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Original Research Article

Efficacy of moringa oleifera as a local drug delivery agent in the treatment of chronic periodontitis: A randomized controlled trial

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Abstract

Aim: To evaluate the efficacy of chitosan chips with Moringa oleifera (MO) and chitosan chips without MO for treating chronic periodontitis.

Materials and Methods: LDD chips were made with 10% MO, 2% chitosanPEG400.A total of 38 sites of chronic periodontitis patients received LDD chips with MO, and another group of 38 sites received LDD chips without MO. The clinical parameters were assessed at baseline, 1 month, and 3 months. Radiographic alveolar bone levels were assessed at baseline and 3 months in Group I only.

Results: Group I exhibited a mean plaque index of 0.45 at one month, improving significantly to 0.45 by the third month ($P < 0.001$), while Group II's mean decreased from 0.65 to 0.29. The mean gingival index for Group I improved from 0.39 to 0.45, and for Group II from 0.08 to 0.29, both showing significant changes ($P < 0.001$). Notably, Group I had a significant reduction in probing pocket depth, from 5.26mm at one month to 4.26mm at three months ($P < 0.001$), outperforming Group II's results.

Conclusion: Moringa oleifera in chitosan chips may be an effective natural treatment for chronic periodontitis. The study shows significant improvements in clinical parameters such as plaque index, gingival index, and probing pocket depth in the Moringa group, suggesting its potential to enhance treatment outcomes in periodontal care. Further exploration of its applications is encouraged.

Clinical Significance: This study underscores the potential of Moringa oleifera in chitosan chips for treating chronic periodontitis. Significant improvements in clinical parameters, such as plaque index, gingival index, and probing pocket depth in the Moringa group, suggest it may enhance treatment outcomes. This natural agent offers a promising adjunct option for dental professionals, supporting better management of chronic periodontitis and encouraging further exploration of natural products in periodontal care.

Keywords: Complete Denture, Remedies, Troublesome dentures

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1. Introduction

Local drug delivery (LDD) is made to administer the medication directly into the periodontal pocket, allowing it to penetrate the periodontal tissues and stay at the therapeutic level there for a longer amount of time.[1] In LDD, several antibiotics have been used to eradicate resident periodontal pathogens and serve as an additional therapeutic option to mechanical treatment.[2] Tetracycline fibers,[3] 10% doxycycline,[4] 2% minocycline,[5] metronidazole,[6] and chlorhexidine gluconate[7] are the most researched local delivery agents. All of them have the potential to cause unfavorable side effects, including toxicity, drug hypersensitivity, drug resistance, opportunistic infection development, and drug interactions.[8]

Herbs, plants, and plant-derived products have long been recognized for their medicinal properties in treating various illnesses, including infectious diseases, across different cultures. Their anti-inflammatory and antimicrobial qualities contribute to their effectiveness.[9] The formulation of a controlled-release drug delivery system (LDD) using herbal extracts could enhance treatment outcomes, reduce side effects, and serve as a complementary therapeutic option to

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mechanical debridement for managing periodontal disease.[10] The investigation into the potential medicinal benefits of natural products like pomegranates, cork bark, tulsi, neem, turmeric, and aloe has grown as a result of this field's research.[11]

