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Effect of oral administration and topical gel application of thymol and low-level laser therapy on oxidative stress, inflammatory biomarkers and dermatitis in patients with type 2 diabetes mellitus

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ABSTRACT

Background: Unmanaged diabetes mellitus, as a chronic metabolic disease, has dangerous consequences. The consequences of diabetes can be delayed and controlled by using antioxidants and anti-inflammatory substances in the food compounds.

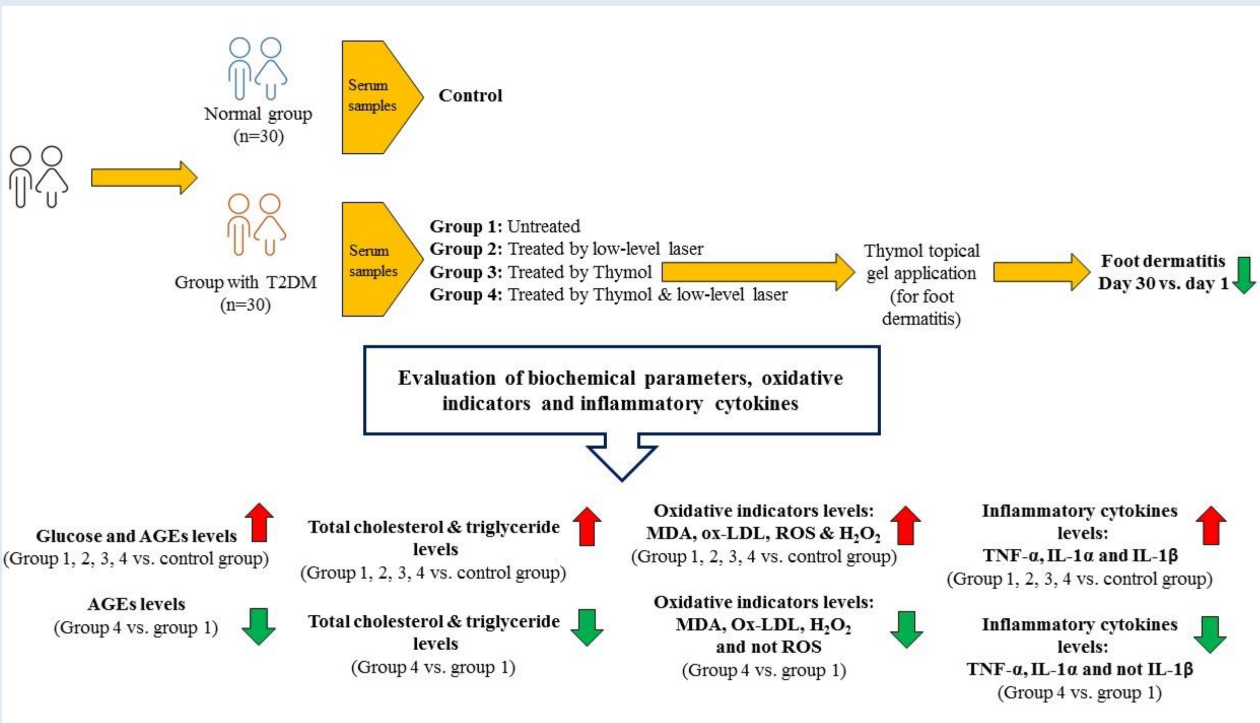
Objective: One of the main objectives of this study was to evaluate thymol administration and low-level laser therapy on the change of inflammatory and, oxidative indicators, and lipid profiles in patients with type 2 diabetes. Another aim was to study the effect of thymol oil extract on dermatitis.

Methods: Thirty volunteers with type 2 diabetes and thirty healthy volunteers as controls were selected. Blood samples were taken from all subjects before the study. The diabetic group was divided into four groups: untreated, treated with low-level laser, treated with thymol (25 mg/kg/30 days) and treated with thymol and laser. Glucose, advanced glycation end products, malondialdehyde, oxidized low level laser, reactive oxygen species, peroxide hydrogen, total cholesterol, triglyceride, and inflammatory cytokines such as tumor necrosis factor alpha, interleukin-1 beta and interleukin-1 alpha were measured and compared between diabetic and control groups and within diabetic groups. Thymol gel oil extract (0.5%) was studied in reduction of dermatitis in the feet of the diabetic group.

Results: Thymol administration, along with low-level laser therapy, reduced levels of cytokines except for interleukin-1 alpha, total cholesterol, triglycerides, advanced glycation end products, hydrogen peroxide, malondialdehyde, and oxidized low density level lipoprotein (P value < 0.05). The effect of 0.5% thymol oil as a gel on the reduction of dermatitis was not significant.

