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Effect of Resveratrol as an Antioxidant in the Treatment of Smokers Patients with Stage III Periodontitis

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Abstract:: **Background:** The present study aimed to evaluate the antioxidant effect of resveratrol gel in the treatment of periodontitis in smokers patients. **Material and Methods**: Fifteen smoker patients with ages ranging from 31–50 years, suffering from stage III periodontitis were included in the study. Using a split-mouth design, sites were randomly allocated into two groups; the Control group (Group I) received only subgingival scaling and root planning (SRP) + placebo gel. Test sites (Group II) received locally delivered resveratrol gel in all sites with probing depth ≥ 5 mm after SRP. Gels application was repeated at 7, 14 and 21 days. Clinical parameters and gingival crevicular fluid samples for evaluation of superoxide dismutase (SOD) enzyme were collected at baseline, 3 and 6 months' evaluation periods. **Results:** Results showed a statistically significant decrease in PI and BI from baseline to 6 months in both groups compared to their baseline value $P<0.05$. Control group showed a statistically significant decrease in PPD and CAL up to 3 months only followed by an increase in their mean scores reaching the baseline value while test group showed significant decrease up to 3 months followed by a slight increase at 6 months but still statistically significant reduction compared to the baseline values $P<0.05$. SOD levels were significantly improved in test sites when compared with control sites. **Conclusion:** The study demonstrated the potential benefits of resveratrol, as an adjunctive treatment to SRP.

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1. Introduction:

Periodontitis is an inflammatory disease of the supporting tissues of the teeth results in the progressive destruction of periodontal tissues. Treatment of periodontitis is challenging and time-consuming procedure especially in smokers because smoking represents a very significant epigenetic risk factor for not only the development of periodontitis but also influences its severity and reduces treatment response.[1]

The negative effects of cigarette smoking on periodontal tissues include immunosuppressive effect on the host, impaired peripheral blood polymorphonuclear leukocyte motility, decreased antibody production, chemotaxis, and phagocytosis, alterations in the subgingival vascular oxygen tension, increased adhesion of bacteria to epithelial cells, reduced proliferation, migration, and attachment of fibroblast to the root surface, and impaired collagen synthesis. It is also known that smoking increases reactive oxygen species (ROS) production and at the same time reduce antioxidant production thereby leading to a magnification of the effects of ROS on tissue.[2,3]

ROS plays a dual role in periodontitis by promoting cell death or blocking apoptosis in infected cells. Oxidative stress is known to cause DNA damage, peroxidation of lipid membranes, and protein inactivation, that is why smoking is considered to be a major risk factor for periodontitis increasing its prevalence and severity[4]. Smoking is not only exacerbate periodontitis but also obtunds treatment effects and jeopardizes the healing process following for this condition.[5]

Antioxidants are groups of substances that prevent the oxidation of substrate by these ROS and offering protection. Currently, there is a great interest in the linkage between antioxidants and periodontal disease. A significant antioxidant enzyme within mammalian tissues is superoxide dismutase.[6] Superoxide dismutase has also been localized within the human periodontal tissues and may represent an important defense mechanism within gingival cells against superoxide release.[7]

The objective of treatment of periodontitis is to prevent progression, recurrence of disease and to regenerate the lost tissues, this can be achieved by various non-surgical and surgical therapies depending

on the specific treatment goal.[8] Resveratrol can be used as a supplemental method for non-surgical treatment of periodontitis due to its anti-inflammatory effects and stimulation to osteoblastic cells. [9]

Resveratrol (3, 4,5-trihydroxystilbene), a pleiotropic molecule, is a polyphenol not flavonoid, an antifungal plant-derived substance that also is present in food like grapes, cranberries, and peanuts. It has several biological properties as improvement of metabolic control of diabetes[10], anti-cancer activity11, antioxidant enzyme activities[12], protection against neural degeneration[13], and prevention of cardiovascular diseases[14]. Additionally, resveratrol may positively interfere with osteoblastogenesis, contributing to new bone formation[15]. Hence, the present studies used resveratrol gel as an adjunct to scaling and root planing (SRP) plus oral hygiene measures in managing moderate to moderate periodontitis (Stage III) in smoker patients.

2. Material and Methods

This study was conducted at the Department of Periodontology, Faculty of dentistry Tanta University. Patients were first briefed about the study and written consent was obtained. The study was performed in compliance with the principles of the Declaration of Helsinki and was conducted from November 2017 to June 2019.

