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LIQUORICE AS AN ADJUNCT TO SCALING AND ROOT PLANING IN THE TREATMENT OF CHRONIC PERIODONTITIS: A CLINICO-MICROBIOLOGICAL STUDY

Dental Science

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ABSTRACT

BACKGROUND: Liquorice is a natural anti-inflammatory compound derived from root extract of Glycyrrhiza glabra. The effect of liquorice on periodontitis and its mechanism of action were determined in the present study.

AIM: The aim of the present study was to compare the clinical and microbiological benefits of routine scaling and root planing with adjunctive use of liquorice as a local drug delivery (LDD) agent in the management of periodontitis.

MATERIALS AND METHODS: Thirty patients diagnosed with chronic periodontitis having periodontal pockets greater than 5 mm and clinical attachment loss greater than 3 mm in more than 20 teeth with moderate to severe bone loss were included in the present study. 30 sites were randomly divided into two groups with 15 sites in each group. The control sites received scaling and root planing alone and the test sites received scaling and root planing with locally delivered liquorice. The clinical parameters were evaluated at the selected sites and subgingival plaque samples were collected at baseline, 15 days and one month. The plaque samples were cultured anaerobically for the keystone periodontal pathogen Porphyromonas gingivalis. Mann Whitney U test and Wilcoxon matched pairs test was performed to compare between the test and control group.

RESULTS: Statistically significant improvement was observed in both the groups. Intergroup comparison of prevalence of Porphyromonas gingivalis also revealed statistically significant difference (p=0.0002) at 15 days and 1 month.

CONCLUSION: Subgingivally delivered liquorice as an adjunct to scaling and root planing in the treatment of chronic periodontitis showed anticipative results revealing slow and constant releasing property of liquorice.

KEYWORDS:

Targeted drug delivery, periodontal disease, periodontal pathogen.

INTRODUCTION

Periodontitis is a serious, progressive form of periodontal disease which involves inflammation of periodontium. Diet, hygiene and hereditary factors can contribute to periodontitis and its precursor, gingivitis.[1-3] The treatment for periodontitis is aimed at removing dental plaque and calculus with or without various surgical modalities.[4,5] The use of local and systemic antibiotics, anti-inflammatory drugs, like sub-anti-microbial low dose doxycycline have shown promising results.[6,7]

Different drugs used for sustained release are tetracyclines including doxycycline and minocycline, metronidazole and chlorhexidine. Inflammation and infection associated with periodontal diseases can be eliminated using herbs like aloe vera, clove oil, chamomile, turmeric, eucalyptus, blood root, neem leaves.[1]

Liquorice (Glycyrrhiza glabra), synonym being sweet wood is inherent to the Mediterranean and few areas of Asia. Liquorice, is a perennial herb which holds sweet taste and has widespread pharmacological effects for human being. Liquorice is employed widely in medical field for treating upper respiratory infections including coughs, hoarseness, sore throat and bronchitis.[8-10]

The advantageous properties of liquorice can be ascribed to several mechanisms. Liquorice and its metabolic end products prevent hepatic metabolism of aldosterone and suppress 5-[beta]- reductase.[11] In-vitro studies have also established that liquorice prevents cyclooxygenase activity and prostaglandin synthesis as well as indirectly inhibiting platelet aggregation, all components in the inflammatory cascade. Liquorice components possess important antioxidant and hepatoprotective properties. Liquorice hinder the synthesis of reactive oxygen species (ROS) by neutrophils at the site of inflammation.[4]

Many literature reviews [4,6,8] state that liquorice extracts and their bioactive ingredients have potential role on both the oral microbial pathogens and the host immune response involved in common oro-dental diseases like dental caries, periodontitis, candidiasis, and recurrent aphthous ulcers. The capacity of liquorice to lessen development of dental plaque adds to its role in periodontitis management. In addition liquorice root extracts exert an anti-bacterial activity, which is the main etiology for dental caries.[4-9]

Evidence suggests that liquorice and its bioactive ingredients may represent potential phytochemicals for the development of a new natural therapy to treat or prevent periodontitis. [9] The data regarding the use of liquorice as a local drug delivery agent is very narrow. Therefore this study was aimed to assess the clinical and microbiological effect of liquorice as a local drug delivery agent in treating periodontitis.

