BMC Oral Health

RESEARCH

Open Access

Evaluation of melatonin gel as local drug delivery system for the treatment of periodontitis: a split-mouth randomized controlled trial

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Abstract

Background Periodontitis is a polymicrobial, multifactorial infection that affects the supporting structures of teeth. Melatonin, a biomolecule with anti-inflammatory, antibacterial, and antioxidant properties, has demonstrated promising results in various medical fields, including dentistry.

Objective This study aimed to evaluate the effectiveness of 1% (w/v) melatonin gel as an adjunct to Non-Surgical Periodontal Therapy (NSPT) in improving clinical periodontal parameters, reducing antimicrobial activity against *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*, and increasing superoxide dismutase (SOD) levels in gingival crevicular fluid (GCF) among patients with stage II periodontitis.

Methods A split-mouth randomized controlled trial was conducted on 24 periodontitis patients. Two sites per patient were randomly assigned: the test site underwent scaling and root planing (SRP) followed by intra pocket application of 1% melatonin gel, while the control site received SRP alone. Clinical parameters, including the Plaque Index (PI), Gingival Index (GI), Gingival Bleeding Index (GBI), Periodontal Pocket Depth (PPD), and Clinical Attachment Loss (CAL), were assessed at baseline, 1 month, and 3 months. Subgingival plaque samples and GCF were collected to evaluate microbial and biochemical changes.

Results Both groups showed statistically significant improvements in clinical parameters from baseline to the 3rd month post-therapy. A quantitative reduction in *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* was observed at both sites. Additionally, the test site exhibited a greater increase in SOD levels compared to the control site.

Conclusion The adjunctive application of melatonin gel with SRP demonstrated enhanced antioxidant potential and improved clinical outcomes in patients with stage II periodontitis.

Trial registration trial registry ISRCTN. Trial registration number ISRCTN40460432. Date of Registration: 22/10/2024. "Retrospectively registered".

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Keywords Clinical attachment loss, Gingival index, Melatonin, Periodontopathogens, Periodontitis, Superoxide dismutase

Introduction

Periodontitis is a significant public health concern due to its high prevalence and potential to cause tooth loss, which negatively affects chewing ability, aesthetics, and overall oral health-related quality of life of a person [1]. Globally, an estimated 40–90% of people are affected by periodontitis, despite the availability of various surgical and nonsurgical treatment options [2]. The condition is primarily attributed to bacteria within dental plaque biofilm, which serve as the main cause of inflammatory periodontal diseases. In addition to bacterial involvement, the inflammatory cascade plays a critical role in disease progression. Heightened pathogenic activity and the migration of a large number of neutrophils to inflamed areas lead to the production of proteases and reactive oxygen species, resulting in increased oxidative stress and tissue damage [3].

Certain compounds, such as vitamin C, flavonoids, carotenoids, Aloe Vera, and melatonin, have demonstrated effectiveness in managing periodontal inflammation [4]. Among these, melatonin, a naturally occurring compound primarily secreted by the pineal gland, is notable [5]. It regulates key physiological and pathological processes, including the circadian rhythm, body temperature, and exhibits strong anti-inflammatory, antioxidant, and free radical scavenging properties [6]. Within the oral cavity, melatonin exerts paracrine effects on cells [7], acts as an antioxidant and anti-inflammatory agent, modulates immune responses [8, 9], stimulates antioxidant enzyme activity, and plays a pivotal role in bone formation, type I collagen synthesis and reducing bone resorption [10].

Currently SRP is a widely practiced nonsurgical treatment for periodontitis, but it is insufficient in completely mitigating the excessive immune-inflammatory response or re-establishing a balanced oral microenvironment. Consequently, progressive attachment loss persists in some patients following SRP. This limitation underscores the need for adjunctive treatments, such as host modulation therapy, to achieve more effective outcomes. Recently, considerable attention has been directed toward the potential use of melatonin as a host-modulation agent in periodontology. Several in vitro and clinical studies have reported positive results regarding therapeutic application of melatonin in managing periodontal disease [11–14]. While most studies have evaluated the effects of oral melatonin supplements on periodontal health, only a limited number have investigated its application as a gel in varying concentrations alongside nonsurgical periodontal therapy (NSPT). Furthermore, no standardized clinical protocol for its administration currently exists and further research is necessary to facilitate its widespread use.

The present study was designed to assess the efficacy of 1% melatonin gel as an adjunct to NSPT in improving periodontal parameters, including PI, GI, PPD, CAL, antimicrobial activity against *Aggregatibacter Actinomycetemcomitans* and *Prevotella Intermedia* and SOD levels in GCF, in patients with stage II periodontitis. The study hypothesizes that combining 1% melatonin gel with NSPT will result in superior periodontal outcomes, enhanced antibacterial effects, and increased SOD levels when compared to NSPT alone.

Materials and methods

Study design and population

A split-mouth randomized clinical trial was carried out in the Department of Periodontology at the College of Dental Sciences, Davangere, Karnataka, India. The study involved a total of 24 patients diagnosed with stage II periodontitis who were recruited from the same department. All participants were thoroughly informed about the clinical procedures and the follow-up requirements before their enrolment in the study.

Eligibility criteria

The study included systematically healthy individuals aged between 30 and 45 years who had been diagnosed with stage II periodontitis. The diagnostic criteria included interdental CAL of 3–4 mm at the site of greatest loss, a maximum probing pocket depth of ≤ 5 mm, and horizontal bone loss. Exclusion criteria consisted of smokers, pregnant or lactating women, individuals used antibiotics within six months or using melatonin for sleep disorders, and those who had recently undergone any form of periodontal therapy.

