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Original Article

Dentistry Section

Preparation of Nutmeg Gel and Evaluation of its Efficacy as Local Drug Delivery in Stage II Grade A Periodontitis Patients: A Prospective Interventional Study

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ABSTRACT

Introduction: Periodontitis is defined as a chronic inflammatory disease caused by specific microbes, which triggers the production of inflammatory mediators. This process leads to the loss of tissue structure and function. Nutmeg has been found to consist of a number of chemical components that have been linked to antioxidant, health promoting, and disease-prevention activity.

Aim: To prepare nutmeg gel and to assess its anti-inflammatory, antioxidant, and cytotoxic activities, and its effectiveness as a locally delivered drug in the management of stage II grade A periodontitis.

Materials and Methods: The present study was conducted in Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India. A 30 mg nutmeg gel was prepared and subjected to anti-inflammatory, antioxidant, and cytotoxic assays. Followed by in-vitro analysis, 40 patients with stage II grade A periodontitis patients who reported between June 2023 and September 2023 to the Department of Periodontology were enrolled. A total of 20 patients were subjected to Scaling and Root Planing (SRP) alone (Group A - Control), and the remaining 20 patients were subjected to SRP + placement of 0.3% nutmeg gel (Group B - Test). Probing Depth (PD) and Clinical Attachment Level (CAL) were recorded at baseline and after three months. The data were analysed for statistical significance using Statistical Package for the Social Sciences (SPSS) software, version 23.0. Intergroup and intragroup comparison was done using independent t-test and paired t-test, respectively, with a p-value <0.05 considered statistically significant.

Results: In vitro analysis revealed that the anti-inflammatory and antioxidant activity of nutmeg gel was comparable to the control. Also, the survival rate of nauplii fell within acceptable ranges. Followed by in-vivo analysis, in-vivo analysis was done. A total of 13 females and seven males with a mean age of 29.45±3.98 years were treated with SRP (Group A), and eight females and 12 males with a mean age of 28±3.22 years were treated with SRP along with 0.3% nutmeg gel (Group B). PD and CAL showed significant reduction from baseline to three months in test and control groups and was statistically significant (p<0.05). On intergroup comparison in terms of PD and CAL statistically significant difference was present (p<0.05) at the end of three months, favouring Group B.

Conclusion: The developed nutmeg gel was found to be effective when used in addition to SRP as a Local Drug (LD) among patients with stage II grade A periodontitis.

Keywords: Herb, Mechanical therapy, Non surgical periodontal therapy, Phytotherapy

INTRODUCTION

Periodontitis is defined as a chronic inflammatory disease affecting the tooth-supporting structures, resulting in periodontal pockets, clinical attachment loss, recession, mobility, and bone loss eventually leading to tooth loss [1]. It is a multifactorial disease that is caused by the complex interplay between microorganisms present in plaque and the host immune system [2]. The SRP is the fundamental periodontal therapy that aims to disrupt the biofilm responsible for periodontal inflammation. However, the limitations of SRP include its inability to reach deep pockets and areas with limited accessibility. Therefore, LD therapy has emerged as a promising approach for treating periodontitis, offering targeted delivery of therapeutic agents directly to the affected periodontal tissues, reaching areas that may be difficult to access through conventional methods. Various antibiotics, such as tetracycline, minocycline, and doxycycline, have been used as LD agents in periodontal pockets. However, the main concern is that some bacteria can become resistant to antibiotics, making the therapy challenging [3]. Natural or herbal agents in the treatment of periodontitis present several advantages over antibiotic-based LD delivery systems. While antibiotics have been conventionally employed to combat bacterial infections associated with periodontal diseases, the use of natural compounds offers a reduced risk of antibiotic resistance; therefore, herbs are a safer and more sustainable approach to managing periodontitis [4-6]. Nutmeg, derived from the seed of *Myristica fragrans*, has been traditionally used for its aromatic and culinary properties. Recent studies have unveiled its potential therapeutic benefits, including antimicrobial and anti-inflammatory properties [7,8]. The active compounds in nutmeg, such as myristicin and macelignan, exhibit antibacterial and anti-inflammatory effects. This dual action of targeting both bacterial pathogens and inflammation contributes to a more comprehensive approach in managing periodontitis. The main focus of the study was to formulate nutmeg gel and to assess its anti-inflammatory, antioxidant, and cytotoxic activities, and its effectiveness as a locally delivered drug in the management of stage II grade A periodontitis.

MATERIALS AND METHODS

In-vitro analysis: In-vitro analysis was done under the guidance of researchers in the Pharmacology laboratory at Department of Periodontology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha

University, Chennai, Tamil Nadu, India. Patients who reported between June 2023 and September 2023 were enrolled.