Conclusion: Thymol administration and thymol gel as well as low-level laser therapy, as adjunctive methods, through the reduction of free radicals and oxidative stress can be useful in controlling and reducing the diabetes complications.

Keywords: Diabetes mellitus, Thymol, Topical gel, Low-level laser therapy, Dermatitis



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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that is on the rise today due to an unhealthy lifestyle [1]. T2DM affects more than 85% of diabetics [2]. In addition to hyperglycemia, individuals with T2DM have to deal with other complications, if it is not controlled and managed. Because T2DM can damage organs such as the heart, eyes, and kidneys, as well as cause neuropathy, coronary artery disease, and stroke [3]. In addition to these complications, T2DM also affects patients' skin in various ways. In fact, diabetes can lead to skin inflammation (dermatitis), infection and skin diseases such as sores, dry skin and itching and in this way it can lead to a reduction in the quality of life of patient [4-5]. It has been reported that diabetes can cause skin manifestations in 30% of patients [6-7]. Skin problems may even be the first indicator of diabetes in some patients with diabetes [8]. An imbalance between the production of free radicals and antioxidant mechanisms in diabetes can lead to oxidative stress. This imbalance plays an important role in the skin disorders of diabetes [9]. Today, people are becoming more interested in using herbs to treat diabetes and its complications, as well as taking therapeutic drugs [10].

Thyme is one of the most important and widely used medicinal plants with many healing properties [11]. One of the most important properties of thyme, which is particularly important in studies of T2DM, is its antioxidant activity, which has been attributed to thymol [12]. As Figure 1 shows, thymol (5-methyl-2-isopropyl phenol) is a phenolic compound that is present in thyme [13].

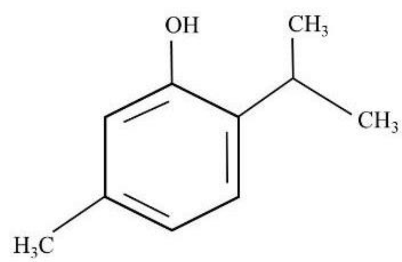

Figure 1. Thymol structure

Other properties of thyme that result from phenols include anti-hyperglycemic and anti-lipid activities [14]. Studies have shown that thymol affects the parameters of blood glucose, triglyceride, low density lipoprotein (LDL) and plasma malondialdehyde (MDA) levels in diabetes [15]. MDA, oxidized low density lipoprotein (ox-LDL), reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) are biomarkers of oxidative stress. MDA is a biomarker of lipid damage while advanced glycation end products (AGEs) are derived from glucose [16-17]. In addition to the antioxidant properties of thymol [18], which has made it important in studies related to diabetes, its anti-inflammatory properties have been proven by several studies [19-21]. Phenols have been reported to be effective in improving skin irritation or allergies by inhibiting pro-inflammatory mediators [22]. Studies have highlighted the effect of thymol on human health as a food compound. In addition to the role of thymol in reducing inflammation and increasing antioxidant activity, which is very important in controlling diabetes, it has been reported that the use of thymol in combination with other foods products can have a protective effect against coronavirus disease [23-24].

In addition to the role of phenols in improving diabetes-related parameters as well as inflammatory factors, low-level laser therapy (LLLT) is of particular importance today. LLLT has been shown to have significant effects on inflammatory markers including tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and ROS modulation and antioxidant enzyme activity [25-26]. In this study, we aimed to investigate the antioxidant and anti-inflammatory synergistic effects of thymol and LLLT by measuring some biochemical parameters and inflammatory factors. In addition, the effect of thymol oil extract as a gel on dermatitis (that can be the consequences of T2DM) was investigated.

MATERIALS AND METHODS

Materials: Thymol (T0501), as a trans isomer, was purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Total cholesterol and triglyceride kits were purchased from Pars Azmoun Company, Tehran, Iran. Triglyceride and total cholesterol levels were measured by enzymatic-colorimetric (GPO-PAP) and enzymatic-colorimetric (CHOD-PAP) methods, respectively. The enzyme-linked immunosorbent assay (ELISA) kit to assay human glucose (with an intra- and inter-assay CV < 8% and < 10%, respectively) and ROS levels were purchased from My BioSource Inc. (San Diego, USA). The H_2O_2 assay kit was bought from ZellBio (ZellBio GmbH, Ulm, Germany) to measure the levels of H_2O_2 in the blood.

