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Review

Effects of Melatonin in the Non-Surgical Treatment of Periodontitis: A Systematic Review

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Featured Application: Melatonin is a powerful antioxidant; it can bring numerous advantages to periodontal diseases, pursuing homeostasis, such as restoration of the concentration of antioxidants, reduction of periodontal inflammation with the regulation of inflammatory cytokines, reduction of oxidative stress, and significant reduction of bone resorption through the modulation of osteoclastic and osteoblastic activities. It was considered a reliable and feasible option as an adjunctive to the classical NSPT, obtaining a significative improvement of the periodontal parameters (PD, CAL, BOP, PI, and GI), a significative reduction of the pro-inflammatory proteins (IL-1b, IL-6, and TNF-α), and a better response for other biomarkers.

Abstract: Background: Melatonin is a hormone produced by the pineal gland, an endocrine gland located at the base of the brain. It acts as a powerful antioxidant; it can bring numerous advantages to periodontal diseases, pursuing homeostasis, such as restoration of the concentration of antioxidants, reduction of periodontal inflammation with the regulation of inflammatory cytokines, reduction of oxidative stress, and significant reduction of bone resorption through the modulation of osteoclastic and osteoblastic activities. Then, the goal of this integrative review was to evaluate the literature to better understand whether the use of melatonin is feasible to improve the non-surgical treatment of periodontitis. Methods: The integrative review was based on PICO strategy and PRISMA methodology. The focus question was: "Are there significant benefits in applying melatonin for the non-surgical treatment of periodontitis?" The PubMed, B-On, and Cochrane Library databases were enrolled, using the keywords melatonin, periodontal therapy, non-surgical treatment, and periodontitis, as associated with the Boolean connectors. The inclusion criteria were (i) CCT or RCT, (ii) adult population, (iii) full-text articles available, and (iv) in the last 10 years (2012–2022). The exclusion criteria were (i) animal studies, (ii) systematic review, and (iii) no other languages than English, Spanish, Portuguese, and Italian. A risk of bias was performed to assess the articles. Results: Initially, 2705 articles were identified. However, only six articles were included. From a total of 228 patients (109F and 119M) diagnosed with periodontitis, 22 patients dropped out (9.65%). The follow-up period varied between 8 weeks, 3 months, and 6 months. For clinical and molecular parameters, the melatonin group had significant and greater improvement (intragroup) and better data than the control group, which also had favorable results. There was low risk of bias for all studies. Conclusions: Within the limitation of this study, melatonin is a reliable and feasible option as an adjunctive to the classical NSPT, obtaining a significative improvement of the periodontal parameters (PD, CAL, BOP, PI, and GI), a significative reduction of the pro-inflammatory proteins (IL-1b, IL-6, and TNF-α), and a better response for other biomarkers.

Keywords: melatonin; therapeutics; biomarkers; periodontitis

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1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine produced mainly by the pineal gland (an endocrine gland) and located at the base of the brain, also called the epiphysis. Additionally, it can be produced by various other organs such as retina, bone marrow, and intestines in a circadian pattern, which it is not considered a hormone. Besides, it does not exert any effect on a specific target [1]. Nevertheless, extra pineal sites do not contribute so much to the production of melatonin, depending on specific stimuli, in order to secrete it [2]. Inside the pineal gland, its production occurs in cells, pinealocytes, until the age of 30, in which, after that, its synthesis decreases [3]. The production and release of this hormone depends on the conditions of exposition to light, increasing in the darkness during the night and decreasing in the sunlight during the day [4].

Briefly, the mechanism of the formation of melatonin is that free tryptophan from the blood is taken up by the pinealocytes and converted into serotonin, with the help of the enzymes tryptophan-5-hydroxylase and 5-hydroxytryptophan decarboxylase, that successfully hydroxylate and decarboxylate tryptophan, respectively. During the night, by the action of N-acetyltransferase, serotonin is converted to N-acetylserotonin, on which the enzyme hydroxyindole-O-methyl transferase acts on, causing its methylation and then forming melatonin.

It is considered a pleiotropic molecule and was characterized and isolated in 1958 [5]; its binding to membrane receptors mediates numerous physiological functions [6,7], playing several important roles for living organisms, including regulating the circadian cycle, controlling body temperature, and having great anti-inflammatory, antioxidant, and free radical scavenging properties [8]. Being a highly lipophilic molecule, it has the capacity to enter the subcellular compartment, easily spreading in tissues. It exists in high concentrations in the nucleus and the mitochondria [1,9] and can bind with serum albumin and some cytosolic proteins as kinase-C [10], calmodulin [11], and calreticulin [12]. It reaches the oral cavity through the bloodstream, passively spreading in the saliva [10]. Nevertheless, melatonin in the saliva is about one-third of that found in blood [3]. Authors have shown that melatonin released in the saliva has numerous protective effects against various oral conditions, such as periodontal disease, oral cancer, herpes, and local inflammatory processes [13]. Moreover, it exerts positive regulation of homeostasis, with relevant inhibitory action on bone resorption, which can be used as a biomarker for periodontitis and peri-implantitis [14].

In mammals, melatonin level reaches its maximum at the middle of the night and decreases to low levels during the day [15]. Then, because of the association of the synthesis of melatonin during night, it is referred to as the chemical expression of darkness [16]. Otherwise, melatonin production decreases with advancing age [17]. In the body, it can rapidly cross the blood–brain barrier and placenta [18], control the circadian rhythm [19,20], regulate body temperature [21], sexual development, and the reproductive cycle, and activate the immune system [22,23].

Specifically in the oral cavity, melatonin has paracrine effects on cells [24], acts as an antioxidant and an anti-inflammatory agent, has an immunomodulatory role [22,25], stimulates antioxidative enzymes, and plays an essential stimuli and role in bone formation, the synthesis of type I collagen [5], and the reduction of bone resorption.

The use of melatonin as drug therapy for periodontal treatment has the advantage over other drugs, due to being inexpensive and having a larger margin of safety, a broad tissue impact, and practically no side effects, suggesting its potential as a primary or complementary treatment strategy for a wide range of diseases, such as bone injury, osteoporosis, osteoarthritis, and periodontitis, by exerting multiple effects.

The use of melatonin, which acts as a powerful antioxidant, can bring numerous advantages to periodontal diseases pursuing homeostasis, such as restoration of the concentration of antioxidants, reduction of periodontal inflammation with the regulation of inflammatory cytokines, reduction of oxidative stress, and a significant reduction of bone resorption, through the modulation of osteoclastic and osteoblastic activities [14,26].

The anti-inflammatory property of melatonin allows us, in fact, to eliminate ROS (exogenous and endogenous reactive oxygen species) and RNS (reactive nitrogen species), which are the causes of tissue damage [3]. In addition, melatonin also stimulates the synthesis of type I collagen fibers through the receptor located in pre-osteoblasts, which leads to the production, in these cells, of bone sialoprotein, alkaline phosphatase (ALP), osteopontin, and osteocalcin, significantly reducing the time required for their differentiation into mature osteoblasts, from 21 to 12 days [27].

Thus, the goal of this systematic review was to evaluate the literature, in order to verify if the use of melatonin improves the non-surgical treatment of periodontitis. The positive hypothesis was that there would be an improvement in the periodontal parameters when using melatonin for non-surgical periodontal therapy. In contrast, the null hypothesis was that no difference would be found using melatonin.