Preparation of nutmeg gel: A 5 g of nutmeg powder were mixed with 100 mL of distilled water. The obtained solution was heated at 65 degrees centigrade for 15 minutes, resulting in nutmeg extract. Then, 2 mL of propylene glycol (humectant), 4 mL of 95% ethanol (antiseptic), 0.12 g methyl paraben (preservative), 0.01 mL triethanolamine (buffer), 0.05 mg piperine (anti-inflammatory and antioxidant), and 150 mg of carbopol 934 (gelling agent) were added to 20 mL of distilled water, and a thick mixture was obtained. To this mixture, 1 mL of nutmeg extract was added to obtain 30 g of nutmeg gel [Table/Fig-1] [9].

[Table/Fig-1]: Nutmeg gel [9].

Anti-inflammatory activity: To evaluate the anti-inflammatory property of the nutmeg gel, a protein denaturation method called Bovine Serum Albumin (BSA) assay [9] was done. Initially, 10 mg of nutmeg gel was shifted into a 100 millilitre flask, which underwent pre-washing with distilled water and dimethylformamide. This volume was subsequently adjusted using a phosphate buffer (0.2 M, pH 7.4). Next, 1.5 millilitres of the above solution was shifted into a test tube, then 1.5 milligrams of BSA (1.329 g in 10 millilitres of phosphate buffer). This solution was kept at 37°C for 10 minutes and then further incubated at 27±1°C for 15 minutes. After cooling, absorbance was measured at 660 nm. Positive control was Diclofenac gel. The percent inhibition of protein denaturation, conducted in triplicate, was calculated using the formula:

% Inhibition= 100× (Vt/Vc-1)

Where,

Vt= absorbance of the test sample

Vc= absorbance of the control

Cytotoxic analysis: The cytotoxic effect of nutmeg gel was evaluated with the brine shrimp lethality assay [10].

Brine shrimp eggs (*Artemia salina*), sourced locally from Aquatic Remedies in Chennai, were incubated in artificial seawater. This solution consisted of 40 g/L of sea salt, enriched with six mg/L of dried yeast, and was oxygenated with an aquarium aerator. After 48 hours of incubation in a warm atmosphere (22°C to 29°C), the nauplii were harvested by drawing them to one side of the tank using a light source and collecting them using a Pasteur pipette. Using a pipette, the nauplii were then removed from the eggs and placed two or three times into small beakers filled with seawater. Then, 30 µg of nutmeg gel was applied to each well which was filled with Sodium Chloride (NaCl) solution, and 10 nauplii were added. For comparison, a control group comprising solely of nauplii and NaCl solution was employed. The wells to remain undisturbed for 24 hours, then the number of surviving nauplii in each well was counted and documented. The lethality of the extract was then calculated using the following formula: {number of dead nauplii/(number of dead nauplii+number of live nauplii)}×100.

Antioxidant activity: A test for antioxidant activity using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was carried out [11]. After 30 minutes of incubation, 30 µg of nutmeg gel was combined with 450 µL of 50 mM Tris-HCl buffer (pH 7.4) and 1 mL of 0.1 mM DPPH in methanol. After incubation, the absorbance at 517 nm was used to measure the reduction in DPPH free radicals, with Butylated Hydroxytoluene (BHT) acting as the control. Using the following formula, the % inhibition, which was measured in triplicate, was then calculated: (sample absorbance - absorbance of control) / control absorbance × 100. This measurement was conducted in triplicate.

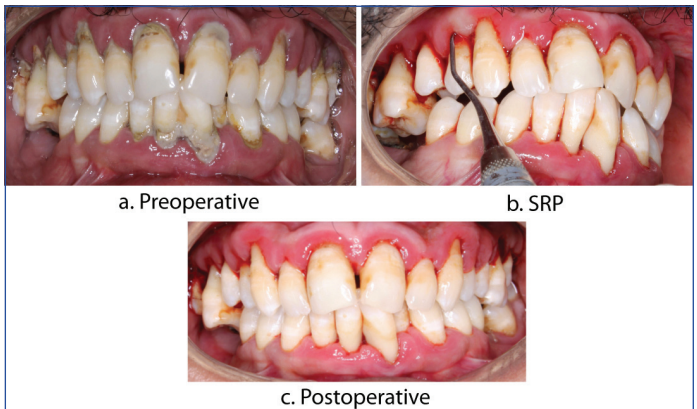
In-vivo analysis: The present study included 40 chronic periodontitis patients from the study Institute. The research protocol received approval from the Institutional Review and Ethical Committee of Saveetha Dental College and Hospitals, Chennai, India (Reference no: IHEC/SDC/PERIO-2201/24/007).