Ethical procedure

This study adhered to the principles outlined in the Declaration of Helsinki and received approval from the Ethical Committee of the College of Dental Sciences, Davangere, under approval number CODS/3230/2019–2020. Before initiating the study, all participants were provided with detailed information about the clinical procedures and follow-up requirements in clear and simple language. Voluntary written informed consent was obtained for clinical examinations, charting, radiographic assessments, clinical procedures, and photography of the treated sites.

Clinical examination

Eligible participants underwent a comprehensive periodontal evaluation, which included complete periodontal charting and periapical radiographs of the selected sites for the diagnosis of stage II periodontitis. SRP of the entire mouth were performed on all participants using ultrasonic scalers and Gracey curettes. Additionally, oral hygiene instructions were provided to ensure proper maintenance of periodontal health.

Sample size estimation: The sample size was calculated using the G*Power software for Windows (version 3.1.9.7). A total of 24 patients was determined to be sufficient to detect an effect size of 0.4, with a study power of 80% and a significance level of 5%, accounting for a 20% dropout rate over a 3-month period.

Random allocation: A random allocation sequence was generated using computer software, with an allocation ratio of 1:1. To ensure masking, both the melatonin gel and placebo gel were packaged in identical syringes. Each pre-packed syringe was numbered based on the randomization schedule. The sequentially numbered syringes were concealed by an individual not involved in the study.

Intervention

Two sites in each patient were randomly selected using computer software to receive one of the following treatments: the test site received an intra pocket application of 1% melatonin gel after SRP, while the control site received an intra-pocket application of placebo gel (without active ingredient) after SRP. Both products were applied once a week for a duration of four weeks. Figure 1 illustrates the flow diagram of clinical trial phases.

Preparation of melatonin gel

The melatonin gel was prepared at the Department of Pharmacognosy, Bapuji Pharmacy College, Davanagere. Carbopol 934 (1% w/v), a gelling agent, was weighed and soaked overnight in Milli-Q water at 4 °C. A specific amount of melatonin (1% w/v) was dissolved in an appropriate volume of polyethylene glycol-400 (PEG-400) solvent. The melatonin-containing solution was then added to the carbopol solution under magnetic stirring. Methyl paraben (0.18% w/v) and propyl paraben (0.02% w/v) were incorporated into the mixture as preservatives. An emulsifier, 1 N NaOH/triethanolamine solution, was gradually added dropwise to the dispersion until it formed a gel. The final gel was stored in airtight containers in a refrigerator for future use. Prior to the study, the minimum inhibitory concentration of the gel was determined using the serial dilution technique.

Blinding and calibration

A single investigator, trained and calibrated to record clinical parameters, conducted all assessments. Blinding was maintained for all participants, the investigator, and the statistician regarding the allocation of melatonin and placebo gels in the intervention groups. The investigator received calibration from a periodontist for evaluating plaque, gingivitis, and periodontitis using standardized indices. Inter-examiner reliability was assessed using kappa statistics, resulting in values of 0.80 for the gingival index and 0.84 for the plaque index.

Outcomes

All outcome measures were evaluated at baseline, as well as 1 month and 3 months after periodontal therapy.

Clinical outcomes

Various periodontal parameters were recorded, including the PI, GI, GBI, CAL, and PPD. PPD and CAL were measured using a UNC-15 probe. PPD was recorded as the distance from the crest of the gingival margin to the base of the pocket, while CAL was measured from the cementoenamel junction (CEJ) to the base of the pocket.

Biochemical analysis– Assay of superoxide dismutase enzyme using GCF.

The GCF sample was obtained from the single site exhibiting the greatest PD and CAL. To ensure accuracy, the chosen site was isolated using a cotton roll and gently air-dried to prevent salivary contamination. Pre-weighed filter paper strips (No. 1), measuring 2 mm × 8 mm, were employed to collect the samples using the intra crevicular method. For each individual, two strips were placed for one minute at the sulcus or pocket entrance to collect an adequate volume of GCF. Any strips contaminated with blood were discarded, and the process was repeated after a 30-minute interval. The GCF volume was determined by comparing the pre- and post-weighing of the filter paper strips. The six collected strips were pooled with 1 ml of Tris-hydrochloride buffer (pH 6.5), eluted for 30 min, and stored until the SOD enzyme assay. Samples were collected at baseline and three months post-periodontal treatment. Following collection, the strips were promptly placed in sterile Eppendorf tubes and stored in liquid nitrogen (– 80 °C) for future biochemical analysis.

