sites have been shown in Table 2. As shown in Table 2. The number of sites affected by plaque following the use of the calendula toothpaste was decreased compared to the placebo group.

At the beginning of trial, no significant differences were observed in baseline data between the two treatment groups. Analysis of the calendula treatment group, shows significant differences between $PI_{baseline}$ and PI_{week4} ($p < 0.0001$). Inter-group comparative analysis shows a significant decrease between the placebo and calendula groups at week 4 ($p < 0.0001$). The effects of calendula toothpaste at the end of week 2, 3 and 4 compared to the placebo group shows significant differences (Table 3). After 28 days, the calendula group presents an average 33.4% reduction in plaque, whereas the control group shows a mean 9.8% reduction.

A significant difference in the GI can be seen at the end of weeks 2, 3 and 4 between the two groups. Group data analysis in calendula toothpaste users shows significant differences between $GI_{baseline}$ and GI_{week4} .

Gingival Index (GI)

There was no significant difference in the Gingival Index (GI) between the groups at baseline. After 28 days, a mean 46.3 and 18.4% reduction in GI was observed in the calendula and control groups, respectively (Table 4).

Table 1: Mean±SD baseline data for placebo and drug treatment groups analyzed on a subject-wise basis

Treatment groups	PI	GI	BOP
Placebo (n = 20)	1.67±0.12	1.21±0.31	1.17±0.33
Calendula (n = 20)	1.63±0.07	1.23±0.21	1.29±0.28

Table 2: Plaque-covered surface ratios on all observed sites in two treatment groups during weekly intervals of clinical examination

Treatment groups	Baseline	Week		
		2	3	4
Placebo (base tooth paste)	1435/2144	1351/2144	1248/2144	1300/2144
Calendula tooth paste	1477/2352	1207/2352	1137/2352	978/2352

Table 3: Mean±SD and comparison between groups according to the PI data

Treatment groups	Baseline	Week		
		2	3	4
Placebo	1.672±0.124	1.629±0.099	1.528±0.116	1.606±0.073
Calendula	1.628±0.069	1.515±0.128	1.486±0.134	1.418±0.102
p-value	0.127	0.001	0.018	0.0001

Table 4: Mean±SD and comparison between groups according to the GI

Treatment groups	Baseline	Week		
		2	3	4
Placebo	1.480±0.258	1.360±0.278	1.231±0.362	1.208±0.307
Calendula	1.226±0.204	0.911±0.194	0.760±0.259	0.658±0.261
p-value	0.73	0.004	0.0001	0.0001

Table 5: Mean±SD and comparison between groups according to the bleeding on probing (BoP)

Treatment groups	Baseline	Week		
		2	3	4
Placebo	1.491±0.253	1.425±0.282	1.246±0.354	1.166±0.335
Calendula	1.290±0.279	0.867±0.277	0.727±0.265	0.635±0.291
p-value	0.25	0.001	0.0001	0.0001

Bleeding on Probing (BOP)

The BOP results are shown on Table 5. Bleeding was observed in most of the oral regions at the starting of the study and there was no significant difference in the BoP between the groups at baseline. But the BoP was significantly decreased in two groups particularly those receiving calendula toothpaste applications during the 28 days of present investigation ($p < 0.0001$). Comparative analysis between groups shows a significant decrease between placebo and calendula groups at the end of week 2, 3 and 4 (Table 5).

After 28 days, the calendula group presents a mean 50.8% reduction in BoP, whereas the control group shows a mean 21.8% reduction.

During investigation two of the volunteers reported an un-pleasant taste related to the calendula toothpastes. No serious adverse reactions were observed during the trial.

DISCUSSION

Up to until now, local delivery of drug- therapy has not been used widely in maintenance patients with gingivitis. In this trial we examined a dentifrice form containing *Calendula officinalis* flower extracts to evaluate its effects on gingivitis. The results of this randomized double-blind two arm, placebo-controlled study suggests that the calendula toothpaste is sufficiently safe and effective in the treatment of gingivitis as compared to the placebo group. This study demonstrates a significant reduction in the mean PI, GI and BoP when comparing results of the calendula with the placebo group. Regular home care by patients in addition to professional removal of plaque generally ensures adequate plaque and gingivitis control in healthy adults.