Inclusion and Exclusion criteria: Patients aged between 25 to 55 years, diagnosed with stage II grade A periodontitis (PD of ≤5 mm, interdental clinical attachment loss of 3-4 mm, and horizontal bone loss with slow rate of disease progression) [12], patients with a minimum of 20 natural teeth, systemically healthy patients were enrolled as study participants. Whereas, systemically compromised patients, patients on long-term medications, tobacco users, pregnant and lactating women were excluded from the study. Informed consent was obtained from all participating individuals prior to the start of the study.

Sample size calculation: For the calculation of sample size, G*Power software was used, considering a power of 80% and an alpha error at a 95% confidence level based on mean and standard deviation values from previous research (2.702±0.12) [13]. The sample size was calculated to be 40.

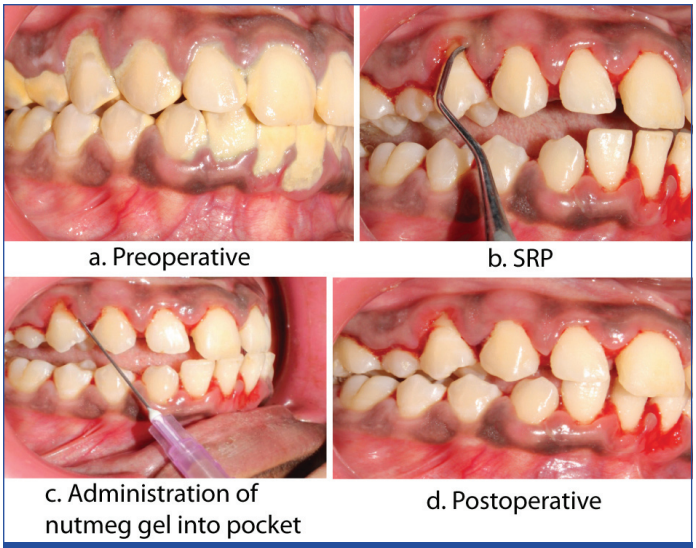
Study Procedure

A total of 20 patients were subjected to SRP alone (Group A - Control [Table/Fig-2a-c]), and the remaining 20 patients received SRP combined with 0.3% nutmeg gel (Group B - Test [Table/Fig-3a-d]).



[Table/Fig-2a-d]: Pre- and postoperative images of Group-A.
group A- Control (SRP)

Clinical parameters: Clinical parameters, including Pocket Probing Depth (PPD) and CAL, were assessed at baseline and after a span of three months using the University of North Carolina probe (UNC-15 probe). PPD was measured from the tip of the marginal gingiva to the base of the pocket at six sites (mesiobuccal, mid-buccal, distobuccal, mesiopalatal/lingual, mid-palatal/lingual, distopalatal/lingual), and the average was obtained. CAL was estimated from the cementoenamel junction as a reference point to the base of the pocket at the same



[Table/Fig-3]: Pre- and postoperative images of Group-B.
Group-B- test (SRP+0.3% nutmeg gel) (images from left to right)

six sites (mesiobuccal, mid-buccal, distobuccal, mesiopalatal/lingual, mid-palatal/lingual, distopalatal/ lingual) and the average was obtained. All the measurements were done by a single examiner (AR).

Periodontal therapy: All participants in present study underwent single-visit SRP, using a piezoelectric scaler and hand instruments (Gracey curettes, Hu-Friedy®, Chicago, IL, USA).

In Group B patients, along with SRP, 1 mL of 0.3% nutmeg gel was applied. The gel was injected into the periodontal pocket with the help of a syringe immediately after SRP. Participants received instructions to avoid hard or sticky foods, and brushing or using interdental aids was refrained from near the treated areas for one week. Patients were scheduled for a review after three months.

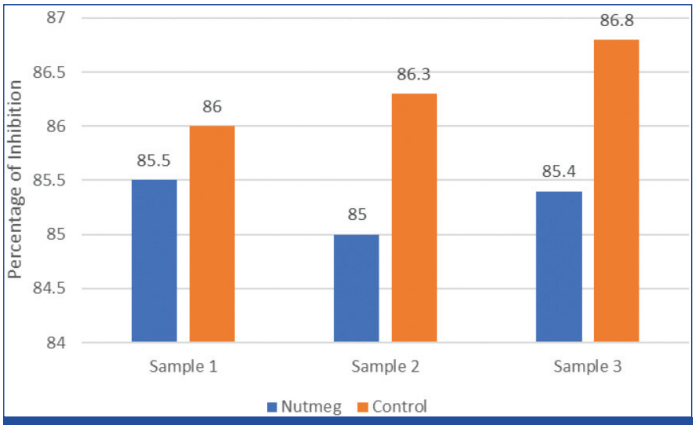
STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software, version 23.0. To assess the normality of the data distribution Shapiro-Wilk test was performed. Intergroup comparison of age, PPD, and CAL was done by independent t-test. Intergroup comparison of gender was done by Chi-square test. And for comparing within the group between baseline and three months paired t-test was utilised. A p-value<0.05 indicated a statistically significant difference.

