Determination of efficacy of curcumin and Tulsi extracts as local drugs in periodontal pocket reduction: A clinical and microbiological study

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INTRODUCTION

The usage of antimicrobial agents such as tetracycline, metronidazole, and minocycline[4,5] as local drug delivery (LDD) agents in the treatment of periodontitis has posed limitations due to development of bacterial resistance, high cost, unavailability, thus indicating need of safer and economic alternatives. Certain herbal agents have been found to be better alternatives in this regard.

Curcumin derived from herb Curruma longa (Haldi/Turmeric) has proven its anti-inflammatory,[6] antimicrobial action when used alone and also in combination with other antibiotics.[5,6] It is found effective in the treatment of periodontitis when used in the form of lozenges,[6] topical application,[5] LDD agent into periodontal pocket and mouth wash.[6] The three curcuminoids, i.e., curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin, as well as volatile oils (turmerone, atlantone, and zingiberone), sugars, proteins, and resins present in turmeric modulate the inflammatory response by down-regulating the activity of cyclooxygenase-2, lipoxygenase, inducible nitric oxide synthase enzymes, and inhibiting the production of the inflammatory cytokines.[9] Curcumin inhibits the growth of Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, and Treponema denticola almost completely at very low concentrations of 10–15 µg/ml, and possesses antibacterial activity against periodontal pathogens.[10] Its plasma half life of around 6.77 h on oral administration of 10–12 mg curcumin makes it more suitable as local drug rather than for oral consumption.[11]

The other commonly used herb in mouthwashes is Ocimum sanctum (Tulsi) which is employed because of its anti-inflammatory and broad spectrum antimicrobial action.

Background: The aim of our study is to assess the effectiveness of Curcumin and Tulsi in the control of periodontal parameters when delivered in the form of local drug delivery (LDD) agents. Methods: Curenex gel® and Tulsi gel were used as the two LDD agent. A split mouth randomized clinical trial was carried out in 15 patients. Three sites in different quadrants were assigned treatment modality of scaling and root planing (SRP) alone, SRP with LDD of curcumin and SRP with LDD of Tulsi extract, respectively. Clinical parameters Probing Pocket Depth, Clinical Attachment Level, Plaque Index, Gingival Index, and modified Sulcus Bleeding Index were recorded on baseline followed by LDD with extracts in the assigned group. The parameters were recorded at baseline and on 30th day postoperatively. Unpaired and Paired-t test were used for intergroup and intragroup comparison of recorded clinical and microbiological parameters. Results: All the treatment modalities showed statistically significant reduction in clinical and microbiological parameters on intragroup comparison. Intergroup comparison showed statistically significant reduction in Plaque Index in curcumin group and BAPNA assay in Tulsi group when compared to SRP. Conclusion: Both the herbs were effective in improving periodontal parameters and may develop as an alternative to currently used LDD agents in near future.

Key words: Curcumin, local drugs, microbiological study, Tulsi

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Submitted: 08-Mar-2020
Revised: 18-Aug-2020
Accepted: 15-Sep-2020
Published: 03-May-2021

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its antimicrobial, anti-inflammatory, wound healing, and immunoregulatory properties. The essential oils contained in Tulsi are eugenol and methyl eugenol. The caracrol, Tetrpe, bcaryophyllene, and sesquiterpene b-caryophyllene present in Tulsi contribute to antibacterial activity of Tulsi extract and are FDA approved.[13] Till date, there have been no studies reporting the effectiveness of Tulsi as a LDD agent in the treatment of periodontal pockets. Hence, in this study, Tulsi extract was included for assaying its efficacy as LDD agent and was compared to that of curcumin which is already in use, in the treatment of periodontal pockets.

METHODS

The present study was conducted after clearance from institutional ethical committee. Curcumin has already been available in its effective concentration as Curenext gel®. However, at what concentration does Tulsi extract exhibit antimicrobial efficacy when delivered as LDD agent is unknown. Hence, the study was carried out in two phases. Phase I was meant to determine the Minimum Inhibitory Concentration of Tulsi extract for its antimicrobial action which could be later used in Phase II of the study as LDD agent. Phase II was meant for the employment of Tulsi extract and curcumin gel (Curenext gel®) as LDD agents in the treatment of periodontal pockets.

Phase I

Different concentrations of Tulsi extracts (2%, 4%, 6%, 8%, and 10%) were prepared[13] using distilled water in gel base and tested for their effectiveness on periodontal pathogens, i.e., Aggregatibacter actinomycetemcomitans, P. gingivalis, and Tannerella forsythia with time kill curve method. The results showed that antimicrobial action was profound when Tulsi extract reached the concentration of 10%. Thus, 10% Tulsi extract was employed in the study as LDD agent.