The MDA assay kit was procured from Cayman chemical company (Michigan, USA). Test kits for IL-1α and IL-1β were purchased from Diaclone (Besançon, France). The TNF-α analytical kit was procured from R&D Systems, Inc. (Minneapolis, USA). AGEs, oxidant parameters including, MDA, and ox-LDL and Inflammatory parameters were measured by ELISA method according to their assay kits and ELISA reader apparatus (MR-96A, Mindary Co, Germany).

METHODS

Participants: Among the individuals referred to the Vali-Asr medical diagnostic laboratory in Tehran, Iran, thirty healthy volunteers, without any disease, were randomly selected as the control group. Thirty volunteers with T2DM were also selected as a diabetic group. Informed consent was obtained from all volunteers participating in the study and they were informed about the study. The serum sample of subjects in the diabetic group were divided into four groups as follows:

Serum sample of diabetic patients before any treatment (as group 1). Serum samples treated with LLL irradiations (as group 2). After that, all subjects of the diabetic group were treated with thymol. (at a dose of 25 mg/kg per day for 30 days), after treatment with thymol, a serum sample was obtained. The biochemical and inflammatory factors were checked on these samples twice, once before and once after they had been exposed to LLL irradiation (as group 3 and group 4).

Examination of changes in dermatitis: Thymol gel was prepared in a pharmaceutical company with a composition of 0.5% (w/v) thymol (contains 0.5% thymol oil extract). Patients in the diabetic group with dermatitis used the gel for 30 days in the area of inflammation in the foot. The study of dermatitis in diabetic patients was performed by viewing photos taken from the area of inflammation and calculating the percentage of possible changes in inflammation. The following formula was used to obtain the percentage of changes in the inflammatory region [27].

$$\text{Percentage of observed changes} = (A_0 - A_t)/A_0 \times 100$$

A_0: Area of inflammation (cm^2) on the first day of using thymol gel

A_t: Area of inflammation (cm^2) on the 10^{th}, 15^{th}, and 30^{th} days of using thymol gel

General Features and Sampling: The age, sex, weight, height, and body mass index (BMI) of all study subjects were recorded. Blood samples were taken from all participants after 12 hours of overnight fasting. After collecting the blood sample, they were centrifuged (250 g for 10 min). Following this, the serum was separated from the centrifuged samples. To look at the biochemical endpoints and inflammatory parameters in both diabetic and healthy samples, isolated serums were used.

Laser Irradiation: A laser pointer was used in the irradiation. The laser type was a low-level green laser diode with a wavelength from 532 nm to 100 mw in continuous mode with divergence < 1.5 mRad, beam mode (TEMoo), aperture beam diameter ~1.5, crystal type Nd:VYO4:KTP, and power supply 1× 3V CR2 alkaline batteries. Power density was 509.55 mW/cm^2 at a distance of 6.5 cm between the laser device and specimens' tube, and the diameter of the laser spot was fixed to 0.5 cm. Like our previous study irradiation was applied for 8 seconds [26]. Green diode pumped solid state (DPSS) Laser Pointer (model RLP-532, 1040 Vienna, Austria) was used for LLLT.

Statistical Analysis: All results were expressed as mean ± standard deviation. The Kolmogorov–Smirnov test was used to check the normal distribution of results. Statistical significance was analyzed by one-way ANOVA by comparing the mean of the obtained data. After that, we used Tukey post hoc. P-values < 0.05 were considered significant. The graphs were drawn with originPro 2019b Build 9.6.5.169 for windows.

RESULTS

General Features: Voluntary contributors were between 65 and 80 years old. In the control group and group of people with T2DM (diabetic group), 50% were male (n = 15) and 50% were female (n = 15). Results on the general characteristics of the control and diabetes groups are presented in Table 1. Stature, weight, and BMI of controls were compared with those of diabetics. Statistically, there was no significant difference in comparing the overall characteristics between control and diabetic groups.

Table 1. Anthropometric data

	Groups				
	Control group (n = 30)		Diabetics group (n = 30)		
Feature	Male (n=15)	Female (n=15)	Male (n=15)	Female (n=15)	P-value
Weight (Kg)	88.0 ± 4.7	83.0 ± 4.6	85.4 ± 4.5	86.0 ± 4.8	0.23
Height (Cm)	166.7 ± 7.4	168.0 ± 6.9	169.1 ± 6.2	166.5 ± 5.9	0.69
BMI (Kg/m^2)	31.9 ± 4.3	29.4 ± 3.7	29.8 ± 3.2	31.0 ± 3.6	0.51

Data are given as mean ± SD. BMI, Body mass index

Biochemical Parameters: In this study, we also evaluate some important biomedical parameters in serum samples of control and diabetic groups. The biochemical factors included blood glucose, total cholesterol, triglyceride, inflammatory biomarkers (IL-1α, IL-1β, and TNF-α), AGEs and some oxidative indicators such as H_2O_2, ROS, MDA, and ox-LDL.

