

Preparation and Characterisation of Flaxseed Microspheres and Assessment of its Anti-inflammatory and Antioxidant Properties: An In-vitro Study

VAZEEHA AFRIN SYED¹, ANANYA RAVIKUMAR², ARVINA RAJASEKAR³, RAJESHKUMAR SHANMUGAM⁴

ABSTRACT

Introduction: Flaxseed (*Linum usitatissimum*), commonly known as flax or linseed, is a member of the Linaceae family and is high in calories, proteins, carbohydrates, fibres, fatty acids, vitamins and minerals. Flaxseed is a source of phytochemicals with possible health advantages. Given flaxseed's rich phytochemical content with antioxidant and anti-inflammatory properties, it holds potential as a therapeutic agent for managing periodontitis.

Aim: The present in-vitro study aimed to formulate flaxseed microspheres and to evaluate its anti-inflammatory and antioxidant potential.

Materials and Methods: The present in-vitro research was done in Nanomedicine lab, Saveetha Dental College and Hospitals, Tamil Nadu, India, between May 2024 and July 2024. The flaxseed microspheres were prepared using the water-in-oil-in-water

(w/o/w) double emulsion method. The obtained microspheres were subjected to topographic analysis via Scanning Electron Microscope (SEM). Also, anti-inflammatory and antioxidant properties were analysed using protein denaturation and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assays, respectively. Intergroup comparison was done using an independent t-test and p-value <0.05 was considered statistically significant.

Results: SEM images revealed spherical microspheres with a smooth, dense surface and slight aggregation. Protein denaturation and DPPH assays revealed that there was a significant increase in the percentage of inhibition with relation to the increase in the concentration of flaxseed microspheres.

Conclusion: The fabricated flaxseed microspheres exhibited superior anti-inflammatory activity and antioxidant activity that gives promising view for future researches to further explore its therapeutic role in oral healthcare.

Keywords: Free radical, Inflammation, Phytochemicals, Phytotherapy

INTRODUCTION

Periodontitis is a chronic inflammatory disease that primarily affects the tissues that support teeth, such as the gingiva, periodontal ligament, and alveolar bone [1]. If untreated, it causes a progressive destruction of the periodontal tissues and is caused primarily by microbial plaque biofilm. The relationship between particular microbial species and the pathogenesis of periodontal disease was firmly established in earlier research [2]. Periodontitis is influenced by various risk factors, such as inadequate oral hygiene, smoking, cardiovascular issues, genetic predisposition, diabetes mellitus and stress [3]. Current treatment for periodontitis focuses on minimising infection, resolving inflammation and establishing a clinical state conducive to optimal periodontal health.

As a preventive strategy, non surgical periodontal therapy plays a crucial role in halting the advancement of periodontal disease. Incorporating systemic antibiotics as a supplement to non surgical periodontal therapy enhances treatment outcomes by addressing residual periodontal pockets and ceasing the progression of periodontal disease [4]. Systemic antibiotics assist the host by targeting and inhibiting specific microbial species. The objective of utilising systemic antibiotics alongside non surgical periodontal therapy is to eradicate pathogenic bacteria. The development of resistant bacterial strains and documented cases of antibiotic resistance, however, present obstacles to the efficacy of antibiotics. Therefore, to inhibit particular pathogenic bacteria and to avoid development of antibiotic resistance, local drug delivery utilising herbs have gained popularity in recent days [5-7].

Within phytotherapeutic formulations, flaxseed holds a significant position due to its robust anti-inflammatory and antioxidant

characteristics, largely attributed to its high content of omega-3 Polyunsaturated Fatty Acids (PUFAs). Flaxseed (*Linum usitatissimum*), commonly known as flax or linseed, is a member of the Linaceae family and is high in calories, proteins, carbohydrates, fibres, fatty acids, vitamins and minerals [8]. Investigation has shown that omega-3 PUFAs regulate gene expression, inflammatory pathways, and cell signaling [9]. Flaxseed consists of notable levels of cysteine and methionine which contribute to its well-known abilities in scavenging hydroxyl radicals and exhibiting antioxidant properties [10]. Microspheres are small spherical particles (1-1000 µm) used as drug delivery systems for controlled release. Their ability to deliver drugs in precise, fixed amounts makes them ideal for sustained therapeutic applications [11]. Literature evidence suggests that the pharmacological properties of flaxseeds have been previously assessed [12]. The present study is novel, as no previous research has explored flaxseeds in the form of microspheres. The present in-vitro study aimed to formulate flaxseed microspheres and to evaluate the anti-inflammatory and antioxidant potential of flaxseed microspheres. In this context, the primary objective of the present study was to prepare flaxseed assisted microspheres and the secondary objective was to assess its anti-inflammatory and antioxidant properties.