Moringa oleifera (MO), also referred to as the drumstick tree, is a highly significant and valuable medicinal plant that grows throughout tropical climates and is used to treat a variety of illnesses.[12] More than 90 different types of nutrients, including vital vitamins, minerals, and amino acids, are found in *moringa oleifera*, which has also been shown to have anti-aging and anti-inflammatory properties.[13] Because it contains phytosterols, flavonoids, tannins, glycosides, and amino acids like quercetin and kaempferol, the majority of its parts—seeds, leaves, flowers, roots, & fruits—may have anti-inflammatory, antitumor, & antibacterial properties.[14] Traditionally, the leaves of MO have been used to treat sores, fever, bronchitis, sore throats, eye and ear infections, and glandular swelling.[15] MO leaves' ability to heal wounds has been well-documented over time in both in vitro and in vivo models.[16] The anti-inflammatory, anti-cancer, antibacterial, antiviral, immunomodulatory, antithrombotic, and osteoprotective properties of flavonoids are found in the leaves.[17] Vitamins A, B, C, calcium, iron, and protein are among the many nutrients found in *moringa leaves*.[18] *Moringa oleifera* leaf extract may inhibit the inflammatory pathway, inhibit bone resorption, and increase the proliferation and differentiation of osteoblast cells.[19] Saponins, terpenoids, and alkaloids found in *Moringa oleifera* contain anti-inflammatory transcription factors that work against NF-kB (nuclear factor kappa B) ligand and nuclear factor erythroid-derived 2, two transcription factors that are frequently seen and implicated in the pathophysiology of chronic inflammatory diseases like periodontitis.[20] The plant is used in dentistry for its antibacterial properties, including toothpaste, mouthwash, root canal irrigation, wound healing, and prevention of dental caries.[21-23] Based on our literature search, there are currently no reports on *Moringa oleifera* utilized as a local drug delivery agent. Therefore, this study aims to assess the efficiency of using Moringa oleifera as a LDD agent in the treatment of chronic periodontitis. As far as we are aware, this is the first investigation.

2. Materials and Methods

2.1. Study design and ethical approval

This study was a randomized split-mouth, a single-blinded trial conducted in the Department of Periodontology at SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India over the period from 09-2-2024 to 10-8-24. The study received institutional ethical approval from the ethical council (IEC number: 2022/PG/PERIO/101) and was registered in the clinical trial registry (ClinicalTrials.gov ID: CTRI/2024/01/061913). The study adhered to the principles outlined in the Helsinki Declaration of 1975, as revised in 2013. Each patient was provided with an information sheet detailing the purpose and methodology of the study, and consent was signed by them.

2.2. Preparation of biodegradable chitosan chips

The process commenced with the careful collection of approximately 100 grams of fresh Moringa oleifera leaves from a single plant in agricultural fields and taxonomically verified by the biologist. Subsequently, the leaves underwent a meticulous drying process lasting between 3 to 4 days while being shielded from direct sunlight. After the filtering and drying process, the final quantity of Moringa oleifera extract obtained was around 15 grams, which was carefully stored in a refrigerator at 4 degrees Celsius to ensure its stability and integrity were maintained.

Later, the water-soluble 2% chitosan and polyethylene glycol 400 were dissolved in distilled water. This solution was stirred for an hour, and then Moringa oleifera extract, ascorbic acid, and saccharine were mixed thoroughly for 30 minutes. After stirring the solution, the mixtures were combined and homogenized. The resulting solution was then poured onto a suitable petri dish and placed in a hot air oven at 60 degrees Celsius for an hour to aid in the drying process. Once the chip had solidified, it was removed from the glass plate, cut into 2mm x 5mm chips, wrapped in aluminum foil, sterilized in a UV chamber, and stored in sterile vials at room temperature. Plain chitosan chips were also prepared using the same method, excluding the addition of Moringa oleifera extract. The chips with and without Moringa oleifera exhibited a yellowish hue, opacity, firm texture, and smooth surface, with 0.002mm thickness, 0.027mg weight, and 6.8 pH at 37°C.

2.3. Study sample and selection criteria

Among 38 systemically healthy chronic periodontitis patients, two contralateral sites from each patient were selected for the study, resulting in a total of 76 sites with a pocket depth of 5-7 millimeters. The patients ranged in age from 18 to 60 and had at least 20 teeth in their mouths. Smokers, pregnant and breastfeeding women, and individuals who had undergone dental surgery or taken antibiotics within the last three months were excluded from the study.