2. Materials and Methods

2.1. Protocol and Focus Question

This systematic study was conducted following the PRISMA guidelines, diagram methodology for article selection [28,29], with the focus question: "Are there significant benefits in applying melatonin for the non-surgical treatment of periodontitis?" PICO strategy was applied and presented according to Table 1, which shows the population (P), intervention (I), comparison (C), and outcomes (O).

Table 1. PICO strategy for focus question formulation.

Population	Patients over 18 years old, both genders, with periodontal disease
Intervention	Periodontal treatment with the application of melatonin
Comparison	Periodontal treatment without the application of melatonin or healthy control
Outcomes	Clinical parameters and biomarkers after melatonin application in non-surgical periodontal treatment

2.2. Search Strategy

Based on the methodological objectives outlined, the electronic research was performed between 16 December 2021 and 20 February 2022 and carried out through three databases: Medline/PubMed, On-Line Knowledge Library (B-On), and Cochrane Library. It applied specific keywords associated with the Boolean connectors "AND" and "OR" to combine them, achieving a more significant number of articles: melatonin, methoxytryptamine, "periodontal therapy", "periodontal treatment", "non-surgical treatment", periodontitis, "clinical attachment level", "pocket depth", and "bleeding on probing".

2.3. Inclusion and Exclusion Criteria

The articles included were (i) clinical controlled trials and randomized controlled trials, (ii) adult population (females and males), (iii) full-text articles available, and (iv) within the last 10 years (2012–2022). The exclusion criteria were (i) in vitro and animal studies, (ii) systematic or narrative review, (iii) reports based on questionaries, interviews, and case series/report, and (iv) no other languages than English, Spanish, Portuguese, and Italian.

2.4. Selection of Articles and Data Extraction

The analysis was carried out independently by two reviewers (SP and FPO), and a third reviewer (FCC) helped in case of disagreement in selecting the articles to include. The results obtained were discussed by integrating the inclusion/exclusion criteria, analyzing the title and abstract, and after reading the full text. Duplicate articles were removed.

The main domains extracted from the included articles will be the following: author, type study, follow-up, sample size and sample characterization, clinical features, treatment done, adverse effects observed, and results found.

2.5. Risk of Bias

All selected studies were subjected to a qualitative assessment. Two independent investigators (FPO and FCC) performed the biases assessment. This analysis was used to assess the risk of bias of randomized and controlled clinical trials focusing on: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias, where each group is classified as "low risk", "unclear", and "high risk". In the case of divergences, a third researcher was consulted (GVOF).

3. Results

Initially, 2705 articles were identified (2668 through B-On, 16 in the PubMed database, and 21 in the Cochrane). After the first analysis, 2550 articles were excluded due to being reviews, animal studies, periods, restricted access, or being in other languages, 9 were repeated, and 114 had titles and abstracts out of the criteria. Then, for full-text reading, 32 articles were eligible, 26 out of them were removed (due to the eligibility criteria), and the remaining 6 articles were finally included (k = 0.9) (Figure 1), which demonstrated the efficacy of melatonin in subjects with periodontitis, with improvement in periodontal clinical parameters. However, Bazyar et al. (2019) and Javid et al. (2020) had the same cohort groups; otherwise, they reported different parameters that were gathered and included in Tables 2–6. The results are presented below and are described and summarized in Tables 2–6.

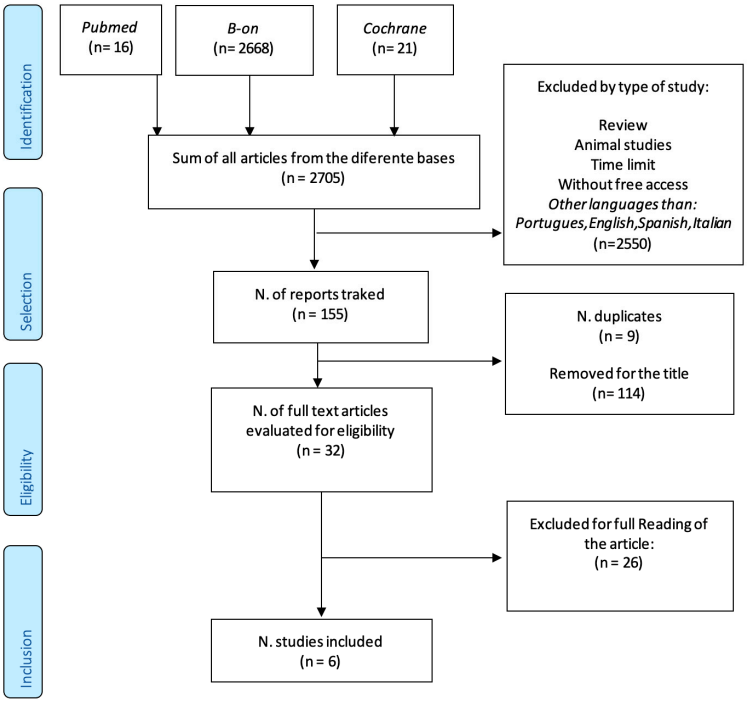

Figure 1. Flow diagram for Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA).

All studies were randomized or controlled clinical trials that treated patients with NSPT and melatonin. A total of 228 patients (109F and 119M) diagnosed with periodontitis were included. Twenty-two patients dropped out (9.65%). According to the systemic conditions, two studies included only healthy patients, two others included patients with type-2 diabetes, and another included individual with primary insomnia, which is not related to any other systemic causes or drug intake, according to the International Classification of Sleep Disorders (ICSD-3).

Table 2. General data of the included article.

Authors (Year)	n Control	n Test Group	Patients	Diagnosis	Systemic Condition	Type of Study	Dropout	NSPT Description	Exclusion Criteria	Period of Treatment	Follow-Up	Side Effects
Ahmed et al. (2021) [30]	24	24	30 patients. In the end, 24 patients (17F and 7M with age range 32–55)	Stage II periodontitis (interdental CAL is detectable at ≥2 nonadjacent teeth, or buccal or oral CAL ≥ 3 mm with pocketing >3 mm is detectable at ≥ teeth and CAL 3–4 mm and maximum probing depth ≤ 5 mm)	Systemically free according to the modified Cornell Medical Index	Split-mouth randomized controlled clinical trial	6	Test: NSPT + Intrapocket application of 5% melatonin gel using a plastic disposable syringe. Control was treated by nonsurgical therapy, followed by intrapocket application of placebo gel using a plastic disposable syringe.	Smokers, pregnant and lactating, patients who received any periodontal treatment in the past 6 months prior to the examination, patients who used antibiotic or anti-inflammatory drugs or antioxidants within the 6 months preceding the beginning of the study, and patients working in night shifts or received any medication that known to alter melatonin levels (e.g., for sleeping disorders).	Weekly once received the application, for 4 weeks	3 months	NR
Anton et al., 2021 [31]	27	27	54 patients. In the end, 50 patients (21F and 29M) Test: 53.24 ± 3.4 Control: 52.21 ± 3.1 years old	With periodontal disease	Type 2 diabetes (Glycated hemoglobin A1c (HbA1c)	A double-blind, placebo-controlled, single-center study	4	Non-surgical periodontal debridement that involved ultrasonic scaling (Woodpecker UDS-A-LED, Guilin Woodpecker Medical Instrument Co., Ltd., Guilin, China) and manual root planing (Gracey Standard and Mini curettes—Hu-Friedy, Chicago, IL, USA) (SRP) in one session.	Subjects who had undergone periodontal or anti-inflammatory treatment in the last 6 months, insulin treatment, significant change in drug use, and treatment of their diabetes or diet.	Daily for 8 weeks, 1 h before bedtime	8 weeks	No adverse effects of melatonin were observed during the study.

