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CLINICAL EFFICACY OF INTRAPOCKET APPLICATION OF CYMBOPOGON CITRATUS GEL AS ADJUNCTIVE TO NON-SURGICAL THERAPY IN EGYPTIAN PATIENTS WITH MODERATE PERIODONTITIS (A SINGLE-BLIND RANDOMIZED CONTROLLED CLINICAL TRIAL)

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ABSTRACT

INTRODUCTION: Mechanical removal of dental biofilm is the gold standard therapy in treatment of moderate periodontitis. Local delivery drugs as adjunctives to conventional treatment are widely used to modulate inflammatory host response and eliminate microbes. Nowadays, herbal therapies have been used as safe alternative agents in place of antibiotics to overcome antibiotics side effects.
OBJECTIVE: To evaluate the clinical effectiveness of intra-pocket application of Cymbopogon citratus (lemongrass) gel on periodontal status.
MATERIALS AND METHOD: Forty patients with moderate periodontitis, divided equally into two groups. Group-I was managed by SRP with the intra-pocket application of 2% lemongrass oil gel. Group- II was managed by SRP with the intra-pocket application of a placebo gel. Bleeding on probing (BOP), plaque index (PI), probing pocket depth (PPD), and clinical attachment level (CAL) were measured for each group at baseline before and twelve weeks after treatment.
RESULTS: All assessed parameters showed improvement at the end of the study period in both groups compared to baseline. CAL, BOP, and PI showed a significant decrease from baseline to the end of the study in lemongrass gel group when compared to placebo group.
CONCLUSION: Combining intrapocket lemongrass gel with SRP was more effective in improving the clinical periodontal status than using SRP alone in the treatment of moderate periodontitis.
KEYWORDS: Lemongrass oil, Moderate periodontitis, Nonsurgical treatment, Phytotherapy.
RUNNING TITLE: Clinical efficacy of lemongrass oil gel.

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INTRODUCTION

Periodontal disease is an inflammatory disease that damages the supporting tissue surrounding the teeth (1). Periodontitis causes irreversible destruction when causative agents are not removed (2).

Depending on the new classification scheme for periodontal and peri-implant diseases and conditions, periodontitis has two categories divided into stages and grades. The first category consists of four stages based on the severity and complexity of management. In this category, stage II is moderate periodontitis with a clinical attachment loss of 3-4 mm and mostly horizontal bone loss (3,4).

Proper diagnosis of periodontitis is important for achieving the best treatment plan. Several diagnostic procedures such as assessing of probing depth, bleeding on probing, mobility and plaque index together with radiographs to evaluate alveolar bone level are cost-effective, easy to use, and provide clinically useful information concerning the location of disease and the presence or absence of damaged tissues (5,6).

Non-surgical therapy is the proper treatment of moderate periodontitis and removing dental biofilm by the conventional periodontal therapy is still the gold standard therapy (7). However, there are many reasons that decrease the effectiveness of mechanical therapy such as rate of progression, extent and type of periodontal pocket, and response to therapy. Thus, systemic host modulation and antimicrobial therapy are used as adjunctive therapy to nonsurgical treatment to overcome these limitations (8,9).

Systemic antimicrobials proved their effectiveness in treating periodontitis as an adjunctive agent, but they cause several side effects, the most important of which is the development of resistant species. Therefore, localized drug delivery systems were developed to overcome these side effects (10).

Several clinical investigations reported that local antibiotics have advantageous over systemic antimicrobials in periodontal

therapy. They provide proper concentrations of active ingredients at the targeted site. Moreover, they have controlled-release ability for prolonged periods of time. However, the use of local delivery antibiotics could not cope with bacterial resistance to antibiotics especially if used for a long term (7,11,12).

Phytotherapy (phytoscience), using phytochemicals of plants as medicinal agents, has evolved as an alternative option for antibiotics to reduce side effects (13).

According to Federal Regulations Section U.S.A in 1982, (14) stated that *Cymbopogon citratus* (lemongrass) extract/essential oil is safe for human consumption. Lemongrass essential oil has established antibiotic, antifungal, and antiviral properties (15–17), and it was added to creams and ointments to treat topical inflammation in medicine (18). Accordingly, it is used as an adjunctive agent to nonsurgical treatment of periodontitis (19).

To the best of our knowledge, there are no previous clinical studies conducted on Egyptian patients to evaluate the clinical effectiveness of intrapocket application of lemongrass oil gel in treatment moderate periodontitis patients.

The null hypothesis of this research is that there will be no difference in clinical improvement in moderate periodontitis patients treated by *Cymbopogon citratus* (lemongrass) gel in comparison to those with non-surgical treatment only.

