Original Article

The Impact of *Nigella sativa* Oil on the Salivary Levels of Interleukin-17, and Periodontal Status among Adult Diabetic Patients

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Abstract

Background: Diabetes and periodontitis are distinct disorders. Several inflammatory diseases have been treated with extracts derived from *Nigella sativa* seeds. **Objective:** To investigate the effects of *Nigella sativa* oil on salivary interleukin-17 levels and clinical periodontal parameters in type 2 diabetic patients with periodontitis after scaling and root planning. **Materials and Methods:** A double-blind, randomized clinical trial was carried out. A confirmed diagnosis of periodontitis stage III, grade B was made in a total of 44 male Iraqi patients between the age of 55 and 65 participated; 30 of them had type 2 diabetes, and 14 were non-diabetes. The baseline visit included taking unstimulated salivary samples, assessing periodontal indices plaque (PLI), bleeding on probing (BOP), periodontal pocket depth (PPD), and clinical attachment level (CAL), injecting Nigella sativa oil or a placebo material into the pocket twice-weekly for two weeks following scaling and root planing. The collections of unstimulated saliva and periodontal indices (PLI, BOP, PPD, and CAL) were measured again after 2 months. The collection of salivary samples for interleukin-17 assay was done. For diabetic patients, baseline measures of glycated hemoglobin (HbA1c) and fasting blood glucose were repeated 3 months later. **Results:** After 2 months of treatment, the average values of PLI in the non-diabetic group represent a highly significant difference $P = 0.000$. PLI and CAL show statistically a significant difference in the diabetic group $P = 0.040$, and $P = 0.048$, respectively, whereas the average values of (BOP, PPD) show a highly significant difference in the diabetic group, $P = 0.000$. Although the concentration of IL-17 decreased in the diabetic and non-diabetic Nigella sativa group, there was no significant difference among groups. **Conclusion:** Nigella sativa oil can reduce salivary levels of IL-17 and, improve periodontal health state when used in conjunction with root planing following scaling.

Keywords: Clinical parameters, interleukin-17, Nigella sativa oil, periodontitis, type 2 diabetes mellitus

Introduction

The tooth's supporting structures are destroyed by the chronic inflammatory condition known as periodontitis. Periodontal disease's onset and progression depend on complicated interactions between immune cells and periodontal pathogens.[1]

In addition to being the most common chronic disease and a difficult global health issue with an active inflammatory state type 2 diabetes, however, the exact etiology of the disease is unknown, it is generally accepted that dietary factors, psychological stress, obesity, lack of physical exercise, genetic predisposition, immunological factors, and oxidative stress have all played a role in the development of the condition.[2]

Type 2 diabetes and periodontitis are linked in a "two-way" manner, the risk of periodontitis may double or treble in people with type 2 diabetes, according to the

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epidemiological research.[3] Periodontitis is more common in diabetic patients, as well as lower glycemic control in those who have untreated periodontitis. People who have poor glycemic control are at risk for both short-term and long-term problems.[4,5] Potential molecular connections between diabetic mellitus (DM) and periodontitis include changes in polymorphonuclear cell activity, increased adipokine secretion, and altered apoptosis. Both patients with periodontitis and those with DM may produce more inflammatory cytokines as a result of these modifications. Recent studies have identified inflammation as a major contributor to the pathogenesis of DM. Clinical studies have linked increased levels of a number of inflammatory cytokines, such as, interleukin-1, tumor necrosis factor, interleukin-6, and interleukin-18, with a variety of diabetes complications.[6]

Many components of saliva contribute significantly to the preservation of dental homeostasis. Additionally, many people agree that saliva is a good indicator of how well the body is doing.[7] As a result, salivary molecules may be helpful for detecting, monitoring, and treating both systemic and local illnesses.[8] It has been demonstrated that a number of salivary biomarkers are connected to both systemic and oral illnesses.[9]