The SOD enzyme activity was measured using the Kakkar et al. (1984) method, which relies on the formation of NADH-phenazine methosulphate-nitro blue tetrazolium formazan. The assay mixture comprised 1.2 ml sodium pyrophosphate buffer, 0.1 ml phenazine methosulfate, 0.3 ml NBT, 1 ml of appropriately diluted enzyme preparation, and water, making a total volume of 3 ml. The reaction was initiated with the addition of 0.2 ml NADH. After a 90-second incubation at 30 °C, the reaction was terminated by adding 1 ml glacial acetic acid. The reaction mixture was then shaken with 4.0 ml n-butanol and allowed to stand for 10 min. The butanol layer was subsequently separated by centrifugation and its absorbance

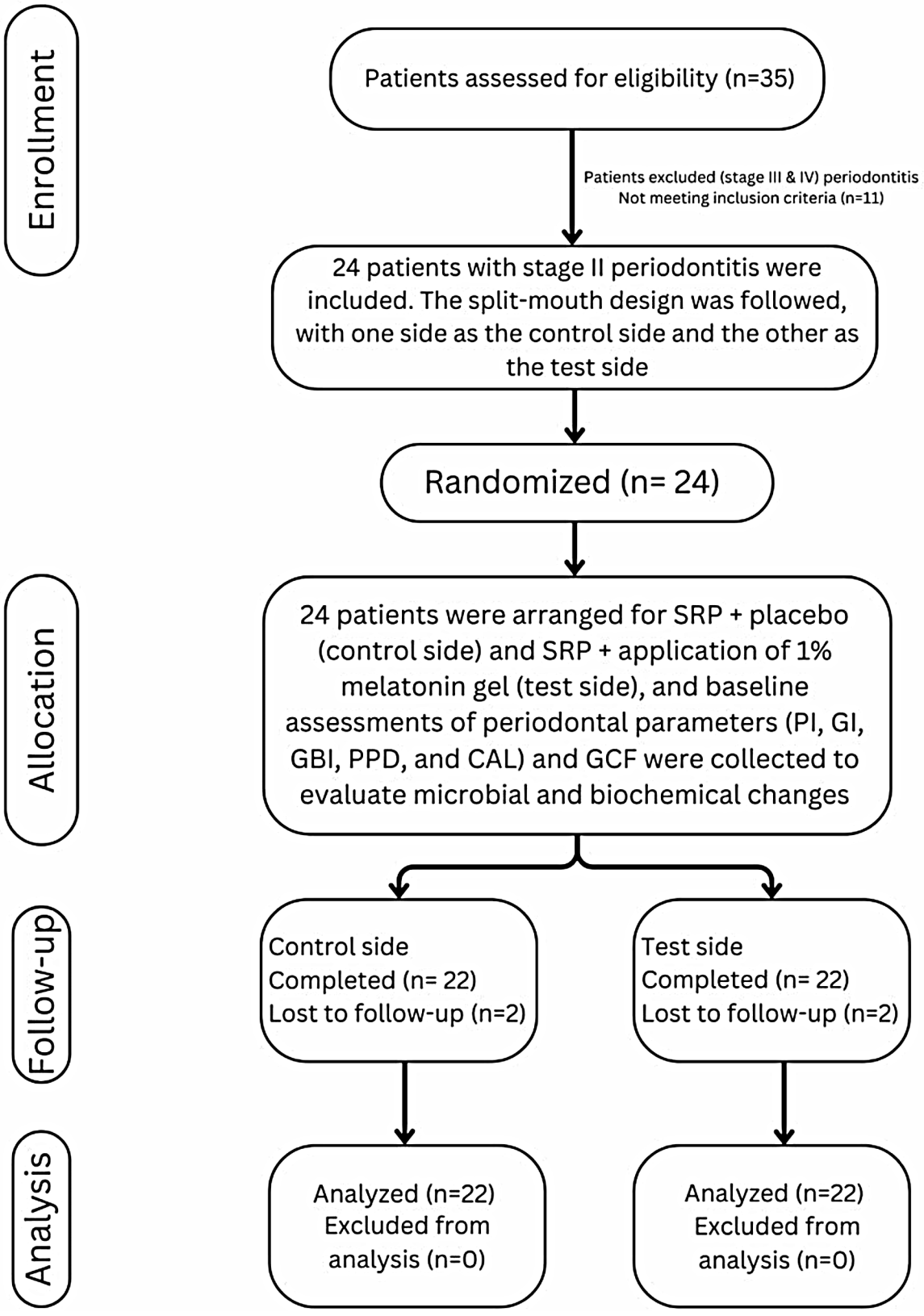

Fig. 1 CONSORT flowchart of the study

measured at 560 nm. For the control, water was used in place of the enzyme preparation and processed identically to the test assay. The enzyme activity was expressed in units/ml [15].

Molecular analysis

The subgingival plaque from the periodontal pocket was collected using a sterile periodontal curette. The sample was then centrifuged at 10,000–16,000 g for 15 min at 4℃ to isolate the sediment for subsequent analysis. Samples were collected and processed from the selected sites at baseline (T0) and three months (T3) following periodontal treatment. These samples were homogenized for molecular analysis. Genomic DNA was extracted through cell rupture and subsequently purified using affinity columns (Presto™ Mini gDNA Bacteria Kit, Geneaid). The endpoint PCR technique was utilized to identify two periodontopathic bacterial species. Specific primers for *Aggregatibacter actinomycetemcomitans* (Aa) and *Prevotella intermedia* (Pi) were synthesized based on the design by Ashimoto et al. Amplification products were visualized via electrophoresis in 2% agarose gel prepared with TAE buffer, using GelRed® Nucleic Acid Gel Stain as the intercalating fluorophore. The results were visualized with the Gel-Doc XR Gel Imaging System (BIO-RAD) [16].