MATERIALS AND METHODS

The randomized, controlled, parallel, double-blind study (examiner and microbiologist) was conducted at Department of Periodontics, Rajarajeswari Dental College and Hospital, Bangalore, India. Forty patients, 19 males and 21 females, aged 25 to 50 years (mean age 37.5±17.67 years) were included in the study out of which ten patients were lost to follow up. Type II errors may be termed as 'statistical power', which is simply 1-b: i.e. having a 10% type II error is the same

as the study having 90% 'power'. So, based on this formula, thirty patients were sufficient for conducting the study with power analysis (90%). The study was conducted between April and June 2016. Prior to the execution of the treatment, written informed consent was obtained after explaining the treatment procedure. Ethical clearance was obtained from Institutional Ethical Committee Review Board, Rajarajeswari Dental College and Hospital, Bangalore.

The study inclusion criteria were as follows: (1) patients with a noncontributory medical history (2) Chronic periodontitis were diagnosed based on the criteria of American Academy of Periodontology classification of periodontal diseases (1999)[10]. Chronic periodontitis patients had periodontal pockets greater than 5 mm and clinical attachment loss greater than 3 mm in more than 20 teeth with moderate to severe bone loss. The exclusion criteria were as follows: (1) smokers (2) pregnant and lactating women (3) patients with any other systemic conditions like diabetes that can alter the course of periodontal disease progression and treatment (4) patients who had undergone periodontal therapy in last 6 months. Control group was treated by scaling and root planing (SRP) alone and in the test group, the sites were treated by SRP followed by subgingival placement of liquorice [100% concentrated gel pre-made by the manufacturer - Jain ayurvedic pharmacy]. In every patient, the selected sites were marked and assigned randomly either to the control group or test group by a flip of a coin. [Table-1].

TABLE : 1



After enrolment by a chief investigator (SPB), the clinical measurements were performed by a single examiner (NS). At baseline, all patients were examined in order to register the Gingival index (GI) (Loe and Silness, 1964)[11], Sulcus bleeding Index (BI) (Muhlemann and Son, 1971)[12], Probing pocket depth (PPD) and clinical attachment level (CAL). The investigated parameters were measured at six sites per tooth. The criteria for GI were: 0- normal gingiva; 1- mild inflammation, slight change in color, slight odema, no bleeding on probing; 2- moderate inflammation, redness, odema and glazing, bleeding on probing; 3- severe inflammation, marked redness and odema, ulceration, tendency to spontaneous bleeding[11]. The probing pocket depth were measured using a graduated Williams periodontal probe which was inserted parallel to the long axis of the tooth at 3 buccal (mesiobuccal, midbuccal and distobuccal) and 3 lingual (mesiolingual, midlingual and distolingual) sites for all maxillary and mandibular teeth. CAL was measured as the distance from the cemento-enamel junction (CEJ) to the base of the gingival sulcus using the periodontal probe. These parameters were reassessed at 15 days and one month after therapy. Liquorice is believed to show its maximum potency by 15 days and effect continues till 1 month. Later, the overlapping effect of oral hygiene as confounding factor is observed. Thus the recall period of 1 month was considered for the study[4].

MICROBIAL SAMPLING

Isolation of site was done using sterile cotton rolls and dried prior to sampling. At baseline, subgingival plaque samples were collected from the deepest portion of the pocket by means of sterile curettes from both test and control sites. Subgingival samples were collected with a single stroke after gentle insertion of curette into the bottommost part of the sampling site. After baseline examination and plaque sample collections, test sites were treated with SRP followed by subgingival administration of liquorice, which was preweighed (~5mg) and control sites were treated with SRP alone. Subgingival plaque samples were collected at 15 days and one month post treatment in similar manner.