RESULTS

In-vitro Analysis

Anti-inflammatory activity: The mean percentage inhibition of nutmeg gel and control were 85.47±0.37 and 86.53±0.45, respectively. Therefore, anti-inflammatory property of nutmeg gel was comparable to that of the control [Table/Fig-4].



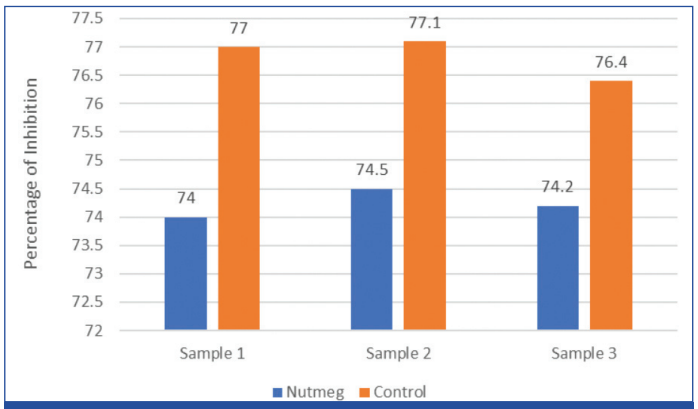
[Table/Fig-4]: Bovine Serum Albumin (BSA) assay.

Cytotoxic analysis: Observations were made over a span of two days. At the end of 1st day, no death of nauplii were noted in both the groups (nutmeg gel and control group) and on 2nd day, death of 2 nauplii was seen in the nutmeg group and no mortality was seen in the control group [Table/Fig-5]. Therefore, results showed that nauplii survived in nutmeg extract. The survival rate of nauplii fell within acceptable ranges.

Groups	Survival of naupauli (percentage) at baseline n (%)	Survival of naupauli (percentage) after 24 hours n (%)	Survival of naupauli (percentage) after 48 hours n (%)
Test	10 (100)	10 (100)	8 (80)
Control	10 (100)	10 (100)	10 (100)

[Table/Fig-5]: Cytotoxic analysis.

Antioxidant activity: The mean percentage inhibition of nutmeg gel and control were 74.05±0.55 and 77.56±0.34, respectively. Therefore, antioxidant activity of nutmeg gel was comparable to that of the control [Table/Fig-6].



[Table/Fig-6]: The 2,2-Diphenyl-1-Picrylhydrazyl assay.

In-vivo Analysis

The demographic characteristics of both the groups is summarised in [Table/Fig-7]. Patients treated with SRP (Group A) presented with a mean age of 29.45±3.98 years, while patients treated with SRP along with 0.3% nutmeg gel (Group B) presented with a mean age of 28±3.22 years. There were 13 female and seven male participants in Group B comprised eight female and 12 male patients. No statistical significance was seen between both the groups regarding age and gender (p-value>0.05).

Parameters	Group A (SRP) n=20	Group B (SRP+0.3% Nutmeg gel) n=20	p-value
Age (in years)#	29.45±3.98	28±3.22	0.59
Gender§ (Female/Male)	13/7	8/12	0.45

[Table/Fig-7]: Demographic characteristics of both the groups.
#: Independent t-test; §: Chi-square test

Clinical parameters: There was a statistically significant decrease (p-value<0.05) seen in PD from baseline to three months in both the groups. On intergroup comparison at the end of 3rd month, there was a statistically significant difference (p-value=0.025) favouring Group B. Also, in both the groups CAL also reduced from baseline to three months and was statistically significant (p-value<0.05). On intergroup comparison at the end of 3rd month, statistically significant difference (p-value=0.045) favouring Group B was observed [Table/Fig-8].

Clinical parameters	Groups	Baseline (Mean±SD)	3 months (Mean±SD)	p-value[a]
PD (mm)	Group A Group B	7.24±2.13 7.25±2.20	5.05±0.90 4.32±1.05	0.04* 0.03*
p-value[b]		1.997	0.025*	
CAL (mm)	Group A Group B	8.56±1.70 8.45±1.55	5.45±0.9 4.39±1.25	0.05* 0.01*
p-value[b]		1.887	0.045*	

[Table/Fig-8]: Inter- and intragroup comparison of clinical parameters.
a: Intragroup comparison (paired t-test); b: Intergroup comparison (independent t-test); *Statistically significant

The clinical images from the test and control groups are shown in [Table/Fig-2,3].