Table 1 Comparison of periodontal parameters at baseline, 1 month and 3 months between test and control site

Variables	Intervention	Test site (*N* = 22) (Mean ± SD)	Control site (*N* = 22) (Mean ± SD)	*P* value
Plaque index (PI)	Baseline	2.02 ± 0.40	2.17 ± 0.49	$p > 0.05$, NS
	1 month	1.98 ± 0.34	2.01 ± 0.36	$p > 0.05$, NS
	3months	1.00 ± 0.44	0.81 ± 0.49	$p > 0.05$, NS
	pvalue	< 0.05,S	< 0.05,S	
Gingival Index (GI)	Baseline	2.00 ± 0.00	2.00 ± 0.00	$p > 0.05$, NS
	1 month	1.41 ± 0.33	1.50 ± 0.44	$p > 0.05$, NS
	3months	0.74 ± 0.35	0.62 ± 0.36	$p > 0.05$, NS
	pvalue	< 0.05,S	< 0.05,S	
Gingival Bleeding Index (GBI)	Baseline	73.86 ± 12.99	66.81 ± 15.77	$p > 0.05$, NS
	1 month	60.50 ± 10.12	62.50 ± 11.72	$p > 0.05$, NS
	3months	27.00 ± 13.83	29.63 ± 15.85	$p > 0.05$, NS
	pvalue	< 0.05,S	< 0.05,S	
Periodontal Pocket Depth (PPD)	Baseline	5.81 ± 0.73	5.95 ± 0.78	$p > 0.05$, NS
	1 month	5.40 ± 0.59	5.13 ± 0.71	$p > 0.05$, NS
	3months	4.63 ± 0.79	4.59 ± 0.79	$p > 0.05$, NS
	pvalue	< 0.05,S	< 0.05,S	
Clinical Attachment Loss (CAL)	Baseline	7.09 ± 1.30	6.72 ± 1.51	$p > 0.05$, NS
	1 month	6.18 ± 1.00	5.77 ± 1.06	$p > 0.05$, NS
	3months	4.00 ± 0.72	4.14 ± 0.94	$p > 0.05$, NS
	pvalue	< 0.05,S	< 0.05,S	

NS, non-significant, S, significant, SD, standard deviation, GBI, gingival bleeding index, PPD, probing pocket depth, CAL, clinical attachment level

Statistical analysis

The data was organized using Microsoft Excel and analyzed statistically using SPSS version 20 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp. Version 20). To assess the normality of the data distribution, Kolmogorov–Smirnov and Shapiro–Wilk tests were employed. The variables are presented as mean and standard deviation, with the significance level set at $p < 0.05$. For clinical parameters, repeated measures ANOVA was performed for within-site comparisons, while a paired t-test was conducted for between-site comparisons. Regarding microbiological and biochemical parameters, a paired t test was applied to evaluate differences between the test and control sites, as well as changes following treatment.

Results

The current study involved 24 participants, with two subjects lost to follow-up, leaving 22 participants at the conclusion of the trial. Of these, 14 were female and 10 were male, aged between 30 and 45 years. None of the participants who received either the 1% melatonin gel or the placebo gel reported any adverse events. The clinical parameter outcomes, measured at baseline, 1 month, and 3 months post-treatment for both sites, are summarized in Table 1. Significant reductions in PI, GI, PPD, and CAL scores were observed from baseline to the third month in both groups. However, by the end of the third month, there were no statistically significant differences between the test and control sites for PI, GI, PPD, and CAL scores.

Table 2 presents the mean SOD levels at baseline and at 3 months for both sites. At the test site, the mean SOD levels increased significantly from 4.62 ± 2.01 at baseline to 7.74 ± 2.54 at 3 months ($p < 0.05$). In the control site, the mean SOD levels also showed a significant increase, from 4.89 ± 2.29 at baseline to 5.64 ± 1.65 at 3 months ($p < 0.05$). Although both groups exhibited a statistically significant rise in mean SOD levels, the increase at the test site (7.74 ± 2.54) was greater than that at the control site (5.64 ± 1.65), with the difference being statistically significant ($p < 0.05$).

Table 2 Comparison of intergroup and intragroup comparison of colony forming units at baseline and after 3 months

Variables	Groups	Baseline (Mean ± SD)	3 months (Mean ± SD)	*P* value
A. Actinomycetemcomitans	Test	158.18 ± 47.77	30.00 ± 18.11	$p < 0.05$,S
	Control	123.63 ± 79.97	35.45 ± 3.62	$p < 0.05$,S
pvalue		$p > 0.05$ NS	$p > 0.05$ NS	
P. Intermedia	Test	139.54 ± 68.06	38.63 ± 20.45	$p < 0.05$,S
	Control	113.63 ± 69.31	25.00 ± 11.14	$p < 0.05$,S
pvalue		$p > 0.05$ NS	$p > 0.05$ NS	

S, Significant, NS, Not Significant, SD, Standard Deviation, p, p value

Table 3 Comparison of intergroup and intragroup comparison of Superoxide dismutase levels (U/L) at baseline and after 3 months

Sites	Baseline ($N = 22$) Mean ± SD	3 months ($N = 22$) Mean ± SD	*P* value
Test	4.62 ± 2.01	5.64 ± 1.65	$p < 0.05$,S
Control	4.89 ± 2.29	7.74 ± 2.54	$p < 0.05$,S
pvalue	$p > 0.05$,NS	$p < 0.05$,S	

NS, non-significant, S, significant, SD, standard deviation, p, p value

Table 3 presents the mean colony-forming units (CFU) of *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* at both sites before and after treatment. At baseline, the mean CFU of *A. actinomycetemcomitans* was 158.18 ± 47.77 at the test site and 123.63 ± 79.97 at the control site. By the third month post-treatment, these values decreased to 30.00 ± 18.11 and 35.45 ± 3.62, respectively, with both reductions being statistically significant ($p < 0.05$). Similarly, the mean CFU of *P. intermedia* at baseline was 139.54 ± 68.06 at the test site and 113.63 ± 69.31 at the control site. After 3 months, these values dropped to 38.63 ± 20.45 at the test site and 25.00 ± 11.14 at the control site, also showing statistically significant reductions ($p < 0.05$). Although significant reductions in bacterial counts were observed in both sites, the differences between the test and control sites at the third month were not statistically significant.