Table 2. *Cont.*

Authors (Year)	n Control	n Test Group	Patients	Diagnosis	Systemic Condition	Type of Study	Dropout	NSPT Description	Exclusion Criteria	Period of Treatment	Follow-Up	Side Effects
Bazyar et al., 2018 [32], and Javid et al. (2020) [33]	25	25	50 patients (30F, 20M) with T2DM M and F (aged 30–60 years) Control: 51.45 ± 5.03 Test: 53.72 ± 6.68	Severity periodontitis of mild to moderate (PD ≥ 4 mm and CAL = 1–4 mm)	Type-2 diabetes mellitus (T2DM) and body mass index of 18.5–30 kg/m^2	Double-blinded, placebo-controlled, and single-center trial	6	Both groups underwent the NSPT, and instructions for dental hygiene (how to brush and use dental floss correctly) were explained to patients. The patients were asked to avoid using mouthwash.	Patients with kidney failure, pregnancy, breastfeeding, thyroid disease, traveling more than two weeks, smoking, using any immuno-suppressive medications, antioxidants, anti-inflammatory agents, insulin, any mouthwash and antibiotic, and noticeable change in consumption of drugs, patients with severe periodontitis, and following a specific diet over the past six months.	8 weeks (melatonin and placebo tablets were recommended to use one hour before sleeping at night)	8 weeks	NR
Tinto et al., 2020 [34]	10	10	12M and 8F, with a mean age of 45.6 years (between 30 years and 70 years old)	Untreated severe stage III (interdental CAL ≥ 5 mm, ≤4 teeth lost, maximum PD ≥ 6 mm) periodontitis	Healthy adult patients	Randomized, placebo-controlled, triple-blind, monocentric clinical trial	0	NSPT was performed following a one-stage full-mouth protocol under local anesthesia with ultrasonic instruments and periodontal curettes. The time spent per quadrant was nearly 45 min. Patients were motivated toward oral hygiene and plaque control and instructed to rinse twice per day for 14 days with a 0.20% solution of chlorhexidine.	• Smoking >20 cigarettes per day • Uncontrolled diabetes; • Immunosuppres-sion (pathological or drug-induced); • Current therapy with antiresorptive drugs (bisphosphonates); • Pregnancy and breastfeeding; • In need of antibiotic therapy or treatment with antibiotics in the previous month; • Therapy with mood modulators or sedatives.	30 days	6 months	Sleepiness (20%); headache (10%); symptoms resolved sponta-neously after a few days.

Table 2. *Cont.*

Authors (Year)	n Control	n Test Group	Patients	Diagnosis	Systemic Condition	Type of Study	Dropout	NSPT Description	Exclusion Criteria	Period of Treatment	Follow-Up	Side Effects
El-Sharkawy et al., 2019 [35]	40	40	41M and 33F, with mean age: Control: 46.7 ± 8.3 Test: 45.6 ± 7.1	At least 20 teeth were diagnosed to have moderate to severe generalized chronic periodontitis (i.e., radiographic evidence of bone loss and presence of PD ≥ 5 mm and at least three sites in each quadrant with attachment loss ≥ 4 mm)	AIS score ≥ 6 (provided that individuals have primary insomnia, which is not related to any other systemic causes or drug intake according to the International Classification of Sleep Disorders, ICSD-3)	Randomized Controlled Trial	6	All patients received meticulous thorough SRP with a standard ultrasonic scaler and hand curettes in two visits by an experienced periodontist (MA) prior to giving the capsules. For all participants, strict oral hygiene instructions were given, and 0.12% chlorhexidine mouthwash was prescribed for 2 weeks following SRP.	Diabetes mellitus, smokers, individuals having night work shifts, patients with AIS score <6, cancer patients, patients with autoimmune diseases or osteoporosis, users of antibiotics or non-steroidal anti-inflammatory drugs within the last 3 months, and patients who were subjected to any periodontal therapy during the previous year.	2 months	3 and 6 months	Mild adverse reactions observed ranged from zero to a maximum of two cases of melatonin and placebo. - Headache; - Dizziness; - Nausea; - Constipation; - Diarrhea; - Abdominal cramp. Daily oral melatonin intake before bedtime has improved sleep quality and daily life activities for insomniac patients by using the AIS.

Non-surgical periodontal therapy (NSPT); body mass index (BMI); clinical attachment loss (CAL); plaque index (PI); gingival index (GI); full mouth bleeding score (FMBS %); full mouth plaque score (FMPS %); Total Antioxidant Capacity (TAC); matrix of metalloproteinase (MMP); interleukin (IL), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx); high-sensitivity C-reactive protein (hs-CRP).

Table 3. General data and results of the included article.

Authors (Year)	Clinical Features	Treatment	General Results
Ahmed et al., 2021 [30]	-Patients with periodontitis stage II were diagnosed as having interdental CAL, which is detectable at ≥2 nonadjacent teeth or buccal, CAL ≥ 3 mm with pocketing >3 mm, which is detectable at ≥2 teeth, CAL 3–4 mm, and maximum probing depth ≤ 5 mm	**-TG**: NSPT + OHI + with 5% melatonin gel **-CG**: NSPT + OHI + placebo gel administration 4 weeks for once a week	**Baseline vs. After treatment** **-TAC: TG** (284.5 ± 33.2 vs. 584.4 ± 64.1), **CG** (283.2 ± 30.9 vs. 437.3 ± 60.4) **-MMP-9: TG** (77.71 ± 2.86 vs. 31.20 ± 2.3), **CG** (77.53 ± 3.4 vs. 47.1 ± 1.8)
Anton et al., 2021 [31]	-Patients with type 2 diabetes and chronic periodontitis -Subjects with fastening blood glucose levels higher than 126 mg/dL and glycated hemoglobin higher than 6.5% were defined as diabetic	-One a day for 8 weeks **-TG**: NSPT + OHI + 2 melatonin tablets (250 mg) with 3 mg melatonin **-CG**: NSPT + OHI + 2 placebo tablets (250 mg)	**Baseline vs. After treatment** **-PD: TG** (4.65 ± 1.04 vs. 2.27 ± 0.7), **CG** (4.53 ± 1.01 vs. 4.40 ± 1.02) **-CAL: TG** (3.05 ± 0.56 vs. 1.24 ± 0.45), **CG** (3.02 ± 0.93 vs. 2.98 ± 0.96) **-NSPT therapy: CG** decrease without reaching statistical significance **-PI%: TG** (100 vs. 24), **CG** (100 vs. 48) **-BOP%: TG** (100 vs. 20), **CG** (100 vs. 40) **-Periodontitis severity**: TG significant changes were observed for all severity categories (superficial, moderate, and severe) **CG** a slight decrease in the number of teeth with moderate and severe periodontitis ($p > 0.05$) and a significant increase in the number of teeth with superficial periodontitis ($p < 0.05$).
Javid et al., 2020 [33]	-Patients with diabetes 2 with mild to moderate periodontal disease (PD 4 mm and CAL = 1–4 mm) with body mass index of 18.5–30 kg/m^2	**-TG**: NSPT + OHI+ 250 mg per day (2 tablets) of sodium starch glycolate, magnesium stearate, and 3 mg melatonin **-CG**: NSPT + OHI+ 250 mg per day placebo tablets with cellulose, silicon dioxide, magnesium stearate, starch, and a few tastes of peppermint oil 1 once/day for 8 weeks	**Baseline vs. After treatment** **-SOD: TG** (13.91 ± 2.75 vs. 15.53 ± 4.37 with $p = 0.008$), **CG** (14.27 ± 2.52 vs. 14.49 ± 2.58 with $p = 0.1$) **-CAT: TG** (24.23 ± 4.54 vs. 27.47 ± 4.12 with $p = 0.004$), **CG** (23.14 ± 3.52 vs. 22.72 ± 5.58 with $p = 0.77$) **-GPX: TG** (243.04 ± 68.37 vs. 262.04 ± 62.45 with $p = 0.004$), **CG** (231.18 ± 67.28 vs. 233.18 ± 62.66 with $p = 0.71$) **-TAC: TG** (0.289 ± 0.04 vs. 0.313 ± 0.05 with $p = 0.02$), **CG** (0.318 ± 0.06 vs. 0.327 ± 0.08 with $p = 0.48$) **-MDA: TG** (17.2 ± 1.82 vs. 16.13 ± 1.76 with $p < 0.001$), **CG** (17.49 ± 1.38 vs. 17.17 ± 1.39 with $p = 0.1$) **-IL-1b: TG** (2.41 ± 0.55 vs. 2.06 ± 0.48 with $p = 0.008$), **CG** (2.47 ± 0.48 vs. 2.33 ± 0.54 with $p = 0.12$).
Tinto et al., 2020 [34]	-Diagnosis of untreated severe stage III periodontitis (interdental CAL ≥ 5 mm, ≤4 teeth lost, maximum PD ≥ 6 mm)	**-TG**: NSPT + OHI+ 1 mg oral melatonin capsules for 30 days **-CG**: NSPT + OHI+ 1 mg oral placebo capsules for 30 days	**Baseline Vs. After treatment** **-PD: TG** (3.72 ± 0.90 vs. 2.45 ± 0.91 with $p = 0.2$); **CG** (3.40 ± 0.81 vs. 2.67 ± 0.85 with $p = 0.2$) **-PD 4–5 mm: TG** (1.86 (0.81)), **CG** (1.04 (0.69)), **-PD ≥ 6 mm: TG** (3.33 (1.43)), **CG** (2.11 (0.96)) **-FMBS% and FMPS%**: No differences were found