MATERIALS AND METHODS

The present in-vitro research was done in Nanomedicine lab, Saveetha Dental College and Hospitals, Tamil Nadu, India, between May 2024 and July 2024 (SRB/SDC/PERIOD-2201/24/418).

Study Procedure

Firstly, the flaxseeds were finely powdered. The w/o/w double emulsion method was used to generate microspheres. A w/o

emulsion was created by emulsifying 250 μ L of 100 μ M flax seed in 2.5 mL of 10% polymer solution in Dichloromethane (DCM) for 10 seconds over an ice bath using a tip sonicator with an output power of 15 W [13]. The w/o emulsion containing the dispersed flaxseed droplets in DCM was gradually added to 20 mL of 1% polyvinyl alcohol while being vigorously stirred to form the w/o/w double emulsion. To harden the microspheres, the solution was swirled for thirty minutes at room temperature. After that, DCM was removed using a water suction method, and the mixture was centrifuged to separate the solid microspheres [Table/Fig-1]. The microspheres obtained underwent three rounds of washing with distilled water, freeze-dried, and then subjected to further analysis.



[Table/Fig-1]: Preparation of flaxseed microspheres.

Topography analysis: Flaxseed microspheres were analysed for their morphological features using SEM. To prepare the samples for imaging, a sputter-coater was employed to apply a thin layer of gold at room temperature. Following this gold coating process, the flaxseed microspheres were subjected to SEM analysis using a field-emission SEM (JEOL JSM-IT800; JEOL USA, Peabody, MA) [Table/Fig-2] at specifically 350X and 500X magnifications, to assess their overall structure.



[Table/Fig-2]: Scanning Electron Microscope (SEM).

Anti-inflammatory activity: Anti-inflammatory activity of the prepared flaxseed microspheres was done by protein denaturation

assay [14]. The experiment was carried out using Bovine Serum Albumin (BSA). Of the total protein in animal serum, about 60% is composed of BSA. BSA denatures and expresses antigens when heated. Different amounts (50, 100, 200, 400 μ g/mL) of the produced flaxseed microspheres (test) were combined with 2 mL of the 1% BSA. To bring the pH of the reaction mixture down to 6.8, 1N hydrochloric acid was then used. The reaction mixture was incubated for 20 minutes at room temperature in a water bath. After letting the mixture cool to room temperature, the absorbance at 660 nm was measured. The control used was diclofenac sodium in various concentrations. This activity was done in triplicates. Percentage of inhibition was calculated using the following formula: % inhibition = (Absorbance of control - Absorbance of sample) / Absorbance of control $\times$ 100.

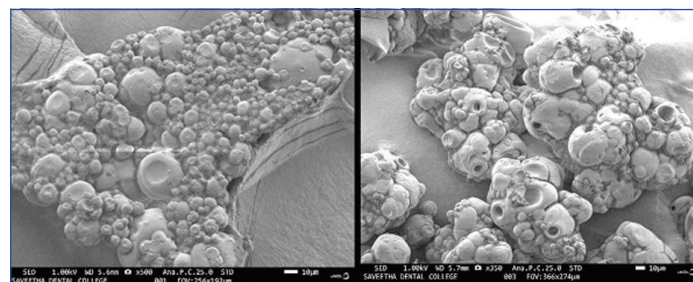
Antioxidant activity: To evaluate the flaxseed microspheres' antioxidant activity, the 2,2-DPPH assay was utilised [15]. Flaxseed microspheres (test) at different concentrations (50, 100, 200, 400 μ g/mL) were mixed with 1 mL of 0.1 mM DPPH in methanol solution and 450 μ L of 50 mM Tris HCl buffer (pH 7.4). The mixture was then incubated for 30 minutes. Afterwards, the decrease in DPPH free radicals was gauged by measuring the absorbance at 517 nm. As a control, Butylated Hydroxytoluene (BHT) was used. This activity was done in triplicates. The percentage of inhibition was determined using the following formula: % inhibition = (Absorbance of control - Absorbance of sample) / Absorbance of control $\times$ 100.

