EVALUATION OF THE EFFECT OF TOPICAL MELATONIN GEL AS AN ADJUNCT TO NONSURGICAL PERIODONTAL THERAPY ON THE CLINICAL PARAMETERS AND TOTAL OXIDATIVE CAPACITY LEVEL IN GCF OF CHRONIC PERIODONTITIS PATIENTS: A SPLIT MOUTH RANDOMIZED CLINICAL TRIAL

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Abstract

Periodontal diseases are a group of bacterial infections and inflammatory diseases that result in the destruction of tooth-supporting tissue, including the gingiva, cementum, periodontal ligament and alveolar bone. The progression of these destructive disease is likely to be related to host response to the subgingival plaque biofilm with strong evidence has emerged to implicate oxidative stress in the pathogenesis of periodontitis.

In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidants defense capacity and when that equilibrium shifts in favour of ROS, oxidative stress results and the damage can occur. So to prevent or decrease that damage it's necessary to elevate the antioxidant defense mechanism and using potent antioxidants such as melatonin may have a beneficial effect.

The present investigation was evaluated the effect of intrapocket melatonin gel combined with non-surgical therapy versus non-surgical therapy combined with placebo gel on the clinical parameters (PI, GI, PD and CAL) and GCF level of total oxidant capacity (TOC) in patients with moderate chronic periodontitis.

This study was conducted on a total of 24 patients 17 females and 7 males in age range from 25 to 55 years having moderate chronic periodontitis and 15 subjects with healthy periodontium.

KEY WORDS: Melatonin, Periodontal Therapy, Periodontitis, Oxidative Capacity Level.

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INTRODUCTION

Chronic periodontitis was recognized as the most frequently occurring form of periodontal disease **(Slade et al., 2000)**. It is a chronic inflammatory process that occurs in response to a predominantly gram-negative bacterial infection originating in dental plaque and characterized by loss of clinical attachment due to destruction of the periodontal ligament and loss of the adjacent supporting bone **(Heitz-Mayfield et al., 2003).** The majority of periodontal tissue destruction is caused by an exaggerated host response to those organisms and their products **(Delima et al., 2002)**.

The neutrophils play an important role in host defence and are the first line of defence against periodontal disease **(Dennison and Dyke, 1997)**. The oxidative killing mechanism of neutrophils and other phagocytes involves the formation of reactive oxygen species (ROS) **(Roos et al., 2003)**.

In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidants defence capacity and when that equilibrium shifts in favour of ROS, either by reduction in antioxidant defences or an increase in ROS production or activity, oxidative stress results. This imbalance between the ROS and antioxidant has been implicated as one of the progressive or pathogenic factors for periodontal tissue destruction **(Waddington et al., 2000)**.

Protection against ROS is provided by antioxidants. Many of which, are released locally at sites of inflammation by polymorphonuclear leukocytes (PMNs) and other cells **(Akalin et al. 2005).** Antioxidants are those substances which when present at low concentrations, compared to oxidizable substrate will significantly delay or inhibit oxidation of that substrate **(Chapple and Matthews, 2007)**.

Melatonin (MT: N-acetyl-5-methoxytryptamine) is a neuroendocrine hormone secreted mainly by the pineal gland in the brain **(Ravindra et al., 2006)**. It is produced with a circadian rhythm characterized by elevated blood levels during the night **(Mor et al., 1999)**.

Melatonin and its metabolites are highly effective free radical scavengers **(Tan et al., 2007; Peyrot and Ducrocq, 2008; Gitto et al., 2009)** and act as potent antioxidant **(Macchi and Bruce, 2004)**, indirect antioxidant **(Reiter et al., 2002; Rodriguez et al., 2004),** and stimulators of antioxidative enzymes. Arising out of its antioxidative actions, melatonin protects cells during severe inflammatory processes and reduces oxidative damage **(Rodriguez et al., 2004)**.

Melatonin is present in Gingival Crevicular Fluids (GCF) in a concentration; 60% less than that of serum melatonin (14 to 60 pg/ml). When the levels of melatonin in GCF were analyzed,

there was a reduction in melatonin levels in subjects with periodontitis than the healthy subject. These values ran inversely proportional to the values of clinical indices supporting the antioxidant and anti-inflammatory effects of melatonin **(Srinath et al., 2010)**.

