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Therapeutic Potential of Melatonin in Periodontitis: A Randomised, Placebo Controlled, Double Blind Study.

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ABSTRACT

Until now, primary clinical weapons against periodontal disease have been scaling and root planning (SRP), antibiotics and surgery. Antioxidants, if given, can act systemically to support the body's natural immune system. **Aim:** This study was designed to evaluate the ability of melatonin supplementation to reduce the periodontal inflammation.This was randomised, double blind, placebo controlled study. It was conducted on 160 patients of periodontitis. Patients were randomly divided into 2 different groups. Group A: The patients underwent SRP & received placebo & Group B: Patients underwent SRP & supplemented with tablet melatonin 3 mg daily at night for 4 weeks. Study visits included clinic visits on day 0, day 30, day 60 and day 90. Patients underwent the clinical examination, i. e., Gingival Index (GI), Periodontal Disease Index (PDI), Community Periodontal Index (CPI) during each visit. We observed that there was significant improvement in all the indices in group B as compared to group A. Melatonin is a potential antioxidant and the clinical improvement it showed was significantly superior to the standard control group.

Keywords: Free radical, melatonin, periodontitis, reactive oxygen species.

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INTRODUCTION

Chronic periodontitis is an inflammatory disease that affects 10–15% of the developed world population [1]. The human inflammatory periodontal diseases are amongst the most common of chronic diseases to affect adults [2]. Periodontal infection may significantly enhance the risk for certain systemic conditions like coronary heart disease (CHD), atherosclerosis, stroke, diabetes mellitus, preterm labour, low birth weight delivery and chronic obstructive pulmonary disease (COPD) [3].

Chronic periodontitis is the commonest of all the forms of diseases frequently observed in adults. It is defined as "an infectious disease resulting in inflammation within the supporting tissue of the teeth, progressive attachment loss and bone loss".[3] These changes associated with periodontitis are irreversible, resulting in tooth loss and substantial morbidity in medically compromised patients, when focus of infection and subsequent bacteremia may present a major risk [2].

Periodontal disease is considered an inflammatory disease initiated and perpetuated by a small group of predominantly gram-negative, anaerobic, or micro-aerophilic bacteria that colonize the subgingival area.[4] Bacteria cause the observed tissue destruction directly by toxic products and indirectly by activating the host defense systems [5] i.e. inflammation. The characteristic of inflammation features a burst of non-mitochondrial O_2 consumption, which generates superoxide anion radicals, hydrogen peroxide, hydroxyl radicals, and hypochlorous acid, all capable of damaging either cell membranes or associated biomolecules [4,5]. It is likely that the role of reactive oxygen species (ROS) is common to both bacterial and host-mediated pathways of tissue damage. Free radical species have been implicated in the pathogenesis of over 100 conditions [6].

Melatonin is a ubiquitous natural neurotransmitter like compound produced primarily by pineal gland [7]. The role of endogenous melatonin in circadian rhythm disturbances and sleep disorders is well established.[8] Subsequently, melatonin was shown to have significantly broader action including oncostatic effects, immune system stimulation and anti-inflammatory functions. And now, melatonin is identified as a powerful direct free radical scavenger and indirect antioxidant [9]. Melatonin reduces oxidative stress by several means. It is an active scavenger of both the highly toxic hydroxyl radical (.OH), produced by 3 electron reduction of oxygen and peroxy radical which is generated during unsaturated lipid peroxidation.

Furthermore, many studies have proved that salivary melatonin level varies according to the degree of periodontal disease indicating that salivary melatonin may act to protect the body from external body insults. Therefore, melatonin supplementation, i.e., synthetic version of hormone melatonin may be potentially valuable in the treatment of periodontal diseases [10].

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Considering the above mentioned functions of melatonin, this study was designed to evaluate the ability of melatonin supplementation to raise the antioxidant levels and thereby to reduce the periodontal inflammation.

MATERIAL AND METHODS

This was a randomised, double blind, placebo controlled, comparative clinical study. The subjects of age 18 years and above were selected from the outpatient department of Periodontics, Rural Dental College and Hospital, Loni. After obtaining an informed consent form, a total of 160 patients suffering from periodontitis were enrolled in the study. Patients were randomly divided into 2 different groups.