2.4. Study design details

After patient selection, phase I therapy was completed for all participants. The split-mouth study design allowed for the placement of treatment chips at the chosen contralateral sites in each patient. The selected sites were divided into the following groups: Group 1 (38 sites) received chitosan chips with Moringa oleifera, and Group 2 (38 sites) received chitosan chips without Moringa oleifera. An impartial investigator, who had no direct involvement in the clinical aspects of the trial, expertly devised the allocation sequence using a computer random number generator with a 1:1 allocation ratio. To maintain the study's integrity, the

allocation sequence was kept hidden from both the patient and the lead investigator inside opaque sealed envelopes. A different researcher, not involved in treatment or data gathering, managed patient enrollment and the distribution of the sealed envelopes containing the treatment modalities for each site. Each patient's registration number was initialed on these opaque envelopes, ensuring precise identification and distribution. Each envelope was opened promptly before the start of the respective procedure, adhering to strict procedural guidelines. A solitary investigator carried out every procedure. Baseline measurements included the plaque index (PI), gingival index (GI), and modified Sulcus Bleeding Index (mSBI).[24-26] Customized acrylic stents were made to record pocket depth (PPD) and relative attachment level (RAL) at baseline, one month, and three months. Radiovisiography (RVG) equipment was employed to measure radiographic alveolar bone levels (RBL) in Group I at baseline and three months into the treatment using a paralleling/long-cone approach at 70 KVp, 10 mA, and an exposure period of 0.8 seconds. The paralleling technique was employed with the sensor holder. Using the most recent version of the ImageJ processing and analysis software, the radiographic bone levels were compared.

2.5. Study procedure

The designated site was isolated before inserting the chip into the pocket. The chip's rounded end was pointed towards the bottom of the pocket. A periodontal dressing (Coe-pak) was placed. The dressing was removed after one week, and the patients were retrieved after one and three months to record their parameters. There were no adverse effects identified in any patient.

2.6. Statistical analysis

SPSS (28 version) was used to analyze the data. To determine if every variable was distributed normally, the Shapiro-Wilk test was employed. Two independent groups were compared using the Friedman test, and pairwise comparisons were made using the t-test and the Dunn-Bonferroni test. A p-value of less than 0.05 was considered to be statistically significant.

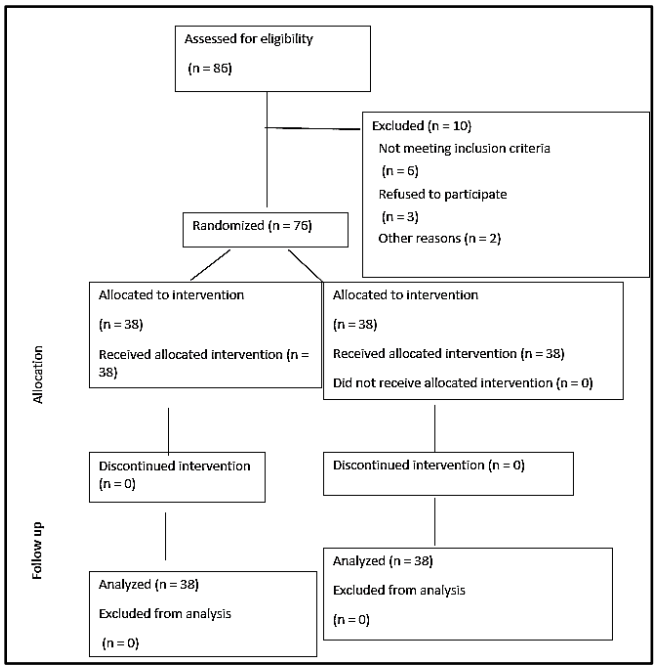

Figure 1: Consort diagram showing the flow of participants through each stage

Table 1: Comparison of PI, GI, and mSBI at various time intervals in groups I and I $^{*}p \leq 0.05$ (statistically significant)

Parameters	Groups	Period	Mean	SD	P Value
	Group I	Baseline	1.21	0.62	0.001*
		1 month	0.45	0.69	
PI		3rd month	0.18	0.39	
	Group II	Baseline	1.05	0.7	0.001*
		1 month	0.61	0.75	
		3rd month	0.34	0.58	
	Group I	Baseline	1.05	0.7	
		1 month	0.39	0.64	0.001*
GI		3rd month	0.08	0.27	
	Group II	Baseline	0.84	0.72	0.003*
		1 month	0.45	0.69	
		3rd month	0.29	0.57	
	Group I	Baseline	0.79	0.41	
mSBI		1 month	0.34	0.48	0.001*
		3rd month	0.08	0.27	
	Group II	Baseline	0.68	0.47	0.034*
		1 month	0.45	0.5	
		3rd month	0.29	0.46	