Interleukin-17 is recognized more frequently in diabetes patients than in non-diabetic individuals, as well as in periodontitis patients compared to gingivitis patients.[10] Many theories suggest that interleukin-17 has a role in the etiology of periodontitis. First, it can activate the osteoclastogenesis-promoting receptor activator of nuclear factor kap-B (RANK)-RANK ligand (RANKL) signaling pathway. Second, it functions as a regulatory cytokine that encourages the production of more inflammatory cytokines from macrophages, epithelial cells, and fibroblastic cells, such as interleukin-6, interleukin-8, and interleukin-1, resulting in inflammatory reactions. Finally, it helps control the synthesis of some matrix metalloproteinases, which may damage periodontal tissue.[11]

Nigella sativa, sometimes known as "Kalonji," is a fragrant plant with strong anti-inflammatory properties.[12] Locally, oro-dental problems have also been treated with Nigella sativa oil. Its active component, thymoquinone, has also been studied for its potential use in periodontal therapy. Current chemotherapeutic agents are highly successful in treating periodontal disease, but they come with a number of undesirable side effects, such as taste changes, tooth and tongue discoloration, antimicrobial resistance, and greater costs for these substances.[13] Consequently, the use of natural and herbal remedies for treating periodontitis, such as Nigella sativa and thymoquinone, has recently attracted more attention and may have significant advantages, especially for individuals with lower socio-economic level worldwide. The lack of prior research on the anti-inflammatory benefits of *N. sativa* oil on type 2 diabetics with periodontitis led to the conduct of this study.

Materials and Methods

A randomized clinical interventional study was conducted. The participants were assigned into four groups (two diabetic and two non-diabetic); 44 Iraqi men, aged 55-65 years old, with a confirmed diagnosis of periodontitis stage III, grade B, 30 of them with type 2 diabetes, fasting blood glucose (FBG) was more than 126 mg, HbA1c 7-9, and 14 of them were a non- diabetic. Excluded from the study were participants with a history of smoking, systemic diseases, female gender, drug use within previous fifteen days, and periodontal treatment within previous 6 months. Diabetic patients were examined in the Diabetic Center at Imam Al-Hussein Medical Hospital in Karbala, while 14 non-diabetic patients were examined in the Department of Periodontology at Al-Hour Al-Riahy Dental Special Center. *N. sativa* oil extract or placebo bottles were encoded (one A and the other B) by an external dentist who was not involved in the study for purposes of concealment.

Evaluation of fasting blood glucose and HbA1c

Individuals with type 2 diabetes were picked from the diabetic center at Imam Al-Hussein Medical Hospital, submitted to the laboratory for testing of FBG and HbA1c, and the procedure was repeated 3 months later.

Saliva collection

Morning salivary samples were collected from each patient 9-11 a.m., at the baseline day and 2 months later. The patients were asked to refrain from eating, drinking, and performing teeth brushing one hour before saliva collection. In order to avoid blood contamination, this procedure was performed before any other clinical procedure. The collected samples of saliva were centrifuged for 5 min at 13,000 rpm and 4°C. The supernatant was subsequently transferred to a new eppendorf tube and labeled appropriately, and all samples were stored at -80°C until analysis.

Periodontal examination

The following indices were used to assess periodontal health: plaque index (PLI), according to the O'Leary method, this index takes into consideration the presence of supragingival plaque on all four tooth surfaces (buccal, lingual, mesial, and distal); bleeding on probing (BOP); probing pocket depth (PPD), the distance (in mm) from the gingival edge to the bottom of the pocket; and clinical attachment level (CAL), the distance in millimeters from the cement-enamel junction to the bottom of the pocket. These indices (PPD and CAL) were recorded at six surfaces; Buccal, lingual, mesio- buccal, mesio-lingual, disto- buccal, and distao- lingual sites were considered.

Scaling and root planing

A universal curette and an ultrasonic instrument were used in non-surgical periodontal care. Study subjects received oral hygiene instructions for at-home care methods. The researcher carried out a clinical assessment and periodontal therapy.

