Original Research Article

Evaluation of the efficacy of 2% *Ocimum sanctum* gel in the treatment of experimental periodontitis

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Abstract

Introduction: One of the options for the treatment of periodontitis is local drug delivery systems (LDD). Tulsi (*Ocimum sanctum*), a traditional herb, has many uses in medicine. It could be a suitable agent as LDD for the treatment of periodontitis. **Aim:** The aim was to formulate, evaluate the anti-inflammatory activity; assess duration of the action and the efficacy of 2% tulsi (*O. sanctum*) gel in the treatment of experimental periodontitis in Wistar Albino rat model. **Settings and Design:** Thirty six Wistar albino rats were randomly assigned to 3 groups. Periodontitis was induced using ligature model. Group 1-control; Group 2-Plain gel and Group 3-2% tulsi (*O. sanctum*) gel. **Materials and Methods:** 2% tulsi (*O. sanctum*) gel were prepared. The anti-inflammatory activity and duration of action were assessed. Silk ligature 5-0 was used to induce periodontitis. Gingival index (GI) and probing pocket depth were measured. Treatment was done. The rats were sacrificed. Morphometric analysis was done using Stereomicroscope and ImageJ software. **Statistical Analysis Used:** ANOVA followed by Bonferroni's test, Wilcoxon's test for intergroup comparison, Mann-Whitney test for *P* value computation was used. The observations are mean ± standard deviation and standard error of the mean. $P < 0.01$ as compared to control was considered as statistically significant. **Results:** 2% tulsi (*O. sanctum*) gel showed 33.66% inhibition of edema and peak activity was noted at 24 h. There was statistically significant change in the GI and probing pocket depth. Morphometric analysis did not show any significant difference between groups. No toxic effects were seen on oral administration of 2000 mg/kg of Tulsi extract. **Conclusions:** 2% tulsi (*O. sanctum*) gel was effective in the treatment of experimental periodontitis.

Key words: Anti-inflammatory activity, experimental periodontitis, local drug delivery, morphometric analysis, tulsi gel

INTRODUCTION

The Babylonians, Sumerians, and Assyrians had suffered from periodontal disease and used clay tablets for gingival massage along with various herbal medicines. The *Susrutha Samhitha* and the *Charaka Samhitha*, the medical works of ancient India, contain numerous descriptions of severe periodontal disease with loose teeth and purulent discharge from the gingiva or gums. Chronic periodontitis is a destructive disease that affects the supporting structures of the teeth namely, periodontal ligament, cementum, and alveolar bone.[1] It may develop in any age group, but is most common in adults. The prevalence and severity of the disease increase with age. The number of teeth affected may vary, and the rate of progression also varies. Bacteria accumulate around the teeth in the form of biofilm and initiate the disease.[2] The main clinical features of chronic periodontal disease include supra and subgingival plaque accumulation (associated with calculus formation), gingival inflammation, bleeding on probing, periodontal pocket formation, attachment loss, and alveolar bone loss. If treatment is not given, the disease progresses and results in tooth loss.[3]

Mechanical instrumentation is the first treatment of choice; however, remnants of plaque and calculus left behind can cause the treatment to fail. Complete removal of plaque and calculus is difficult in deep pockets. The remnants of plaque and calculus can cause failure in periodontal treatment.[4] The drugs can act as valuable adjuncts to treat plaque-associated periodontal diseases. Many studies have addressed the need for the local application of antimicrobial agents to the subgingival area for the treatment of periodontitis. Many studies have extensively

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studied the efficacy of the tetracycline group of drugs as local drug delivery (LDD) systems and found to be effective in the treatment of periodontitis.[5-7] Most of these LDD systems are difficult to procure and expensive. India being a developing country, the cost of the treatment is a matter of concern for our patients. Hence, the use of indigenous herbal drugs has been an area of significant interest in the treatment of periodontal diseases. Several research studies have established their efficacy in the treatment of periodontal diseases.[8]