Discussion

Periodontitis is a multifactorial infectious disease characterized by the destruction of periodontal tissues, bone resorption, and eventual tooth loss, primarily triggered by pathogenic microorganisms [17]. Alongside effective non-surgical periodontal therapy, local drug delivery into the periodontal pocket stands out as one of the most promising treatment options due to its ability to maintain high drug concentrations in the GCF for an extended period [18, 19]. Melatonin, an indolamine predominantly secreted by the pineal gland, possesses immunomodulatory, antioxidant, and bone remodeling properties [20, 21]. Increasing evidence from both in vitro and clinical studies has highlighted potential effect of melatonin as a host-modulating agent in periodontology [14, 22]. In vitro studies have shown its antimicrobial effects against *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and Methicillin-resistant *Staphylococcus aureus*. However, only a limited number of studies have explored its antibacterial activity against periodontal pathogens [23]. In this context, the present study aimed to evaluate the efficacy of 1% melatonin gel as an adjunct to NSPT in improving periodontal parameters, such as PI, GI, PPD, CAL, antimicrobial activity against *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*, and SOD levels in GCF, in patients with stage II periodontitis. In this study, melatonin was applied in gel form within the periodontal pocket to enhance its bio adhesive properties and thereby prolong its biological effects.

Clinical analysis

In the present study, within-group analysis revealed significant reductions in PI, GI, PPD, and CAL from baseline to the 3-month postoperative period. SRP effectively reduced the bacterial load and disrupted the bacterial biofilm [24]. These findings are consistent with previous studies that reported short-term clinical improvements in PI, GI, and PPD following phase I therapy [25, 26]. Additionally, several studies, including those by Cugini et al. [26], Haffajee AD et al. [27], Lui J et al. [28], and G. A. van der Weijden et al. [29], observed improvements in CAL within one month of SRP.

Although both the test and control sites demonstrated similar reductions in clinical parameters, a previous study by Ahmed et al. (2021) [3], which used a 5% melatonin gel, reported decreases of 0.7 ± 0.6, 0.6 ± 0.5, and 2.9 ± 0.7 in PI, GI, and PPD, respectively, along with an increase of 3.5 ± 0.6 in CAL after three months. Similarly, Montero et al. (2017) found that topical melatonin significantly improved gingival index and pocket depth in diabetic patients with periodontal disease [30].

Microbiological analysis

Subgingival plaque analysis is widely considered the most reliable method for evaluating the microbial profiles associated with periodontitis. Although ideal studies involve sampling from individual sites, pooled subgingival plaque samples are often used for logistical and financial reasons [31]. In the current investigation, pooled plaque samples collected from periodontal pocket sites were microbiologically analyzed for *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*.

To identify the most effective method for sampling subgingival plaque to detect periodontal infections, researchers used sterile curettes to collect samples both before and after periodontal therapy. A study by Pia-Merete Jervoe-Storm et al. [32] compared two methods of subgingival bacteria collection: curettes and paper

points. The findings revealed that the curette method consistently yielded higher levels of subgingival bacteria compared to paper points, regardless of the sample order. However, the composition of the plaque samples in terms of specific target pathogens was found to be similar for both methods, despite curettes capturing a greater total bacterial load. Consequently, both methods are considered suitable for microbiological testing of periodontal lesions in clinical practice.

Most existing research on this topic has relied on in vitro analyses. In the present study, both groups demonstrated a significant reduction in pathogen counts. However, the percentage of bacterial counts at three months showed no significant difference between the groups. To the best of our knowledge, no clinical trials have been conducted to evaluate the antibacterial effectiveness of melatonin within periodontal pockets. The bilayer membrane of Gram-negative organisms, with its variable lipid composition, forms a permeability barrier that restricts the entry of certain medications and antibiotics. However, melatonin, being more lipophilic, can readily cross cell membranes. *P. intermedia* utilizes heme to generate metabolic energy by breaking down hemoglobin through the proteinase–adhesin complex. In contrast, melatonin binds to metal ions such as iron, copper, and zinc, thereby inhibiting bacterial uptake and survival [33, 34]. Additionally, *P. intermedia* and *A. actinomycetemcomitans* activate NOS and calcium-dependent peptidylarginine deiminases, leading to hypercitrullination [35, 36]. Melatonin, apart from scavenging NO and peroxynitrite, is also known to inhibit NOS activity at physiological doses [37]. In a separate study conducted by Zhou et al. [38], melatonin was tested at concentrations ranging from 3.13 to 1600 µg/mL, with the minimum inhibitory concentration and minimum bactericidal concentration determined to be 100 µg/mL and 1600 µg/mL, respectively. Similarly, Ganganna et al. [39] used a smaller dilution range but still observed a strong bactericidal effect, indicating that melatonin remains effective even at lower concentrations. This finding is promising for its potential clinical application.

Biochemical analysis

GCF is a highly specific biological fluid that reflects the unique characteristics of its collection site, making it a valuable resource for diagnosing, treating, and managing various diseases. The primary advantage of GCF as a diagnostic marker lies in its site-specific nature, which allows laboratory analysis of its constituents to be directly linked to clinical evaluations performed at the sample collection site.