Injection of Nigella sativa oil extract/placebo materials inside the pockets

Inside the pocket, Nigella sativa oil extract or liquid paraffin were applied twice per week for two weeks. It was injected into the pocket with a blunted, 25-gauge needle bent at about 130 degrees along its shank and held in a disposable 5 mL syringe. The needle was inserted at the base of the pocket for the first and second applications, but for the third and fourth applications, the oil was applied at the entrance of the pocket to prevent slowing down the healing process.

Biochemical testing

Interleukin-17 level in saliva was measured using cytokine ELISA kits (BT LAB, Shanghai, China) according to the manufacturer's instructions at 450 nm using an ELISA plate reader device (Awareness, Statfax-2100 model, USA).

Statistical study

Utilizing Statistical Package for the Social Sciences, statistical analysis was performed (SPSS version 22, Chicago, Illinois).

Analysis descriptive

Minimum, maximum, range, mean, and standard deviation for quantitative variables.

Analysis inferential

- Independent sample *t* test: a parametric examination of the distinction between two groups.
- Paired *t* test: examines the difference between two related points (two measurements by a single subject or two subjects for a single measurement).

Ethical approval

The study was carried out according to with the ethical standards found in the Helsinki Declaration. Before a sample was taken, it was done with the patient's verbal and analytical approval. A regional ethics committee examined and approved the study protocol, subject information, and permission form on April 4, 2021 based on the document number (324321).

Results

All of the 55-65 year-old participants in this randomized clinical trial were enrolled in this study.

Table 1 depicts the age distribution of the diabetic and non-diabetic patient populations. Statistically these groups did not differ significantly.

Table 2 shows the mean values and standard deviation of the plaque index at the baseline visit and 2 months later. After 2 months of treatment, the mean values and standard deviation of the plaque index reduced in both diabetes and non-diabetic (control and NSO) groups.

Statistically there was a highly significant difference after 2 months of treatment, in the non-diabetic group $P = 000$, beside that there was a significant difference after 2 months of treatment, in the diabetic group $P = 0.040$. The effect size in the non-diabetic group was more than in a group with diabetes. Statistically there was no significant difference between a diabetic and a non-diabetic group.

The mean and a standard deviation of bleeding on probing at the baseline and after treatment for non-diabetic and diabetic groups are shown in Table 3.

In the group without diabetes, although the average value of bleeding on probing decreased from 63.186 to 46.471 in the control group and from 82.114 to 32.857 in the NSO group at $P = 0.209$. There was no statistically significant difference between the control and NSO groups before and after treatment.

The average value of bleeding on probing decreased from 83.733 to 59.867 in the diabetic control group, and from 66.460 to 39.573 in the diabetic NSO group. Statistically a highly significant difference between the diabetic (control and NSO) groups following treatment at $P = 0.000$.

After treatment with NSO, the effect size was greater in the non-diabetic group than in the diabetic group, but there was no difference between the two groups pre- and post-treatment.

Table 1: Distribution of patients according to age (in years) among groups

Groups	Mean	±SD	Minimum	Maximum	*F*	*P* value
DM + Placebo	58.933	3.494	55.000	65.000	0.775	0.515 NS
DM + NSO	57.733	3.283	55.000	65.000		
NDM + Placebo	58.286	3.904	55.000	65.000		
NDM + NSO	56.571	3.735	55.000	65.000		

SD: mean standard deviation, NS: not significant, DM: diabetes mellitus, NDM: non-diabetes mellitus, NSO: Nigella sativa oil

Table 2: The mean and SD of plaque index among a diabetic and the non-diabetic pre- and post-treatment

		Treatment						*t*-test	*P*-value
		Control			NSO				
		Range	Mean	±SD	Range	Mean	±SD		
NDM	Baseline	10.000	97.429	4.429	19.000	92.857	8.295	1.286	0.223
	After	16.000	75.571	6.321	20.000	52.571	6.268	6.836	**0.000**
Paired *t*-test			10.651			23.128			
P-value			**0.00004**			**0.00000**			
ES			4.026			8.742			
DM	Baseline	25.000	95.533	9.296	20.000	96.000	7.368	0.152	0.880
	After	40.000	64.400	17.381	30.000	53.667	8.338	2.156	**0.040**
Paired *t*-test			7.681			23.298			
P-value			**0.00000**			**0.00000**			
ES			1.983			6.016			