Changes in biochemical variable concentrations were investigated in serum samples from the control group and diabetics (groups 1 to 4). Evaluation of total cholesterol and triglyceride results as well as results of inflammatory agents are shown in Figures 2 and 3, respectively and Table 2. As can be seen from the figures, in comparison of lipid profile levels (total cholesterol and triglyceride levels) and the mentioned inflammatory biomarkers, there was a statistically significant difference (P-value < 0.05) between control groups and samples of diabetic groups in untreated, laser treated, thymol treated, and laser and thymol treated conditions (groups 1 to 4).

A comparison of other biochemical variables such as AGEs, H_2O_2, ROS, MDA, and ox-LDL is shown in Table 2. There was a statistically significant difference (P-value < 0.05) in the results from groups 1 to 4 relative to the control group.

Biochemical parameter level assessments were studied in samples of patients with T2DM under untreated conditions, laser irradiation, treated with thymol, as well as thymol-treated and laser irradiation (groups 1 to 4). To assess the effects of laser and thymol alone, and the synergistic effects of laser and thymol on biochemical parameters, the results were compared between groups and are shown in Table 3. Glucose levels did not show a significant difference (P-value > 0.05) between the diabetic groups. That is, thymol and LLLT together and separately have no effect on lowering glucose in diabetics. Comparison of the results of total cholesterol levels in group 4 to 1, group 4 to 3 and group 3 to 1 showed a significant decrease (P-value < 0.05). The results showed a significant decrease (P-value < 0.05) in triglyceride levels in group 4 compared to group 1 and group 2 compared to group 1. Regarding the results of AGEs, a significant change in the levels of this parameter was observed in group 4 compared to group 1, group 4 compared to group 3 and group 3 compared to group 1 (P-value < 0.05). Significant changes were also observed in H_2O_2 (as an oxidative indicator) levels between group 4 compared to 1 and group 2 compared to 1 (P-value < 0.05). At ROS levels, as another oxidative indicator, no significant change (P-value > 0.05) was observed between groups. Significant changes were observed in MDA and ox-LDL levels between groups 4 compared to group 1, group 4 compared to group 2, group 4 compared to group 3, and group 2 compared to group 1 (P-value < 0.05). The two mentioned biochemical parameters are also oxidative indicators.

Significant changes were also observed in the comparison of IL-1α and TNF-α levels in different groups. IL-1α levels in group 4 compared to group 1, group 3 compared to group 1, and group 2 compared to group 1 showed a significant decrease (P-value < 0.05). TNF-α levels were significantly lower in group 4 compared to group 1, and group 2 compared to group 1 (P-value < 0.05). Comparison of Il-1β levels between groups did not show a significant decrease (P-value > 0.05).

Table 2: Comparison between the levels of glucose, AGEs and some oxidative indicators in the control group with other groups

	Groups					
Biochemical parameters	Control	Group 1	Group 2	Group 3	Group 4	P-value
Glc (µg/ml)	213.4 ± 20.1	392.9 ± 22.4	382.3 ± 19.8	387.2 ± 20.5	378.6 ± 19.7	< 0.001
AGEs (AU)	46.1 ± 5.1	86.1 ± 5.9	75.0 ± 5.9	78.8 ± 5.6	71.6 ± 5.9	< 0.001
H_2O_2 (µM/ml)	2590.4 ± 244.1	3659.1 ± 126.9	3528 ± 127	3557 ± 127	3506.1 ± 126.7	< 0.001
ROS (U/l)	236.6 ± 18.6	345.3 ± 25.4	335.8 ± 15.1	337.3 ± 25.4	331.3 ± 25.4	< 0.001
MDA (µM/ml)	2.4 ± 0.1	3.4 ± 0.1	3.1 ± 0.2	3.2 ± 0.08	2.9 ± 0.2	< 0.001
ox-LDL (mU/l)	11.9 ± 0.8	19.4 ± 1.1	15.0 ± 0.8	16.0 ± 1.3	13.1 ± 0.9	< 0.001
IL-1α (pg/ml)	487.5 ± 32.5	928.8 ± 27.9	899.4 ± 28.8	902.0 ± 26.4	894.8 ± 26.6	< 0.001
IL-1β (pg/ml)	289.4 ± 41.3	433.9 ± 36.1	418.4 ± 36.2	423. 4 ± 44.4	416.8 ± 41.5	< 0.001
TNF-α (pg/ml)	472.3 ± 50.5	928.4 ± 24.2	908.6 ± 26.5	912.5 ± 30.4	901.6 ± 23.8	< 0.001
Total Chol (mg/dl)	180.0 ± 9.4	202.7 ± 6.2	194.8 ± 6.2	196.8 ± 6.2	192.7 ± 6.2	< 0.001
TG (mg/dl)	122.6 ± 9.5	142.8 ± 6.1	136.8 ± 6.1	138.9 ± 6.0	134.8 ± 6.1	< 0.001