MATERIALS AND METHOD

A. Materials:

The study was accepted by the Research Ethics Committee of the faculty of Dentistry, Alexandria University (IRB NO: 00010556-IORG0008839). Registration of the study was done at U.S. National Institutes of Health Clinical Trials Registry (NCT04605289). It also followed the principles of the modified Helsinki code for human clinical studies (2013) (20) and CONSORT 2010 guidelines for reporting randomized clinical trials (21).

Sample size:

This study was a single-blind randomized, placebo-controlled, parallel group, clinical trial conducted during the period between December 2019 and September 2020. Twenty moderate periodontitis patients per group (number of groups=2) were included in the study. All subjects approved to participate in the clinical trial and signed a written informed consent. The sample size was calculated based on a previous study aimed to evaluate the clinical and microbiologic effects of the adjunctive use of photodynamic therapy to non-surgical periodontal treatment (22). They were recruited from the outpatient clinic of the Department of Oral Medicine, Periodontology, Diagnosis and Oral Radiology Faculty of Dentistry, Alexandria University, Egypt. This sample size was the enough required sample as statistically significant with 80% power and at a significance level of 95% (accepted α error = 0.05).

Study design:

The selected forty patients were randomly assigned by using a computer-generated list of random numbers and divided into the following groups:

Group I: twenty patients were treated with scaling and root planning (SRP) and intra-pocket application of 2% lemongrass oil gel.

Group II: twenty patients were treated with SRP and intra-pocket application of placebo gel.

Inclusion criteria included systemically healthy patients of both sexes having moderate periodontitis (stage II), CAL 3-4mm, with an age ranging from 25 to 45 years old, with no history of previous periodontal therapy or the intake of antibiotics during the six months preceding the trial. While the exclusion criteria included patients, who have a history of smoking, previous adverse reaction to the products (or similar products) used in this study, grade C category that has a rapid rate of progression, and pregnant or lactating women.

Materials:

1. 2% *Cymbopogon citratus* (lemongrass) oil gel:

The gel was prepared at the Department of Pharmacology, Alexandria University by a method similar to that applied in the preparation of other anti-inflammatory gel formulations. Carbopol 934 was soaked in water for a period of two hours. Carbopol is consisting of mucoadhesive polymers and has been added to increase the contact time of gel to the tissue, in the periodontal pocket (23). Then neutralized with triethanolamine (TEA) by stirring. In a pre-weighted amount of propylene glycol and ethanol in which 2% lemongrass essential oil was dissolved. The solvent blend was transferred to the carbopol container and agitated for an additional twenty minutes. Then, the dispersion was allowed to hydrate and swell for sixty minutes. pH was adjusted with 98% TEA until it reached (6.8-7). During pH adjustment, the mixture was stirred gently with a spatula until a homogeneous gel was formed (24). (Fig.1)

Figure 1: Showing lemongrass essential oil gel.

2. Placebo gel:

It was prepared with the same handling and physical properties of the test gel, without adding the active ingredients of lemongrass.

B. Methods:

1. Clinical examination included standard clinical periodontal parameters to whole mouth for each participant: bleeding on probing (BOP) (25), plaque index (PI) (26), periodontal probing depth (PPD), and clinical attachment loss (CAL) (27). Michigan-O periodontal probe with Williams calliberations (Medizintechnik GmbH & Co. KG, Dorsten, Germany) was used to measure PPD and CAL. (Fig.2)

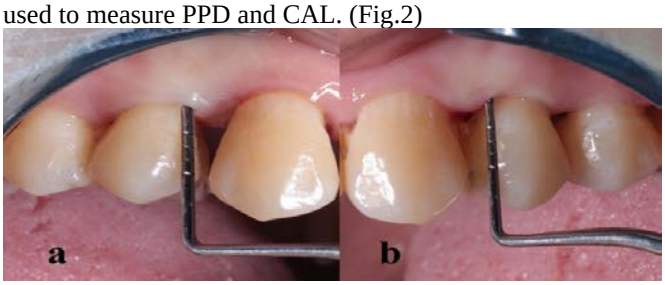

Figure 2: Showing baseline PPD a) Placebo group and b) Test group.