Difference between NDM and DM

		t-test	*P*-value
Baseline	Control	0.648	0.525
	NSO	0.857	0.410
After	Control	2.197	0.40
	NSO	0.342	0.737

SD: mean standard deviation, DM: diabetes mellitus, NDM: non-diabetes mellitus, NSO: Nigella sativa oil

Table 3: The mean and standard deviation of BOP among diabetic and non-diabetic groups before and after treatment

Groups		Treatment						*t* test	*P* value
		Control			NSO				
		Range	Mean	±SD	Range	Mean	±SD		
NDM	Baseline	66.700	63.186	27.829	39.200	82.114	15.766	1.566	0.143
	After	46.700	46.471	17.728	60.000	32.857	20.525	1.328	0.209
Paired *t* test			4.205			6.780			
P value			**0.006**			**0.001**			
ES			1.590			2.563			
DM	Baseline	35.000	83.733	13.403	68.700	66.460	17.782	3.004	0.051
	After	36.000	59.867	13.228	35.000	39.573	8.603	4.981	**0.000**
Paired *t* test			12.734			7.657			
P value			**0.000**			**0.000**			
ES			3.288			1.977			

Difference between NDM and DM

		t test	*P* value
Baseline	Control	1.856	0.104
	NSO	1.988	0.061
After	Control	1.988	0.061
	NSO	1.099	0.285

SD: mean standard deviation, DM: diabetes mellitus, NDM: non-diabetes mellitus, NSO: Nigella sativa oil, ES: effect size

Table 4 displays the mean value and a standard deviation of PPD at the baseline visit and after treatment for a non-diabetic and diabetic groups.

The average value of probing pocket depth decreased from 4.586 ± 0.406 into 4.243 ± 0.351 in the control non-diabetic group and from 4.551 ± 0.430 into 3.800 ± 0.443 in the NSO group. Statistically there was no significant difference between the control and NSO groups before and after treatment.

The average value of probing pocket depth decreased from 4.997 ± 0.407 into 4.473 ± 0.403 in the diabetic control group, and from 5.033 ± 0.447 into 3.933 ± 0.346 in the diabetic NSO group. Statistically there was a highly significant difference between the diabetic control and diabetic NSO groups following treatment at $P = 0.000$.

After treatment with NSO, the effect size was greater in non-diabetic group than in the diabetic group, but there was no difference between the two groups before and after treatment.

Table 4: The mean and standard deviation of probing pocket depth (PPD) among diabetic and anon-diabetic groups before and after treatment

Groups		Treatment						*t* test	*P* value
		Control			NSO				
		Range	Mean	±SD	Range	Mean	±SD		
NDM	Baseline	1.000	4.586	0.406	1.300	4.551	0.430	0.153	0.881
	After	1.000	4.243	0.351	1.400	3.800	0.443	2.073	0.060
Paired *t* test			3.361			4.662			
P value			**0.015**			**0.003**			
ES			1.270			1.762			
DM	Baseline	1.350	4.997	0.407	1.800	5.033	0.447	0.235	0.816
	After	1.200	4.473	0.403	1.600	3.933	0.346	3.941	**0.000**
Paired *t* test			11.377			16.619			
P value			**0.000**			**0.000**			
ES			2.938			4.291			

Difference between NDM and DM

		t test	*P* value
Baseline	Control	1.750	0.089
	NSO	1.819	0.145
After	Control	1.299	0.209
	NSO	0.771	0.450

SD: mean standard deviation, DM: diabetes mellitus, NDM: non-diabetes mellitus, NSO: Nigella sativa oil, ES: effect size

Table 5: The mean and standard deviation of clinical attachment level among diabetic and a non-diabetic groups before and after treatment