Cutando et al. (2013) evaluate the effect of topical application of melatonin to the gingiva and he found that; it was associated with an improvement in the gingival index and pocket depth, with reduction in salivary concentrations of receptor activator of nuclear factor kappa B ligand (RANKL) and increase in salivary concentrations of Osteoprotegerin (OPG), Furthermore, there was a reduction in salivary concentrations of acid phosphatase, alkaline phosphatase, osteopontin and osteocalcin.

All of these properties of melatonin suggest that melatonin can be used with great benefit in the treatment of patients with chronic periodontitis.

AIM OF THE STUDY

The aim of the current study was to evaluate of the effect of topical melatonin gel as an adjunct to non-surgical periodontal therapy on the clinical parameters and total oxidative capacity level in GCF of chronic periodontitis patient.

MATERIAL AND METHODS:

PICO:

P: Patients with moderate chronic periodontitis candidate for non- surgical periodontal therapy.

I: non-surgical therapy combined with intra pocket application of melatonin gel.

C: non-surgical therapy combined with intra pocket application of placebo gel.

O: Primary outcome: GCF level of total oxidative capacity (TOC). Secondary outcome: -Plaque index (PI) -Gingival index (GI) -Probing depth (PD) -Clinical attachment level (CAL)

Trial design:

A split mouth, prospective randomized clinical trial.

Sample size calculation:

Total sample size of 24 patient was sufficient to detect medium effect size (f=0.3) that estimated by Cohen (1992) and Cutando et al. (2013), with power 85% and 5% significance level. This number had to be increased to 30 patient to overcome for losses during follow up done by G power (Faul et al., 2007).

Patient Selection:

This study was conducted on a total of 30 patients (6 patients did not show up at follow up) ending in 24 patients (17 females and 7 males with age range 32-55) having moderate chronic periodontitis and 15 subject with healthy periodontium (9 females and 6 males with age range 34-48). Patients were recruited form the outpatient clinic of Oral diagnosis, Faculty of Oral and Dental Medicine, Oral Medicine and Periodontology Department, Cairo University.

Eligibility criteria:

Patients eligible for the trial must be complied

with all of the following:

i) Inclusion Criteria:

1- Patients were selected to be systemically free according to modified Cornell Medical Index. (Brightman, 1994).

2- Patients were diagnosed with moderate chronic periodontitis according to the American Academy of Periodontology (AAP) with the following criteria: Slight to moderate destruction is generally characterized by PDs up to 6 mm with CAL of up to 4 mm. Radiographic evidence of bone loss and increased tooth mobility may be present (American academy of Periodontology. 2000).

ii) Exclusion Criteria:

1. Smokers.
2. Pregnant and lactating females.
3. Patients received any type of periodontal treatment in the past 6 months prior to examination
4. Patients who used antibiotic or anti-inflammatory drugs or antioxidants within the 6 months preceding the beginning of the study.
5. Patient working in night shifts, or received any drug that known to alter Melatonin levels (e.g., for sleeping disorders).

Ethics:

The study was approved by the Ethics committee of Cairo University faculty of oral medicine and periodontology. After full explanation of the procedure to be done each patient signed a written consent. The patients were instructed not to take any medication that may interfere with the trial during the course of the study and were informed that they are free to leave the study at any time without any conflicts. Patients were informed about the follow up visit needed which was one visit after one month from the treatment.

Setting:

- Dental units: Adec / knight.
- Operator: department. Masters' degree student in Oral Medicine and Periodontology.

I. Preoperative Evaluation:

Patients eligible for the study were screened by comprehensive periodontal examination and full periodontal charts were obtained. Periapical radiographs were taken for chronic periodontitis patients, at sites of attachment loss for diagnosis of the case.

II. Interventions:

• After proper examination and diagnosis, full mouth supra- and sub-gingival scaling and root planing were performed in all patients over 2 weeks using scalers and Gracey Curettes from Hu-Friedy and all patients received an oral hygiene instruction.

• Two sites in each patient were randomly assigned using random allocation computer software to receive one of the following

treatment modalities as follows:

• Test site: was treated by non-surgical therapy followed by intrapocket application of 5 % melatonin gel using a plastic disposable syringe with plastic flexible tip preloaded to deliver the melatonin inside the pocket.