Numerous studies have analyzed markers of bone turnover and periodontal disease progression in GCF samples from patients with periodontitis. GCF analysis has proven to be a valuable diagnostic tool in both periodontology and orthodontics. In 2021, Bibi et al. [40] published a comprehensive review on host defense mediators and their expression in crevicular fluid, further highlighting the utility of GCF as a universal diagnostic tool.

To maintain periodontal tissue homeostasis and prevent tissue damage during an immune response triggered by periodontal pathogens, a proper balance between the production of reactive oxygen species (ROS) and the total antioxidant capacity of host tissue is essential [41, 42]. In the present study, a statistically significant increase in SOD levels was observed three months postoperatively, indicating the strong antioxidant properties of melatonin. These findings are consistent with the study by Ahmed et al. [3] and align with earlier research showing that melatonin administration significantly reduces oxidative stress in patients with periodontitis. Both melatonin and its endogenously produced metabolites act as effective free radical scavengers [43, 44]. Similarly, studies have demonstrated that melatonin can protect periodontal tissues from ROS-induced damage caused by inflammation and prevent alveolar bone loss by reducing elevated oxidative stress levels in these patients [43].

Studies have shown that oxidative stress triggers a rapid increase in SOD activity. Research indicates that the human periodontal ligament possesses the antioxidant enzyme SOD, which may play a protective role against ROS, particularly O2, during inflammation. This is further supported by the current study's observation of heightened gingival SOD activity levels in cases of periodontitis [45].

In the current study, SOD levels were recorded at 4.62 ± 2.01 and 4.89 ± 2.29 in group I and group II, respectively. After three months of therapy, SOD levels increased to 7.74 ± 2.54 at the test site and 5.64 ± 1.65 at the control site. A more significant reduction in SOD levels was observed at the test site compared to the control site after three months ($P < 0.05$). These findings align with previous studies that investigated the antioxidant effects of systemic melatonin (3 mg) in periodontitis patients. Those studies demonstrated that treatment with melatonin resulted in greater improvements in serum SOD and Glutathione Peroxidase levels compared to vitamin E supplementation in chronic periodontitis patients [44, 45]. While there is limited clinical research on the impact of melatonin on SOD levels, both the referenced study and the current findings highlight melatonin as a powerful antioxidant. However, further clinical trials are needed to fully explore the antioxidant potential of this remarkable molecule. This study has several limitations, including a small sample size and a short follow-up duration. Additionally, radiographic evaluation of bone was not conducted to verify improvements in clinical parameters. Future research should prioritize longitudinal

studies encompassing different stages of periodontal disease, particularly in middle-aged populations.

Conclusion

The study demonstrated a notable increase in Superoxide Dismutase levels within the test group following the intra pocket application of 1% melatonin gel, highlighting its strong antioxidant properties. Based on this observation, it can be concluded that the adjunctive use of locally delivered 1% melatonin gel alongside non-surgical periodontal therapy exhibits antioxidant potential in managing stage II periodontitis, offering a novel perspective in periodontitis treatment.

Abbreviations

CFU	Colony forming units
CAL	Clinical attachment loss
GCF	Gingival crevicular fluid
SRP	Scaling and root planing
NSPT	Non-surgical periodontal therapy
PPD	Probing pocket depth
SOD	Superoxide dismutase

Author contributions

Conceptualization, A.P.; R.S., and P.N.C.; methodology, A.P., R.S., and P.N.C.; software, A.P.; R.S.; and P.N.C.; validation, P.S. and M.S.; formal analysis, P.S., M.S., and R.S.; investigation, R.S., and P.S.; resources, S.A., I.G., and K.G.; data curation, A.P., and R.S.; writing—original draft preparation, I.G., S.A., and K.G.; writing—review and editing, K.G.; supervision, R.S.; and P.N.C.; visualization, I.G., A.P., and K.G. All authors have read and agreed to the published version of the manuscript.

Funding

The authors extend their appreciation to Prince Sattam bin Abdulaziz University for funding this research work through the project number (PSAU/2024/01/99528).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate

"The study was conducted in accordance with the Declaration of Helsinki and this study was approved by ethical committee of College of Dental Sciences, Davangere, with the approval number CODS/3230/2019–2020. All patients have signed the informed consent to participate in the study.

Consent for publication

All the participants gave consent for the publication of the data.

Competing interests

The authors declare no competing interests.

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Received: 12 October 2024 / Accepted: 3 February 2025
Published online: 13 February 2025