Groups		Treatment						*t* test	*P* value
		Control			NSO				
		Range	Mean	±SD	Range	Mean	±SD		
NDM	Baseline	6.100	4.029	2.770	2.100	5.471	0.725	1.333	0.207
	After	5.700	3.786	2.600	1.200	4.729	0.482	.943	0.364
Paired *t* test			3.545			5.392			
P value			**0.012**			**0.002**			
ES			1.340			2.038			
DM	Baseline	2.300	6.210	0.792	3.700	6.127	1.005	0.2520	0.803
	After	2.700	5.573	0.829	3.300	4.920	0.903	2.064	**0.048**
Paired *t* test			3.988			14.525			
P value			**0.001**			**0.000**			
ES			1.030			3.750			

Difference between NDM and DM

		t test	*P* value
Baseline	Control	2.045	0.083
	NSO	1.539	0.139
After	Control	1.778	0.121
	NSO	0.523	0.607

SD: mean standard deviation, DM: diabetes mellitus, NDM: non-diabetes mellitus, NSO: Nigella sativa oil, ES: effect size

Table 5 displays the mean and SD of CAL at the initial visit and after treatment for both the non-diabetic and diabetes groups.

In the non-diabetic group, average value of CAL decreased from 4.029±2.770 to 3.786±2.600 in the control group and from 5.471±0.725 to 4.729±0.482 in the Nigella sativa group. Statistically there was no significant difference between control and NSO groups before and after treatment.

The average value of CAL decreased from 6.210±0.792 to 5.573±0.829 in the diabetic control group, from 6.127±1.005 to 4.920±0.903 in the diabetic NS group. Statistically there was a significant difference between

Table 6: The mean and standard deviation of IL-17 among diabetic and non-diabetic groups before and after treatment

Groups		Treatment						*t* test	*P* value
		Control			NSO				
		Range	Mean	±SD	Range	Mean	±SD		
NDM	Baseline	52.510	83.611	20.742	217.321	141.233	77.733	1.895	0.101
	After	32.103	76.739	9.811	158.557	113.275	51.991	1.827	0.114
Paired *t* test			0.950			0.932			
P value			0.379			0.387			
ES			0.359			0.352			
DM	Baseline	151.278	95.794	37.491	193.247	147.260	63.654	1.876	0.053
	After	75.564	99.488	24.534	173.670	114.667	49.715	1.060	0.301
Paired *t* test			0.562			2.999			
P value			0.583			0.010			
ES			0.145			0.774			

SD: mean standard deviation, DM: diabetes mellitus, NDM: non-diabetes mellitus, NSO: Nigella sativa oil, ES: effect size

diabetic control and diabetic NSO groups following treatment at $P = 0.048$.

After treatment with NSO, the effect size was more in the group with diabetes than in the group without diabetes, but there was no difference between the two groups pre and post-treatment.

Table 6 represents the mean value and SD of the concentration level of interleukin-17 at the baseline and after treatment. The mean and SD of interleukin-17 in the non-diabetic control group decreased from 83.611 ± 20.742 to 76.739 ± 9.811 and from 141.233 ± 77.733 to 113.275 ± 51.99 in the non-diabetic NSO group after treatment. Statistically, there was no significant difference between the two groups. The mean and standard deviation decreased from 147.260 ± 63.654 to 114.667 ± 49.715 in the diabetic NSO and in the diabetes NSO group at baseline, and 99.488 ± 24.534 in the diabetes control group and in the diabetic NSO group after treatment. Despite a decrease in IL-17 levels following treatment, statistically there was no significant difference between the control and NSO groups.

Discussion

Evaluation of clinical indices (BOP, CAL, PPD, and PLI) is an essential component in periodontitis diagnosis. Cytokines are considered a key component to the pathogenesis of any inflammatory diseases and play an important role in the initiation, progression, and alternation in host modulation of any inflammatory disease including periodontal inflammation.[14] The re-evaluation should occur after about 6 weeks of scaling and root planing, because during this time, both epithelial and connective tissue healing occurred.[15] The gradual reductions in inflammatory cell infiltration, crevicular fluid flow, and repair of connective tissue resulted in decreased clinical signs of inflammation.[16]

A small number of therapeutic studies have previously used nigella sativa. The main active ingredient in NS oil is thought to be thymoquinone. From 18.4% to 24% of *N. sativa* volatile oil is made up of it.[17] Investigations on the pharmacological properties of NS seed extracts reveal a wide range of activities, including antioxidant,[18] anti-inflammatory,[19] and antimicrobial[20] properties.