Medicinal plants can be natural composite sources that act as new anti-infectious agents. The emergence of bacterial resistance has necessitated the search for new and effective antimicrobial compounds. Globally, plant extracts are employed for their anti-inflammatory, antibacterial, antifungal, and antiviral activities.[9] Eugenol-based gels are available for the treatment of periodontits.[10] Free eugenol can cause bone resorption. Literature report is available where osteoradionecrosis has occurred when there is an excess free eugenol.[11] Low concentrations of eugenol exert anti-inflammatory and local anesthetic effects on the dental pulp, on the other hand, high eugenol concentrations are cytotoxic. Direct application of eugenol to pulp tissue may result in extensive tissue damage.[12] Whole tulsi extract, on the other hand, have been widely used in traditional medicine and human clinical trial without significant side effects.[8,13] Hence, we used supercritical fluid extract of tulsi leaves for 2% *Ocimum sanctum* gel preparation.

MATERIALS AND METHODS

Institutional Animal Ethical Committee, approval was obtained. *O. sanctum* gel was formulated. Dosage of the gel was selected based on the results of Acute Oral Toxicity Study of *O. sanctum* done in Wistar Albino Rats as per the OECD Guidelines No. 420. The rats were housed in polypropylene cages under standard conditions with access to food and water *ad libitum*.

The aim of the present study was to formulate, evaluate the anti-inflammatory activity, to assess the duration of action and the efficacy of 2% *O. sanctum* gel in the treatment of experimentally induced periodontitis in Wistar Albino rat model.

PREPARATION OF 2% *OCIMUM SANCTUM* GEL BY SIMPLE DISPERSION METHOD

Two percentage *O. sanctum* gel were prepared in the following manner. Carbopol-940 was soaked in purified water containing 0.2% w/v sodium benzoate overnight. Using tissue homogenizer hydroxypropyl methyl cellulose (HPMC) solution was mixed with propylene glycol. 2 ml of tulsi extract (Supercritical fluid extract, procured from Sami labs, Bengaluru) was transferred into HPMC solution and homogenized. This drug solution was later transferred to Carbopol solution and homogenized. Triethanolamine was added to neutralize the pH [Table1]. Control gel was prepared in the same manner. The gel was stored at ambient temperature. This gel was stable over a period of 6 months. Slight pH changes were noted and corrected.[14] The formulation was done in NSGM institute of Pharmaceutical sciences, NITTE university, Mangalore [Figure 1].

EVALUATION OF PHYSICOCHEMICAL PARAMETERS OF 2% *OCIMUM SANCTUM* GEL

The formulations were subjected to various tests like physical evaluation, homogeneity, spreadabilty, grittiness, extrudability, and pH measurement.

Physical evaluation

Physical observations such as color and appearance were checked.

Spreadability

Spreadability was determined by an apparatus that consists of a wooden block with a pulley at one end. The basis for this method was the slip and drag characteristics of gels. 2 g of the gel was placed on the ground slide. The gel was sandwiched between the ground slide and a glass slide of similar dimensions with an attached hook. 1 kg weight was placed on the top of the two slides for 5 min to remove air bubbles and to provide a uniform gel film between the slides. Excess gel was removed from the edges. The upper plate was then subjected to pull of 80 g by the help of string attached to the hook and the time (in seconds)

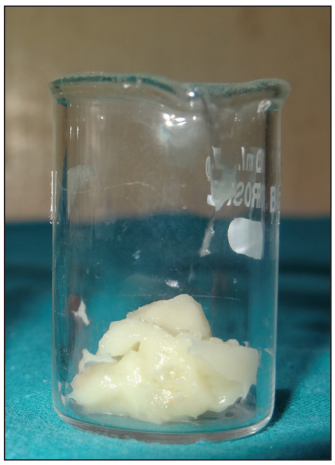

Figure 1: 2% tulsi gel

Table 1: Formula used to prepare 2% Tulsi (*Ocimum sanctum*) gel

Ingredients	Quantity
Carbapol	2 g
Polymer (HPMC)	2 g
Tulsi SCF extract	2 ml
Propylene glycol	5 ml
Sodium benzoate	0.2 ml
Triethnolamine	q.s.
Distilled water	q.s. to make100 ml