• Control site: was treated by non-surgical therapy followed by intrapocket application of placebo gel using a plastic disposable syringe with plastic flexible tip

Application of Melatonin

Melatonin oral gel preparation:

• Pure melatonin was purchased from Bulk Supplements (USA) Company and prepared as oral gel 5% by Al Ezaby Pharmacy.

• The used base was 1% carboxymethyl cellulose in distilled water.

• The placebo gel was also prepared by Al Ezaby Pharmacy and consisted of 1% carboxymethyl cellulose in distilled water alone.

III. Outcomes of the trial:

1. Primary outcome:

• **GCF level of total oxidative capacity:** collection of gingival crevicular fluid (GCF) sample: In all patients, GCF samples were collected at the baseline and 4 weeks after treatment from the most periodontally affected site and from subjects with healthy periodontium at baseline only as follows (Griffiths, 2003):

1. At the selected area, all clinically detected supragingival plaque was removed carefully without touching the marginal gingiva to prevent inducing bleeding and minimize contamination of the paper strips by blood.
2. The sites under study were isolated with cotton rolls to prevent contamination with saliva and gently dried with an air syringe.
3. One filter paper strip was used for each collection site. Two sites were selected (controlled site and exposure site) in each participants in different quadrants.
4. Filter paper strips were carefully inserted until resistant is felt into the gingival crevice and left there for 30 seconds.
5. Any filter paper contaminated with blood was discarded.
6. The samples from the individual sites were stored in eppendorff at -80°C for later processing.
7. Filter paper strips for each participant were

pooled, and the GCF was extracted and assayed for measuring TOC level.

• Determination of Total Oxidant Capacity (TOC) in GCF using Enzyme-Linked Immunosorbant Assay (ELISA):

Phosphate buffer saline (100 ul) was added to the paper point and vortexed. Centrifugation was done at 3000 xg. Supernatants were separated for determination of TOC using PerOx (TOS/TOC) Kit provided by Immun Diagnostik, Germany. It is a photometric test system used for the determination of the total oxidative status/capacity (TOS/TOC) in EDTA- plasma, serum and other biological samples.

• Principle of the test:

The determination of the peroxides was performed by the reaction of a peroxidase with peroxides in the sample followed by the conversion of Tetra- methyl Benzidine (TMB) to a colored product. After addition of a stop solution, the samples were measured at 450 nm in a microtiter plate reader. The quantification was performed by the delivered calibrator.

• Preparation of the reagents:

- The reaction buffer mixture: 5 ml reaction buffer A (REABUF A) + 100 µl reaction buffer B (REABUF B) 5 µl enzyme solution (ENZ)The amounts were sufficient for 40 tests.

- Reconstitution of calibrator and controls: The calibrator (CAL) and controls (CTRL1, CTRL2) were reconstituted in 250 µl reconstitution solution (RECSOL). After 5 minutes, homogenization of reconstituted calibrators (CAL) and controls (CTRL1, CTRL2) were done on a vortex mixer.

• Assay procedure:

1. 10 µl of sample, calibrator (CAL), controls (CTRL1, CTRL2) and Blank /Reconstitution solution (RECSOL) were added in duplicates in appropriate wells.

2. 100 µl of reaction buffer A (REABUF A) was added to each well.

3. Measurement 1: The absorption of the samples was read in the ELISA reader at 450 nm.

4. 100 µl of reaction buffer mixture was added to each well.

5. Incubation was done for 15 min at 37°C. 6. 50 µl stop solution (STOP) was added.

7. Measurement 2: was performed immediately after addition of the stop solution (STOP) at 450 nm in the ELISA reader.

• Calculation:

The difference between measurement 1 and 2 was directly proportional to the peroxide content of the sample: For evaluation, the estimated optical density (OD) values of the 1st measurement were subtracted from the OD of the 2nd measurement to obtain the OD values of sample, calibrator, controls and blank. The concentrations of samples and controls (CTRL1, CTRL2) were calculated.

2. Secondary outcomes:

The following clinical parameters were recorded in both exposure and control sites at baseline and again 1 month after treatment.

1. Gingival index (GI): (Loe and Silness, 1963):

The degree of gingival inflammation was assessed by means of GI. It was developed for assessment of the qualitative changes in the gingiva related to the four areas of the tooth which make up the total circumference of the marginal gingiva and has four scored as follows:

0: Normal gingiva.

1: Mild inflammation = slight change in color, slight edema, no bleeding on probing.

