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ORIGINAL RESEARCH ARTICLE

Efficacy and Safety of Tulsi Extract Mouthwash on Periodontal Health Status of Well-Controlled Type 2 Diabetes Mellitus Patients in India: A Concurrent Parallel Pilot Trial

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ABSTRACT

Background: One of the main risk factors for periodontitis is Diabetes Mellitus (DM). In patients with DM, maintaining optimal oral hygiene is crucial to managing the advancement of periodontitis. The adverse effects of chlorhexidine limit its long-term use. Tulsi is gaining more popularity due to its anti-inflammatory and antimicrobial properties. Studies related to Tulsi mouthwash in Type 2 DM patients are lacking.

Objective: The aim of the study is to evaluate the efficacy of 6% Tulsi extract and 0.2% chlorhexidine mouthwashes on the periodontal health status of well-controlled type 2 DM patients.

Materials and Methods: The present study is a concurrent, parallel, randomized controlled trial. A total of 38 participants aged 45–55 years were randomly allocated to two different groups. Group A and B participants were treated with 0.2% chlorhexidine and 6% Tulsi extract mouthwashes, respectively. The assessments of gingivitis (gingival Index), dental plaque (plaque Index), and periodontal pocket depth (Community Periodontal Index) were done at baseline and on the 15th day. An unpaired t-test was used to compare the clinical conditions of the two groups.

Results: Both groups have individually shown a statistically significant reduction in gingivitis, dental plaque, and periodontal pocket depth. When both groups were compared on the 15th day, chlorhexidine mouthwash showed a statistically significant reduction in PI scores (Group A 0.79 vs. Group B 0.86).

Conclusion: Tulsi mouthwash is as efficacious as chlorhexidine in reducing dental plaque, gingivitis, and pocket depth among well-controlled Type 2 DM patients.

1. INTRODUCTION

Diabetes Mellitus (DM) is one of the earliest metabolic disorders mentioned in the ancient manuscript. The characteristic feature of DM is hyperglycemia, resulting from impaired secretion or activity of insulin.[1] According to the International Diabetes Federation, India currently has 77 million diabetic subjects. By 2045, the figure is expected to rise to 134 million.[2]

Individuals diagnosed with type 2 DM are vulnerable to both systemic and oral complications. Epidemiological investigations have consistently proven the bidirectional relationship between periodontal infection and DM.[3] The magnitude of the risk for periodontitis depends on the level of glycemic control and serum high-sensitivity C-reactive protein (hsCRP) levels.[4] The formation of Advanced Glycation End Products also plays a key role in the upregulation of periodontal inflammation, the production of ROS, and vascular endothelial injury.[5,6] Type 2 DM individuals with severe periodontal disease also show increased levels of inflammatory mediators such as interleukin-6, tumor necrosis factor-α, CRP, and MMPs. The effectiveness of periodontal therapy in lowering HbA1C percentages in individuals with type 2 DM has been demonstrated by numerous studies.[7-9]

Non-surgical therapy in type 2 DM patients has been shown to be effective in controlling periodontal disease progression.[10,11] Chlorhexidine in the

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form of mouthwash is considered as a gold standard adjunctive treatment in the management of periodontal disease due to its substantivity and broad-spectrum antibacterial activity.[12-14] Unfortunately, the extensive and continuous usage of 0.2% chlorhexidine mouthwash is restricted due to its adverse effects. The most reported side effects include brown staining of teeth, alteration of taste, soreness, irritation, mild desquamation, ulceration, and erosion of the oral mucosa.[12] Recently, in developing nations, complementary and herbal medicines have gained more popularity due to their anti-inflammatory and antimicrobial properties, minimal side effects, and most affordable treatment protocols.