Inclusion criteria:

Fifteen smoker male patients with an age range of 31–50 years and suffering from moderate periodontitis (attachment loss of ≥ 5 mm on at least three teeth), with clear medical history were included in the study. The subject was classified as a current smoker if he or she regularly smoked more than 10 cigarettes/day for a minimum of 5 years. Subjects were with no history of any periodontal treatment 6 months before the study.

Exclusion criteria for patients included pregnancy or lactation, systemic antibiotics or NSAIDs taken within the previous 3 months, systemic illnesses (i.e., diabetes mellitus, diseases or disorders that compromise wound healing).

Preparation of the Resveratrol-Containing Gel

The gel was prepared by dissolving 5 g of sodium carboxymethyl cellulose and 10 g of 85% glycerol in 85 g of deionized water under stirring. Resveratrol was dissolved in deionized water at a concentration of 0.01% weight in volume. Then, 1 g of this aqueous solution was incorporated into 10 g of the vehicle gel under stirring in the absence of light. The vehicle gel and resveratrol containing gel were stored at 4 C.

Clinical protocol

Initial therapy was performed on all patients and consisted of full mouth scaling and root planing on 2 sessions (24 hours), by hand and ultrasonic instrumentation, with oral hygiene instructions reinforcement and proper brushing technique (modified Bass technique) instructions.

Using a split-mouth design, sites were randomly allocated using a coin-flip method into two groups; one is a test group including 15 sites that were thoroughly dried to get rid of blood and debris, then received locally delivered resveratrol gel in all sites with probing depth ≥ 5 mm at this time. The applicator tip was gently advanced to the deepest point of the pocket till resistance is felt and the cartridge content was expelled by gently pressing the plunger till some material overflowed, fig (1c) (Fig 1a). The periodontal dressing was applied after the placement of the drug (Fig 1 d) (Fig 1b). 15 control sites received only subgingival SRP plus placebo gel. Application of resveratrol gel and placebo gel were repeated at 7, 14 and 21 days

Patients were advised to postpone brushing for 12 hours, not eating hard or sticky foods for 1 week and not using interproximal cleaning aids for 10 days. No antibiotics or anti-inflammatory agents were prescribed after treatment.

All patients who were enrolled in the study returned for scheduled maintenance visits every second week during the first 2 months after application and once a month for 4 months. There were no inflammatory reactions observed following the application of the gel.

The following clinical parameters at baseline, 3 and 6 months, using a color-coded periodontal probe (PQWBR - Hu Friedy Mfg. Inc. Chicago, IL, USA): Plaque Index (PI)16, Bleeding index (BI) 17, Probing Pocket Depth (PPD)[18] (Fig 1a,e, & f), Clinical Attachment Level (CAL[18]);

Sites for GCF sample collection were selected based on the sites showing the greatest amount of attachment loss. The area was isolated with cotton rolls with attention to eliminating salivary contamination, and the site gently air-dried. The samples were collected by paper point size 30 using Brill's, 1962[19] intracellular techniques. The paper points were inserted into the pockets until a slight resistance was felt and held in the sulci for 30 s with delicate care to avoid irritation of pocket/sulcus epithelium (fig 1 b). Any paper contaminated with blood was discarded and the collection was repeated at another point. The GCF paper points were pooled in:1 mL phosphate buffer solution and eluted for 30 min and samples were immediately stored at –20 °C until superoxide dismutase analysis.

3. Results

All participants completed the study without any recorded side effects. The study population of 30 sites

in 15 males Patients was between the ages of 30 and 50 years. Fifteen of the enrolled patients were assigned to the SRP plus resveratrol group, while the others were in the SRP plus placebo control group. There were no significant differences found between the 2 groups concerning age, (table 1).

Table 1: Mean± SD of age among the study groups

Variable	Group I (Control group) mean± SD	Group II (Test group) mean± SD	P- value
Age	41.46± 6.16	42.25 ± 5.49	0.852 ^{ns}

The distribution of mean and standard deviation values of all the clinical and biochemical parameters of both groups was illustrated in tables (2,3,4, and 5). Baseline values showed no significant differences between the two groups for all the studied parameters. After applying the student's Paired t-test, results showed a statistically significant decrease in PI and BI scores from baseline to 6 months in both groups as compared to their baseline value P<0.05. While PPD and CAL in the control group showed statistically significant decrease up to 3 months only followed by an increase in their mean scores reaching the baseline value while in group I there is significant decrease up to 3 months followed by a slight increase at 6 months but still statistically significant reduction as compared to the baseline values P<0.05.