Group A: The patients underwent SRP & received placebo.
Group B: Patients underwent SRP & supplemented with tablet melatonin 3 mg daily at night for 4 weeks.

Study visits included clinic visits on day 0, day 30, day 60 and day 90. Patients underwent the clinical examination, i. e., Gingival Index (GI), Periodontal Disease Index (PDI), Community Periodontal Index (CPI) during each visit. Subjects with the history of significant cardiovascular, neurological, metabolic, endocrinal, hematological, psychiatric, autoimmune, liver disorder were excluded from the study.

The patients were assessed for gingival inflammation using the Gingival Index, periodontal disease severity by the Periodontal Disease Index, and attachment loss by Community Periodontal Index.

Scoring criteria for indices

Gingival Index (GI) [11]:

Table 1: Scoring criteria for Gingival Index (GI)

0	Normal gingiva.
1	Mild inflammation: Slight change in colour and slight edema. No bleeding on probing.
2	Moderate inflammation: Redness, edema and glazing. Bleeding on probing.
3	Severe inflammation: Marked redness and ulceration. Tendency towards spontaneous bleeding.

Total GI score

0.1 to 1.0	Mild inflammation
1.1 to 2.0	Moderate inflammation
2.1 to 3.0	Severe inflammation

The teeth selected as index teeth were 16, 12, 24, 44, 32 and 36. The tissues surrounding each tooth were divided into four gingival scoring units: distal-facial pailla, facial margin, mesia-facial papilla and the entire lingual margin. A blunt instrument such as periodontal probe was used to assess the bleeding potential of the tissues. The indices for each of the teeth were added and then divided by total number of teeth examined. This gave the gingival index for the individual. Table 1 shows the scoring criteria.

Periodontal Disease Index (PDI) [11]:

PDI is concerned with an accurate assessment of the periodontal status of the individual person. Assessment of degree of periodontal disease includes a subjective assessment of colour, form, consistency and bleeding tendency of the gingival tissue. The most important feature of PDI is measurement of the level of periodontal attachment related to the cemento-enamel junction (CEJ) of the teeth. Among the three components of PDI, gingival and periodontal components were taken into consideration. Teeth 16, 21, 24, 36, 41, 44 were selected. The PDI score for the individual was obtained by totalling the scores for each tooth and dividing it by the number of teeth examined. PDI scores ranged from 0-6. Table 2 shows the scoring criteria.

Table 2: Scoring criteria for Periodontal Disease Index (PDI)

0	Absence of inflammation.
1	Mild to moderate inflammatory gingival changes not extending all around the tooth.
2	Mild to moderate gingivitis, extending all around the tooth.
3	Severe gingivitis, characterized by marked redness, tendency to bleed and ulceration.
4	Gingival crevice in any of the measured areas (mesial, distal, facial, lingual) extending less than 3 mm apical to CEJ.
5	Gingival crevice in any of the four measuring areas extending 3-6 mm apical to CEJ.
6	Gingival crevice in any of the four measuring areas extending more than 6 mm apical to CEJ.

Community Periodontal Index (CPI) [11]:

Two indicators of periodontal status are used for this assessment; gingival bleeding and periodontal pockets. A specially designed light weight CPI probe having 0.5 mm ball tip is used. The mouth is divided into sextants derived by tooth no. 18-14, 13-23, 24-28, 38-34, 33-43 and 44-48.

Introduction of loss of attachment is collected from index teeth in order to obtain an estimate of the lifetime accumulated destruction of the periodontal attachment. Probing pocket depths gives some indication of loss of attachment.

The index teeth and all remaining teeth in a sextant where there is no index tooth should be probed and the highest score is recorded in the appropriate box. The codes are given in Table 3.