Among all medicinal plants, Tulsi (*Ocimum sanctum*) is considered an "elixir of life" that is without equal for both its medicinal and spiritual properties.[15] It has been observed that tulsi leaves exert hypocholesterolemic, hypotriglyceridemic, and hypophospholepidemic effects in animal and human studies.[16,17] Recent studies have shown anti-oxidative, antihyperglycemic, anti-inflammatory, and analgesic effects of Tulsi with no genotoxic or organotoxic effects among diabetic patients.[17,18] Thus, Tulsi can act as a common therapeutic agent in the maintenance of periodontal health as well as DM.

A thorough literature search revealed that there are no studies showing the effect of Tulsi mouthwash on the periodontal health status of diabetic patients. This study is an attempt to evaluate the efficacy of Tulsi mouthwash in the maintenance of periodontal health status after scaling among well-controlled diabetic patients aged 45–55 years in Davangere City. Hence, we hypothesize a difference in the efficacy and safety of 6% Tulsi extract and 0.2% chlorhexidine mouthwash on gingivitis, plaque, and periodontal pocket depth among well-controlled type 2 DM patients.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

A randomized concurrent parallel-group trial was conducted for a period of 4 months, from August to November 2018. The protocol was approved by the Ethical Review Board of Bapuji Dental College and Hospital, Davangere. The trial was registered in the Clinical Trial Registry of India with trial registry number CTRI/2019/01/016960. A list of all the registered, well-controlled type 2 diabetic patients was taken from two diabetic clinics. Steps involved in the study are comprehensively represented in the form of flow chart (Figure 1).

2.2. Eligibility Criteria

Subjects with HbA1c <7.0%.[19,20] well-controlled diabetes at least for 3 months, and the presence of at least 20 scorable teeth in the oral cavity with a mean gingival index (GI) score of at least 1.0 (Loe-Silness Gingival Index) and a mean plaque index (PI) score of at least 1.5 (Sillness and Loe Plaque Index)[21] and superficial pocket depth. Subjects with a history of hypersensitivity, systemic conditions, and diseases other than diabetes affecting salivary flow rate, were unable to comply with the study appointment schedules were excluded from the study. Subjects with a habit of tobacco and alcohol consumption were also excluded from the study.

2.3. Sample Size Estimation

The sample size estimation was done using G*Power software for Windows version 3.1.9.2. After considering an anticipated dropout rate of 20%, type I error ($\alpha = 0.05$), power of the study ($1-\beta = 0.20$), and medium effect size (0.5), a total of 38 well-controlled type 2 DM patients, aged 45–55 years, who fulfilled the eligibility criteria were randomly selected.

2.4. Preparation of Tulsi Extract and Mouthwash[22]

Sundried black Tulsi leaves (Shyama tulsi) were selected and prepared into a fine powder. Three hundred grams of Tulsi powder were then macerated with 100% ethanol, and Whitman filter paper was used to get a clear filtrate. A clear filtrate was subjected to a temperature of <60° Celsius to obtain a solid residue. The final residue of 18 g of extract was obtained by dissolving 300 g of Tulsi powder in 1 l of ethanol, which yielded 6% w/v. Three hundred grams (300 g) of 6% Tulsi extract was dissolved in 10 l (10000 mL) of distilled water and gently stirred with a stirrer till it was completely dissolved. The prepared mouth rinse was transferred to plastic bottles.

2.5. Random Allocation

Computer-generated random numbers were used to allocate the mouthwashes to two groups. The concealed randomization method was followed. Pre-coded bottles similar in shape and size containing mouth rinses were given to participants by a person who was not directly involved in the study. Group A received 0.2% chlorhexidine mouthwash, and Group B received 6% Tulsi mouthwash.

2.6. Intervention Details

After scaling and root planning, all the selected participants, along with the mouthwashes, received an oral hygiene kit containing a soft-bristled toothbrush and toothpaste. During the trial phase, all the participants brushed their teeth twice a day for 4–5 min using the modified Bass technique. They were strictly instructed to refrain from any other oral hygiene measures, such as flossing and interdental brushing, for the next 15 days. All the participants swished their mouths using 10 mL of their respective mouthwash for 1 min, twice daily in the morning and night after toothbrushing.[23] Soon after the rinse, they were not allowed to eat or drink anything for 30 min.