2. Phase I therapy (Non-surgical)
Oral hygiene instructions were given to the patients and SRP was performed using ultra-sonic scalers (DREAMKER/VRN/K3, Foshan, China) and Gracey curettes (Kohler. Medizintechnik GmbH & Co. KG, Dorsten, Germany).
The gels (lemongrass oil gel and placebo) were applied by a syringe with a bent, blunt-end needle. The needle was carefully entered into the periodontal pocket, and the gel was administered once in all pockets in a gentle probing manner, trying to fill the entire extent of the pocket. The excess gel was eliminated with sterile gauze. (24,28). (Fig.3)

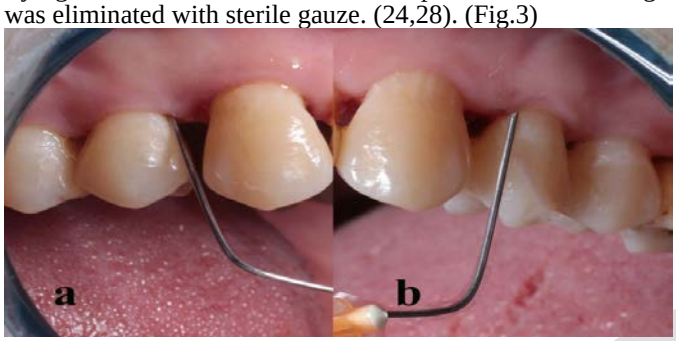

Figure 3: Showing intrapocket application of gel a) Placebo group and b) Test group.

Periodontal dressing was applied after the delivery of the drug, and the patients were asked not to eat for thirty minutes after treatment. The patients were instructed to follow a strict oral hygiene protocol and not chew sticky or hard foods at the gel placement sites for the rest of the week (29).
3. Follow up
Patients were recalled at first week to reassess the oral hygiene status, and recalled at the twelfth week after phase I therapy to measure PI, BOP, PPD and CAL. (Fig.4)
Follow up visits were measured by the same two blinded and calibrated examiners and their mean was recorded. Both the examiners and the participants were blinded, but the outcome assessor was un-blinded. The examiners were calibrated before starting the study by repeated measurements in four patients. The agreement was good (weighted kappa=0.89).

Statistical Analysis

Data were collected per patient for whole mouth then fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was set at $P \leq 0.05$.
For normally distributed variables, intergroup comparisons were analyzed using Student t-test, and intragroup comparisons were analyzed by Paired t-test. For variables that were not normally distributed, intergroup comparisons were analyzed using the Mann-Whitney U test, and intragroup comparisons were analyzed by Wilcoxon Sign Rank test. Friedman test was used to compare between more than two periods and Post Hoc Test (Dunn's) for pairwise comparisons (30).
Regarding PI, there was significant decrease in test group from (2.68±0.48) to (0.053±0.23) and in control group from (2.67±0.49) to (0.94±0.73) with statistically significant intergroup difference (P value<0.001*), while BOP is decreased in test group from 100% to 4.01%, and in control group decreased from 100% to 12.6%. (P<0.001). (Fig.5)

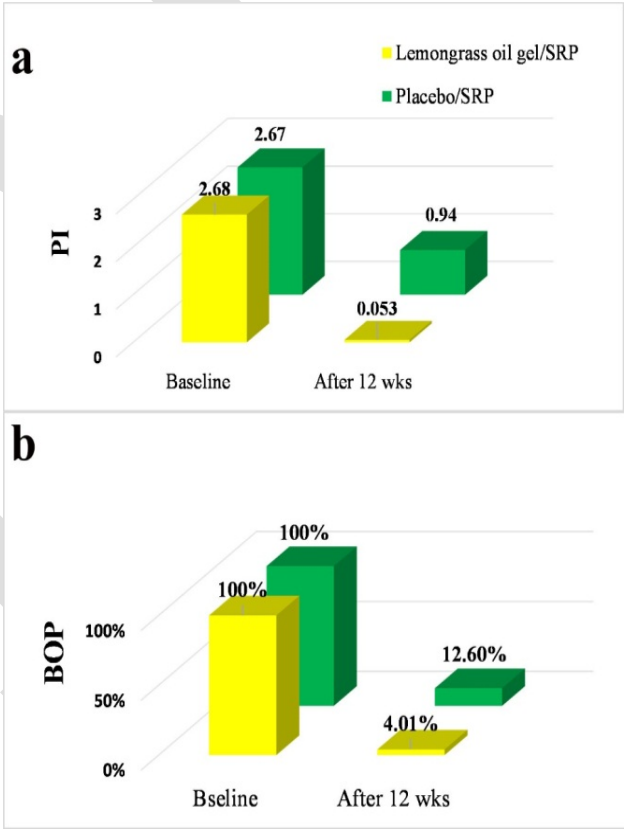

Figure 5: Showing comparison between the two studied groups a) PI and b) BOP throughout the study period.