Phase II

Fifteen systemically healthy controls (5 males and 10 females) in the age range of 37–57 years, with pocket depth ≥5 mm and ≤8 mm at three nonadjacent sites in different quadrants of the mouth and with at least 20 teeth remaining were selected. Informed consent was obtained. Patients under systemic antibiotic treatment, antibiotic mouthwash, orthodontic treatment, prosthesis, pregnant or lactating females, or allergic to the drugs used in the study were excluded from the study.

Patients were subjected to a split-mouth randomized clinical trial. Maxillary and mandibular first molars were selected for study to obtain homogeneity in study sites; 45 out of 60 sites were selected for the study by lottery method.

The study sites were divided in to three groups, each group comprising of 15 sites; Group 1 sites were subjected to Scaling and Root Planing (SRP) alone, Group 2 sites to LDD of curcumin extract in periodontal pocket in adjunct to SRP and Group 3 sites to LDD of Tulsi extract in periodontal pocket in adjunct to SRP.

Probing pocket depth (PPD) and clinical attachment Level (CAL) were recorded at six sites around each tooth using the stent that helped reproduce unbiased measurements during 30th day posttreatment. GI (Loe and Silness, 1963),[18] PI (Silness and Loe, 1964),[19] and mSBI (Mombelli et al., 1987)[20] were recorded.

Subgingival plaque samples were collected with the help of sterile curettes[21] [Figure 1] and transported to laboratory in Reduced Transport Fluid for N-benzoyl-L-arginine-p-nitroanilide (BAPNA) assay [Figure 2]. BAPNA assay was used to study the effectiveness of LDD agents in reducing the red complex bacteria by measuring trypsin like enzyme activity of red complex bacteria and BAPNA assay can easily measure and quantify the red complex bacteria activity present in plaque samples by measuring trypsin like enzyme level into units of nano moles of product per minute per milligram of dental plaque wet weight[22,23] [Figure 3].

After collection of plaque samples, full mouth single sitting SRP was performed without pre rinsing with antimicrobial mouth wash. The sites allotted to group 1 were treated with SRP alone [Figure 4]. Sites belonging to Group 2 were treated with SRP followed by LDD with Curenext gel® containing Curcumin and sites belonging to Group 3 were treated with SRP followed by LDD with Tulsi extract. The pocket was sealed with periodontal dressing following therapy[24] [Figures 5 and 6]. Patients were advised to brush by modified Bass technique and refrain from mouth wash or any drug during entire study duration.

The sites were reassessed for clinical and microbiological parameters at 30th day posttreatment.

Statistical analysis

The data were analyzed using SPSS for Windows, version 17.0 Trial. The prevalence of an outcome variable along with 95% confidence limits was calculated. Assuming that PPD, CAL, PI, GI, mSBI, and BAPNA assay have followed a normal distribution and normality of data for the three groups, a parametric test was carried out.

Intergroup comparisons of data from baseline to 30th day posttreatment were carried out using Unpaired-‘t’ test and the intragroup comparisons of data from baseline to 30th day post treatment were carried out using Paired-‘t’ test.

RESULTS

Tables 1-3 depict statistically significant reduction in PPD, CAL, PI, GI, mSBI, and BAPNA assay with P < 0.001 for all the parameters in all the groups.

Table 4 shows greater reduction in the values of PPD, CAL, GI, mSBI, and BAPNA assay in Group 2 than in Group 1 on 30th day posttreatment. However, difference was statistically insignificant.

The only statistically significant difference was found to be in PI at 30th day posttreatment (P = 0.007) in Group 2 implying that LDD with Curcumin is more effective in plaque reduction compared to SRP alone.

Table 5 shows that except for GI which showed no difference, greater reduction was found in the values of PPD, CAL, PI, and mSBI assay in Group 3 than in Group 1 on 30th day posttreatment. However, difference was statistically not significant.
The mSBI showed considerable reduction in Group 3 reaching the $P = 0.07$ suggesting that LDD of 10% Tulsi gel as an adjunct to SRP has a better potential to reduce bleeding on probing than SRP alone. This could be attributed to the finding that there was statistically significant reduction in BAPNA values of Group 3 ($P = 0.001$) in comparison to Group 1, implying significant reduction in the levels of red complex bacteria (responsible for bleeding on probing) at sites treated by LDD with Tulsi gel. This denotes that LDD with Tulsi gel as an adjunct to SRP is more effective in reducing red complex bacteria levels than SRP alone.
**Table 1: Intragroup assessment of improvements in periodontal parameters from baseline to 30th day postintervention in Group 1**

| Parameters | Sampling stages | Mean±SD     | LOS  |
|------------|-----------------|-------------|------|
| PPD        | Baseline        | 5.12±0.46   | 0.001*|
|            | 30th day        | 4.30±0.61   |      |
| CAL        | Baseline        | 4.75±0.32   | 0.001*|
|            | 30th day        | 4.10±0.57   |      |
| PI         | Baseline        | 2.25±0.41   | 0.001*|
|            | 30th day        | 1.01±0.30   |      |
| GI         | Baseline        | 1.66±0.26   | 0.001*|
|            | 30th day        | 0.70±0.25   |      |
| mSBI       | Baseline        | 1.80±0.41   | 0.001*|
|            | 30th day        | 1.13±0.74   |      |
| BAPNA      | Baseline        | 3.54±1.21   | 0.001*|
|            | 30th day        | 1.52±0.93   |      |