2: Moderate inflammation= redness, edema, glazing, bleeding on probing.

3: Severe inflammation = marked redness and edema, ulceration, tendency to spontaneous bleeding.

2. Plaque index (PI): (Silness and Loe, 1964):

The amount of dental plaque was scored according to the plaque index (PI) with scores from 0-3 as follows:

0. No plaque.

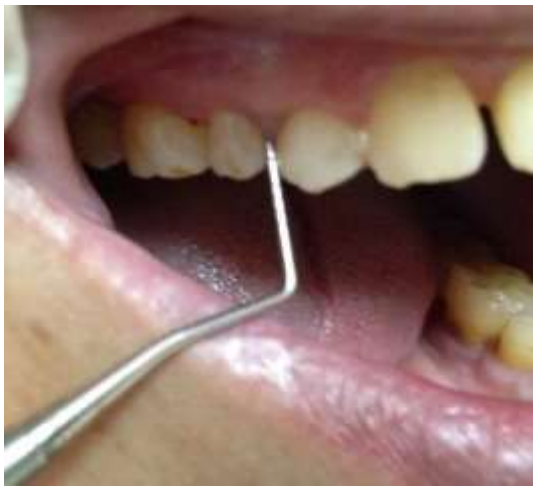
1. A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.

2. Moderate accumulation of soft tissue deposits within the gingival pocket, or the tooth and gingival margin which can be seen with naked eye.

3. Abundance of soft matter within the pocket and /or on the tooth and gingival margin.

3. Probing depth (PD):

PD was measured as the distance from the base of pocket to free gingival margin using 0.5 mm periodontal probe with William's graduations. It was recorded at six location points around the circumference of each tooth. These location points were mesio-buccal, mid buccal, disto-buccal, mesio- lingual, mid-lingual and disto-lingual.



Measuring the pocket depth

4. Clinical attachment level (CAL): (Glavind and Leo, 1967)

CAL was measured as the distance from the base of the pocket to cemento- enamel junction at the same locations of probing depth using the same periodontal probe.

IV. Statistical analysis:

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Pocket depth (PD) and clinical attachment level (CAL) data showed parametric distributionwhile gingival index (GI), plaque index (PI) as well as total oxidative capacity (TOC) data showed non-parametric distribution. Numerical data were presented as mean, median, standard deviation (SD), minimum, maximum and 95% Confidence Interval (95% CI) values. For parametric data; paired t-test was used to compare between exposure and control sides as well as to study the changes after treatment. For non-parametric data; Wilcoxon signed-rank test was used to compare between exposure and control sides as well as to study the changes after treatment. Kruskal- Wallis test was used to compare between TOC of the two sides and healthy subjects. Mann-Whitney U test with Bonferroni's adjustment was used for pair- wise comparisons when Kruskal-Wallis test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Results:

This study was conducted on a total of 30 patients (6 patients did not show up at follow up) ending in 24 patients (17 females and 7 males with age range 32-55) having moderate chronic periodontitis and 15 subject with healthy periodontium (9 females and 6 males with age range 34 48). No adverse effects were reported by the patients who received 5 % of melatonin oral gel or placebo gel.

A. Clinical Parameters:

I. Plaque Index (PI):

1. Changes within each site:

- At baseline, mean and SD values of PI in the test site were 2.0 (±0.7) and reached 0.7 (±0.6) 4 weeks after treatment. While in the control site, mean and SD values of PI were 1.96 (±0.6) and reached 0.7 (±0.6) 4 weeks after treatment.
- In both sites, there was a statistically significant decrease in the mean PI scores 4 weeks after treatment.

2. Comparison between both sites:

- At baseline, mean and SD values for PI in test and control sites were 2.0 (±0.7) and 1.96 (±0.6) respectively.
- 4 weeks after treatment, mean and SD values for PI in test and control sites were 0.7

(±0.6) and 0.7 (±0.6) respectively.

- At baseline as well as 4 weeks after treatment, there was no statistically significant difference between mean PI in both sites .

II. Gingival Index (GI):

1. Changes within each site:

- At baseline, mean and SD values of GI in the test site were 2.5 (±0.5) and reached 0.6 (±0.5) 4 weeks after treatment. While in the control site, mean and SD values of GI at baseline were 2.4 (±0.5) and reached 0.7 (±0.5) weeks after treatment.
- In both sites, there was a statistically significant decrease in the mean GI scores 4 weeks after treatment.