HPMC: Hydroxypropyl methyl cellulose, SCF: Supercritical fluid

taken by the upper slide to cover a distance of 7.5 cm was noted. A shorter interval indicates better spreadability.[15] Spreadability was calculated using the following formula:

$S = M \times L/T$
Where, S = Spreadability,
M = Weight in the pan (tied to the upper slide),
L = Length moved by the glass slide and
T = Time (in seconds) taken to separate the slide completely each other.

Homogeneity

The formulation was tested for homogeneity by visual observation after it set in a container. We checked for any aggregates. Grades were allotted as +++ Good, ++ fair, + Poor.[16]

Extrudability

The formulation was filled in a clean, lacquered aluminum collapsible one ounce tube with a nasal tip of 5 mm opening. The extrudability was then determined by measuring the amount of gel extruded through the tip when a constant load of 1 kg was placed. The extruded gel was collected and weighed. The percentage of gel extruded was calculated, and grades were allotted.[17]

Determination of viscosity

Viscosity of the formulation was measured at 25°C using Brookfield digital viscosimeter. The measurements were made over the whole range of speed settings from 10 rpm to 100 rpm with 30 s interval between two successive speeds and then in a descending order.[18]

Determination of pH

2.5 g of the gel was accurately weighed and dispersed in 25 ml of water. It was stored for 2 h. The pH was measured using a pH meter.[17]

Evaluation of anti-inflammatory activity of 2% *Ocimum sanctum* gel

18 healthy Wistar albino rats of either sex were randomly allocated to test (2% *O. sanctum gel*), standard (1% Voveron® Emulgel® gel, Novartis, India) and control group (plain gel) with six animals ($n = 6$) in each group. The anti-inflammatory activity was assessed by Carrageenan induced Paw edema method. The average weight of the rats was 237.50 ± 22.305 g in the test group, 227.33 ± 62.199 g in the standard group and 228.33 ± 9.832 g in the control group. Inflammation was induced in the paws by sub plantar injection of 0.1% Carrageenan. After 1 h, 50 mg of the 2% gel was divided into two equal parts of 25 mg. The first part of 25 mg gel was applied on the plantar surfaces of their left hind paw surface by gentle rubbing with the index finger approximately 50 times until no gel was seen or felt on the skin. After 5 min, 25 mg gel was applied in a similar manner.[18] The control gel base and the standard gel were applied by the same mode of application. This was followed by paw thickness measurement using Vernier Caliper method. This reading was the 0th h reading. Then rats were housed in cages without bedding to minimize the chances of the bias of drug being washed out by the bedding material. After 3 h readings were repeated. The rats were then transferred back to cages with bedding.

Percentage inflammation was calculated using the formula

Percentage inflammation = $(V - Vi \div Vi) \times 100$. Where $V_i = 0$ h reading and V = 3 h reading. The average paw thickness in the drug-treated group was compared with the control group.

Percent inhibition of the edema was calculated using the formula

Percent inhibition of the edema = $(Vt \div Vc) \times 100$ Where Vt is percentage swelling of the drug treated group, and Vc is percentage swelling of the control group.

Duration of anti-inflammatory activity of 2% *Ocimum sanctum* gel

36 healthy Wistar albino rats of either sex were divided into six groups ($n = 6$) based on the time duration at which 2% *O. sanctum* gel was applied prior to injection of 0.1% carrageenan. In Group 1-6, 50 mg of 2% *O. sanctum* gel was applied at 0, 2, 4, 6, 12, 24, and 48 h prior to administration of Carrageenan.