Samples collected in coded sterile vials comprising of thioglycollate broth were transported to the laboratory for microbiological investigation at Rajarajeswari Medical College and Hospital. The samples were mixed meticulously and were inoculated with a sterile loop onto enriched blood agar.(Figure 1)

TECHNIQUE FOR DRUG DELIVERY

Liquorice gel was carried into the sulcus using a plastic carrying instrument after completion of SRP. The drug was completely submerged such that it was not exposed to the oral cavity. Periodontal pack was placed at the test site in order to secure the gel in place and patients were advised to follow routine oral hygiene measures. Patient was instructed to avoid proximal cleaning of the test sites until seven days post treatment. Clinical parameters were evaluated at 15 days and one month post treatment.

STATISTICAL ANALYSIS

The statistical analysis was done using SPSS version 15.0 software. The values were represented in number (%) and mean ± standard deviation (SD). The intragroup comparison was performed using Wilcoxon's signed rank test to evaluate the difference between the two treatments or conditions where the samples were correlated. The intergroup comparison was performed using the Mann-Whitney U-test to determine if a difference exist amongst the groups. In the present study p value was set as less than 0.05 to consider the difference as significant.

RESULTS

Controlled release delivery of anti-microbials like liquorice directly into periodontal pocket has received great interest in periodontal therapy. In the present study 20 sites were allocated to control group and 20 sites to test group, of which 15 sites were finally analyzed per group. Patient did not experience any adverse effects at any point during the study by the use of liquorice. Any sort of local allergic reaction, pain, swelling, or other side effects were not observed throughout the study.

Intragroup evaluation at baseline, 15 days and 1 month in the test group showed that the changes were statistically significant at all the time intervals ($p < 0.05$) with request to GI, BI and PPD. A statistically significant difference for all the parameters between test and control group was observed in clinical parameters at one month with the test group showing significantly higher change as compared to the control group ($p < 0.05$) as shown in [Table 2,3,4].

[Table-2,3,4,5] shows the mean GI, BI, PPD and CAL scores in both test and control sites at different points of time and the percentage change.

MICROBIOLOGICAL ANALYSIS

Reduction of the most common periodontal pathogen, Porphyromonas gingivalis (Pg) at the follow-up visits was evaluated. The quantities of colonies are expressed as colony forming units per ml (CFU/ml). Mean values of colony count/ml for Porphyromonas gingivalis (Pg) in test and control groups is shown in [Table 6] at different time intervals. An intragroup comparison at the follow-up intervals, revealed a statistically significant reduction in both the groups with $p = 0.0007$ ($p < 0.05$), wherein the test group showed lower prevalence of Porphyromonas gingivalis compared to the control group.

Overall, both the groups showed statistically significant reduction in relation to the clinical parameters. Also, the groups showed reduction in the microbial count of Porphyromonas gingivalis with p = 0.0269 at 15 days and p = 0.0002 at 1 month revealing slow and constant releasing property of liquorice.



Figure 1: Culture of *Porphyromonas gingivalis* in blood agar

Table 2: Comparison of test and control sites with respect gingival index scores at baseline, 15days and 1 month by Mann-Whitney U test and Wilcoxon matched pairs test

Sites	Baseline to 15 days		Baseline to 1 month		15days to 1 month	
	Mean difference	SD	Mean difference	SD	Mean difference	SD
Test site	0.65	0.45	1.40	0.59	0.75	0.44
Control site	0.67	0.45	1.24	0.77	0.58	0.66
Total	0.66	0.44	1.32	0.68	0.67	0.56
% of change in test site	34.64%# p=0.0022*		75.00%# p=0.0007*		61.75%# p=0.0007*	
% of change in control site	34.48%# p=0.0033*		64.31%# p=0.0007*		45.53%# p=0.0106*	
Z-value	0.0001		-0.8329		-1.2932	
p-value	0.9999		0.4049		0.1960	