Data are given as mean ± SD. Group 1: Diabetic. Group 2: Diabetic + Laser. Group 3: Diabetic + Thymol. Group 4: Diabetic + Thymol + Laser. Glc, glucose; AGEs, advanced glycation end products; H_2O_2, hydrogen peroxide; ROS, reactive Oxygen species; MDA, malondialdehyde; ox-LDL, oxidized Low-Density Lipoprotein; IL-1α, Interleukin 1 alpha; IL-1β, Interleukin 1 beta; and TNF-α, tumor necrosis factor alpha; Glc, glucose; Chol, Cholesterol; TG, Triglyceride

Table 3: Multiple comparisons of some biochemical parameters and inflammatory factors among samples of diabetic group

Groups	Group 1			Group 2			Group 3			Group 4		
Biochemical parameters	Group 2	Group 3	Group 4	Group 1	Group 3	Group 4	Group 1	Group 2	Group 4	Group 1	Group 2	Group 3
Glc (µg/ml)	0.34	0.85	0.10	0.34	0.91	0.94	0.85	0.91	0.43	0.10	0.94	0.43
AGEs (AU)	<0.0001	0.001	<0.0001	<0.0001	0.12	0.24	0.001	0.12	0.001	<0.0001	0.24	0.001
H_2O_2 (µM/ml)	0.01	0.09	0.002	0.01	0.95	0.98	0.09	0.95	0.72	0.002	0.98	0.72
ROS (U/l)	0.48	0.64	0.11	0.48	0.99	0.93	0.64	0.99	0.84	0.11	0.93	0.84
MDA (µM/ml)	0.0002	0.0002	<0.0001	0.0002	0.170	0.0003	0.0002	0.17	<0.0001	<0.0001	0.0003	<0.0001
ox-LDL (mU/l)	<0.0001	<0.0001	<0.0001	<0.0001	0.028	<0.0001	<0.0001	0.02	<0.0001	<0.0001	<0.0001	<0.0001
IL-1α (pg/ml)	0.005	0.012	0.0003	0.005	0.99	0.97	0.012	0.99	0.83	0.0003	0.97	0.83
IL-1β (pg/ml)	0.34	0.83	0.51	0.34	0.99	0.99	0.83	0.99	0.9	0.51	0.99	0.94
TNF-α (pg/ml)	0.04	0.089	0.0006	0.04	0.97	0.8	0.089	0.9	0.61	0.0006	0.88	0.61
Total Chol (mg/dl)	0.0005	0.0045	<0.0001	0.0005	0.70	0.46	0.0045	0.70	0.03	<0.0001	0.46	0.03
TG (mg/dl)	0.01	0.18	<0.0001	0.0136	0.20	0.61	0.18	0.20	0.05	<0.0001	0.61	0.05
P-value												

P-value < 0.05 is significant. Group 1: Diabetic. Group 2: Diabetic + Laser. Group 3: Diabetic + Thymol. Group 4: Diabetic + Thymol + Laser. AGEs, advanced glycation end products; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; MDA, malondialdehyde; ox-LDL, oxidized Low-Density Lipoprotein; IL-1α, Interleukin 1 alpha; IL-1β, Interleukin 1 beta; and TNF-α, tumor necrosis factor alpha; Glc, glucose; Chol, Cholesterol; TG, Triglyceride