Table 3. *Cont.*

Authors (Year)	Clinical Features	Treatment	General Results
Bazyar et al., 2019 [32]	-Patients with type 2 diabetes and chronic periodontitis with mild and moderate periodontitis (PD $\geq$ 4 mm and CAL = 1–4 mm). -Body mass index of 18.5–30 kg/m^2, confirmed DM2 (no more than 5 years since diagnosis)	**-TG**: NSPT + OHI+ 6 mg of melatonin (2 capsules) once daily. **-CG**: NSPT + OHI+ 6 mg placebo (2capsules) 1 a day.	**Baseline vs. After treatment** **-PD: TG** (4.45 $\pm$ 0.96 vs. 2.59 $\pm$ 1.04 with $p < 0.001$), **CG** (4.54 $\pm$ 1.01 vs. 4.36 $\pm$ 1.04 with $p = 0.1$) **-CAL: TG** (3.04 $\pm$ 0.78 vs. 1.59 $\pm$ 0.59 with $p < 0.001$), **CG** (3 $\pm$ 0.75 vs. 2.77 $\pm$ 0.68 with $p = 0.021$) **-IL-6: TG** (2 $\pm$ 0.92 vs. 1.42 $\pm$ 0.73 with $p = 0.008$), **CG** (2.16 $\pm$ 0.91 vs. 2.08 $\pm$ 0.87 with $p = 0.58$) **-TNF-α: TG** (9.05 $\pm$ 3.56 vs. 8.24 $\pm$ 3.45 with $p = 0.1$), **CG** (8.65 $\pm$ 3.87 vs. 8.5 $\pm$ 3.95 with $p = 0.81$) **-MELATONIN: TG** (4.52 $\pm$ 1.78 vs. 5.03 $\pm$ 1.68 with $p = 0.005$), **CG** (4.32 $\pm$ 1.93 vs. 4.07 $\pm$ 1.91 with $p = 0.43$)
El-Sharkawy et al., 2019 [**stage II**] [35]	-Patients with insomniac individuals with generalized chronic periodontitis and have at least 20 teeth, diagnosed moderate to severe periodontitis chronic (radiographic evidence of bone loss and presence of PD $\geq$ 5 mm and at least three sites in each quadrant with attachment loss $\geq$ 4 mm)	**-TG**: NSPT+ OHI+ 2-month regimen of 10 mg oral melatonin capsules 1 time daily **-CG**: NSPT + OHI + 2 months regiment of oral placebo capsules	**Baseline vs. After treatment** **-PD: TG** (4.3 $\pm$ 0.8 vs. 2.3 $\pm$ 0.9), **CG** (4.4 $\pm$ 0.7 vs. 3.0 $\pm$ 0.8) **-PI: TG** (2.35 $\pm$ 0.45 vs. 0.81 $\pm$ 0.23), **CG** (2.44 $\pm$ 0.67 vs. 0.95 $\pm$ 0.17) **-GI: TG** (2.14 $\pm$ 0.36 vs. 0.68 $\pm$ 0.17), **CG** (2.21 $\pm$ 0.24 vs. 0.69 $\pm$ 0.15) **-BOP%: TG** (63 $\pm$ 21 vs. 12 $\pm$ 2.1), **CG** (59 $\pm$ 19 vs. 18 $\pm$ 2.8) **-CAL: TG** (4.8 $\pm$ 0.9 vs. 2.6 $\pm$ 1.0), **CG** (4.7 $\pm$ 1.0 vs. 3.4 $\pm$ 1.2)

CG = control group; TG = test group; OHI = oral hygiene instruction; PD = pocket depth; BOP = bleeding on probing; Non-surgical periodontal therapy (NSPT); body mass index (BMI); clinical attachment level (CAL); plaque index (PI); gingival index (GI); full mouth bleeding score (FMBS %); full mouth plaque score (FMPS %); Total Antioxidant Capacity (TAC); matrix of metalloproteinase (MMP); interleukin (IL), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx); high-sensitivity C-reactive protein (hs-CRP).

Table 4. Detailed clinical parameters found in the included studies.