2. Comparison between both sites:

- At baseline, mean and SD values for PI in test and control sites were 2.5 (±0.5) and 2.4 (±0.5) respictively.
- 4 weeks after treatment, mean and SD values for PI in test and control sites were 0.6 (±0.5) and 0.7 (±0.5) respectively.
- At baseline as well as 4 week after treatment, there was no statistically significant difference between mean GI in both sites .

III. Probing depth (PD) :

1.Changes within each site:

- At baseline, mean and SD values of PD (mm) in the test site were 4.3 (±0.8) and reached 2.9 (±0.7) 4 weeks after treatment. While in the control site, mean and SD values of PD mm were 4.0 (±0.6) and reached 3.1 (±0.7) 4 weeks after treatment.
- In both sites, there was a statistically significant reduction in the mean PD (mm) 4 weeks after treatment.

2. Comparison between both sites:

- At baseline, mean and SD values for PD (mm) in test and control sites were 4.3 (±0.8) and 4.0 (±0.6) respectively.
- 4 weeks after treatment, mean and SD values for PD (mm) in test and control sites were 2.9 (±0.7) and 3.1 (±0.7) respectively.
- At baseline**,** test site showed statistically significantly higher mean PD (mm) than control site. However**,** 4 weeks after treatment, there was no statistically significant difference in mean PD (mm) between both sites.

IV. Clinical attachment level (CAL):

1. Changes within each site:

- At baseline, mean and SD values of CAL (mm) mm in the test site were 4.7 (±0.9) and reached 3.5 (±0.6) 4 weeks after treatment. While in the control site, mean and SD values of CAL (mm) were 4.3 (±0.6) and reached 3.7 (±0.6) 4 weeks after treatment.
- In both sites, there was a statistically significant gain in the mean CAL (mm) 4 weeks after treatment.

2. Comparison between both sites:

- At baseline, mean and SD values for CAL (mm) in test and control sites were 4.7 (±0.9) and 4.3 (±0.6) respectively.
- 4 weeks after treatment, mean and SD values for CAL (mm) in test and control sites were 3.5 (±0.6) and 3.7 (±0.6) respectively.
- At baseline**,** test site showed statistically significantly higher mean CAL (mm) than control site. However**,** 4 weeks after treatment, there was no statistically significant difference in mean CAL (mm) between both sites.

B. Total Oxidative Capacity (TOC):

1. Changes within each site:

- At baseline, mean and SD values of TOC µmol/l in the test site were 16.6 (±3.1) µmol/l and reached 6.6 (±2.5) µmol/L 4 weeks after treatment. While in the control site, mean and SD values of TOC µmol/l were 15.4 (±2.6) µmol/l and reached 9.8 (±2.0) µmol/L 4 weeks after treatment.
- In both sites, there was a statistically significant decrease in mean TOC µmol/l 4 weeks after treatment.

2. Comparison between both sites:

- At baseline, mean and SD values for TOC µmol/l in test and control sites were 16.6 (±3.1) and 15.4 (±2.6) respectively.
- 4 weeks after treatment, mean and SD values for TOC µmol/l in test and control sites were 6.6 (±2.5) and 9.8 (±2.0) respectively.
- At baseline, test site showed statistically significantly higher mean TOC µmol/l level than control site. 4 weeks after treatment**,** test site showed statistically significantly lower mean TOC µmol/l level than control site.

3. Comparison in TOC (µmol/l) between test, control sites and subjects with healthy periodontium:

- At baseline, mean and SD values for TOC in test site, control site and subjects with healthy periodontium were 16.6 (±3.1) µmol/l, 15.4 (±2.6) µmol/l and 7.8 (±3.3) µmol/l respectively. Both sites showed statistically significantly higher mean TOC than subjects with healthy periodontium.
- However, 4 weeks after treatment, there was no statistically significant difference between mean TOC in test site 6.6(±2.5) µmol/l and subjects with healthy periodontium 7.8(±3.3) µmol/l. While the control site showed statistically significantly higher mean TOC 9.8(±2.0) µmol/l than subjects with healthy periodontium.