DISCUSSION

Periodontitis, a prevalent oral disease, poses significant treatment challenges due to its anatomical location and the involvement of anaerobic organisms. To enhance the effectiveness of mechanical therapy, various adjunctive treatments are commonly employed. In recent years, herbal agents have gained substantial importance in this context. Nutmeg, in particular, has been well-documented for its beneficial biological properties, making it a promising candidate in the adjunctive treatment of periodontitis. In this context, the present study aimed to prepare nutmeg gel and assess its anti-inflammatory, antioxidant, and cytotoxic properties. Following this, the nutmeg gel was evaluated for its efficacy as a local drug delivery system in the management of stage II grade A periodontitis.

In the present study, it was observed that nutmeg gel at a concentration of 30 µg/mL displayed anti-inflammatory and antioxidant activities as compared to standard drugs and was also found to be non cytotoxic. Zhang CR et al., evaluated for its bioactive components using in vitro antioxidant and anti-inflammatory assays and found out it has both the properties. Nutmeg's anti-inflammatory effects are attributed to compounds like myristicin, which inhibit Interleukin-1 beta (IL-1β) and Tumour Necrosis Factor-alpha (TNF-α), which are proinflammatory mediators [14]. Nutmeg also contains limonene, β-pinene, α-pinene, and sabinene, which are 5-lipoxygenase inhibitors [15]. Limonene acts as Cyclooxygenase-2 (COX-2) selective inhibitor, significantly inhibiting the production of prostaglandin production. α-Pinene reduces IL-6, while sabinene inhibits IL-1β and IL-6 [16]. According to Morita T et al., out of 21 spices, myristicin, the main component of nutmeg oil, prevented macrophages from releasing TNF-α [17]. Malabaricone C, found in nutmeg, irreversibly inhibits Arg-gingipain (virulence factor of *P. gingivalis*) [18]. This substantiates the usage of nutmeg in periodontitis.

Furthermore, in present study, it was also shown that there was more improvement in clinical parameters, such as PPD and CAL, among patients treated with SRP and nutmeg gel compared to those treated with SRP alone. The results of the current research cannot be directly compared to those of any other study, as it is the first study of its type to assess the efficacy of nutmeg gel as a locally delivered agent in the management of periodontitis. However, these results in the current research are indirectly in line with previous studies where other herbs have been used in addition to SRP as local delivery agents in periodontitis patients.

Padol MV et al., evaluated nutmeg mouthwash for patients with plaque and halitosis and compared it with 0.2% Chlorhexidine (CHX) gluconate mouthwash, observed that nutmeg mouthwash is natural, economical, and produces effects similar to those of 0.2% CHX gluconate mouthwash [19]. Ashouri Moghaddam A et al., evaluated efficiency of local delivery of aloe vera gel along with SRP in chronic periodontitis and observed a significant decrease in pocket depth at 30th and 60th day [20]. Deepika BA et al., reported the efficiency of 2% Ocimum sanctum gel and CHX gel for treating gingivitis, and reported that there was a significant improvement in CAL [21]. Sanghani NN et al., reported that both clinical and microbiological parameters significantly improved in the test sites in comparison of control sites when subgingival delivery of propolis was done in addition to SRP in patients with chronic periodontitis [22]. Similar results were obtained when 2% whole turmeric gel was used in addition to SRP in chronic periodontitis [23]. The present study findings are in accordance with the previous studies.

Limitation(s)

Overall, the developed nutmeg gel is effective when used in addition to SRP as a LD among stage II grade A periodontitis patients. However, present study has few limitations. The study duration, although sufficient to observe significant changes, may limit insights into the long-term efficacy and sustainability of nutmeg gel. Future research should explore the optimal concentration of nutmeg in the gel formulation, evaluate its efficacy in diverse populations, and investigate its long-term effects on periodontal health. Mechanistic studies at the molecular and microbial levels would provide a more nuanced understanding of nutmeg's impact. Large-scale, multicentre trials with extended follow-up periods could further validate the clinical utility of nutmeg gel.

CONCLUSION(S)

The study findings suggest that nutmeg gel has anti-inflammatory, antioxidant properties, and it is non cytotoxic; therefore, it can be used for patients with stage II grade A periodontitis and locally administered nutmeg gel seems to be effective as an adjunct to SRP.

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