ISSN: 0975-8585

Table 3: Scoring criteria for Community Periodontal Index (CPI)

0	Healthy.
1	Bleeding observed, directly or by using mouth mirror, after probing.
2	Calculus detected during probing, but the entire black band on the probe visible.
3	Pocket 4 – 5 mm (gingival margin within the black band on the probe).
4	Pocket 6 mm or more (black band on the probe not visible).
X	Excluded sextant.
9	Not recorded.

Statistical Analysis

Z test was used to measure the differences among the drugs and the result is expressed in the form of 'p' value.

RESULTS

Table 4 shows a comparison of the mean values of the gingival index at four visits in Group A and B. We observed that there was a highly significant decrease in the mean values of GI in Group B when compared with Group A at all visits ($p<0.01$). (Figure 1).

Table 4: Comparison of mean values of Gingival Index at four visits in Group A and B.

Clinical index /Visits	Group A	Group B	'z' value	'p' value
	Mean ± SD	Mean ± SD		
Base line visit	1.86 ± 0.60	1.77 ± 0.55	**1.0**	**p>0.05**
Visit 1st	1.73 ± 0.59	1.52 ± 0.59	**2.25**	**p<0.01**
Visit 2nd	1.65 ± 0.60	1.24 ± 0.55	**4.50**	**p<0.01**
Visit 3rd	1.56 ± 0.58	1.01 ± 0.56	**6.33**	**p<0.01**

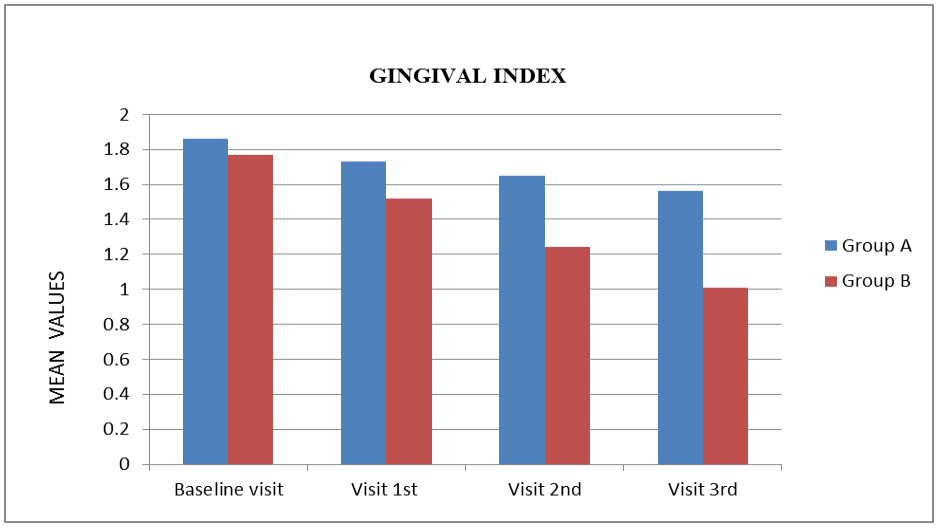

Figure 1: Comparison of mean values of Gingival Index at four visits in Group A and B.

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Table 5 shows a comparison of the mean values of the periodontal disease index at four visits in Group A and B. We observed that there was a highly significant decrease in the mean values of PDI in Group B when compared with Group A at all visits ($p<0.01$), except difference between mean values of PDI at 2nd visit. (Figure 2).

Table 5: Comparison of mean values of Periodontal Disease Index at four visits in Group A and B.

Clinical index /Visits	Group A	Group B	'z' value	'p' value
	Mean ± SD	Mean ± SD		
Base line visit	4.73 ± 0.52	4.67±0.43	0.7	p>0.05
Visit 1st	4.71±0.50	4.51±0.48	3.12	p<0.01
Visit 2nd	4.53±0.49	4.49±0.34	0.60	p>0.05
Visit 3rd	4.49±0.49	4.27±0.33	3.33	p<0.01