In control sites, the mean value was 2.20± 0.41 whereas at 3 and 6 months were 0.80± 0.41and 1.20± 0.56 respectively. There was a significant reduction in PI scores at both 3 and 6 months' interval. The mean PI for test group at baseline was 2.40 ± 0.50 in resveratrol gel treated sites whereas the mean value at 3 and 6 months were 0.66 ± 0.48 and 1.06 ± 0.59 respectively. However, between the groups, the difference was not statistically significant at any period (P>0.05) (Table 2).

Table 2: Shows the effect of the treatment modalities on the PI score at the study evaluation periods.

Groups Time	Group I (Control group) (n= 15) mean±SD		Group II (Test group) (n=15) mean±SD		P
Baseline	2.20± 0.41		2.4±0.50		T= 1.183 P=0.24
3months	0.80± 0.41	t=10.69 P=0.000***	0.66 ±0.48	t=11.30 P= 0.000***	T=-0.80 P =0.42
6 months	1.20± 0.56	t=5.91 P=0.000***	1.06±0.59	t= 5.73 P=0.000***	T=-0.63 P =0.53

There was a significant reduction in overall mean bleeding index scores in both groups from baseline (2.20±0.41) in resveratrol gel treated sites and (2.43±0.49) in control sites, to three months (0.66±0.48) in resveratrol gel treated sites and (1.13±0.51) in control sites. This improvement was maintained until the end of the study, with (0.73± 0.70) in resveratrol gel treated sites and (1.63±0.54) in control sites. Between the groups, the difference was statistically significant at 3and 6 months' evaluation periods (P> 0.05) (Table 3).

Table 4 shows the clinical probing depth parameters of the resveratrol gel treated sites and control sites at different time intervals. At baseline the probing depths mean value of 6.80±0.77 for in resveratrol gel treated sites and 6.66± 0.72 for control sites. There was no statistical difference between the two sites at baseline (P>0.05). At 3 months both sites showed significant improvement in probing depths over baseline. For in resveratrol gel treated sites, probing depths mean value of 4.53± 0.51 mm. For control, sites mean value of 5.73±0.18 mm. There was a statistically significant difference between the two sites (P < 0.05). At 6 months the probing depths mean value of 5.33 ±0.81 mm in resveratrol gel treated sites. For control sites, a mean PPD was 6.46±0.88 mm. There was a statistically significant difference when the two groups were compared (P < 0.05).

Concerning the clinical attachment levels at baseline, both in resveratrol gel treated sites and control sites with mean values of 6.40 ±0.63 mm for in resveratrol gel treated sites and 6.00±0.53 mm; in control sites, there was no statistical difference between the two sites at baseline (P>0.05). At 3 months, both sites showed significant improvement of clinical attachment levels over baseline measurement with the mean value for in resveratrol gel treated sites of 4.53±0.35 mm. For control sites, the mean value was 5.06±0.59 mm. There was a statistically significant difference between the two groups (P< 0.05). At 6 months the mean CAL value of 4.66±0.25 mm in resveratrol gel treated sites and 6. 6 ±0.48 mm

for control sites; there was a statistically significant difference when both groups were compared at 3, and 6 months' evolution periods (P< 0.05) table 5.

Regarding the effect of the treatment modalities on the GCF level of SOD, results are summarized in Table 6 as follows: In the control group, at baseline, the SOD level was 99.80 ± 0.47. At 3 months after treatment SOD level improved to 102.4 ± 3.32 which is statistically significant as compared to baseline value P=0.004 while at 6 months the SOD level was (99.87 ± 0.46) which is statistically insignificant as compared to baseline P>0.05. In the test group, the SOD level at baseline was 99.91 ± 0.63 and 3 and 6 months after treatment SOD level improved to 114.9 ± 4.92, 106.04±5.33) which are statistically significant as compared to baseline value p =0.000,0.001 respectively as shown in table 6. Comparison of SOD levels postoperatively in both groups showed that there are statistically significant differences at 3 and 6 months' period with a P =0.000, 0.000 respectively.