Table 2: Comparison of PPD, RAL at various time intervals in groups I and II and RAL at baseline and 3 months in group I

Parameters	Groups	Period	Mean	SD	P Value
	Group I	Baseline	6.26	0.977	0.001*
		1 month	5.26	1.201	
PPD		3rd month	4.26	0.446	
	Group II	Baseline	6.26	0.86	0.072
		1 month	5.58	0.889	
		3rd month	4.76	0.634	
	Group I	Baseline	8.28	1.55	
		1 month	6.81	1.64	0.98
RAL		3rd month	5.28	0.92	
	Group II	Baseline	7.76	1.46	1
		1 month	6.84	1.30	
		3rd month	5.89	0.95	
	Group I	Baseline	2.84	0.31	
RBL		3rd month	2.85	0.32	0.001*

*$p \leq 0.05$ (statistically significant)

3. Results

The study procedure was detailed through a CONSORT flowchart, providing a comprehensive description of the various stages of the study.is given in **Figure 1** ("Additional file 1")

3.1. Plaque index

The intra-group comparison revealed that Group I had a mean Plaque Index (PI) of 0.45 at 1 month, while Group II had a mean PI of 0.65. Both groups demonstrated significant improvements in PI at the 3-month mark, with a notable reduction in the mean PI from 1 to 3 months ($P < 0.001$). Intergroup comparison showed that the PI in Group I was significantly lower in the 3rd month compared to Group II (**Table 1**).

3.2. Gingival index

The mean Gingival Index (GI) in the 1st month for Group I was 0.39 and for Group II, it was 0.45. By the 3rd month, Group I had a mean GI of 0.08, and Group II had a mean GI of 0.29. Significant improvement was observed in both groups at the 3rd month when compared to their respective baseline and 1-month values ($p < 0.001$). Furthermore, intergroup comparison indicated a statistically significant lower GI index at 3 months for both groups compared to their baseline and 1-month measurements ($P < 0.01$) (**Table 1**).

4. Modified Sulcular Bleeding Index

In the 1st month, the mean Modified Sulcular Bleeding Index (mSBI) for Group I was 0.34 and for Group II was 0.45. By the 3rd month, Group I showed a mean mSBI of 0.08, while Group II had a mean score of 0.29. Both groups exhibited positive changes in mSBI at the 3rd month compared to baseline and 1 month ($p < 0.001$). Intergroup comparison indicated a statistically significant reduced mSBI index for Group I compared to Group II at both baseline and 1 month ($P < 0.001$) (**Table 1**).

4.1. Probing pocket depth

The mean probing pocket depth at 1 month was 5.26 mm for Group I and 5.58 mm for Group II. At the 3-month assessment, Group I had a mean probing pocket depth of 4.26 mm, while Group II had a mean of 4.76 mm. Both groups demonstrated improvement in mean probing pocket depth from baseline to 1 month, with intergroup comparison using the Bonferroni test revealing a greater reduction in probing pocket depth in Group I ($P < 0.001$) compared to Group II at the end of three months (**Table 2**).

4.2. Relative attachment level

Comparison of the relative attachment level (RAL) between the groups showed no discernible variation at 1 month or 3 months. By the 3rd month, Group I's mean RAL improved significantly, but the findings were statistically non-significant ($p = 0.98$ for Group I and $p = 1$ for Group II) (**Table 2**).

5. Radiographic Bone Level in Group I

Group I's mean radiographic bone level (RBL) at baseline was 2.84, which increased slightly to 2.85 by the 3rd month. A statistically significant difference was observed at the 3rd month when compared to baseline ($p < 0.001$, 95%). (**Table 2**)