Authors (Year)	Clinical Parameters											
	PD mm (Baseline) (±SD)	**PD mm (Final) (±SD)**	***p*-Value (%)**	**CAL mm (Baseline) (±SD)**	**CAL mm (Final) (±SD)**	***p*-Value (%)**	**PI (Baseline) (± SD)**	**PI (Final) (±SD)**	***p*-Value (%)**	**GI (Baseline) (± SD)**	**GI (Final) (±SD)**	***p*-Value (%)**
Ahmed et al. (2021) [30]	Melatonin 4.3 ± 0.8 Placebo 4.0 ± 0.6 (p = 0.1485)	2.9 ± 0.7 3.1 ± 0.7 (p = 0.3275)	<0.001, −32.56% <0.001, −22.5%	Melatonin 4.7 ± 0.9 Placebo 4.3 ± 0.6 (p = 0.07)	3.5 ± 0.6 3.7 ± 0.6 (p = 0.254)	<0.001, −25.53% <0.001, −13.95%	Melatonin 2 ± 0.7 Placebo 1.96 ± 0.6 (p = 0.83)	0.7 ± 0.6 0.7 ± 0.6 (p = 0.83)	<0.0001, −65% <0.0001, −64.29%	Melatonin 2.5 ± 0.5 Placebo 2.4 ± 0.5 (p = 0.5)	0.6 ± 0.5 0.7 ± 0.5 (p = 0.5)	<0.001, −80% <0.001, −70.83%
Anton et al. (2021) [31]	Melatonin 4.65 ± 1.04 Placebo 4.53 ± 1.01 (p = 0.15)	2.27 ± 0.7 4.40 ± 1.02 (p < 0.001)	<0.001 0.12	Melatonin 3.05 ± 0.56 Placebo 3.02 ± 0.93 (p = 0.1)	1.24 ± 0.45 2.98 ± 0.96 (p < 0.001)	<0.001 0.08	Melatonin 100% Placebo 100% (p = 0.9)	24% 48% (p = 0.07)	<0.001 <0.05	NR	NR	-
Bazyar et al. (2019) [32] and Javid et al. (2020) [33]	Melatonin 4.45 ± 0.96 Placebo 4.54 ± 1.01 (p = 0.76)	2.59 ± 1.04 4.36 ± 1.04 (p < 0.001)	< 0.001 0.1	Melatonin 3.04 ± 0.78 Placebo 3 ± 0.75 (p = 0.84)	1.59 ± 0.59 2.77 ± 0.68 (p < 0.001)	< 0.001 0.021	Melatonin 100% Placebo 100%	59.1% 81.8%	0.09	NR	NR	-
Tinto et al. (2020) [34]	Melatonin 3.72 ± 0.90 Placebo 3.40 ± 0.83	2.45 ± 0.91 2.67 ± 0.85	0.02 0.02	NR	NR		NR	NR		NR	NR	-
El-Sharkawy et al. (2019) [35]	Melatonin 4.3 ± 0.8 Placebo 4.4 ± 0.7 (p = 0.679)	3/6 months: 2.4 ± 1.0 2.3 ± 0.9 3.1 ± 0.9 3.0 ± 0.8 (p < 0.01)	p < 0.001 p < 0.001	Melatonin 4.8 ± 0.9 Placebo 4.7 ± 1.0 (p = 0.538)	3/6 months: 2.7 ± 1.1 2.6 ± 1.0 3.5 ± 0.9 3.4 ± 1.2 (p < 0.01)	p < 0.001 p < 0.001	Melatonin 2.3 ± 0.5 Placebo 2. 4 ± 0.7 (p = 0.424)	3/6 months: 0.84 ± 0.26 0.81 ± 0.23 0.92 ± 0.14 0.95 ± 0.17	p < 0.001 p < 0.001	Melatonin 2.14 ± 0.36 Placebo 2.21 ± 0.24 (p = 0.736)	3/6 months: 0.73 ± 0.19 0.68 ± 0.17 0.67 ± 0.14 0.69 ± 0.15	p < 0.001 p < 0.001

Table 5. Biochemical parameters found in the included studies (Part I).

Authors (Year)	Biochemical Parameters																		
	TAC ± SD % (µmol/L) Baseline	TAC ± SD % (µmol/L) Final	*p*-Value (%)	MMP-9 ± SD (pg/µL) Baseline	MMP-9 ± SD (pg/µL) Final	*p*-Value (%)	IL-1b (pg/mL) ± SD Baseline	IL-1b (pg/mL) ± SD Final	*p*-Value	IL-6 (pg/mL) ± SD Baseline	IL-6 (pg/mL) ± SD Final	*p*-Value	TNF-α (pg/mL) Baseline	TNF-α (pg/mL) Final	*p*-Value	MDA (µM) Baseline	MDA (µM) Final	*p*-Value	SOD (U/mL) Baseline	SOD (U/mL) Final	*p*-Value
Ahmed et al. (2021) [30]	Melatonin 284.5 ± 33.2 Placebo 283.2 ± 30.9	584.4±64.1 437.3 ± 60.4	<0.001, 109.21 ± 41.79% <0.001, 56.25 ± 27.64%	Melatonin 77.71 ± 2.86 Placebo 77.53 ± 3.4	31.20 ± 2.3 47.1 ± 1.8	<0.001, −59.57 ± 14.42% <0.001, −39.25 ± 16.46%	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-
Anton et al. (2021) [31]	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-
Bazyar et al. (2019) [32] and Javid et al. (2020) [33]	Melatonin 0.289 ± 0.04 Placebo 0.318 ± 0.06 (p = 0.09)	0.313 ± 0.05 0.327 ± 0.08 (p = 0.53)	0.02 0.48	NR	NR	-	Melatonin 2.41 ± 0.55 Placebo 2.47 ± 0.48 (p = 0.67)	2.06 ± 0.48 2.33 ± 0.54 (p = 0.1)	0.008 0.12	Melatonin 2 ± 0.92 Placebo 2.16 ± 0.91 (p = 0.58)	1.42 ± 0.73 2.08 ± 0.87 (p = 0.01)	0.008 0.58	Melatonin 9.05 ± 3.56 Placebo 8.65 ± 3.87 (p = 0.72)	8.24 ± 3.45 8.5 ± 3.95 (p = 0.81)	0.1 0.81	Melatonin 17.2 ± 1.82 Placebo 17.49 ± 1.38 (p = 0.56)	16.13 ± 1.76 17.17 ± 1.39 (p = 0.03)	<0.001 0.1	Melatonin 13.91 ± 2.75 Placebo 14.27 ± 2.52 (p = 0.65)	15.53 ± 4.37 14.49 ± 2.58 (p = 0.34)	0.008 0.1
Tinto et al. (2020) [34]	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-
El-Sharkawy et al. (2019) [35]	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-	Melatonin 14Placebo 14(p>0.05)	69.5(p<0.01)	-	NR	NR	-	NR	NR	-

Table 6. Biochemical parameters found in the included studies (Part II).

Authors (Year)	CAT (U/mL) Baseline	CAT (U/mL) Final	*p*-Value	GPx (U/mL) Baseline	GPx (U/mL) Final	*p*-Value	Melatonin (pg/mL) Baseline	Melatonin (pg/mL) Final	*p*-Value	hc-CRP (mg/L) Baseline	hc-CRP (mg/L) Final	*p*-Value
Ahmed et al. (2021) [30]	NR	NR		NR	NR		NR	NR		NR	NR	
Anton et al. (2021) [31]	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-
Bazyar et al. (2019) [32] and Javid et al. (2020) [33]	Melatonin 24.23 ± 4.54 Placebo 23.14 ± 3.52 (p = 0.37)	27.47 ± 4.12 22.72 ± 5.58 (p = 0.003)	0.004 0.77	Melatonin 243.04 ± 68.37 Placebo 231.18 ± 67.28 (p = 0.56)	262.04 ± 62.45 233.18 ± 62.66 (p = 0.13)	0.0040.71	Melatonin 4.52 ± 1.78 Placebo 4.32 ± 1.93 (p = 0.72)	5.03 ± 1.68 4.07 ± 1.91 (p = 0.08)	0.005 0.43	Melatonin 2.53 ± 0.77 Placebo 2.31 ± 0.96 (p = 0.41)	1.6 ± 0.91 2.4 ± 0.94 (p = 0.08)	0.017 0.45
Tinto et al. (2020) [34]	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-
El-Sharkawy et al. (2019) [35]	NR	NR	-	NR	NR	-	NR	NR	-	NR	NR	-

3.1. Groups of Treatment: Control vs. Test

There were two groups of treatments in all studies, the control group and the test group. All patients received NSPT as a treatment modality. In all studies, the control group (CG) used a placebo [30–35]. Javid et al. [33] applied 250 mg per day of placebo tablets with the ingredients containing cellulose, silicon dioxide, magnesium stearate, starch, and a few tastes of peppermint oil matching with the melatonin tablets for shape, color, and size, always administered one hour before bedtime for 8 weeks. Ahmed et al. [30] used placebo gel administration, whereas El-Sharkawy et al. [35] used a capsule, Tinto et al. [34] used 1 mg oral placebo capsules, and Bazyar et al. [32] used 6 mg placebo (2 capsules, 1 per/day).