*p<0.05, * applied Mann-Whitney U test

applied Wilcoxon matched pairs test

Table 3: Comparison of test and control sites with respect bleeding index scores at baseline, 15days and 1 month by Mann-Whitney U test and Wilcoxon matched pairs test

Sites	Baseline to 15 days		Baseline to 1 month		15days to 1 month	
	Mean difference	SD	Mean difference	SD	Mean difference	SD
Test site	1.20	0.41	1.98	0.57	0.78	0.45
Control site	1.00	0.00	1.68	0.53	0.68	0.53
Total	1.10	0.31	1.83	0.56	0.73	0.49
% of change in test site	48.65%# p=0.0007*		80.27%# p=0.0007*		61.58%# p=0.0007*	
% of change in control site	40.54%# p=0.0007*		68.11%# p=0.0007*		46.36%# p=0.0007*	
Z-value	-1.7951		-2.0644		-1.1902	
p-value	0.0727		0.0390*		0.2340	

*p<0.05, * applied Mann-Whitney U test

applied Wilcoxon matched pairs test

Table 4: Comparison of test and control sites with respect PPD scores at baseline, 15days and 1 month by Mann-Whitney U test and Wilcoxon matched pairs test

Sites	Baseline to 15 days (mm)		Baseline to 1 month (mm)		15days to 1 month (mm)	
	Mean difference	SD	Mean difference	SD	Mean difference	SD
Test site	0.87	0.52	2.00	0.76	1.13	0.74
Control site	0.93	0.46	1.13	0.74	0.20	0.56
Total	0.90	0.48	1.57	0.86	0.67	0.80
% of change in test site	14.77%# P=0.0022*		34.09%# P=0.0007*		22.67%# P=0.0022*	
% of change in control site	16.67%# P=0.0015*		20.24%# P=0.0015*		4.29%# P=0.9879	
Z-value	-0.3933		-2.8725		-3.3849	
p-value	0.6941		0.0041*		0.0007*	

*p<0.05, * applied Mann-Whitney U test

applied Wilcoxon matched pairs test

Table 5: Comparison of test and control sites with respect CAL scores at baseline, 15days and 1 month by Mann-Whitney U test and Wilcoxon matched pairs test

Sites	Baseline to 15 days (mm)		Baseline to 1 month (mm)		15days to 1 month (mm)	
	Mean difference	SD	Mean difference	SD	Mean difference	SD
Test site	0.80	0.56	1.87	0.64	1.07	0.70
Control site	1.20	0.56	1.60	0.63	0.40	0.83
Total	1.00	0.59	1.73	0.64	0.73	0.83
% of change in test site	20.69%# P=0.0033*		48.28%# P=0.0007*		34.78%# P=0.0022*	
% of change in control site	29.03%# P=0.0010*		38.71%# P=0.0007*		13.64%# P=0.0679	
Z-value	-1.8655		-1.1602		-2.7032	
p-value	0.0621		0.2460		0.0069*	

*p<0.05, * applied Mann-Whitney U test

applied Wilcoxon matched pairs test

Table 6: Comparison of test and control sites with respect *P gingivalis* scores at baseline, 15days and 1 month by Mann-Whitney U test and Wilcoxon matched pairs test

Sites	Baseline to 15 days (CFU/ml)		Baseline to 1 month (CFU/ml)		15days to 1 month (CFU/ml)	
	Mean difference	SD	Mean difference	SD	Mean difference	SD
Test site	35.27	19.50	59.47	19.23	24.20	9.82
Control site	24.07	6.51	53.87	14.90	29.80	13.37
Total	29.67	15.37	56.67	17.15	27.00	11.87
% of change in test site	52.3%# P=0.0007*		88.23%# P=0.0007*		75.31%# P=0.0007*	
% of change in control site	35.57%# P=0.0007*		79.61%# P=0.0007*		68.35%# P=0.0007*	
Z-value	-1.7171		-0.7475		-1.2883	
p-value	0.0860		0.4548		0.1977	

*p<0.05, * applied Mann-Whitney U test
applied Wilcoxon matched pairs test

DISCUSSION

The aim of the present study was to compare the clinical and microbiological benefits of routine scaling and root planing with adjunctive use of liquorice as a local drug delivery (LDD) agent in the treatment of chronic periodontitis. It decreases the subgingival bacterial flora and the clinical signs of periodontitis[8]. In cases wherein localized recurrent and/or residual probing depth of ≥ 5 mm with inflammation is still persisting, LDD may be used as an adjunct to SRP after conventional therapies.