50 mg of the 2% gel was divided into two equal parts of 25 mg. The first part of 25 mg gel was applied on the plantar surfaces of their left hind paw surface by gentle rubbing with the index finger approximately 50 times until no gel was seen or felt on the skin. After 5 min, 25 mg gel was applied in a similar manner at 2, 4, 6, 12, 24, and 48 h prior to injection of 0.1 ml of 1% Carrageenan solution.[18] Three hours after the injection the 3rd h reading was noted. The percentage inflammation and the percent inhibition of edema at each dosing interval were calculated using the above-mentioned formulae. In the control group, ($n = 6$) the plain gel and standard (Voveron® gel) group ($n = 6$) gel was applied 3 h prior to the Carrageenan injection.

Experimental periodontitis

36 Wistar albino rats (5-10 weeks old) weighing between 150 and 250 g were used in the study. 5 rats weighing 150-250 g were taken in a polypropylene cage each day of the experiment for 9 days. In order to carry out dental procedures in the mouth of rats, a profound and safe general anesthesia is a must.[19] Use of inhalational anesthesia and the equipment hamper the visual field and maneuverability during oral surgical procedures. Using single drug intraperitoneally to achieve all three components of anesthesia will make the procedure easy. Ketamine (Anket, Lupin Ltd., India), a cyclohexamine, was used in the current study as it is easy to use and has a greater margin of safety for most laboratory animals.[20] High doses of ketamine induce catalepsy and is not accompanied by central nervous system depression. Respiratory functions are depressed, but cardiovascular function is maintained. The swallowing reflex is maintained, and this might help to prevent aspiration pneumonia if the animal regurgitates. The

chance of animal morbidity and mortality is low. Hence, we used Ketamine anesthesia.[21]

The rats were anesthetized with ketamine anesthesia using intraperitoneal route. Pre-periodontal examination was done, and the upper second molars were ligated using a sterile braided silk suture (5-0) [Figure 2]. Soft tissue indicators were measured.

Treatment of experimental periodontitis

4 weeks after ligature placement the rats were divided into three groups. The rats in the group 1-control group did not receive any treatment, group 2 received plain gel, group 3 received 2% Tulsi gel. The gels were applied with a tuberculin syringe with a blunt tip. The application was done every alternate day for 6 days. The soft tissue indicators were measured prior to euthanizing the rats.[22]

Soft tissue indicators of periodontitis

Gingival index

Gingival index (GI) was recorded on maxillary 2nd molar on four surfaces - Mesial, Buccal, Distal, and Palatal surface. GI was recorded 1 week after ligature placement and 1 week after treatment.[23]

Pocket probing depth

Pocket probing depth (PPD) was taken from the gingival margin to the bottom of the pocket using a modified graduated silver cone with round-ended tip of approximately 0.4 mm in diameter. Six values were recorded and averaged (mesiobuccal, midbuccal, distobuccal, mesiopalatal, mid palatal, and distopalatal). PPD was examined 1 week after ligature placement and 1 week after treatment.[22]

Sacrificial indicator

Morphometric analysis of alveolar bone loss

After the rats were euthanized with ketamine overdose the specimens were dissected carefully to maintain their integrity then, immersed in sodium hypochlorite for 4 h and manual scavenging of the remaining tissue was done. The specimens were stained with methylene blue dye (1 g/100 mL, Sigma-Aldrich, Saint Louis, MO, USA) for 1 min to demarcate the cemento enamel junction [CEJ] and examined under a stereomicroscope. In order to ensure reproducibility of the alignment of the image, the buccal cusp tip of the first and second molars were placed such that they superimposed on the corresponding lingual/palatal cusp tip. Photographs were obtained with a 6.1-megapixel digital camera (Nikon D100, Ayutthaya, Thailand). Measurements were made on the maxillary second molar in a blinded fashion, three times using ImageJ, image-analysis software (Java based image processing software, NIH, USA), and the mean values were used in statistical analysis.[24] The distance method applied by Crawford, Taubman and Smith on digitalized images was used to perform linear measurements from the CEJ to the alveolar bone crest, on half of each root following the axis. Six measurements were obtained for the maxillary second molar [Figures 3 and 4]. The data were subjected to statistical analysis.