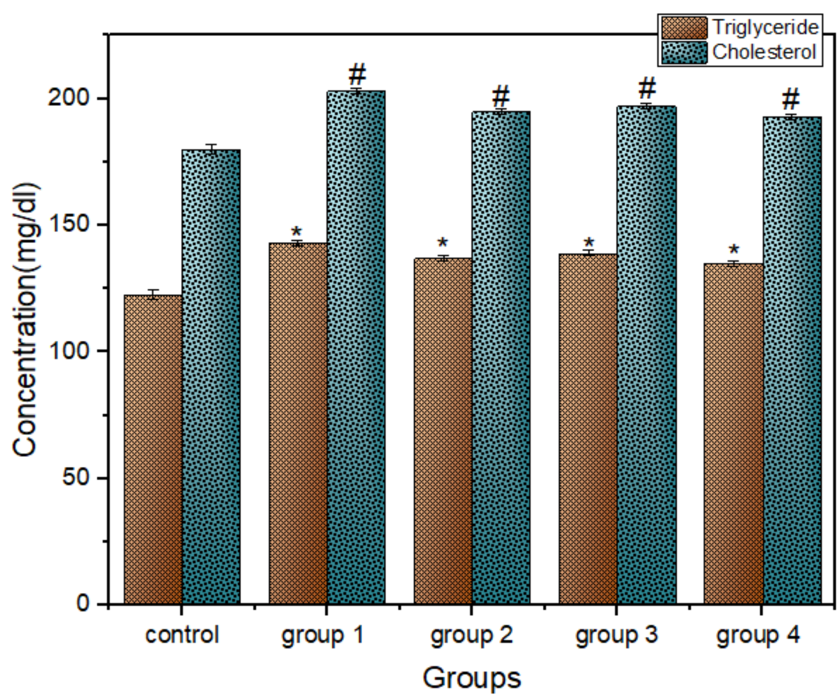

Figure 2. Changes in total cholesterol and triglyceride levels in the control group and diabetic samples in different conditions. *and # Significances of data ($p < 0.001$). Group 1: Diabetic. Group 2: Diabetic + Laser. Group 3: Diabetic + Thymol. Group 4: Diabetic + Thymol + Laser.

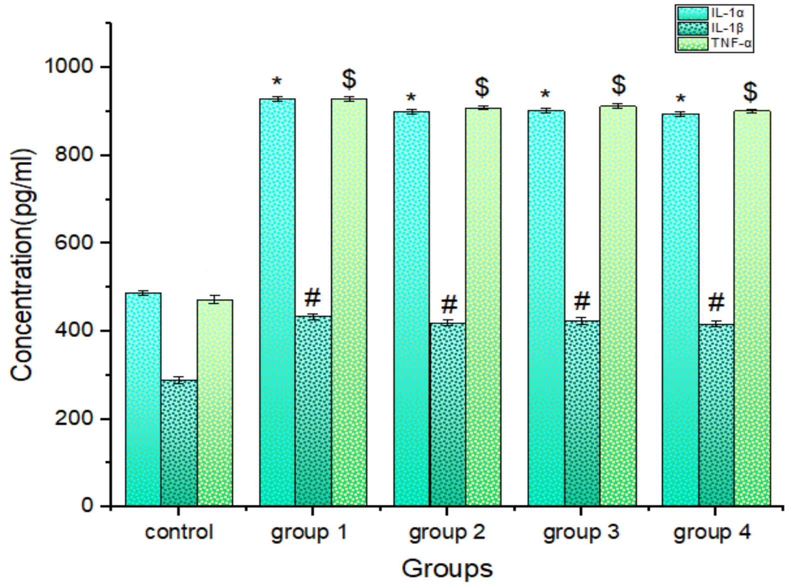

Figure 3. Changes in TNF-α, IL-1α and IL-1β levels in the control and diabetic groups (in different conditions). *and #and $ Significances of data comparing diabetic samples vs. the control group (Pvalue < 0.05). Group 1: Diabetic. Group 2: Diabetic + Laser. Group 3: Diabetic + Thymol. Group 4: Diabetic + Thymol + Laser.

Evaluation of the effect of topical thymol gel on the skin: Due to the antioxidant and anti-inflammatory effects of thymol, the effect of 0.5% of thymol oil extract, which was prepared as a gel, was given to the diabetic group. All subjects of the diabetic group used topical thymol gel for one month while simultaneously taking oral thymol. All diabetic volunteers selected for the study suffered from skin problems including dermatitis and itching (pruritus) in the foot area. The part of the foot to which thymol gel was applied was examined and observed every 10 days for dermatitis changes. Reduction of inflammation and itching was observed in a number of diabetic patients, but this reduction was not significant (P-value > 0.05) (Figure 4 and Figure 5).