All test groups received NSPT as a treatment associated with melatonin. Ahmed et al. [30] applied a 5% melatonin gel format for treatment. Bazyar et al. [32] and Javid et al. [33] used 250 mg daily, two tablets of sodium starch glycolate, magnesium stearate, and 3 mg net melatonin. The other three studies used capsules: Tinto et al. [34] treated with 1 mg of oral melatonin capsules; likewise, El-Sharkawy et al. [35] used 10 mg of oral melatonin capsules once daily before bedtime. Anton et al. [31] provided two melatonin tablets (250 mg) containing 3 mg of melatonin for 8 weeks.

3.2. Period of Treatment and Follow-Up

The treatments had different approaches. Ahmed et al. [30] treated once weekly, applying melatonin and a placebo for 4 weeks, similarly to Tinto et al. [34], whereas Bazyar et al. [32], El-Sharkawy et al. [6], Javid et al. [33], and Anton et al. [31] asked patients to intake (melatonin or placebo tablets) daily for 8 weeks, 1h before bedtime.

The follow-up period varied between 8 weeks [31–33], 3 months [30,35], and 6 months [34,35].

3.3. Clinical Parameters

3.3.1. PD and CAL

For all studies, the group melatonin had a significant statistical decrease: Tinto et al. [34] reported a reduction of 1.27 mm after 6 months, from 3.72 $\pm$ 0.90 mm to 2.45 $\pm$ 0.91 mm ($p = 0.02$); Ahmed et al. [30] achieved reduction of 1.4 mm after 3 months, from 4.3 $\pm$ 0.8 mm to 2.9 $\pm$ 0.7 mm ($p < 0.001$), a decrease of 32.56%; Anton et al. [31] observed a reduction of 2.38 mm, from 4.65 $\pm$ 1.04 mm to 2.27 $\pm$ 0.7 mm ($p < 0.001$) in 8 weeks; Bazyar et al. [32] also obtained a reduction in 8 weeks of 1.14 mm, with 4.45 $\pm$ 0.96 mm at baseline and 2.59 $\pm$ 1.04 mm; whereas El-Sharkawy et al. [35] had a decrease of PD in 3 months of 1.9 mm ($p < 0.001$) and in 6 months of 2.0 mm ($p < 0.001$).

For the control groups (CG), placebo, also there was a decrease in PD; therefore, the reduction was lower, compared with the test group: Tinto et al. [34] reported a similar statistical significance ($p = 0.02$) between groups, and the CG had 0.73 mm of reduction after 6 months; Ahmed et al. [30] had 0.9 mm after 3 months, a decrease of 22.5%; Anton et al. [31] and Bazyar et al. [32] had a similar result, with the reduction of 0.13 mm ($p = 0.12$), with statistical significance found between groups ($p < 0.001$) in 8 weeks and with 0.18 mm ($p = 0.1$), and with high difference, compared with the test group after 8 weeks; El-Sharkawy et al. [35] also had significant reduction of PD in 3 months of 1.3 mm ($p < 0.001$) and in 6 months of 1.4 mm ($p < 0.001$), and compared with a test group, a significant result was found with greater reduction for melatonin group, the difference in 3 and 6 months was 0.7 mm ($p < 0.01$).

CAL was not described by Tinto et al. [34]. Nevertheless, this parameter had significant and higher results for the melatonin group, with a reduction in improvement varying by 1.2–2.2 mm ($p < 0.001$). The differences found for the control group were lower, but significant, between 0.04–1.3 mm.

3.3.2. Plaque Index (PI), BOP, and GI

GI was evaluated in two studies [30,35]. There was a similar and significant reduction ($p < 0.001$) for both studies and groups analyzed, without a significant intergroup/period.

BOP was also reported by two articles [31,35]. For the study from 2018, BOP suffered a significant reduction ($p < 0.001$); in the melatonin group, the baseline was 63 ± 21%, and the final results after 3 and 6 months, respectively, were 11 ± 2.3% and 12 ± 2.1%. In contrast, for the placebo group, the baseline result found was 59 ± 19%, with results of 16 ± 2.2% (3 months) and 18 ± 2.8% (6 months), with $p < 0.001$.

Four authors reported PI, all with significant results. Bazyar et al. [32] found, for the test group, a reduction from 100% to 59.1% and, for the control, 100% to 81.8%, without significant results between groups after 8 weeks ($p = 0.09$). Anton et al. [31] reported 100% of PI (initial) for both groups, with reductions to 24% (test group, $p < 0.001$) and 48% (control group, $p < 0.05$); $p = 0.07$ between groups. Ahmed et al. [30] found similar reductions, 64.29% (control group) and 65% (test group), with a significant reduction after 3 months ($p < 0.001$), therefore without a statistically significant results intergroup ($p = 0.83$). El-Sharkawy et al. [35] also reported significant results, with numbers decreasing by 64.78% in the test group and 60.42% in the control group ($p < 0.001$).

3.4. Biomarkers

The main biomarkers associated with periodontal diseases were also evaluated. IL-1beta was only reported by Javid et al. [33]. There was a statistically significant result only for the test group ($p = 0.008$), with values from 2.41 ± 0.55 pg/mL (baseline) to 2.06 ± 0.48 pg/mL after 8 weeks. IL-6 was reported by Bazyar et al. [32], also with significant results found in the test group: 2.0 ± 0.92 pg/mL to 1.42 ± 0.73 pg/mL ($p = 0.008$). Bazyar et al. [32] also reported the analysis of tumoral necrosis factor-alpha (TNF-α), with significative results reported only for the test group, 9.05 ± 3.56 pg/mL at baseline and 8.24 ± 3.45 pg/mL after 8 weeks ($p = 0.1$). Another important parameter found was reported by Ahmed et al. [30], who assessed the levels of the matrix of metalloproteinases-9 (MMP-9); the authors found significant reductions for both groups, the melatonin, and the placebo, with a significant statistical result ($p < 0.001$). Other biomarkers were reported and detailed in Tables 5 and 6.

3.5. Diseases

Tinto et al. [34] and Ahmed et al. [30] included only healthy patients (Ahmed et al. considered systemically free, according to the modified Cornell Medical Index). Three articles [31–33] included patients with type-2 diabetes, observing the glycated hemoglobin A1c (HbA1c), and Bazyar et al. [32] and Javid et al. [33] also evaluated the body mass index (BMI).

One article [35] included patients with an Athens insomnia scale (AIS) score ≥ 6, provided that individuals have primary insomnia, which is not related to any other systemic causes or drug intake, according to the International Classification of Sleep Disorders (ICSD-3); AIS is an anatomically-based injury severity scoring system.

3.6. Risk Assessment

The risk of bias was evaluated, and all studies had a low level of bias. Only two unclear data were found in Anton et al. [31] and one in Ahmed et al. [30], which did not interfere with the final result (Figure 2). The low level for RoB found can be interpreted as the included studies transmit trustworthy and reliable data.