*Significant (P<0.05). LOS – Level of significance; PPD – Probing pocket depth; CAL – Clinical attachment level; PI – Plaque index; GI – Gingival index; mSBI – Modified sulcus bleeding index; BAPNA – N-benzoyl-L-arginine p-nitroanilide; SD – Standard deviation

**Table 2: Intragroup assessment of improvements in periodontal parameters from baseline to 30th day postintervention in Group 2**

| Parameters | Sampling stages | Mean±SD     | LOS  |
|------------|-----------------|-------------|------|
| PPD        | Baseline        | 5.39±0.56   | 0.001*|
|            | 30th day        | 4.28±0.73   |      |
| CAL        | Baseline        | 4.88±0.73   | 0.001*|
|            | 30th day        | 4.06±0.68   |      |
| PI         | Baseline        | 2.15±0.52   | 0.001*|
|            | 30th day        | 0.68±0.31   |      |
| GI         | Baseline        | 1.58±0.29   | 0.001*|
|            | 30th day        | 0.65±0.29   |      |
| mSBI       | Baseline        | 1.73±0.45   | 0.001*|
|            | 30th day        | 0.73±0.59   |      |
| BAPNA      | Baseline        | 3.54±1.69   | 0.001*|
|            | 30th day        | 1.01±0.07   |      |

*Significant (P<0.05). LOS – Level of significance; PPD – Probing pocket depth; CAL – Clinical attachment level; PI – Plaque index; GI – Gingival index; mSBI – Modified sulcus bleeding index; BAPNA – N-benzoyl-L-arginine p-nitroanilide; SD – Standard deviation

**Table 3: Intragroup assessment of improvements in periodontal parameters from baseline to 30th day postintervention in Group 3**

| Parameters | Sampling stages | Mean±SD     | LOS  |
|------------|-----------------|-------------|------|
| PPD        | Baseline        | 5.15±0.41   | 0.001*|
|            | 30th day        | 4.01±0.58   |      |
| CAL        | Baseline        | 4.79±0.41   | 0.001*|
|            | 30th day        | 3.92±0.44   |      |
| PI         | Baseline        | 1.96±0.50   | 0.001*|
|            | 30th day        | 0.83±0.40   |      |
| GI         | Baseline        | 1.61±0.26   | 0.001*|
|            | 30th day        | 0.71±0.31   |      |
| mSBI       | Baseline        | 1.86±0.35   | 0.001*|
|            | 30th day        | 0.66±0.61   |      |
| BAPNA      | Baseline        | 3.50±1.42   | 0.001*|
|            | 30th day        | 0.46±0.41   |      |

*Significant (P<0.05). LOS – Level of significance; PPD – Probing pocket depth; CAL – Clinical attachment level; PI – Plaque index; GI – Gingival index; mSBI – Modified sulcus bleeding index; BAPNA – N-benzoyl-L-arginine p-nitroanilide; SD – Standard deviation

Table 6 depicts greater reduction in the values of PPD, CAL, mSBI, and BAPNA assay (P = 0.07) in Group 3 than Group 2, whereas greater reduction in PI and GI was more evident in Group 2 than Group 3 on 30th day post treatment. Thus, denoting Tulsi extract possessed a better antimicrobial property and Curcumin a better anti plaque property.
DISCUSSION

On extensive search in literature for applications of Tulsi extract in periodontal treatment, no reports were available concerning the use of Tulsi extract as a LDD agent as an adjunct to SRP. However, these evidences of clinical benefits of Tulsi extract as mouthwash certainly denote its possible usefulness as LDD agent. Hence, this study was undertaken to investigate the effectiveness of Tulsi extract as LDD agent. The present study compared the effectiveness of curcumin and Tulsi extracts in the control of periodontal parameters when employed in the form of LDD.

As shown in Table 1, sites treated with SRP alone showed statistically significant reduction of mean values from baseline to 30 days’ postoperative that was in accordance with other researches by Cugini et al.,[23] which showed significant reduction in clinical parameters and periodontal pathogens by checkerboard DNA-DNA hybridization; similarly Yang et al.,[24] by TaqMan real-time PCR and Dhalla et al.,[25] by BANA test showed reduction in periodontal pathogens after SRP alone; thus, denoting that SRP itself is effective in reducing periodontal parameters.