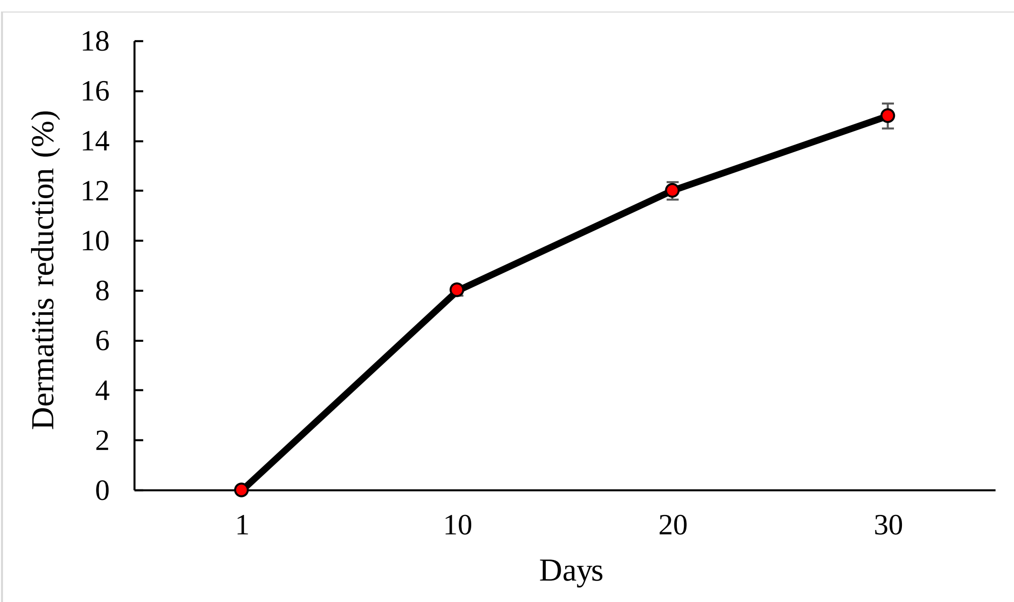

Figure 4. Reduction of dermatitis on days 10, 20 and 30 after using 0.5% thymol oil as topical gel

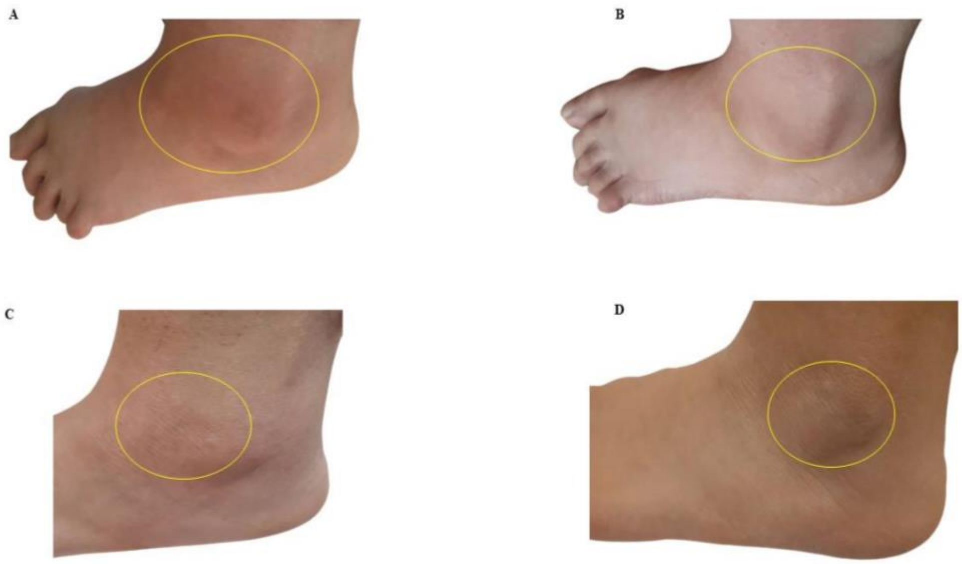

Figure 5. Digital photos of the site of foot dermatitis, in one of the patients in the group with diabetes, on days 1 (A), 10 (B), 20 (C) and 30 (D) after using 0.5% thymol oil as topical gel

DISCUSSION

In the present study, according to the results, we showed that thymol as a phenolic compound as well as a component of functional food and LLLT can play a role in reduction of some oxidative indicators such as H_2O_2. Reducing free radicals and oxidant compounds and subsequently increasing antioxidant activity in the body can play an important role in preventing and controlling diseases and their consequences. Free radicals and ROS play an important role in the consequences of T2DM. According to previous studies, oxidative stress and inflammatory factors may play a role in the development of cardiovascular disease, retinopathy, nephropathy, cardiomyopathy, neuropathy, and skin problems, which can be uncontrollable complications of DM [28-30]. In this study, we focused on the effect of thymol (25 mg daily for 1 month) and LLLT on diabetic patients and their role on changes in levels of oxidative markers (H_2O_2, ROS, ox-LDL and MDA), inflammatory factors (IL-1α, IL-1β and TNF-α), lipid profile (total cholesterol and triglyceride), glucose, and AGEs (glycosylation indicator) were investigated. Due to the lack of toxic and harmful effects of thymol on humans [31], this study was performed for the first time on patients with T2DM.