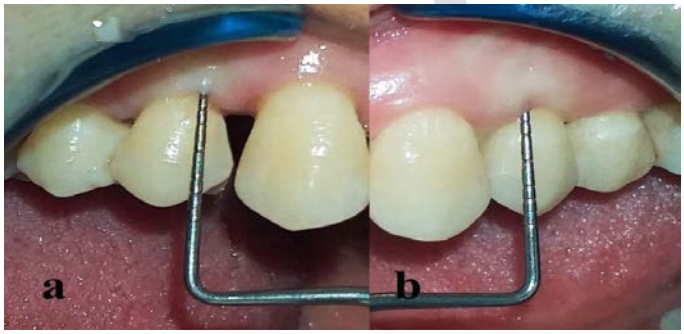

Figure 4: Showing PPD after 12 weeks, a) Placebo group and b) Test group

RESULTS

Forty Egyptian patients with moderate periodontitis were included in the study. Thirty-seven patients completed the study, while three dropped out at the last visit.
At baseline, no significant differences were seen between two groups in all parameters. After 12 weeks, significant improvement was seen in lemongrass gel group manifested by a reduction of plaque index and bleeding on probing with a gain of attachment. Whole mouth clinical periodontal parameters are presented in Table 1.

Table1: Comparison between studied groups with respect to PPD, CAL, PI, and BOP throughout study period.

Evaluated parameters		Lemongrass oil gel/SRP (n = 19)	Placebo/SRP (n = 18)	p
PI	**Baseline** Mean ± SD.	2.68±0.48	2.67±0.49	0.912
	After 12 weeks Mean ± SD.	0.05±0.23	0.94±0.73	<0.001*
	p_0	<0.001*	<0.001*	
	Decrease Mean ± SD.	2.63±0.5	1.73±0.58	<0.001*
BOP %	**Baseline** Mean ± SD.	100%	100%	1.000
	After 12 weeks Mean ± SD.	4.01±9.78	12.6±15.1	<0.001*
	p_0	<0.001*	<0.001*	
	Decrease Mean ± SD.	96%	87.4%	
PPD(mm)	**Baseline** Mean ± SD.	4.65 ± 0.59	4.60 ± 0.41	0.756
	After 12 weeks Mean ± SD.	2.97 ± 0.52	3.21 ± 0.46	0.147
	p_0	<0.001*	<0.001*	
	Decrease Mean ± SD.	1.68 ± 0.74	1.39 ± 0.43	0.150
CAL(mm)	**Baseline** Mean ± SD.	3.72 ± 0.25	3.65 ± 0.27	0.461
	After 12 weeks Mean ± SD.	1.65 ± 0.17	2.59 ± 0.22	<0.001*
	p_0	<0.001*	<0.001*	
	Decrease Mean ± SD.	2.07 ± 0.34	1.06 ± 0.26	<0.001*

*: Statistically significant.
p: p value for comparing between the studied groups.
p0: p value for comparing between baseline and after 12 weeks in each group.

Concerning PPD at end of the study, there was a reduction in lemongrass oil/SRP group from (4.65±0.59mm) to (2.96±0.52mm) and from (4.60±0.41mm) to (3.21±0.46mm) in placebo/SRP group; however, this difference was insignificant between two groups (P value= 0.147). (Fig.6)

In lemongrass oil/SRP group, the mean CAL decreased from (3.72 ±0.25mm) to (1.65±0.17mm), while in the placebo/SRP group it decreased from (3.65±0.27mm) to (2.59±0.22mm) the difference between the two groups was significant (P<0.001). (Fig.6)

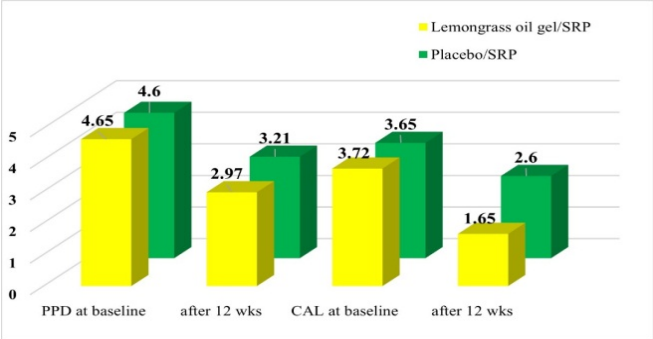

Figure 6: Showing Comparison between the two studied groups according to PPD and CAL throughout the study period.

DISCUSSION

The present study aimed to evaluate the efficacy of intrapocket lemongrass essential oil gel as an adjunctive to the non-surgical treatment of moderate periodontitis on the clinical periodontal status.

