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# The effect of squalene on cellular energy and inflammation in type 2 diabetes patients

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**ABSTRACT**

**Background:** Squalene is a 30-carbon ring hydrocarbon in the triterpene class. Squalene as a bioactive compound has been shown to have several health benefits specifically in the reduction of diabetic symptoms, including anti-inflammatory effects.

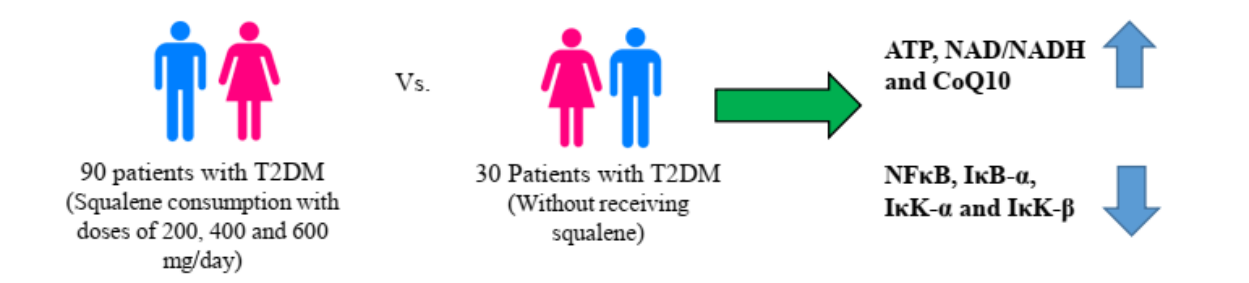
**Objective:** The purpose of the study was to examine effect of squalene on specific parameters regarding energy production and inflammation in those with type 2 diabetes mellitus. These parameters included ATP, NAD/NADH, CoQ10, NFκB, IκB-alpha, IκK-alpha, and IκK-beta.

**Methods**: 150 volunteers were selected for this study split into 5 groups. Group 1 contained 30 healthy participants and served as the control. The remaining 120 participants were split into 4 groups containing 30 volunteers each. All the participants in groups 2, 3, 4, and 5 have type 2 diabetes mellitus. Group 2 did not receive any squalene while group 3, group 4, and group 5 all received an oral supplementation of squalene at 200 mg/day, 400 mg/day and 600 mg/day respectively. Participants were prescribed to take the oral supplementation of squalene for a total of 84 days. Blood samples were taken on days 1, 14, 28, 56, and 84 in five time periods. For all participants ATP, NAD/NADH, CoQ10, NFκB, IκB-α, IκK-α and IκK-β levels of all groups were evaluated.

**Results:** Throughout the 84 days there was a statistically significant difference when comparing the healthy group and the diabetic groups in reducing ATP, NAD/NADH and CoQ10 ($P < 0.05$) and increasing NFκB, IκB-α, IκK-α and IκK-β ($P < 0.05$). When compared, participants in groups 3, 4, and 5 also showed a statistically significant in the changes of ATP, NFκB, IκB-α, IκK-α and IκK-β levels.

**Conclusions:** Squalene as a bioactive compound can play an important role in reducing inflammatory mediators and increases energy production.

**Keywords:** squalene, diabetes mellitus, ATP production, NAD/NADH, Reactive oxygen species (ROS), CoQ10, NFκB, IkB-α, IκK-α, IκK-β