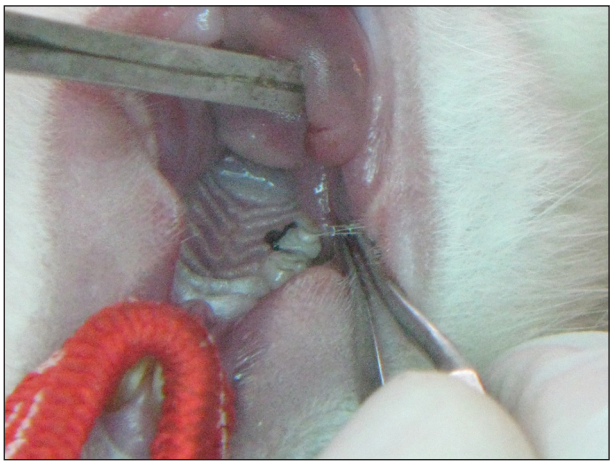

Figure 2: Ligature placement

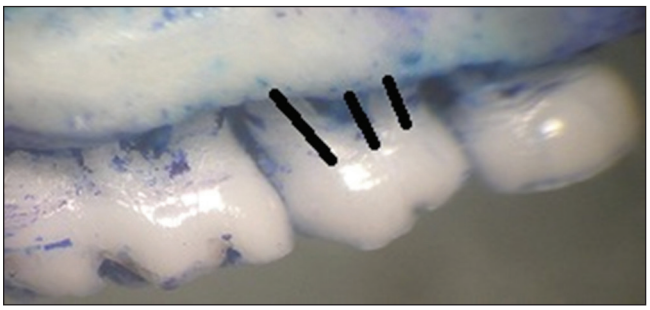

Figure 3: Morphometric analysis: Buccal view

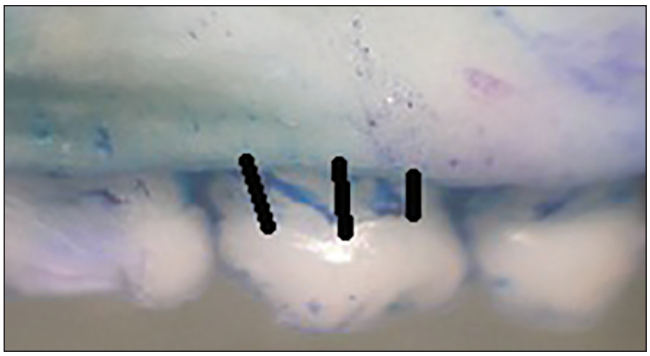

Figure 4: Morphometric analysis: Palatal view

RESULTS

All statistical analysis was done using InStat-GraphPad software (GraphPad software Inc. CA, USA). $P < 0.001$ was considered highly significant and $P < 0.05$ was considered significant.

Evaluation of physicochemical parameters of the 2% *Ocimum sanctum* gel

The developed gel was grayish in color, translucent in appearance and showed good homogeneity with absence of lumps. The gel showed optimum spreadability of 15 g cm/s. Hence, the spreadability was good. The gel showed homogeneity of +++ grade and good extrudability. The pH was within the acceptable range of 6.9-7.2 even at the end of 30 days [Table 2].

Table 2: Physicochemical characters of 2% tulsi gel

Parameters	Results
Homogeneity	++
Grittiness	-
Extrudability	++
Spreadability (s)	15.5
pH	6.9

Table 3: Weight of the animals selected for the study

Weight[gm]	N	Mean	Standard deviation	Standard Error	nfidence Interval fo		ANOVA F	P value
					Lower Bound	Upper Bound		
Standard					162.06			
Test	6	227.3	62.19	25.39	214.09	292.6		
Control	6	237.5	22.30	9.10	228.02	260.9		0.86
	6	238.3	9.832	4.01		248.6	1.51	NS
Total	18	234.3	36.59	8.63	216.19	252.5		