Table3: Effect of treatment modalities on the BI score at the study evaluation periods

Groups Time	**Group I (Control group) (n= 15) mean±SD**		**Group II (Test group) (n=15) mean±SD**		P
Baseline	2.43± 0.49		2.20±0.41		T= 1.40 P=0.172
3 months	1.13± 0.51	t=11.06 P= 0.000***	0.66±0.48	t=9.28 P= 0.000***	T=2.54 P =0.017**
6 months	1.63± 0.54	t=3.88 P=0.02**	0.73±0.70	t= 6.20 P=0.000***	T=3.90 P =0.001***

Table 4: The effect of different treatment modalities on the PPD score at the study evaluation periods

Groups Time	**Group I (Control group) (n= 15) mean±SD**		**Group II (Test group) (n=15) mean±SD**		P
Baseline	6.66± 0.72		6.80±0.77		T= -0,48 P=0.63
3 months	5.73± 0.181	t=7.89 P =0.000***	4.53±0.51	t=14.78 P= 0.000***	T=5.32 P =0.000***
6 months	6.46± 0.88	t=1.14 P=0.27	5.33±0.81	t= 4.03 P=0.001***	T=4.23 P =0.000***

Table 5: The effect of the different treatment modalities on the CAL at the study evaluation period

Groups Time	**Group I (Control group) (n= 15) mean±SD**		**Group II (Test group) (n=15) mean±SD**		P
Baseline	6.00± 0.53		6.40±0.63		T= -1.87 P=0.07
3 months	5.06± 0.59	t=6.08 P=0.000***	4.53 ±0.35	t=11.29 P= 0.000***	T=2.62 P =0.014**
6 months	6.66± 0.48	t=-1.58 P=0.136	4.66±0.25	t= 11.30 P=0.001***	T=5.61 P =0.000***

Table 6: The effect of different treatment modalities on the GCF level of SOD enzyme at the study evaluation periods

Groups Time	**Group I (Control group) (n= 15) mean±SD**		**Group II (Test group) (n=15) mean±SD**		P
Baseline SOD	99.8±0.47		99.91±0.63		T= -0.130 P=0.89
3months SOD	102.4±3.32	t= -3.41 P=0.004**	114.9 ±4.92	t=- 11.54 P= 0.000***	T=- 7.92 P =0.000***
6 months SOD	99.87± 0.46	t= 0.149 P=0.88	106.04±5.33	t= -4.46 P=0.001**	T=- 4.46 P =0.000***

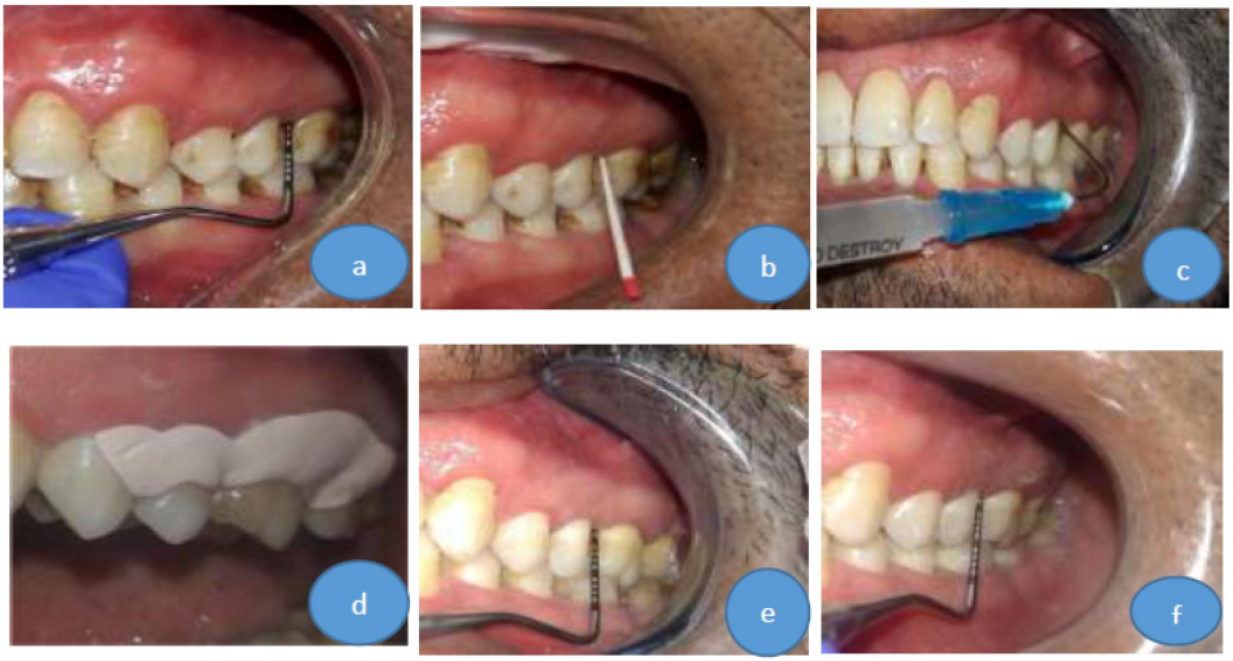

Fig (1) a- shows patient PPD before treatment. b- sampling of GCF with paper point, c- shows a-subgingival application of resveratrol gel. d- periodontal pack inplace to protect the gel, e & f- show follow up of PPD in patient treated with resveratrol gel at 3 and 6 months respectively**.**