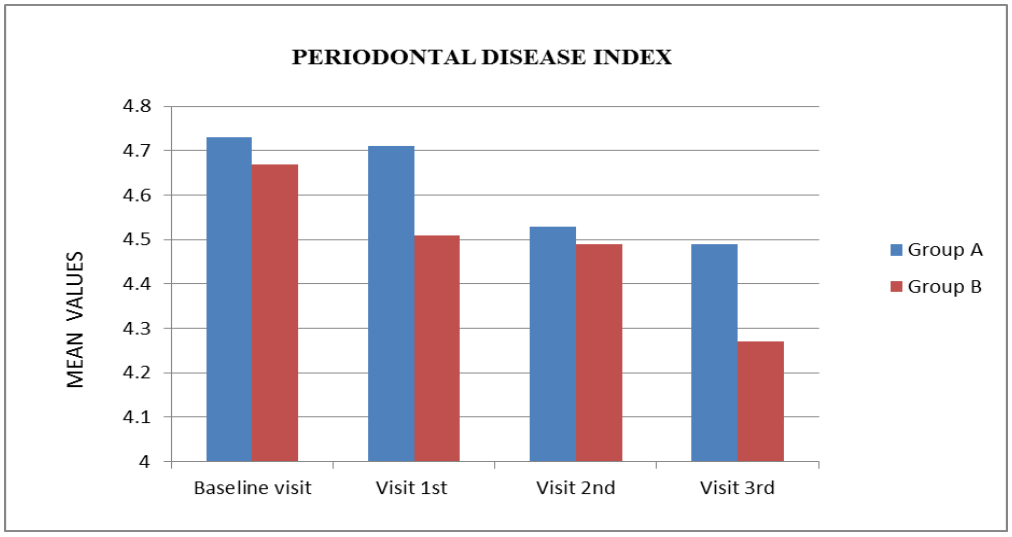

Figure 2: Comparison of mean values of Periodontal Disease Index at four visits in Group A and B.

Table 6 shows a comparison of the mean values of the community periodontal index at four visits in Group A and B. We observed that Group B showed significant improvement in CPI as compared to Group A ($p<0.01$). (Figure 3)

Table 6: Comparison of mean values of Community Periodontal Index at four visits in Group A and B.

Clinical index /Visits	Group A	Group B	'z' value	'p' value
	Mean ± SD	Mean ± SD		
Base line visit	4.69±0.95	4.51±1.06	1.22	p>0.05
Visit 1st	4.56±0.98	2.96±1.03	10.00	p<0.01
Visit 2nd	4.46±0.94	2.68±0.98	11.12	p<0.01
Visit 3rd	4.41±0.91	2.41±0.94	12.50	p<0.01

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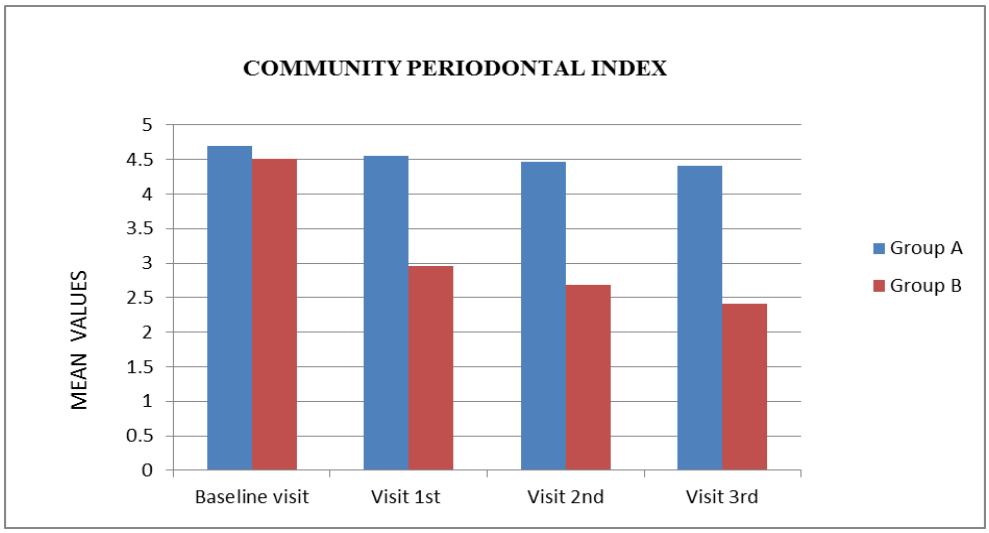

Figure 3: Comparison of mean values of Community Periodontal Index at four visits in Group A and B.