Table 4: Percentage of inflammation in control, standard, and test group

Percentage of inflammation	Control	Standard	Test (mm)
Mean percentage of inflammation ± SEM	68.387±25.05	15.74±4.634	45.36±23.22

SEM: Standard error of mean

Table 5: Percentage of inhibition of edema

Formulation	Dose (mg/paw)	Number of rats	Percentage of swelling (mm)	Percentage of inhibition of edema
Control	50	6	38.99±42.98	—
Standard	50	6	15.74±4.63	76.975
Test	50	6	45.36±23.22	33.66

We could not do determine drug content uniformity and drug release studies, as it was a whole leaf extract, and we had technical difficulties.

Evaluation of anti-inflammatory action of 2% tulsi (*Ocimum sanctum*) gel

The results of the ANOVA show that there was no statistically significant difference in the mean weight of the rats which, in the control, standard, and the test group was 238.33 ± 9.83 g, 227.33 ± 62.19 g, and 237.50 ± 22.30 g, respectively. ($P = 0.861$).

The test group showed moderate anti-inflammatory activity compared to the standard. Percent inhibition of edema in the test group was 33.66%, and the standard group was 76.97% [Tables 3-5].

Duration of the anti-inflammatory activity of 2% Ocimum sanctum gel

Tulsi gel showed peak anti-inflammatory activity during 24 h, and the activity was seen even at the end of 48 h. Percent inhibition of edema at 24 h was 67.58% [Table 6]. Hence, the gel was applied every 24 h to treat periodontitis.

Soft tissue indicators of periodontitis

Gingival index

There was a statistically significant reduction in the mean GI scores in the test group at the end of the treatment when compared to the control and the plain gel group. The mean percentage change was –40.35, –39.62, and 43.23% ($P = 0.001$) [Table 7].

Pocket probing depth

There was no statistically significant difference between the groups in PPD at the baseline. But at the end of the treatment, tulsi gel showed statistically significant different reduction in probing pocket depth, Mean difference was 1. There was no statistically significant difference between the control group and plain gel. Mean difference was 0.054 [Tables 8 and 9].

Table 6: Duration of anti inflammatory action of 2% Tulsi gel

Tulsi gel	2 hours	4 hours	6 hours	12 hours	24 hours	48 hours
Mean percentage of inflammation ± SEM	39.55±8.089	31.08±5.507	28.57 ±10.52	25.29±14.32	22.16±9.08	52.95±17.20
% inhibition	46.15	54.23	58.21	63.02	67.58	22.56

Table 7: Comparison of GI before and after treatment

Group	*n*	Minimum	Maximum	Mean	SD	Median	Wilcoxon signed rank test value	*P*
Control group								
GI at 7 days	12	2.00	3.00	2.19	0.33	2.00	3.24	0.001 HS**
GI at 35 days	12	2.50	3.50	3.08	0.34	3.00		
Plain gel								
GI at 7 days	12	2.00	3.00	2.21	0.33	2.00		0.002 HS**
GI at 35 days	12	2.50	3.50	3.08	0.36	3.00	3.11	
2% tulsi gel								
GI at 7 days	12	12	2.00	3.00	2.58	0.51	3.00	1.12
GI at 35 days	12	12	1.10	1.80	1.47	0.23	1.45	

SD: Standard deviation, GI: Gingival index, *P* value < 0.001, HS: Highly significant

Sacrificial indicators

Morphometric analysis

On the comparison using Bonferroni's multiple comparisons test, there was no statistically significant bone loss between the various groups at the mesiobuccal, midbuccal, distobuccal, mesiopalatal, midpalatal, and distopalatal site [Tables 10-16].