4. Discussion:

Smoking is known as a factor that may negatively affect oral health and patient smoking status has a bad impact on periodontal treatment efficacy as cigarette smoke contains free radicals that induce oxidative stress.[20]

Oxidative stress has been linked with both the onset of periodontal tissue destruction and systemic inflammation[21]. The antioxidant disturbance in smokers may be further enhanced by lower intake of both supplemental and dietary antioxidants.[22]

Many studies showed that SOD (one of the antioxidant enzymes that synthesized by the body and protects within the cell against ROS) level was found to be decreased in smokers than nonsmokers which might be due to the inactivation by hydrogen peroxide,[23,24] this oxidative stress induced by smoking was reflected by the reduced GCF and salivary SOD concentrations in smokers.

Lately, (2011)[25]resveratrol is natural compounds capable of moderating host inflammatory responses have received extensive attention because of its antioxidative and anti-inflammatory properties. Resveratrol can induce activation of antioxidant enzymes and activate the nuclear factor E2–related factor (NRF2) antioxidant defense pathway.[26]

Hence the present study was conducted to evaluate the antioxidant effect of resveratrol gel as an adjunct to scaling and root planing (SRP) plus oral hygiene measures in managing moderate to moderate periodontitis (Stage III) in smoker patients. Additionally, its effect on the SOD level in the GCF.

The results of the present study showed a statistically significant decrease in PI and BI scores from baseline to 6 months in both groups as compared to their baseline value $P<0.05$. Moreover, the group I (control group) showed a statistically significant decrease in PPD and CAL up to 3 months only followed by an increase in their mean scores reaching the baseline value while in group II there is significant decrease up to 3 months followed by a slight increase at 6 months but still statistically significant reduction as compared to the baseline.

The improvement in the selected clinical parameters in group II may be related to the anti-inflammatory effect of resveratrol that inhibits the expression of proinflammatory cytokines, such as interleukin 1 (IL-1) and TNF-α which are involved in the pathogenesis of periodontitis.[15]

In the present study the GCF was collected by paper points although the majority of the studies used paper strips which were considered to be more efficient in GCF collection because they could be inserted easily into gingival sulcus or periodontal pockets, as well as for their ability to absorb fluids[27]. However, few studies used paper points (size 30) to collect GCF samples although it was shown that paper points and paper strips had different absorption rates[28].

Additionally, Correa et al.,[29] showed that resveratrol administered alone led to reduced loss of

alveolar bone in experimental periodontitis when compared with placebo. Moreover, an in vitro study[30], using human periodontal ligament cells stimulated with lipopolysaccharide of Porphyromonas gingivalis, showed that treatment with resveratrol reduced the production of proinflammatory cytokines and nitric oxide. Moreover, using a Porphyromonas gingivalis-ligature-induced periodontitis model in diabetic mice. It has been shown that resveratrol caused decreases in alveolar bone loss and also reductions in the levels of IL-1b, IL-6, IL-8, TNF-a and toll-like receptor compared with the control.[31]

Additionally, Correa et al.,[32] investigated the effect of systemic resveratrol on oxidative stress in surgically induced periodontitis in rats exposed to cigarette smoke inhalation. They found that resveratrol is efficient in reducing the exacerbation of bone loss in experimental animals and this could be mediated by its antioxidant and anti-inflammatory properties that attenuate the effect of oxidative stress induced by smoking.

In the present study, SOD level was significantly improved in test sites when compared with control sites. It is suggested that the beneficial effects of resveratrol are related to the antioxidant properties of resveratrol. It was described as a scavenger of superoxide radicals, hydroxyl radicals, and peroxynitrite.[33]

Recently, a human clinical trial reported by Zare Javid et al[34]. suggested that resveratrol supplement may be beneficial as adjuvant therapy to nonsurgical periodontal treatment in insulin resistance and improving the periodontal status in patients with diabetes and periodontal disease. Hence, using resveratrol as an adjunctive to SRP may help control the periodontal status of smoker's patients especially smokers patients are not good responders to surgery.

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