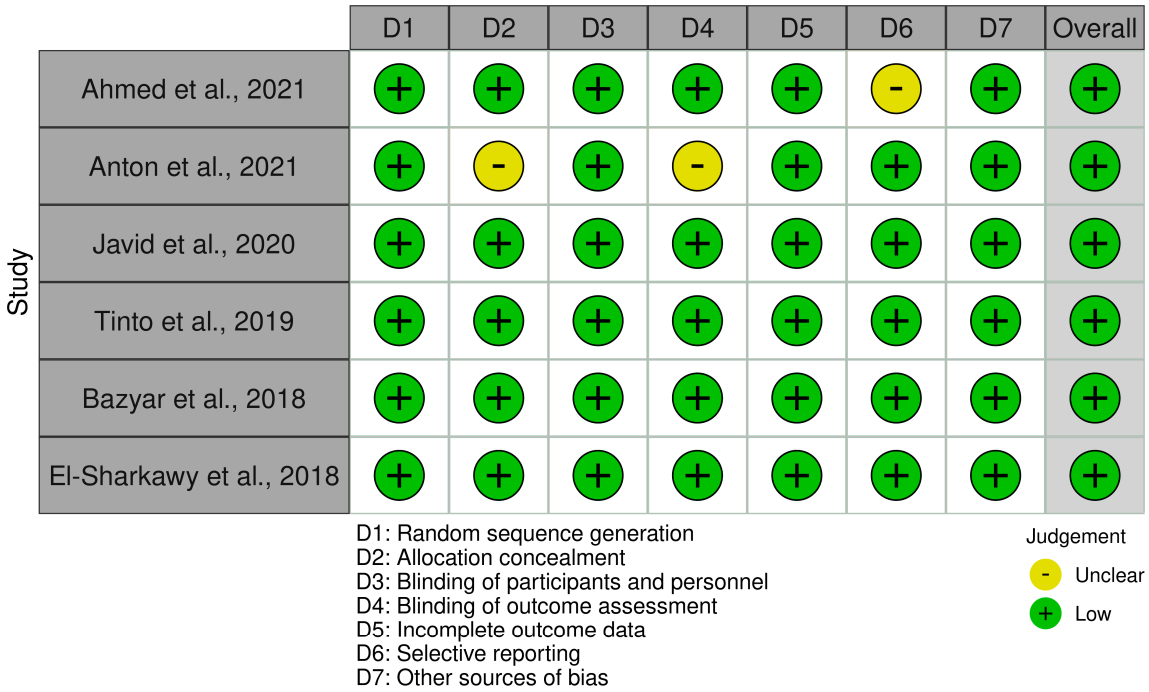

Figure 2. Risk of bias for 6 included articles [30–35].

4. Discussion

This integrative review aimed to analyze the most robust literature (including just controlled and randomized clinical trials) to ground the use of melatonin as an adjuvant in NSPT. Six articles were included to answer the focus question, "are there significant benefits in the application of melatonin for the non-surgical treatment of periodontitis?" Therefore, one of them [33] had the same cohort of patients as Bazyar et al. (2019) [32]; nonetheless, it was considered, due to reports of different findings.

Periodontal medicine terminology has stimulated research to improve the connection between oral and systemic conditions, bringing the interdisciplinary medical approach to patients with systemic and periodontal diseases [36]. Thereby, melatonin has been considered a relevant antioxidant, anti-inflammatory, and immunomodulatory function, beyond exerting a powerful endogenous effect as a free-radical scavenger [37], being a key molecule for periodontal protection. In addition, melatonin can protect cells from oxidative stress and neutralize up to ten molecules of reactive oxygen species (ROS), in contrast to classical antioxidants that neutralize only one molecule [38], which can be hypothesized as a reduction of oxidative stress through its application, which would be effective in the treatment of diabetes and periodontal diseases [39]. Moreover, it plays a crucial role in periodontal homeostasis by preventing and preempting periodontal destruction [14,26]. Additionally, melatonin administration can either be topical (mouthwash or gel) or systemic.

4.1. Proteins Analysis

Bone resorption enrolls osteoclasts that secrete various molecular agents for bone degradation, mainly TNF-a, IL-1b, MMP, and free radicals. Furthermore, monocytes, macrophages, neutrophils, and lymphocytes also accumulate on the site, due to chronic inflammatory processes, which are stimulated to produce free radicals and liberate many types of mediators. Then, melatonin might be an adequate adjunctive therapy associated with NSPT to bring and improve treatment, limiting tissue destruction [14]. In addition, Arabaci et al. [26] obtained excellent results in the melatonin group studying alveolar bone resorption in rats with periodontitis.

It was reported that melatonin reduces the synthesis of prostaglandins (PGE-2) and the up-regulation of a variety of pro-inflammatory cytokines [40], inhibits the adhesion of leuko-

cytes to endothelial cells [41], and attenuates transendothelial cell migration [42]. It also stimulates the release of IL-2 [43] and IL-6 [44]. Additionally, melatonin can inhibit acute inflammatory reactions [42]. Various proteins were analyzed in the included studies, such as IL-6, TNF-α, hs-CRP, MMP-9, IL-1b, MDA, and others. Regarding El-Sharkawy et al.'s [35] assessment, TNF-α level and AIS scores, at 3 and 6-month, a statistically significant reduction in these two parameters was observed in the melatonin group ($p < 0.01$), which is in agreement with Cutando et al.'s study [45], who obtained a reduction (in serum) of TNF-α and other pro-inflammatory cytokines, following topical application of melatonin; in contrast, Bazyar et al.'s study [32] did not report statistically significant results for TNF-α reduction; there was no improvement, probably due to different samples/period of administration. Bazyar et al.'s [32] values coincided with studies conducted by Kara et al. [46] and Köse et al. [47].

On the other hand, Bazyar et al. [32] obtained a significant reduction in the inflammatory biomarkers IL-6 and hs-CRP ($p = 0.008$ and $p = 0.017$, respectively) in the intervention group. At baseline, IL-6 for the control and intervention groups were, respectively, 2.16 ± 0.91 pg/mL and 2.0 ± 0.92 pg/mL, in contrast to the results obtained after 8 weeks in the intervention group, which showed a statistically significant decrease of 1.42 ± 0.73 pg/mL, compared to the control group 2.08 ± 0.87 pg/mL, which had no statistical significance.

Ahmed et al. [30] considered the level of MMP-9 (collagenase), which has significantly involved in connective tissue destruction. After melatonin treatment, compared with the control site, there was a statistically significant reduction in the MMP-9 of $-39.25 \pm 16.46\%$ ($p < 0.001$). That result was supported by Rudra et al. [48], which highlighted the effect of melatonin on MMP-9, in association with other diseases, such as rheumatoid arthritis, atherosclerosis, gastric ulcer, tumor growth, and cancer metastasis, proving significant inhibition of MMP-9 activity in a dose- and time-dependent manner. Other approaches to inhibit MMP-9 expression have been studied, such as developing reversible peptidomimetic hydroxamate inhibitors, concluding with negative results, due to a lack of specificity and toxic effects in physiological systems. This obstacle can be overcome through the medical delivery of melatonin.

Javid et al. [33] evaluated the intervention group's serum levels of IL-1b and MDA. Compared with the baseline results, there were decreased statistically significances (IL-1b with $p = 0.008$ and MDA with $p < 0.001$, respectively). At the same time, serum levels of SOD, GPx, CAT, and TAC significantly increased in the melatonin group. A significant increase in TAC ($p < 0.001$) was also observed by Ahmed et al. [30]. There is a close relationship between diabetes and periodontitis; diabetes increases the oxidative stress level that affects insulin secretion and action, accelerates the progression of the periodontitis, and, consequently, increases ROS and MDA.