In general, the periodontal therapy seeks to reduce or remove the pathogenic microorganisms, arrest the progression of inflammation, and improve the clinical attachment level. Scaling and root planing is the standard treatment for moderate periodontitis (4,7). Presence of pathogens within the tissue or in deep pockets, where instruments cannot reach, causes incomplete eradication the pathogens by conventional treatment alone (31,32).

Combining local delivery antibiotic agents with scaling and root planning became needed to achieve the periodontal therapy goal as reduction periodontal pocket depth and gaining clinical attachment level. Many commercial local delivery antimicrobial agents are available, but the demand for inexpensive, safe, and effective agents has encouraged the use of natural extracts (28) .

In this study, we used 2% concentration of lemongrass essential oil gel as an adjunct to the conventional therapy of moderate periodontitis. The outcome of therapy was measured based on clinical parameters like PPD, CAL, PI, and BOP.

Our results showed a statistically significant difference in CAL, PI, and BOP between two groups, but statistically insignificant reduction in PPD at end of the study.

Regarding PI and BOP, the results showed the statistically significant differences intergroup (P <0.001*)at the end of the study, but it is difficult to compare the present study results with data reported by other authors since there are no data concerning to evaluate the PI and BOP in patients with periodontitis after the intrapocket application of lemongrass essential oil gel, but some authors have stated a decrease in PI after using lemongrass oil mouthwash (33–35). That proved the beneficial effect of using the lemongrass oil as adjunctive to nonsurgical therapy for periodontitis.

The decreasing of PI and BOP observed in the test group can be explained by the antibacterial effects of lemongrass essential oil towards the periodontal pathogens (35,36).

Lemongrass oil has the ability to inhibit growing many kinds of periodontal pathogens especially the strains of Porphyromonas gingivalis and Actinomyces naeslundii which are resistant to tetracycline hydrochloride (37).

Kukkamalla et al (35) proved the ability of lemongrass oil mouthwash to reduce dental plaque accumulation. They concluded that lemongrass oil has an antibacterial as well as an anti-biofilm property.

In the same manner, another study about the antibiofilm activity of lemongrass oil solution confirmed that the solution decreases the ability of bacterial adherence and inhibits biofilm formation (34).

The changes in PPD from baseline to the end of the study were statistically significant in each group individually (P<0.001), but no significant difference was found between the two groups. This is conflicting with the finding of previous studies (24,28). However, the reduction in PPD from baseline to 12 weeks was greater in lemongrass oil gel/SRP group (2.96±0.52mm) than in placebo/SRP group (3.21±0.46mm).

PPD of lemongrass oil gel/SRP group reduced by (1.68 ± 0.74mm), whereas in placebo/SRP group decreased by (1.39 ± 0.43mm). Few investigators have examined the relationship between PPD and CAL changes after nonsurgical intervention. Changes in PPD cannot be a sensitive and reliable predictor for gaining attachment. The former is measured from the gingival margin (GM), which is affected by gingival inflammation and can be changed through treatment course, and this can affect probing depth measures. Studies confirmed that there is poor agreement between the reduction in PPD and the decreasing in CAL after conventional intervention (38,39).
Concerning CAL, statistically significant gain in clinical attachment was seen in the test group as compared to the control group after 12 weeks. This result is in agreement with previous studies (24,28).
The improvement in CAL can be explained by the antioxidant and anti-inflammatory properties of lemongrass oil.
Dany et al. (40) confirmed that 2% lemongrass oil rinse can accelerate the healing process by increasing the salivary glutathione levels. Glutathione, called sulfhydryl glutathione (GSH), has a critical role in defense against oxidative stress. Citral, a main component of lemongrass oil, can promote Vitamin A and C formation, which are secondary antioxidants, and inhibit damage of tissue by blocking chain reactions of lipid metabolism (41).
Moreover, Anand et al (19) estimated the efficacy of lemongrass oil mouthwash on salivary and GCF superoxide dismutase levels. Results of the study confirmed the antioxidant effectiveness of lemongrass oil on the treatment outcome.
The limitations encountered during our study were the shortage of access to literature about lemongrass gel and dropping three patients during the follow up visits.
However, the improvement of clinical periodontal parameters in this study cannot directly be compared to other studies. No previous studies investigated the exact mechanism of 2% lemongrass essential oil gel on subgingival microbiota and its antioxidant activity. Thus, additional studies supported by biochemical and histological analysis to directly evaluate its absolute role in periodontal therapy as a local delivery agent are needed.

CONCLUSION

Within the limitation of this study, we can conclude that both treatment modalities can be used in the management of moderate periodontitis, and the combination of SRP and intrapocket 2% lemongrass oil gel has a better outcome and lead to an improvement in clinical parameters more than SRP alone.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The authors received no specific funding for this work.

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