2.7. Clinical Examination

A single examiner, who had received training and calibration to record clinical findings, examined all the participants. The clinical examination was conducted on hospital premises using artificial lighting. The participants were asked to sit comfortably on a chair, and the examiner was standing on the right side of the patient during the examination of gingivitis (Loe and Sillness GI 1964), dental plaque (Sillness and Loe PI 1967), and periodontal pocket depth (Community Periodontal Index, WHO 2013).[23]

2.8. Assessment of Safety and Other Side Effects of the Mouth Wash

A predesigned questionnaire was given to all the participants to rate their satisfaction, tolerance, and any adverse events during the trial phase.

2.9. Statistical Analysis

Data analysis was done using SPSS software version 20.0 (IBM Corp., Armonk, N.Y., USA). The significance level was fixed at $P < 0.05$. paired t-test (intragroup comparison) and unpaired t- test (intergroup comparison) were used to assess the gingivitis, dental plaque, and periodontal pocket depth at baseline and on the 15th day. A Chi-square test was applied to find out the safety and any side effects of mouthwashes.

3. RESULTS

A total of 38 diabetic patients participated in our study, of whom 58% were 40–50 years of age, and the remaining 42% were in the age

range of 51–55 years. No statistically significant difference in clinical parameters was observed between 0.2% CHX and 6% Tulsi mouthwash at baseline evaluation: GI ($P = 0.11$), PI ($P = 0.69$), and percentage of bleeding sites ($P = 0.40$) [Table 1]. Individually, both mouthwashes showed a statistically significant reduction from baseline to follow-up. When compared between the groups at post-intervention, 0.2% CHX mouthwash showed a statistically significant reduction in PI scores (0.79 ± 0.27 vs. 0.86 ± 0.15) than 6% Tulsi mouthwash ($P = 0.03$) [Table 2]. All the participants used mouthwashes as per the instructions given by the researcher. Few subjects did not use the allocated mouthwashes on some days due to their busy schedules; some subjects said that they had forgotten to use them on a particular day. Few subjects reported a bitter taste for Tulsi mouthwash, and all the participants in Group A reported well tolerance to the 0.2% CHX mouthwash ($P = 0.02$) [Table 3].

4. DISCUSSION

This is the first trial to investigate 6% Tulsi extract in the form of mouthwash for its efficacy on periodontal inflammation among well-controlled type 2 DM patients. The results indicate a statistically significant reduction in gingivitis, dental plaque score, and periodontal pocket depth within the study groups. Our study's findings indicate both mouthwashes are comparable in their ability to reduce periodontal inflammation. The results of our investigation are consistent with a study by Gupta,[24] which revealed the effectiveness of Tulsi mouthwash in lowering gingivitis and plaque during a 15–30 day period. Numerous prior studies conducted by Kelm,[25] Singh[26] Gaur[27] Nadar[28] demonstrated the antigingival action of Tulsi when applied topically in various forms to treat periodontal disease. Major bioactive components found in Tulsi include linolenic acid (43-64%) and eugenol (71.3%), which can block the COX-2 and lipoxygenase pathways. The antigingivitis effect of Tulsi may be explained by the presence of these key ingredients.[18]

The antibacterial effectiveness of Tulsi against harmful oral pathogens has been tested by several *in vitro* and *in vivo* investigations. The growth of periodontal pathogens, including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Escherichia coli*, have been found to be inhibited by the alcoholic extract of Tulsi.[29] Tulsi contains essential oils and bioactive chemicals that can transform silver ions into silver nanoparticles.[30] Some of the antibacterial properties of Tulsi might be explained by this mechanism. Hosadurga *et al*.[31] observed a decrease in periodontal pocket depth when experimental rats were treated with 2% Tulsi gel. When Gaur[27] used 4% Tulsi extract as a subgingival irrigant, there was a significant reduction in plaque scores and bleeding on probing. Similarly, in our study, 6% of Tulsi mouthwash showed a significant reduction in dental plaque scores and periodontal pocket depth over a period of 15 days. This demonstrates unequivocally that Tulsi has antimicrobial properties when administered for brief periods of time.