Pharmacologically active phytochemicals useful for the prevention, treatment and maintenance of periodontal diseases have been extensively acknowledged. They may be tannins, flavonoids, terpenoids, alkaloids.[1] Antimicrobial activities of these have been found to be predominantly useful for periodontal diseases. Conventional antibiotic use has the disadvantage of low benefit to high risk as compared to herbal treatment which possess high benefit to low

risk ratio.[2] Natural products like liquorice are preferred because it is safe, economical and more effective.

Liquorice has been used extensively over the centuries for sore throat and cough in the Indian subcontinent where it is commonly known as "mulethi". The antimicrobial action of liquorice has been reported by several authors.[3,4] Chu Hong Hu et al., revealed that the extract of liquorice roots employed strong antimicrobial action against cariogenic bacteria.[5] It has been proposed that one of its main ingredients, glycyrrhizin, dose-dependently inhibits the glucosyl transferase activity of S. mutans , which is involved in the formation of insoluble glucans required in biofilm formation thus controlling periodontitis which is similar with our study results.[6] . When mixed with an acidulated phosphate-fluoride solution, glycyrrhizin reduces enamel solubility and increases fluoride uptake, most likely due to a surface-coating effect and its deposition in the porous structure of demineralized enamel , as reported by Gedalia et al.[7] Liquorice is an alkaline food with a protective effect in Gastro esophageal reflux disease (GERD) and peptic ulcers.[8] Sasakia H et al. demonstrated that liquorice extract completely inhibits P.gingivalis induced bone loss when delivered in either prophylactic or therapeutic regimens, suggesting results which are in accordance with our study.[9]

At baseline, the majority of the evaluated sites exhibited high levels of P gingivalis [figure 6]. The use of liquorice showed significant reduction in the prevalence of this pathogen in test site(7.93±3.28) compared to control site (13.80±3.08) with p-value 0.0269 at 15 days and 0.0002 at 1 month. Some in vitro and in vivo studies have demonstrated activity of liquorice against periodontal patho gens.[9,13] Proven anti-herpetic action of liquorice root extract has been confirmed and the mechanism maybe the inhibition of HSV attachment process through direct contact between virus and the extract. The HSV-1 was repressed by either directly inactivation of virus or anti-adhesive property of liquorice, which impedes the adhesion of HSV-1 to Vero, cells in vitro.[14]

There was no adverse effect seen in any of the patients treated with liquorice in the present study. The varied benefits of liquorice, such as affordability, easy availability, antibacterial and anti-inflammatory properties make liquorice a potential therapeutic agent in periodontal therapy.

One of the limitation of the study could be the shorter recall interval to check for the efficacy of the drug. But previous studies have proved initial higher efficacy of drug within 1 month followed by constant periodontal parameters in subsequent visits. Another limitation for the present study might be that higher efficacious techniques like PCR could have been performed in the study. Thus, liquorice gel can be administered as local drug delivery as an adjunct to scaling and root planning to improve the periodontal health.

CONCLUSION

The present study confirmed greater reduction of gingival index, bleeding index, probing pocket depth, clinical attachment level and P.gingivalis in the test group treated with scaling and root planing and liquorice when compared to the control sites treated with SRP alone. Subgingivally delivered liquorice as an adjunct to scaling and root planing in the treatment of chronic periodontitis showed anticipative results. Further research focusing on the in vivo and in vitro anti-inflammatory/antimicrobial effects of liquorice done on larger sample size is required for the better understanding of its specific role in the management of periodontitis.

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