STATISTICAL ANALYSIS

The data obtained were analysed using Statistical Package for Social Sciences (SPSS); Version 23 (Armonk, NY, USA: IBM Corp). An independent t-test was conducted to compare the anti-inflammatory and antioxidant activities between the test and control. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Topography analysis: The SEM image shows that the prepared microspheres were spherical in shape with a smooth and dense outer surface with aggregation. Distinct pores were evident on the surface of microspheres, which will be responsible for drug release [Table/Fig-3].



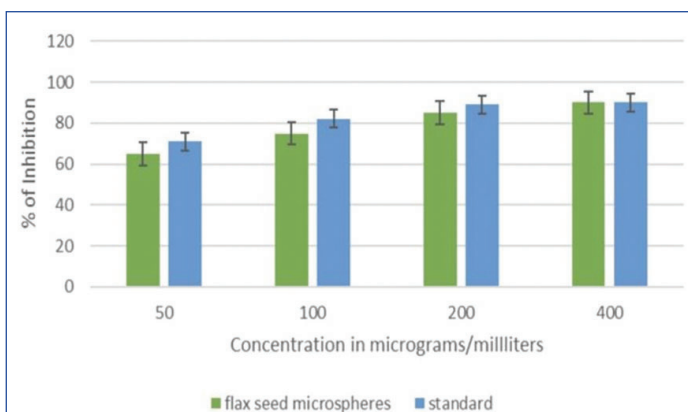
[Table/Fig-3]: SEM image of flaxseed microspheres.

Protein denaturation assay: Flaxseed microspheres demonstrated a concentration-dependent increase in anti-inflammatory activity, with inhibition values ranging from 67.0 $\pm$ 1.2 to 90.1 $\pm$ 1.0%. Although diclofenac sodium showed slightly higher inhibition at all concentrations, the differences were not statistically significant ($p > 0.05$), indicating comparable efficacy between the two groups [Table/Fig-4,5].

DPPH assay: Flaxseed microspheres showed a concentration-dependent increase in antioxidant activity, with values comparable to the standard. Although the standard consistently exhibited slightly higher inhibition, the differences were not statistically significant ($p > 0.05$), indicating similar antioxidant potential [Table/Fig-6,7].

DISCUSSION

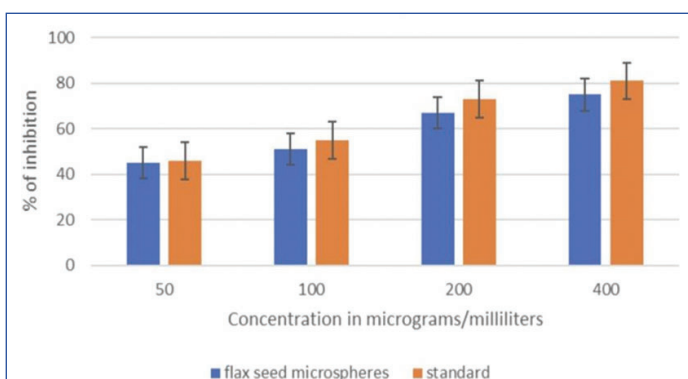
The fabrication of flaxseed microspheres represents a promising advancement in the field of medical research, particularly in the



[Table/Fig-4]: Graphical representation of the anti-inflammatory activity of flaxseed microspheres.

Concentration (µg/mL)	Flaxseed Microspheres (% inhibition, Mean±SD)	Diclofenac Sodium (% inhibition, Mean±SD)	t-value	p-value#
50	67.0±1.2	70.1±1.4	-2.91	0.798
100	78.2±1.5	81.0±1.7	-2.14	0.156
200	85.4±1.3	87.2±1.6	-1.51	0.831
400	90.1±1.0	91.3±1.1	-1.40	0.914

[Table/Fig-5]: Comparison of anti-inflammatory activity between flaxseed microspheres and standard drug at various concentrations. Values are expressed as mean±standard deviation from three independent experiments.



[Table/Fig-6]: Graphical representation of the antioxidant activity of flaxseed microspheres.