Periodontal disease is a complex disease as a result of the interplay between bacterial infection and the host's response to its challenge. It is best managed by controlling immunoinflammatory mediators. Studies have shown modulation in both humoral and cell-mediated immune responses produced by the flavonoids present in the extract of Tulsi.[32,33] This action of Tulsi promises its efficiency in the management of periodontal disease. A meta-analysis of RCTs on the hypoglycemic effect of Tulsi shows a significant reduction in fasting blood glucose.[17] Evidence also suggests the synergistic interaction of bioactive components of Tulsi targeting metabolic and cellular pathways.[34,35] Therefore, there is a possibility that Tulsi extract could be a common treatment for both DM and periodontitis.

In our study, 0.2% chlorhexidine mouthwash showed a statistically significant reduction in plaque score, gingivitis, and periodontal pocket depth from baseline to the 15th day ($P < 0.05$). Similar results were seen in previous studies.[24,27,28,31] Even at shorter durations, the evidence unmistakably points to the antibacterial properties of chlorhexidine mouthwash.

In the present study, a 6% concentration of Tulsi extract was used as mouthwash. At 6% concentration, a maximum (22 mm) zone of inhibition was observed against periodontal pathogens among the ten different concentrations. The participants were instructed to use 10 mL of the assigned mouthwash for 1 min in undiluted form. The antimicrobial effect of mouthwash is dose dependent, and 10 mL (20 mg) for 30–60 s is considered the optimal dose. This way, the volume and duration of rinsing were standardized. A few participants in our study reported a bitter taste for Tulsi mouthwash. This could be attributed to the astringent properties of Tulsi, and the concentration at which Tulsi extract was prepared. The study was conducted on a small sample, requiring greater caution in extrapolating the study findings as the sample was drawn from two diabetic clinics in Davanagere city. Further long-term studies are needed to find out the efficacy of Tulsi in the management of periodontitis among type 2 DM patients.

5. CONCLUSION

The antibacterial and antigingivitis properties of Tulsi mouthwash are comparable to the benchmark mouthwash (0.2% chlorhexidine). Tulsi is inexpensive, culturally acceptable, and often used in traditional medicine for a wide spectrum of diseases, making it a viable substitute for chemical-based mouthwashes.

6. ACKNOWLEDGMENT

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7. AUTHORS' CONTRIBUTIONS

All the authors contributed equally in design and execution of the article.

8. FUNDING

Nil.

9. ETHICAL APPROVALS

The protocol was approved by the Ethical Review Board of Bapuji Dental College and Hospital, Davangere. The trial was registered in the Clinical Trial Registry of India with trial registry number CTRI/2019/01/016960.

10. CONFLICTS OF INTEREST

Nil.

11. DATA AVAIBALITY

This is an original manuscript, and all data are available for only research purposes from principal investigators.

12. PUBLISHERS NOTE

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Table 1: Comparison of clinical parameters at baseline between groups

Variables	0.2% CHX mouth rinse (*n*=19)	6% Tulsi mouth rinse (*n*=19)	*P*-value
GI (Mean±SD)	1.15±0.16	1.17±0.11	0.11
PI (Mean±SD)	1.57±0.08	1.66±0.12	0.69
PPD (Mean±SD)	1.39±0.12	1.41±0.17	0.40