DISCUSSION

Chronic periodontitis is characterized by inflammatory destruction of connective tissue, loss of periodontal attachment and resorption of alveolar bone. Free radicals generation during inflammatory process may be relevant in the development of these alterations. There is increasing evidence that the disease occurs in a predisposed group of the population that has an aberrant inflammatory/immune response to the microbial plaque that accumulates around the gingival margin. This exaggerated response is known to result in inadvertent or collateral host tissue damage [2].

The predominant inflammatory cell within the healthy connective tissues and epithelium of the gingiva is the polymorphonuclear leucocytes (PMNL). In aggressive and chronic forms of periodontitis, PMNLs appear to be functionally activated and exhibit increased production of reactive oxygen species (ROS). These molecules are reported to be capable of inducing periodontal tissue destruction and are associated with osteoclastic bone resorption. The degree to which ROS influence the progression of periodontal diseases is as yet unclear, but their role cannot be considered in isolation, given the range of antioxidant species that protects against excess ROS activity and maintains a delicate equilibrium within host tissues.

The ability of the host to scavenge ROS produced by leucocytes or other cells (for example, fibroblasts) is regarded as a key protective mechanism against inadvertent ROS mediated host tissue damage. This mechanism appears to be crucial in both acute and chronic inflammatory diseases [12].

Melatonin activates several elements of the immune system that reduce tissue destruction during the inflammatory response, either directly by free radical scavenging or indirectly by modulating the action of agents such as cytokines and adhesion molecules, which contribute to the advance of cell damage [13]. In addition; melatonin shows a remarkable

functional versatility exhibiting antioxidant, oncostatic, antiaging and immunomodulatory properties [14]. Melatonin also promotes gene expression of type I collagen, osteoporin, bone sialoprotein and osteocalcin in a dose-dependent manner, and stimulated the mineralized matrix formation in vitro [12].

Considering these facts, this study was conducted to throw light on the effects of melatonin on patients suffering from periodontitis in rural scenario.

The clinical signs or parameters that were considered in our study, were gingival index (GI), by Loe & Silness, periodontal disease index (PDI) by Ramfjord and community periodontal index (CPI). Gingival index was considered as the indicator of inflammatory condition of the gingiva throughout the oral cavity. PDI and CPI were taken into consideration to assess the connective tissue destruction and bone loss. PDI gave an idea of the overall periodontal tissue destruction and clinical attachment level was used to calculate the total periodontal destruction.

There was a highly significant decrease in the mean values of GI, PDI and CPI of group B when compared with group A at all visits ($p<0.01$). Results showed that melatonin treated group was better in improving the clinical status of the patients as compared to the standard group. This finding suggests that the melatonin may possess the ability to fight against infection and inflammation. This finding is similar to the results of the study by Antonio Cutando et al [12] in which they found that salivary melatonin levels varied according to the degree of the periodontal disease. As the degree of the periodontal disease increased, the salivary melatonin level decreased, indicating that melatonin may act to protect the body from external bacterial insults.

One of the experimental study done by Adem Kara et al also showed that when periodontitis was induced, melatonin reduced the oxidative damage in rats' periodontal tissue by reducing inflammatory cytokines, regulating oxidative stress parameters and less periodontal tissue destruction [15].

The findings in our study indicate the important role played by melatonin in combating the oxidative stress in periodontitis and improvement of various clinical parameters, at well tolerated doses. In view of this antioxidant action, a combination of antioxidant vitamins may also be considered in the treatment of periodontitis.

Nevertheless, the treatment duration in our study was one month and since periodontitis can recur in the patient if not treated or the oral hygiene is not maintained properly, it would be worthwhile to conduct a study with prolonged treatment with melatonin, involving a larger sample size. This approach would help to further evaluate the clinical efficacy and safety of melatonin.

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