Clinical periodontal parameter

In this study, statistically significant reduction in the mean of (PLI, PPD, BOP, and CAL) in all groups diabetic and non-diabetic (NS and control) was observed. Statistically significant difference was shown in the mean of PI among non-diabetic and diabetic groups after 2 months of treatment, because scaling and root planing aim to significantly reduce the pathogenicity of dental biofilm, and this may indicate good oral hygiene instructions and motivation as well as an appropriate maintaining of oral hygiene over the period of the study time.[21] A statistically significant difference in non-diabetic and the diabetic Nigella sativa groups might be related to the antimicrobial activity of the Nigella sativa oil against Gram-positive, Gram-negative bacteria, different studies showed that reduction of dental plaque due to the antimicrobial effect, Al-Timimi and AL-Casey[22]; Abed *et al*.[23] found that the effect of different herbs on the reduction of dental plaque.

A statistically significant decrease was observed in the mean of (BOP, PPD, and CAL) in all groups after 2 months of treatment, and this might be due to the fact that scaling and root planing facilitated the re-attachment of periodontal fibers and resolution of the inflammation.[24] Diabetic group treated with Nigella sativa oil exhibited a significant decrease after 2 months of treatment.

In the present investigation, the effects of the treatments were observed in all groups; however, the reduction in PPD observed in the SRP plus Nigella sativa oil (non-diabetic and diabetic) groups was greater than that was observed in the other control groups. The current study is consistent with Kapil *et al*.[25]; Hassan and Ghafoori.[26]

Al-Bayaty *et al*.[27] found that Nigella sativa chip reduced the mean of PPD by about 1.33 mm, so results are also in agreement with studies performed[28-30] using periodontal chips containing chlorhexidine as local delivery agents in the management of chronic periodontitis.

Ashouri Moghaddam *et al*.[31] discovered that SRP combined with aloe Vera as adjunctive therapy resulted in a significant reduction of GI and PD clinical criteria.

In the present investigation, the use of Nigella sativa oil led to a significant increase in CALs compared to the control group. This could be the result of the combined anti-inflammatory and antibacterial effect of thymoquinone.[32]

Santos *et al*.,[33] and Mirinc *et al*.[34] reported that improvement of periodontal parameter in diabetic and non-diabetic groups after 3 months of non-surgical periodontal treatment.

Biomarker

Human periodontitis is associated with higher levels of locally produced IL-17 compared to healthy periodontal tissue, according to numerous studies.[35-37]

In 2019, Parhi and colleagues discovered that the concentration of interleukin-17 was higher in diabetic patients compared to non-diabetic patients, which is consistent with the present findings.

In the present study, the concentration of IL-17 in all groups decreased after 2 months of treatment, except in the diabetic control group, where the concentration of IL-17 increased, this mean that scaling and root planing has been not affected in this group. The diabetic NS group exhibited a statistically significant change after 2 months of treatment as compared to the baseline. Rohaninasab *et al*.[38] found that SRP reduced the concentration of IL-17, which is in agreement with the finding in a non-diabetic control group. The concentration of IL-17 is reduced in Nigella sativa group than control group as a result of combined effect of scaling and root planing with the effect of Nigella sativa oil irrigation, and it might be viewed as a favorable response to therapy due to the drop in the pro-inflammatory level as[39] reported that the decline of IL-17 levels in patients with resolution of periodontitis indicates that IL-17 is engaged in the periodontal inflammatory process. This is in agreement with Aljuboori and Shukri.[40]

The precise mechanism responsible for the anti-inflammatory role of Nigella sativa oil is still poorly understood.[12,27]

Conclusion

Nigella sativa oil decreased the concentration of IL-17, and clinical periodontal parameters, so it can be used adjunct to scaling and root planing to improve periodontal health condition.

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Conflicts of interest

There are no conflicts of interest.

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