Table 2 depicts significant reductions in periodontal parameters with curcumin gel as LDD agent in adjunct to SRP, which is akin to results of several other studies conducted by Varghese et al.,[26] which showed significant reduction in PD, PI, and CAL at sites on intrasulcular application of curenext gel when compared to the ornidazole gel group. The study conducted by Hugar et al.,[27] and Anitha et al.,[28] shows similar results at sites where curcumin gel was placed in periodontal pocket as LDD in comparison to sites where chlorhexidine gel was used. Gottumukkala et al.,[29] also found significant reduction in all clinical parameters, microbial colony counts, and BANA test scores from baseline at sites where LDD with curcumin extract was performed in comparison to LDD with chlorhexidine. Thus, denoting that curcumin in adjunct to SRP is found to be as effective as ornidazole gel and chlorhexidine gel which are proven effective LDD agents.

Table 3 depicts significant reductions in periodontal parameters with Tulsi extract as LDD in adjunct to SRP. The present study being the first of this kind, there are no similar studies available for comparison, thus leaving scope for more of such studies. Gupta et al.,[30] in their study compared the effectiveness of Tulsi extract with gold standard chlorhexidine on PI and gingival inflammation concluded that Tulsi extract was equally effective as 0.12% chlorhexidine mouthwash. Hosamane et al.,[31] in their study found that Tulsi extract was effective in inhibiting growth of P. intermedia and F. nucleatum in vitro, they used Tulsi extract as mouth wash and reported statistically significant reduction in plaque score in groups where Tulsi was used as mouth wash in comparison to sterile water and showed similar reduction in plaque scores when compared to 0.2% chlorhexidine mouthwash indicating that Tulsi is as effective antiplaque agent as chlorhexidine mouthwash which is known for its antiplaque activity. These studies compared and concluded that Tulsi as mouthwash is as effective as chlorhexidine mouthwash; thus, giving scope for the use of Tulsi as LDD agent.

Intergroup comparisons [Table 4] for changes in periodontal parameters and BAPNA assay values between SRP and LDD with curcumin gel imply greater reduction, though statistically insignificant, in the values of PPD, CAL, GI, mSBI, and BAPNA assay on 30th day post treatment in Curcumin group than SRP alone. Although statistically insignificant, curcumin certainly offers an additional advantage over SRP. Significant reduction in plaque scores following LDD with curcumin extract indicates that it has additional benefit in plaque reduction over SRP alone. In a study conducted by Anuradha et al.,[32] and Raghava et al.,[33] statistically significant reduction in periodontal parameters have been reported on LDD with curcumin.

Intergroup comparisons [Table 5] for changes in periodontal parameters and BAPNA assay values between sites treated by SRP alone and those by LDD with Tulsi extract showed improvements in all parameters with latter group, though statistically insignificant but it implies that Tulsi extract as an LDD agent certainly offers an additional benefit to SRP.

Statistically significant reduction was noted only with mSBI (P = 0.07) and BAPNA assay values in the group treated with Tulsi extract as compared to those treated by SRP alone, indicating better efficacy of Tulsi extract in reducing red complex bacteria and thereby reducing gingival bleeding. No similar studies/reports are available in literature that could support these findings. Hence, this mechanism needs to be elucidated further by more of such studies.

Intergroup comparisons [Table 6] of effectiveness of curcumin gel (Group 2) and Tulsi (Group 3) extract in improving periodontal parameters on 30th day postoperatively showed that reductions in PPD, CAL, mSBI, and BAPNA assay were greater when Tulsi was used as LD. The reduction of BAPNA assay value (P = 0.07) is suggestive of Tulsi extract having better efficiency in reducing red complex bacteria than curcumin gel. The reduction in PI and GI, though statistically insignificant, was found to be more when curcumin gel was used as LD suggesting its higher effect in reducing plaque, which ultimately reduced gingival inflammation. Thus, it can be concluded from our study that both the herbs are equally effective in reducing periodontal parameters.

CONCLUSION

All the treatment modalities are effective in periodontal pocket reduction and improve the clinical and microbiological parameters from baseline.

Significant improvement in mean plaque scores at sites where LDD with curcumin gel was performed indicates that curcumin exerts additional plaque control effect than SRP alone. Significant improvement in BAPNA assay values and mSBI scores in group where LDD with Tulsi extract was performed denotes that Tulsi extract is effective in reducing red complex bacterial count and resultant bleeding in periodontal pocket.

Curenext® gel and Tulsi extract are equally effective in controlling clinical and microbiological parameters. Small sample size and short duration were limitations to our study. However, this study opens door to further researches in finding a suitable herbal remedy to periodontitis.
Financial support and sponsorship Nil.

Conflicts of interest There are no conflicts of interest.

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