Table 8: Probing pocket depth

Mean probing depth	7 days postligature placement (mean ± SD)	At the end of the treatment (mean ± SD)
Control	2.75±0.121	2.79±0.137
Plain gel	2.73±0.120	2.73±0.120
Tulsi gel	2.72±0.110	2.10±0.130

SD: Standard deviation

Table 9: Comparison between control and treatment group at the end of treatment

Tukey's multiple comparisons test	Mean difference	95% CI of difference	Summary
Control versus plain gel	0.05455	–0.1094-0.2185	NS
Control versus tulsi gel	1	0.8360-1.164	HS**

CI: Confidence interval, NS: Not significant, HS: Highly significant, *P* value < 0.001

Acute oral toxicity

All the animals survived for the period of 14 days. They appeared healthy throughout the study. All the animals appeared to gain weight during the observation period of 14 days. There were no signs of gross toxicity, adverse pharmacological events or changes in behavior. Gross necropsy findings did not show any abnormalities.

DISCUSSION

Local drug delivery to the periodontal pocket as a method to treat periodontal disease has been extensively studied. Several herbal drugs in the form of LDD have been an area of interest in the treatment of periodontal diseases.[8]

The common methods used to induce periodontitis in the rats are by tying ligature braided silk (2-0-5-0) around the cervix of the maxillary or mandibular molars or by injecting lipopolysaccharides into the papilla or combination of both.[25] The time period used for

Table 10: Mean bone level at sites treated with control, plain gel and 2% tulsi gel

Site	Control (mean ± SD)	Plain gel (mean ± SD)	Tulsi gel (mean ± SD)
Mesiobuccal	0.30±0.056	0.30±0.055	0.24±0.085
Midbuccal	0.28±0.056	0.24±0.064	0.20± 0.054
Distobuccal	0.22±0.057	0.17±0.036	0.16±0.049
Mesiopalatal	0.37±0.063	0.42±0.203	0.34±0.036
Midpalatal	0.29±0.030	0.33±0.152	0.24±0.039
Distopalatal	0.29±0.053	0.31±0.153	0.24±0.051

SD: Standard deviation

Table 11: Comparison of the effect of control, plain gel, and 2% tulsi gel on bone loss (mesiobuccal site)

Bonferroni's multiple comparisons test	Mean difference	95% CI of difference	Statistical significance
Mesiobuccal			
Control versus plain gel	0.0492	–0.05154-0.1499	NS
Control versus tulsi gel	0.0635	–0.03724-0.1642	NS
Plain gel versus tulsi gel	0.0143	–0.08644-0.1150	NS

CI: Confidence interval, NS: Not significant

Table 12: Comparison of the effect of control, plain gel and 2% tulsi gel on bone loss (midbuccal site)

Bonferroni's multiple comparisons test	Mean difference	95% CI of difference	Statistical significance
Midbuccal			
Control versus plain gel	0.0734	–0.02734-0.1741	NS
Control versus tulsi gel	0.0721	–0.02864-0.1728	NS
Plain gel versus tulsi gel	–0.0013	–0.1020-0.09944	NS

CI: Confidence interval, NS: Not significant

Table 13: Comparison of the effect of control, plain gel and 2% tulsi gel on bone loss (distobuccal site)

Bonferroni's multiple comparisons test	Mean difference	95% CI of difference	Statistical significance
Distobuccal			
Control versus plain gel	0.0804	–0.02034-0.1811	NS
Control versus tulsi gel	0.0563	–0.04444-0.1570	NS
Plain gel versus tulsi gel	–0.0241	–0.1248-0.07664	NS

CI: Confidence interval, NS: Not significant

Table 14: Comparison of the effect of control, plain gel and 2% tulsi gel on bone loss (mesiopalatal site)

Bonferroni's multiple comparisons test	Mean difference	95% CI of difference	Statistical significance
Mesiopalatal			
Control versus plain gel	0.0169	–0.08384-0.1176	NS
Control versus tulsi gel	0.0298	–0.07094-0.1305	NS
Plain gel versus tulsi gel	0.0129	–0.08784-0.1136	NS

CI: Confidence interval, NS: Not significant

Table 15: Comparison of the effect of control, plain gel and 2% tulsi gel on bone loss (mid palatal site)