DISCUSSION

Chronic periodontitis is an inflammatory disease of the supporting tissues of the teeth initiated by the plaque biofilm. This led to destruction of the supporting tissues of the teeth; the periodontal ligament, bone, and soft tissues **(Byrne et al., 2009).** While the primary etiological factor of

periodontitis is the bacteria in dental plaque, the majority of periodontal tissue destruction is produced by the abnormal host responses to these microorganism and their products **(Takeuchi et al., 2015)**.

Plaque build-up allows the growth of anaerobic bacteria **(Listgarten. 1986)**, which eventually leads to the recruitment and activation of neutrophils. This further results in the upregulation of pro- inflammatory cytokines and also leads to the release of neutrophilic enzymes and reactive oxygen species (ROS) **(Laine et al., 2012).**

In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidants defense capacity and when that equilibrium shifts in favor of ROS, either by reduction in antioxidant defenses or an increase in ROS production or activity, oxidative stress results. This imbalance between the ROS and antioxidant has been implicated as one of the progressive or pathogenic factors for periodontal tissue destruction **(Waddington et al., 2000).**

To combat the oxidative stress, all the cells in the body are equipped with an intrinsic store of molecules known as "antioxidants." **(Ramesh et al.,2016).** They function by scavenging free radicals (FRs) when they form and thereby preventing oxidative stress **(Ramesh et al., 2016)**.

Numerous studies **(Chapple et al., 1997; Sculley and Langley-Evans,2003; Brock et al., 2004)** have shown that the total antioxidant capacity in periodontitis patients is significantly lower when compared to healthy controls or in subjects who have received periodontal therapy **(Shirzaiy et al., 2014)**. These findings have recommended the use of exogenous antioxidants for the treatment of periodontal disease **(Ramesh et al., 2016)**.

Melatonin is a potent antioxidant and FR scavengers. Melatonin and its secondary and tertiary metabolites are able to neutralize numerous toxic oxygen derivatives. Via this means, one melatonin molecule has the capacity to scavenge up to 10 ROS *vs.* the classic antioxidants including vitamin C, vitamin E, glutathione and NADH that scavenge one or less ROS **(Tan et al., 2015).**

Therefore, the present clinical study aimed to evaluate the effect of topical melatonin gel as an adjunct to non-surgical therapy versus non-surgical therapy combined with placebo gel on the clinical parameters (PI, GI, PD and CAL) and GCF level of total oxidant capacity (TOC) in patients with moderate chronic periodontitis.

This study was conducted on a total of 30 patients (6 patients did not show up at follow up) ending in 24 patients (17 females and 7 males with age range 32-55) having moderate chronic periodontitis and 15 subject with healthy

periodontium (9 females and 6 males with age range 34-48) recruited form the outpatient clinic of Oral diagnosis, Faculty of Oral and Dental Medicine, Oral Medicine and Periodontology Department, Cairo University.

All the patients were selected to be systemically healthy according to modified Cornell Medical Index **(Brightman et al., 1994)**.

Smokers were excluded from the present study as smoking is an exogenous source of ROS **(Greenly, 2004).** Females, who were lactating or pregnant, were also excluded as it was mentioned by **(Sajjan et al., 2013),** that there was a raise in ROS during pregnancy and lactation.

Two sites in each patient were randomly assigned using random allocation computer software to receive one of the following treatment modalities: test site was treated by non-surgical therapy combined with intrapocket application of 5 % melatonin gel and control site was treated by non-surgical therapy combined with topical application of placebo gel.

The non-surgical periodontal treatment has long been considered the gold standard of periodontal therapy achieving an immediate ecological change that favors a facultative anaerobic gingival microflora and depriving the subgingival anaerobic microflora of its anaerobic environment at the base of the pocket **(Suvan. 2005).**

Melatonin has most of the desirable characteristics of good antioxidant and has many positive aspects. It is an endogen and non-toxic when administered in local or systemic forms **(Acuna et al., 2002).** It is a broad spectrum antioxidant which is easily transported across cell membranes and can be regenerated after FR scavenging with its metabolites still have an antioxidant activity and acts also as an immunomodulatory agent **(Galano et al., 2011).**

CONCLUSION:

Both the test and control sites showed significant improvement in clinical parameters and significant reduction in GCF levels of TOC 4 weeks after non- surgical periodontal therapy.

The intrapocket application of 5 % melatonin gel produced more significant reduction in GCF levels of TOC compared to placebo gel 4 weeks after non- surgical periodontal therapy.

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