It has been estimated, however, that even in developed countries, only a minority of the population can be expected to practice adequate mechanical plaque removal (Honkala and Freeman, 1988; Micheelis and Bauch, 1996). Moreover, dental plaque reforms within hours or days after its removal. Therefore, the application of antimicrobial agents is a useful adjunct to mechanical oral hygiene or systemic drug therapy procedures.

In the present study, toothbrushing with an herbal-based toothpaste resulted in a significant reduction in gingival inflammation parameters. Mild GI, PI and BoP reduction in the control group shows the over all potentially positive effects of regular thrice daily toothbrushing without using herbal-based toothpaste.

The beneficial effects, however, were significantly lower than with the calendula toothpaste group. Some studies have demonstrated that different formulations of chlorhexidine such as mouthwash (Aziz-Gandour and Newman, 1986; Southern *et al.*, 2006),

collagen gel (Vinholis *et al.*, 2001), local chip (Grisi *et al.*, 2002), spray (Bozkurt *et al.*, 2004), chewing gum (Cosyn and Verelst, 2006) and toothpaste (Gazi *et al.*, 1987; Jenkins *et al.*, 1993; Yates *et al.*, 1993) are effective in treating gingivitis. In the present study we designed a different formulation of calendula flower extract in the toothpaste base as a vehicle. Based on our knowledge, this formulation may be the first preparation that has been investigated in Iranian subjects. We believe that the application of tooth paste is compatible with daily activities and patients will find its application convenient. On the other hand, the main advantage of the present study is patient compliance and the low risk of systemic side effects.

Present study shows that the application of herbal-based mouthrinses in combination with an herbal-based toothpaste lead to a reduction in plaque accumulation (Willershausen *et al.*, 1991). The plaque-reducing effects of herbal-based active agents has been previously described (Mullally *et al.*, 1995; Yankell *et al.*, 1993). Present data is in agreement with other similar studies.

Calendula extract has been shown to exert an anti-inflammatory action (Della *et al.*, 1994; Schmidgall *et al.*, 2000). At least one *in vivo* study using an herbal mouthrinse containing calendula demonstrates its efficacy in reducing gingival bleeding (Scherer *et al.*, 1998).

The accepted active ingredient in calendula extract is primarily triterpenoid (Della *et al.*, 1994). Calendula extract may exhibit anti-inflammatory action through oxygen free-radical scavenging (Cordova *et al.*, 2002).

Calendula has been used for skin complaints, wounds, burns, conjunctivitis, menstrual irregularities, varicose veins, hemorrhoids, duodenal ulcers etc. (Basch *et al.*, 2006; Cordova *et al.*, 2002). According to the fact that calendula contains polyphenols, the assessment of its anti-oxidant properties is of great interest in the understanding of the positive effects of these compounds, especially in phytotherapy. It seems that, antioxidant effects of calendula extract in the present study may be accountable in some part for its positive effects on gingivitis. On the other hand, the positive effects of calendula toothpaste in the present study could be due to its antimicrobial agents. It is established that *Calendula officinalis* has antimicrobial activity (Cordova *et al.*, 2002; De *et al.*, 2002; Larrondo *et al.*, 1995). Given that periodontal infections can cause more or less serious damage, from gum inflammation to the loss of alveolar bone and eventually the loss of teeth, it is necessary to begin a series of preventative measures in patients at risk. Information on the antibacterial activity of medicinal plants against anaerobic and facultative aerobic bacterial present in the oral cavity that often cause periodontal infections is very scarce. Therefore, it is suggested that more studies must be designed to find out the exact antibacterial effects of calendula on gingivitis.

In conclusion, the results of this study have given useful information about the positive effects of calendula toothpaste on decreasing gingivitis indices. It is suggested that the use of the toothpaste containing calendula flower alcoholic extract for gingivitis could be a useful aid to obtaining a significant reduction of parameters for PI, GI and BoP compared to the placebo paste. It can be recommended as an adjunct treatment to daily oral hygiene procedures.

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