Bonferroni's multiple comparisons test	Mean difference	95% CI of difference	Statistical significance
Midpalatal			
Control versus plain gel	0.0191	–0.08164-0.1198	NS
Control versus tulsi gel	0.0537	–0.04704-0.1544	NS
Plain gel versus tulsi gel	0.0346	–0.06614-0.1353	NS

CI: Confidence interval, NS: Not significant

Table 16: Comparison of the effect of control, plain gel and 2% tulsi gel on bone loss (distopalatal site)

Bonferroni's multiple comparisons test	Mean difference	95% CI of difference	Statistical significance
Distopalatal			
Control versus plain gel	0.0367	–0.06404-0.1374	NS
Control versus tulsi gel	0.0554	–0.04534-0.1561	NS
Plain gel versus tulsi gel	0.0187	–0.08204-0.1194	NS

CI: Confidence interval, NS: Not significant

induction of periodontitis in the maxillary molars was 4 weeks.[26] It was similar to the study period used in our study.

Anti-inflammatory agents have been used in the treatment of periodontitis.[27] The methanol extract and the aqueous extract of *O. sanctum* have shown to inhibit acute as well as chronic inflammation in rats.[18] Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum*, has been found to be largely responsible for the anti-inflammatory property of tulsi. It demonstrated 97% cyclooxygenase-1 inhibitory activity when assayed at 1000 μM concentration.

The results of anti-inflammatory activity of *O. sanctum* support the dual inhibition of arachidonic acid metabolism as indicated by its activity in inflammation models that are insensitive to selective cyclooxygenase inhibitors. Linolenic acid present in *O. sanctum* fixed oil has the capacity to block both the cyclo-oxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti-inflammatory activity of the oil.[28,29] Civsilineol, Civsimavitin, Isothymonin, Apigenin, and Rosavinic acid displayed 37, 50, 37, 65, and 58% cyclooxygenase-1 inhibitory activity, respectively, when assayed at 1000 μM concentrations. The activities of these compounds were comparable to Ibuprofen, Naproxen and aspirin at 10, 100 and 1000 μM concentrations.[30] Literature review is not clear about the active ingredient responsible for anti-inflammatory, analgesic and antimicrobial actions of tulsi. Hence, we used a supercritical fluid extract of *O. sanctum* instead of single active ingredient to prepare a 2% gel by simple gel dispersion method.

The gel showed a standard physicochemical profile. Drug content uniformity and drug release studies were not done, as it was a whole extract, and we faced technical difficulties. Percent inhibition of edema was 33.66%. Maximum inhibition was seen at 24 h. The anti-inflammatory activity lasted for 48 h. Hence, the gel was applied on alternate days to treat experimental periodontitis.

The GI scores in the control group and plain gel group increased indicating that the inflammation progressed. In the 2% tulsi gel group, a highly significant mean percentage decrease was noted. This showed that the test gel had good anti-inflammatory effect.

Morphometric analysis did not show any significant difference between the groups. The effect of LDD system on bone regeneration is controversial. In our study, we did not notice any significant difference in the residual periodontal bone level among various groups.

Limitations of the study

- Pressure sensitive probe and stent needs to be used.
- No scaling and root planing was done.
- Microbial profiling, Biochemical, and immuno-histochemical parameters have to be evaluated.
- Drug content uniformity and drug release studies were not done.
- It is worthwhile to do chronic toxicity study. But, the animals were monitored closely during the entire study period for any signs of toxicity. No signs of chronic toxicity were noted.

CONCLUSION

2% tulsi (*O. sanctum*) gel have shown good anti-inflammatory effect for 24-48 h. The 2% tulsi gel showed good anti-inflammatory effect resulting in a reduction of gingival inflammation and pocket depth. The alveolar bone loss was not statistically significant. LDD systems help in the

disease limitation and its role in bone regeneration is not established. Hence, 2% tulsi (*O. sanctum*) gel can be used as useful adjunct to enhance the results of conventional periodontal therapy.

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