SD: Standard deviation, N: Number participants, *P*: Probability value

Table 2: Comparison of clinical parameters at 15th day between groups

Groups	0.2% CHX mouth rinse (*n*=18)	6% Tulsi mouth rinse (*n*=17)	*P*-value
GI (Mean±SD)	0.74±0.19	0.69±0.18	0.68
PI (Mean±SD)	0.79±0.27	0.86±0.15	0.03*
PPD (Mean±SD)	1.27±0.16	1.30±0.23	0.72

*Statistically significant, SD: Standard deviation, N: Number participants, *P*: Probability value

Table 3: Participants' satisfaction and side effects after using mouth rinses for 2 weeks

	6% Tulsi mouth rinse *n* (%)	0.2% CHX mouth rinse *n* (%)	*P*-value
Satisfaction			
Not good-not bad (so so)	7 (41)	5 (28)	0.52
Mostly satisfied	8 (47)	8 (44)	
Fully satisfied	2 (12)	5 (28)	
Total			
N	17 (100	18 (100)	
Side effects			
Bitter taste	7 (41)	3 (17)	0.26
Staining	1 (6)	0	
Bad odour	1 (6)	0	
Other	1 (6)	0	
No side effects	7 (41)	15 (83)	
Total			
N	17 (100)	N – 18 (100)	
Tolerance			
Easy	12 (74)	18 (100)	0.02*
Moderate	5 (26)	0	
Total			
N	17 (100)	18 (100)	

*Statistically significant, SD: Standard deviation, N: Number participants, *P*: Probability value

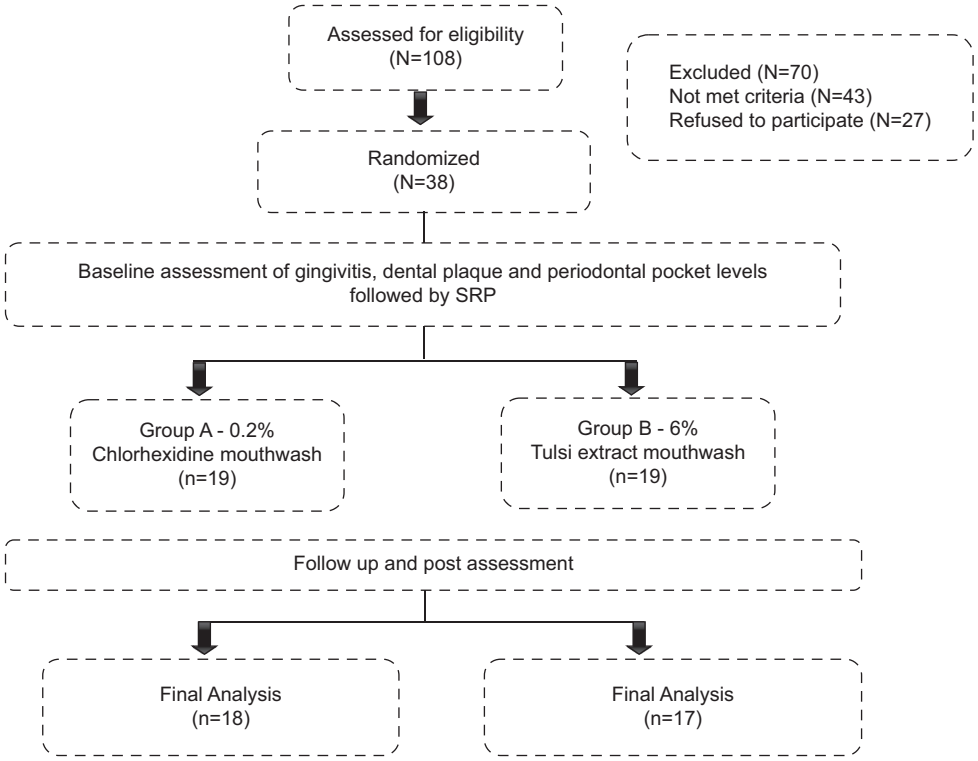

Figure 1: Flow chart of methodology