6. Discussion

Chitosan is one of the most extensively investigated natural polymers. This hydrophilic biopolymer, which is obtained through the deacetylation of chitin, possesses bio-adhesive and permeabilizing characteristics in addition to being non-toxic, biocompatible, and biodegradable.[27] It is a promising

agent in the dental field because it possesses antimicrobial, wound healing, hemostatic, and tissue regenerative properties. These characteristics led to the decision to use chitosan with PEG 400 when making the chip because of its great heat stability and ability to dissolve poorly soluble substances like herbal extracts into the base. The current study is a randomized split-mouth experimental design that was double-blinded to prevent treatment bias and eliminate inter-individual variability. Locally delivered controlled-release drug has a noticeable effect up to 11 weeks after administration.[28] Therefore, the follow-up was scheduled at one- and three-month intervals to assess parameters at these intervals. In the current study, the amount of Moringa oleifera (MO) was ten percent. MO may have antibacterial and antioxidant effects against Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), and Fusobacterium nucleatum (Fn) at doses ranging from 5% w/v to 12.5% w/v, according to Nugraha AP et al.[29]

The results showed a more significant decrease in Plaque Index (PI), Gingival Index (GI), and modified Sulcus Bleeding Index (mSBI) at one month and three months in Group I (chitosan with MO) than in Group II (chitosan without MO). This might have resulted from the anti-inflammatory properties of MO. There exists the possibility of the Hawthorne effect for this reduction in inflammation.[30] MO leaf extract reduces inflammation by decreasing IKBα phosphorylation, NFKB inhibition, nuclear translocation, and IkBα & NFKB dimerization. This process prevents the synthesis of inflammatory proteins, including COX-2, TNFα, IL6, and iNos.[31]

Xu Y et al. reported that M. oleifera contains active compounds in secondary metabolites like saponins, tannins, phenols, terpenoids, flavonoids, and alkaloids, which have anti-inflammatory, antibacterial, and antioxidant characteristics. It can act as an immune booster, helping the wound healing process and improving the mucosal defense system.[32] To investigate M. oleifera's anti-inflammatory properties, Fard MT et al. used the murine macrophage RAW264.7 cell line (ATCC, TIB-71) as an in vitro model. It inhibited the production of various inflammatory indicators, including prostaglandin E2, tumor necrosis factor-alpha, interleukin (IL)-6, IL-1β, and nitric oxide (NO).[33]

The current investigation revealed a reduction in Pocket Probing Depth (PPD) and a gain in Relative Attachment Level (RAL) in the chitosan chip with the Moringa oleifera group when compared to the chitosan chip without Moringa oleifera group at 1 month and 3 months. There was a statistically significant improvement in bone level at the 3rd month compared to baseline. These results align with an animal study by El Soudany KS et al. which investigated Moringa hydrogel and Platelet Rich Fibrin (PRF). CT scans demonstrated a significant increase in bone density, with bone defects filled by newly formed bone, along with a few spots of retarded calcification on histological examination.[34] Moringa oleifera leaf extract may increase the number of osteoblasts and promote bone formation by suppressing osteoclast formation.[35]

6.1. Inference of the current study

The findings of this study indicate that both treatment groups exhibited significant improvements across various indices related to periodontal health. Group I consistently demonstrated greater reductions in the Plaque Index, Gingival Index, and Modified Sulcular Bleeding Index, along with enhanced probing pocket depth and radiographic bone levels. This suggests that the intervention applied in Group I may be more effective in improving periodontal parameters compared to Group II.

6.2. Strength

The study employs a randomized split-mouth design, minimizing treatment bias, and includes a double-blinded approach to reduce inter-individual variability.

6.2. Limitations

The study is limited by the use of whole-leaf extract, which may not provide insights into the effects of specific active components of Moringa oleifera.

6.3. Future directions

Future studies can be conducted with alternative MO extract active components and bio-adhesive polymers. It is important to assess medication release patterns, conduct microbiological analyses, and analyze biomarkers for bone repair. Larger sample size longitudinal clinical trials can be employed to assess the additional benefits of Moringa oleifera in various dosage forms. Additionally, exploring MO's potential for regeneration in periodontal therapy is warranted.

7. Conclusion

Both chips as LDD were effective in the non-surgical treatment of chronic periodontitis, though the chitosan chip with MO was superior to the chitosan chip without MO. Radiographic bone was observed in sites treated with MO. It may be a promising LDD agent and regenerative material in periodontal therapy.

7.1. Data availability

Datasets related to this article will be available upon request to the corresponding author.

8. Source of Funding

None.

9. Conflict of Interest

None.

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