Concentration (µg/mL)	Flaxseed Microspheres (% inhibition, Mean±SD)	Butylated hydroxytoluene (% inhibition, Mean±SD)	t-value	p-value#
50	45.0±1.1	46.3±1.0	-1.51	0.835
100	51.2±1.4	53.0±1.3	-1.63	0.762
200	68.1±1.3	70.2±1.5	-1.83	0.961
400	77.4±1.2	80.1±1.4	-2.54	0.070

[Table/Fig-7]: Comparison of antioxidant activity between flaxseed microspheres and standard across different concentrations. Data are expressed as mean±standard deviation from three independent experiments.

development of anti-inflammatory and antioxidant therapies. This study successfully prepared and characterised flaxseed microspheres, demonstrating their significant anti-inflammatory and antioxidant activities along with the surface characteristics.

The SEM was employed to analyse the surface morphology and structure of the synthesised flaxseed microspheres in this study. SEM is a powerful imaging technique that provides high-resolution, three-dimensional views of sample surfaces, allowing for detailed characterisation at the micro- and nano-scale. SEM analysis revealed the spherical nature of the microspheres, confirming their structural integrity of the flaxseed microspheres in the present study. The microspheres exhibited a mild roughness across their surfaces. This mild roughness is beneficial as it can influence interactions with

gingival tissues and enhance biocompatibility [16]. Additionally, SEM analysis confirmed its suitability for oral or mucosal administration.

Flaxseeds are renowned for their nutritional and medicinal benefits. They are particularly noted for their ability to reduce inflammatory reactions in patients with hypertension and heart disease [17]. Additionally, flaxseeds help to maintain lipid profiles and are rich in dietary fibres, which act as forms of vitamin E. These fibres have the capacity to bind with free radicals, thereby exhibiting significant antioxidant properties [18]. In the current study, the anti-inflammatory properties of the flaxseed microspheres were assessed through a denaturation inhibition assay, where a dose-dependent increase in inhibition was observed. The study findings indicated that higher concentrations of the microspheres effectively reduce protein denaturation, a key marker of inflammation. The potent anti-inflammatory effect can be attributed to the bioactive compounds in flaxseed, such as lignans and omega-3 fatty acids, known for their anti-inflammatory properties [19]. Another study has demonstrated that sufficient dietary intake of omega-3 Polyunsaturated Fatty Acids (PUFAs) elevates tissue concentration of these fatty acids, which aid in reducing inflammation [20]. Dietary intake of flaxseed has been shown to reduce the progression of atherosclerosis caused by high dietary cholesterol or transfat content. This effect is likely due to the anti-inflammatory properties of its alpha-linolenic acid content [21]. Administration of flaxseed to diabetic individuals with mild hypercholesterolemia led to a significant decrease in C-reactive protein levels, a potent inflammatory marker [22]. The present study results are in accordance with the previous studies as the synthesised microspheres from flaxseeds exhibited potent anti-inflammatory properties.

Furthermore, in the present study, varying concentrations of flaxseed microspheres were tested against a butylated hydroxytoluene for assessing antioxidant activity. The assay measured the ability of the microspheres to neutralise or inhibit the activity of DPPH radical, thereby reflecting their antioxidant efficacy. Results indicated a dose-dependent response, with higher concentrations of microspheres demonstrating greater free radical scavenging activity. This antioxidant activity can be attributed to the presence of bioactive compounds in flaxseeds, such as alpha-linolenic acid and phenolic compounds, known for their free radical scavenging abilities [23].

Although research on flaxseeds's role in periodontal disease remains limited, initial studies [24-27] and experimental evidence indicates encouraging outcomes. Farah Shehani A and Kavitha Ramar Sr. highlighted flaxseed's remineralising ability on enamel, while Al-Tememi and Al-Huwaizi demonstrated its antimicrobial effect against *E. faecalis* [24,25]. Gehani G et al., reported anticancer properties in oral carcinoma, and Abdelaziz AG et al., showed its role in bone regeneration via nanofibre scaffolds [26,27]. These findings support the present study, where flaxseed microspheres exhibited notable anti-inflammatory and antioxidant activity, suggesting potential as a local drug delivery system in periodontal diseases. The biological properties demonstrated by flaxseed microspheres in the present study suggest their potential utility in mitigating oxidative stress and reducing associated inflammatory responses. Despite the promising results observed in this study, certain limitations must be acknowledged.