References

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World workshop on the classification of Periodontal and Peri-implant diseases and conditions. J Periodontol. 2018;89(Suppl 1):S173–82.
2. Najeeb S, Khurshid Z, Zohaib S, Zafar MS. Therapeutic potential of melatonin in oral medicine and periodontology. Kaohsiung J Med Sci. 2016;32(8):391–6. https://doi.org/10.1016/j.kjms.2016.06.005. Epub 2016 Jul 25. PMID: 27523451.
3. Ahmed E, Olfat GS, Yussif N, Ghalwash DM. Effect of locally delivered melatonin as an Adjunct to Nonsurgical Therapy on GCF antioxidant capacity and MMP-9 in stage II periodontitis patients: a Randomized Controlled Clinical Trial. Int J Dent 2021:8840167.
4. Kaur G, Kathariya R, Bansal S, Singh A, Shahakar D. Dietary antioxidants and their indispensable role in periodontal health. J Food Drug Anal. 2016;24(2):239–46. https://doi.org/10.1016/j.jfda.2015.11.003. Epub 2016 Jan 8. PMID: 28911576; PMCID: PMC9339570.
5. Radogna F, Diederich M, Ghibelli L. Melatonin: a pleiotropic molecule regulating inflammation. Biochem Pharmacol. 2010;80(12):1844–52. https://doi.org/10.1016/j.bcp.2010.07.041. Epub 2010 Aug 7. PMID: 20696138.
6. Wang C, Wang L, Wang X, Cao Z. Beneficial effects of Melatonin on Periodontitis Management: Far more than oral cavity. Int J Mol Sci. 2022;23(23):14541. https://doi.org/10.3390/ijms232314541. PMID: 36498871; PMCID: PMC9739298.
7. Tresguerres IF, Clemente C, Donado M, Gómez-Pellico L, Blanco L, Alobera MA, Tresguerres JA. Local administration of growth hormone enhances periimplant bone reaction in an osteoporotic rabbit model. Clin Oral Implants Res. 2002;13(6):631-6. https://doi.org/10.1034/j.1600-0501.2002.130609.x. PMID: 12519338.
8. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine. 2005;27(2):189–200. https://doi.org/10.1385/ENDO:27:2:189. PMID: 16217132.
9. García-Mauriño S, Pozo D, Calvo JR, Guerrero JM. Correlation between nuclear melatonin receptor expression and enhanced cytokine production in human lymphocytic and monocytic cell lines. J Pineal Res. 2000;29(3):129– 37. https://doi.org/10.1034/j.1600-079x.2000.290301.x. PMID: 11034109.
10. LERNER AB, CASE JD, TAKAHASHI Y. Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. J Biol Chem. 1960;235:1992-7. PMID: 14415935.
11. Sola VM, Aguilar JJ, Vazquez Mosquera AP, Carpentieri AR. Melatonin is an effective protector of gingival cells damaged by the cytotoxic effect of glutamate and DL-buthionine sulfoximine. J Periodontal Res. 2021;56(1):154–61. https://doi.org/10.1111/jre.12806. Epub 2020 Sep 23. PMID: 32965035.
12. Wu X, Qiao S, Wang W, Zhang Y, Shi J, Zhang X, et al. Melatonin prevents periimplantitis via suppression of TLR4/NF-κB. Acta Biomater. 2021;134:325–36. https://doi.org/10.1016/j.actbio.2021.07.017. Epub 2021 Jul 14. PMID: 34271168.
13. Bazyar H, Zare Javid A, Zakerkish M, Yousefimanesh HA, Haghighi-Zadeh MH. Effects of melatonin supplementation in patients with type 2 diabetes mellitus and chronic periodontitis under nonsurgical periodontal therapy: a double-blind randomized controlled trial. J Res Med Sci. 2022;27:52. https://doi.org/10.4103/jrms.JRMS_927_19. PMID: 36092489; PMCID: PMC9450249.
14. Liu RY, Li L, Zhang ZT, Wu T, Lin S, Zhang XT. Clinical efficacy of melatonin as adjunctive therapy to non-surgical treatment of periodontitis: a systematic review and meta-analysis. Inflammopharmacology. 2022;30(3):695–704. https://doi.org/10.1007/s10787-022-00959-3. Epub 2022 Mar 15. PMID: 35290552.
15. Ramesh KSV, Swetha P, Mohan Kumar P, Sruthima NVS, Naresh Kumar C. Estimation of superoxide dismutase levels in saliva and gingival crevicular fluid among smokers and non-smokers in periodontitis patients - an observational study. Niger Med J 2019 May-Jun;60(3):133–7. https://doi.org/10.4103/nmj.NMJ_56_19. PMID: 31543565; PMCID: PMC6737800.
16. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol. 1996;11(4):266– 73. https://doi.org/10.1111/j.1399-302x.1996.tb00180.x. PMID: 9002880.
17. Martande SS, Kumari M, Pradeep AR, Singh SP, Suke DK. Comparative evaluation of efficacy of subgingivally delivered 1.2% atorvastatin and 1.2% simvastatin in the treatment of intrabony defects in chronic periodontitis: a randomized controlled trial. J Dent Res Dent Clin Dent Prospects. 2017;15(1):18–25.
18. Garg T, Bilandi A, Kapoor B, Kumar S, Joshi R. Tissue engineering and regenerative medicine. Int Res J Pharm. 2011;2(12):37–42.