Other authors obtained the same results [46,47,49] for IL-1beta and MDA levels. Specifically, in the study conducted by Ostadmohammadi et al. [49], melatonin was administered to patients for 12 weeks, and there were not only improvements in MDA (-0.21 mmol/L, 95% CI -0.36 to -0.06; $p = 0.005$) and TAC (253.87 mmol/L, 95% CI 189.18–318.56; $p < 0.001$), but also in HbA1c (-0.58%, 95% CI -1.16 to -0.002; $p = 0.04$) and serum insulin levels (-1.89 mIU/mL, 95% CI -3.34 to -0.45; $p = 0.01$). Through these results, we can understand that melatonin protects against oxidative stress, which is the main cause of cellular and periodontal tissue damage, improves mitochondrial function, and stimulates the expression and activation of antioxidant enzymes, including CAT, SOD, and GPx, as stated by Prado et al. [50] and Konečná et al. [51].

4.2. Clinical Parameters

Practically all studies reported statistically significant results on the effect of melatonin on periodontitis after NSPT. All six included studies that were considered the basic clinical parameters (PD and CAL). Some studies also considered GI, PI, and BOP. There was a positive overall result with different melatonin concentration levels and follow-up,

thus improving the periodontal parameters for the control and melatonin groups, with statistically significant results for the test group.

The systemic use of melatonin was used by El-Sharkawy et al. [35], Bazyar et al. [32], Tinto et al. [34], Javid et al. [33], and Anton et al. [31]. According to Balaji et al. [38], the systemic administration of melatonin was more effective than topical (mouthwash or gel). This may be explained by the GCF (gingival crevicular fluid, periodontal defense fluid) presence, which is an exudate composed of plasma and transports molecules from the bloodstream. The clinical results obtained were better in these studies, supporting the use of melatonin capsules as an auxiliary in treating periodontitis.

For all studies, PD levels statistically significantly decreased, and a considerable gain in CAL was also obtained. Tinto et al. [34] observed that the results were significant for the melatonin groups for 4–5 mm and >5 mm PD sites ($p < 0.001$).

Anton et al. [31] evaluated and correlated BOP and HbA1c. Plaque index (PI) and BOP decreased, after systemic administration of melatonin (patients received two melatonin tablets 250 mg that contained 3 mg of melatonin), showing significantly lower values. There was also a significant reduction in HbA1c during the studies, which was suggested to be matched to the NSPT. Still, a significant decrease was shown in the melatonin-treated group, compared to the control group.

A couple of studies used patients with T2DM and periodontal disease [31–33]. Ostadmohammadi et al. [49], in addition to the HbA1c, also detected the value of serum insulin levels. The results on the effectiveness in glycemic control were also exposed in the study. Thereby, Anton et al. [31] confirmed the veracity of the previous studies, showing that melatonin acted on periodontal parameters and had a relevant effect on glycemic control. Some divergent results from the studies can probably be attributed to the sample studied, follow-up, and the different dosages of melatonin. Javid et al. [33] analyzed the effect of melatonin, in addition to NSPT in patients with T2DM and periodontal disease. The intervention group consisted of 25 patients who received 2 tablets daily of 250 mg for 8 weeks. The results showed that melatonin intake significantly changed various clinical parameters.

All the studies included supported that the use of melatonin helped the NSPT. In contrast, Konečná et al.'s study [51] instructed patients to use 20 mL of melatonin, 5 mg/mL solution, to rinse the oral cavity before sleep and after brushing their teeth for 14 days, without having been subjected to the NSPT. The patients were observed one year after the two-week melatonin treatment and showed no improvement. In fact, the scored parameters and oxidative stress markers did not change. The probable causes of the study failure may depend on various factors, such as the presence of refractory periodontitis that was not assessed, the type of administration (oral rinse), the duration of treatment, and the concentration of melatonin administered. Mouthwash administration is a topical treatment; consequently, its effect depends on the volume and speed of absorption of melatonin from the oral mucosa and the time the patients take to rinse their mouths. In that study, the better and correct way of using mouthwash was not reported to the patients. For the mouthwash acts, the duration of rinsing and the contact time with the oral mucosa must allow the melatonin to be adequately absorbed by the tissues and, thus, produce effects. Indeed, further studies should be carried out to confirm the efficacy of melatonin in the mouthwash because a simple rinse that lasts on average 30 to 40 s or less can probably never give the best time to absorb all melatonin [52].

In another study by Ahmed et al. [30], the test group was treated by NSPT, followed by intrapocket application of 5% melatonin gel (topical administration) with a plastic disposable syringe. The results were positive, in terms of improvement of periodontal parameters, with a reduction in PD of −32.56% (test group) vs. −22.5% (control), while the percentage of CAL was −25.53% in TG and −13.95% in GC, demonstrating that melatonin treatment had significative effects. Although the administration was topical in this study, it was denoted that the intrapocket application gel had a good molecule propagation into the tissue, probably due to the melatonin's direct contact with the affected area, allowing it to have optimal diffusion. Otherwise, other studies that used systemic melatonin also had

significant results and a high percentual of improvement, which did not permit us to say that one method is superior to another.

Regarding the bone–melatonin relationship, studies conducted by Montero et al. [53], Calvo-Guirado et al. [54], Gómez-Moreno et al. [55], Arabaci et al. [26], and Cutando et al. [6], in addition to the report of bone formation improvement, found increased osteoblast differentiation. In particular, Cutando et al. [6] showed that, at 2 weeks of melatonin placement (1.2 mg lyophilized powdered melatonin) in implants, all parameters of osseointegration significantly increased: percentage of bone-implant contact, total peri-implant bone, and percentage of new bone formation. Furthermore, osteoclasts generate high levels of superoxide anions during bone resorption that contribute to the bone degradative process. Melatonin, an important free radical scavenger and a strong antioxidant, eliminate osteoclasts' free radicals during bone resorption by protecting cells from oxidative attacks [55–57].

4.3. Adverse Effects

The use of melatonin has led researchers to verify whether the melatonin regimen is safe, with minimum or no adverse effects. Only two out six articles studied found mild adverse effects: Tinto et al. [34] reported sleepiness (20%) and headache (10%), which were resolved spontaneously after a few days; and El-Sharkawy et al. [35] also had mild adverse reactions, presenting headache, dizziness, nausea, constipation, diarrhea, or abdominal cramp. Otherwise, El-Sharkawy et al. [35] concluded that daily oral melatonin intake before bedtime improved sleep quality and daily life activities for insomniac patients by using melatonin.

4.4. Limitations of the Study

A low number of articles (controlled or randomized) were found, reducing the impact of the study. Additionally, there was a standard of the parameters analyzed, which did not permit the development of a meta-analysis. Some studies included patients with the disease, such as type-2 diabetes, which brought a variable that might impair the possibility of comparison with healthy patients. Moreover, there was no standard for posology and administration pathway, nor a standard to recall the patients and period of intake or use of the melatonin.

5. Conclusions

Within the limitation of this study, melatonin is a reliable and feasible option as an adjunctive to the classical NSPT, obtaining a significative improvement of the periodontal parameters (PD, CAL, BOP, PI, and GI) and a significative reduction of the pro-inflammatory proteins (IL-1b, IL-6, and TNF-α) and better response for other biomarkers. Therefore, further clinical studies must be performed, following a standard period and dosage, with a higher number of patients, in order to demonstrate and confirm the significant results found.

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