Limitation(s)

Firstly, the present study was conducted in an in-vitro setting, which may not fully replicate the complex biological environment of periodontal tissues in-vivo. The lack of clinical trials limits the generalisability of these findings to actual patient care. Also, the stability and long-term release profile of the microspheres were not extensively assessed, which are crucial factors for their application as a local drug delivery system. Future research should address these limitations by conducting in-vivo studies and exploring a broader spectrum of pharmacological effects.

CONCLUSION(S)

In conclusion, the fabrication of flaxseed microspheres presented in this study holds promise as a versatile platform in biomedical research, offering a sustainable and effective approach for delivering therapeutic agents. The fabricated flaxseed microspheres exhibited superior anti-inflammatory and antioxidant properties along with appreciable surface characteristics. Continued research is warranted in future in realising the clinical potential of flaxseed microspheres for improving patient outcomes and advancing healthcare innovation.

REFERENCES

- [1] Flemmig TF. Periodontitis. *Ann Periodontol*. 1999;4(1):32-38.
- [2] Rajasekar A, Varghese SS. Microbiological profile in periodontitis and peri-implantitis: A systematic review. *J Long Term Eff Med Implants*. 2022;32(4):83-94.
- [3] Genco RJ, Borgnakke WS. Risk factors for periodontal disease. *Periodontol* 2000. 2013;62(1):59-94.
- [4] Pretzl B, Sälzer S, Ehmke B, Schlagenhauf U, Dannewitz B, Dommisch H, et al. Administration of systemic antibiotics during non-surgical periodontal therapy-A consensus report. *Clin Oral Investig*. 2019;23(7):3073-85.
- [5] Dharini S, Pandiar D, Rajeshkumar S, Krishnan RP. Evaluation of anti-inflammatory and antioxidant properties of *Persea Americana* and *Syzygium aromaticum*-based herbal mouthwash formulation: An in-vitro study. *Journal of Clinical and Diagnostic Research*. 2024;18(10):ZF01-ZF05.
- [6] Rukmani PA, Shanmugam R, Manigandan P. Anti-inflammatory effect of herbal mouthwash prepared using *andropogon paniculata* and *rosa* formulation. *Journal of Pharmacy and Bioallied Sciences*. 2024;16(Suppl 2):S1345-S1349.
- [7] Krishna KN, Krishnamoorthy K, Veeraraghavan VP, Jayaraman S. Development of anti-inflammatory drug from *Crataeva nurvala*: In silico and in-vitro approach. *Journal of Pharmacy and Bioallied Sciences*. 2024;16(Suppl 2):S1308-S1311.
- [8] Calder PC. Omega-3 fatty acids and inflammatory processes. *Nutrients*. 2010;2(3):355-74.
- [9] Mueed A, Madijrebaye P, Shibli S, Deng Z. Flaxseed peptides and cyclolinopeptides: A critical review on proteomic approaches, biological activity, and future perspectives. *J Agric Food Chem*. 2022;70(46):14600-12.
- [10] Swarna Meenakshi P, Sankari M, Rajeshkumar S. Formulation and evaluation of a novel herbal trio gel containing flax seed extract, carbopol and carboxymethyl cellulose. *Bioinformation*. 2023;19(5):540-45.
- [11] Raj H, Sharma S, Sharma A, Verma KK, Chaudhary A. A novel drug delivery system: Review on microspheres. *J Drug Delivery Ther*. 2021;11(2-S):156-61.
- [12] Meenakshi PS, Malaipappan S. Additive effect of brown flaxseed extract gel on postsurgical periodontal therapy in periodontitis patients: A randomised controlled trial. *Journal of Clinical and Diagnostic Research*. 2024;18(7):ZC16-ZC20.
- [13] Niknam SM, Escudero I, Benito JM. Formulation and preparation of water-in-oil-in-water emulsions loaded with a phenolic-rich inner aqueous phase by application of high energy emulsification methods. *Foods*. 2020;9(10):1411.
- [14] Ameen M, Arumugham M, Ramalingam K, Rajeshkumar S. Evaluation of the anti-inflammatory, antimicrobial, antioxidant, and cytotoxic effects of chitosan thiocolchicoside-lauric acid nanogel. *Cureus*. 2023;15(9):e46003. Doi: 10.7759/cureus.46003.