19. Jain N, Jain GK, Javed S, Iqbal Z, Talegaonkar S, Ahmad FJ, Khar RK. Recent approaches for the treatment of periodontitis. Vol Drug Discov Today. 2008;13:932–43.
20. Madapusi BT, Rao SR. Preliminary Evaluation of Human Gingiva as an Extrapineal Site of Melatonin Biosynthesis in States of Periodontal Health and Disease. J Clin Diagn Res. 2018;12(1):1–7.
21. Shimozuma M, Tokuyama R, Tatehara S, Umeki H, Ide S, Mishima K, Ichiro S, Satomura K. Expression and cellular localization of melatonin-synthesizing enzymes in rat and human salivary glands. Histochem Cell Biol. 2011;135(4):389–96.
22. Bazyar H, Zare Javid A, Zakerkish M, Yousefimanesh HA, Haghighi-Zadeh MH. Effects of melatonin supplementation in patients with type 2 diabetes mellitus and chronic periodontitis under nonsurgical periodontal therapy: a double-blind randomized controlled trial. J Res Med Sci. 2022;27:52.
23. Duarte PM, da Rocha M, Sampaio E, Mestnik MJ, Feres M, Figueiredo LC, et al. Serum levels of cytokines in subjects with generalized chronic and aggressive periodontitis before and after non-surgical periodontal therapy:a pilot study. J Periodontol. 2010;81:1056–63.
24. Becker W, Becker BE, Ochsenbein C, Kerry G, Caffesse R, Morrison EC, et al. A longitudinal study comparing scaling, osseous surgery and modified Widman procedures. Results after one year. J Periodontol. 1988;59:351–65.
25. Boretti G, Zappa U, Graf H, Case D. Short-term effects of phase I therapy on crevicular cell populations. J Periodontol. 1995;66:235–40.
26. Cugini MA, Haffajee AD, Smith C, Kent RL Jr, Socransky SS. The effect of scaling and root planing on the clinical and microbiological parameters of periodontal diseases:12-month results. J Clin Periodontol. 2000;27:30–6.
27. Haffajee AD, Cugini MA, Dibart S, Smith C, Kent RL Jr, Socransky SS. Clinical and microbiological features of subjects with adult periodontitis who responded poorly to scaling and root planing. J Clin Periodontol. 1997;24:767–76.
28. Lui J, Corbet EF, Jin L. Combined photodynamic and low-level laser therapies as an adjunct to nonsurgical treatment of chronic periodontitis. J Periodontal Res. 2011;46:89–96.
29. Van der Weijden GA, Dekkers GJ, Slot DE. Success of non-surgical periodontal therapy in adult periodontitis patients: a retrospective analysis. Int J Dent Hyg. 2019;17:309–17.
30. Montero J, López-Valverde N, Ferrera MJ, López-Valverde A. Changes in crevicular cytokines after application of melatonin in patients with periodontal disease. J Clin Exp Dent. 2017;9:e1081–7.
31. Moussa SG. Evaluation of locally delivered 1.2% atorvastatin gel versus 2% melatonin gel as adjunctive to non-surgical periodontal therapy on GCF osteocalcin level in stage II periodontitis patients: a randomized controlled trial. Fut Dent J. 2021;7(1):Art7.
32. Jervoe-Storm PM, AlAhdab, Koltzscher M, Fimmers R, Jepsen S. Comparison of curette and paperpoint sampling of subgingival bacteria as analyzed by Real Time polymerase chain reaction. J Periodontol. 2007;78:909–17.
33. Srinath R, Acharya AB, Bhat KG. An in vitro evaluation of melatonin as an antibacterial agent on periodontal pathogens. WJPLS. 2018;4:95–8.
34. Gao JL, Kwan AH, Yammine A, Zhou X, Trewhella J, Hugrass BM, et al. Structural properties of a haemophore facilitate targeted elimination of the pathogen porphyromonas gingivalis. Nat Commun. 2018;9:4097.
35. Rana SV. Protection of metal toxicity by melatonin– recent advances. EC Pharmacol Toxicol. 2018;6(9):851–64.
36. Konig MF, Abusleme L, Reinholdt J, Palmer RJ, Teles RP, Sampson K, et al. Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. Sci Transl Med. 2016;8:369ra176.
37. Djordjevic B, Cvetkovic T, Stoimenov TJ, Despotovic M, Zivanovic S, Basic J, et al. Oral supplementation with melatonin reduces oxidative damage and concentrations of inducible nitric oxide synthase, VEGF and matrix metalloproteinase 9 in the retina of rats with streptozotocin/nicotinamide induced pre-diabetes. Eur J Pharmacol. 2018;833:290–7.
38. Zhou W, Zhang X, Zhu CL, He ZY, Liang JP, Song ZC. Melatonin receptor agonists as the Perioceutics agents for Periodontal Disease through Modulation of Porphyromonas gingivalis virulence and inflammatory response. PLoS ONE. 2016;11(11):e0166442.
39. Ganganna A, Rudariah CB, Rao R, Prakash VM. Antibacterial activity of melatonin against prime periodontal pathogens: an in vitro study. J Int Oral Health. 2021;13:164–8.
40. Bibi T, Khurashid Z, Ambreen R, Imran E, Srivastava KC, Srivastava D. Gingival crevicular fluid: a diagnostic tool for the detection of periodontal health and disease. Molecules. 2021;26:1208.
41. Zhang T, Andrukhov O, Haririan H, Muller-Kern M, Liu S, Liu Z, Rausch F. Total antioxidant capacity and total oxidant status in saliva of periodontitis patients in relation to bacterial load. Front Cell Infect Microbiol. 2016;5:97.
42. Permuy M, López-Peña M, González-Cantalapiedra A, Muñoz F. Melatonin: a review of its potential functions and effects on dental diseases. Int J Mol Sci. 2017;18(4):865.
43. Trivedi S, Lal S. Antioxidant enzymes in periodontitis. J Oral Biol Craniofac Res. 2017;7:54–9.
44. Marawar AP, Marawar PP, Nandal DH, Kunkulol RR. Evaluation of antioxidant potential of melatonin in periodontitis with a focus on vitamin C. Int J Basic Clin Pharmacol. 2020;9(3):378–83.
45. Wang Y, Andrukhov O, Raush-Fan X. Oxidative stress and antioxidant system in periodontitis. Front Physiol. 2017;8:910.

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