As shown in Table 2 and Figures 2 and 3, in individuals with T2DM, all the biochemical parameters and inflammatory factors mentioned above had a significant increase compared to controls. In our previous study, that was performed on people with T2DM, this significant increase was reported in some biochemical variables [25]. Several studies have shown that lipid profile levels and inflammatory agents increase in patients with T2DM [19, 32-34]. An important part of insulin resistance and diabetes complications can be caused by factors like TNF-α, which can cause inflammation. The findings of our study agreed with those studies. In studies conducted by Nagoor Meeran et al., the effect of thymol in different doses on the albino Wistar rats, was studied. In their study, glycation, oxidative stress, lipid peroxidation, and inflammatory cytokines were examined. They reported that thymol plays an important role in reduction of lipid peroxidation, glycation, decreased expression of inflammatory cytokines, and oxidative stress [35-37].

In the present study, based on the reported results in Tables 2 and 3 and Figures 2 and 3, decreases in ox-LDL, AGE (as indicators of lipid peroxidation and glycation, respectively), IL-1α (as an inflammatory cytokine) and total cholesterol in groups 3 and 4 were observed in comparison with group 1. In these cases, our study agreed with Nagoor Meeran et al. The synergistic effect of thymol and LLLT was also evident in our results. Thymol reduces oxidative stress through its scavenging activity and elimination of free radicals. It has been reported that thymol prevents obesity from high-fat diets through mechanisms such as limiting visceral fat accumulation, reducing lipid function, improving insulin function, leptin sensitivity, and increasing the antioxidant potential [38]. Thymol reduces the expression of cytokines such as TNF-α and IL-1β by inhibiting the activity of 5-lipoxygenase [39]. A study by Saravana and Pari on diabetic mice showed that thymol increased the activity of antioxidant enzymes. They reported that thymol attenuates total cholesterol and glucose levels [40]. Another aim in this study was to evaluate thymol oil extract, as a topical gel, on dermatitis in patients with T2DM. Several studies have been performed on the anti-inflammatory effect of thymol oil on animal wound models [41-42]. The structure of the skin in rats is in many ways similar to that of human skin. For this reason, in this study, the effect of thymol on the skin of diabetic patients was investigated. It has been suggested that the first and most important phase in wound healing is to reduce and improve inflammation. Thymol reduces TNF-α and IL-1β and some interleukins in the dermis layer of the skin [43]. In a study by Kwon et al., [44] the effect of thymol on dermatitis in BALB/c mice was studied. Dermatitis was induced by Staphylococcus aureus in mice. They reported that thymol reduced the gene expression of some pro-inflammatory factors such as TNF-α and Il-1β. Thymol inhibits the deterioration of dermatitis lesions. In our study, dermatitis may have been reduced by reducing cytokines. Could also suggest studies on the mechanism by which it can reduce dermatitis and to confirm if it's by the reduction of cytokines, given that it is unknown if it is by reducing cytokines.

CONCLUSION

Findings from the study showed that thymol administration and LLLT can play an important role in reduction of cholesterol. triglyceride, MDA, Ox-LDL, H_2O_2, AGEs, and inflammatory biomarkers such as TNF-α and IL-1 α in patients with T2DM. According to the results, thymol oil extract 0.5% as topical gel can also be effective in reducing dermatitis. Polyphenols and phenols such as thymol, which is part of important food compounds, and LLLT can be useful as adjunctive therapies in the control and prevention of diseases such as DM. Controlling and management of diabetes with antioxidant and anti-inflammatory compounds can be done by reducing free radicals and oxidative stress, thus prevent the consequences of diabetes. The use of such compounds can provide a clear perspective on preventing the consequences of DM. However, many studies are needed on the molecular mechanism of thymol.

List of abbreviations: LLLT, low level-laser therapy; T2DM, type 2 diabetes mellitus; DM, diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; CHOD-PAP, cholesterol oxidase phenol 4-aminoantipyrine peroxidase; GPO-PAP, glycerine phosphate oxidase peroxidase; ROS, reactive oxygen species; Glc, glucose; AGEs, advanced glycation end products; MDA, malondialdehyde; ox-LDL, oxidized Low-Density Lipoprotein; Chol, Cholesterol; TG, Triglyceride; IL-1α, Interleukin 1 alpha; IL-1β, Interleukin 1 beta; and TNF-α, tumor necrosis factor alpha

Author's contributions: DM participated in the study design. He also edited the article. MRA participated in the writing. FJ participated in doing experimental works and writing materials and methods section. ASM participated in the abstract writing. SP and SA contributed to data collection and analysis of the results. HM contributed to the original idea of the paper, doing the experimental work and supervision of the research. The authors read and approved the final version before its submission.

Conflict of interest: The authors declare that there are no conflicts of interest.

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