- [15] Amani T, Surentar M. Antimicrobial and cytotoxic activity of citrus macroptera and cucumis sativus herbal formulation: An in vitro study. *Journal of Indian Academy of Oral Medicine and Radiology*. 2024;36(3):274-77.
- [16] Hakim LK, Yazdani M, Alam M, Abbasi K, Tebyaniyan H, Tahmasebi E, et al. Biocompatible and biomaterials application in drug delivery system in oral cavity. *Evid Based Complement Alternat Med*. 2021;2021:9011226.
- [17] Al-Madhagy S, Ashmawy NS, Mamdouh A, Eldahshan OA, Farag MA. A comprehensive review of the health benefits of flaxseed oil in relation to its chemical composition and comparison with other omega-3-rich oils. *Eur J Med Res*. 2023;28(1):240-46.
- [18] Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: Nutrition or pharmacology. *Br J Clin Pharmacol*. 2013;75(3):645-62.
- [19] Bassett CM, McCullough RS, Edel AL, Patenaude A, LaVallee RK, Pierce GN. The α -linolenic acid content of flaxseed can prevent the atherogenic effects of dietary trans fat. *Am J Physiol Heart and Circ Physiol*. 2011;301(6):2220-26.
- [20] Pan A, Demark-Wahnefried W, Ye X, Yu Z, Li H, Qi Q, et al. Effects of a flaxseed-derived lignan supplement on C-reactive protein, IL-6 and retinol-binding protein 4 in type 2 diabetic patients. *Br J Nutr*. 2008;101(8):1145-49.
- [21] Pramanik J, Kumar A, Prajapati B. A review on flaxseeds: Nutritional profile, health benefits, value added products, and toxicity. *eFood*. 2023;4(5):e114.
- [22] Rahimlou M, Jahromi NB, Hasanyani N, Ahmadi AR. Effects of flaxseed interventions on circulating inflammatory biomarkers: A systematic review and meta-analysis of randomized controlled trials. *Adv Nutr*. 2019;10(6):1108-19.
- [23] Pappu R, Varghese J, Koteswara KB, Kamath V, Lobo R, Nimmy K. Evaluation of biodegradable gel containing flax seed extract (*Linum usitatissimum*) as a targeted drug delivery for management of chronic periodontitis. *Int J Herb Med*. 2019;15:100254.
- [24] Farah Shehane A, Kavitha Ramar Sr. Comparative evaluation of the remineralizing potential of flaxseed paste, chicken eggshell paste, and fluoride toothpaste on the enamel of primary teeth using scanning electron microscopy-energy dispersive X-ray analysis: An in-vitro study. *Cureus*. 2024;16(5):e60040.
- [25] Al-Tememi MM, Al-Huwaizi HF. Use of flaxseed oil as a root canal medicament against *enterococcus faecalis* biofilm (in-vitro study). *Frontiers in Biomedical Technologies*. 2024 Sep 9.
- [26] Gehani G, Werfalli S, Elturki F. Anticancer effect of flaxseed and Cisplatin in oral squamous cell carcinoma: An in-vitro study. *Journal of Medical Sciences*. 2024;19(1):47-50.
- [27] Abdelaziz AG, Nageh H, Abdalla MS, Abdo SM, Amer AA, Loutfy SA, et al. Development of polyvinyl alcohol nanofiber scaffolds loaded with flaxseed extract for bone regeneration: Phytochemicals, cell proliferation, adhesion, and osteogenic gene expression. *Frontiers in Chemistry*. 2024;12:1417407.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Student, Department of Periodontology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
2. Postgraduate Student, Department of Periodontology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
3. Associate Professor, Department of Periodontology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
4. Professor, Department of Therapeutics, Nanomedicine, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Arvina Rajasekar,
162, Poonamallee High Road, Velappanchavadi, Chennai-600077,
Tamil Nadu, India.
E-mail: arvinar.sdc@saveetha.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Dec 11, 2024
- Manual Googling: May 27, 2025
- iThenticate Software: May 29, 2025 (6%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: Dec 10, 2024

Date of Peer Review: Feb 05, 2025

Date of Acceptance: May 31, 2025

Date of Publishing: Nov 01, 2025