**INTRODUCTION** Squalene is a bioactive compound found in natural sources such as shark liver and olive oil. It is a 30-carbon ring compound in the triterpene class [1]. The health benefits that squalene provides is a currently emerging field of research as squalene has been suggested to have several health benefits including anti-inflammatory effects. Recent studies have shown that squalene helps to produce changes in inflammatory cytokines, specifically interleukin-1α, interleukin-1β and interleukin-4, as well as immunoglobulin A [2]. According to the definition of functional foods [3], squalene can be considered a part of functional foods. Other studies have shown that squalene has been studied in diabetic patients for effects on various parameters. For example, one such study evaluated the effectiveness of squalene as a method of aiding in reducing proteinuria levels in patients with type 2 diabetes mellitus (T2DM). In this study, it was found that squalene effectively functioned to reduce levels of proteinuria, as well as related parameters such as TGF-beta1, albumin, creatinine, blood urea nitrogen, and systolic and diastolic blood pressure [4]. In another study, focus was on squalene's overall effect on lipid profile, where it was found that with squalene intake in T2DM patients, there had been an overall decrease in low density cholesterol (LDL) and very low-density cholesterol (VLDL) with an increase in high density cholesterol (HDL) [5]. Our current study aims to study squalene as it affects cellular energy in patients with T2DM. In a study focusing on treating aged rats with squalene, it was found that their liver's mitochondrial function was improved, as squalene functioned as a protective agent. It found that ATP levels, TCA enzyme activity, and respiratory marker enzymes showed an observed decline. These respiratory marker enzymes include NADH dehydrogenase and cytochrome-c-oxidase [6]. In another study, it was found that CoQ10, a key molecule in cellular process function, was increased with intake of squalene in rats [7]. This is of key importance as it identified squalene as a potential method or agent in improving cellular processes and function. In a study focused on squalene intake in C57BL/6 mice, it was found that squalene functioned effectively to prevent

activation of dextran sulfate sodium (DSS) induced colitis by NFκB. In this study, it was also found that in the squalene treated rats, IKB alpha breakdown was prevented from occurring [7]. Our study aims to focus on the effect of squalene on parameters including ATP, NAD/NADH, CoQ10, NFκB, IκB-alpha, IκK-alpha, and IκK-beta. These parameters were included in the study due to their connection to cellular energy processes as well as previously published literature indicating a potential connection with squalene. Adenosine triphosphate (ATP) is an organic compound providing a source of energy for cellular processes through the high energy released through the separation of the bonding between phosphorus. This research will allow us to identify squalene as a potential option for improving cellular energy processes within the body.

## MATERIALS AND METHODS:

***Materials:*** Squalene (as a liquid form with code number S3626) was purchased from Sigma Aldrich Company (USA). The colorimetric kits of ATP and NAD/NADH were procured from Abcam Company (USA). The enzyme-linked immunosorbent assay (ELISA) assay kits of IκK-α and IκK-β were procured from antibodies Company (Germany). The kits for measuring CoQ10, NFκB and IκB-α were purchased from MyBiosource Co (USA), CUSABIO Co (USA) and RayBiotech Co (USA), respectively.

***Participants:*** 150 volunteers participated in this study. 30 of these 150 volunteers were healthy subjects and were selected as group 1 (healthy control). The remaining 120 people were divided into four groups. Group 2: Patients with T2DM who didn't receive squalene. Group 3: Patients with T2DM with consumption of 200 mg/day squalene. Group 4: Patients with T2DM with consumption of 400 mg/day squalene. Group 5: patients with T2DM with consumption of 600 mg/day squalene. Groups 3, 4 and 5 consumed squalene (as an oral capsule (liquid filled oral)) for 84 days. Volunteers with diabetes were patients who referred to Vali-Asr medical laboratory (Tehran, Iran). According to World Health Organization (WHO), inclusion criteria contained fasting plasma glucose ≥ 126 mg/dL, glycated hemoglobin (HbA1c) ≥ 6.5% and not taking corticosteroids. Patients with T1DM and other diseases and a history of surgery as well as young patients with T2DM were excluded from the study. In this randomized study, all volunteers were aware of the study process. Informed consent was obtained of all the participants in this study.

***General Features and Sampling:*** After grouping the participating volunteers in the study, anthropometric items including age, sex, weight, height, and body mass index (BMI**)** of all volunteers were recorded. Blood samples were taken from all participants under sterile condition. After 12 hours of overnight fasting, the sampling was performed in five time periods on days 1, 14, 28, 56 and 84. The indicated blood samples were centrifuged to prepare the serum (250 g for 10 min). In each period, ATP, NAD/NADH, CoQ10, NFκB, IkB-α, IκK-α and IκK-β levels of all groups were evaluated.

***Biochemical Measurement:*** In each mentioned time period, the considered parameters were measured in all 5 groups. The measurement of the mentioned parameters was done based on the manual of the purchased kits. Colorimetric method was used to measure ATP and NAD/NADH. ELISA method was used to measure CoQ10, NFκB, IkB-α, IκK-α and IκK-β levels.

***Statistical Analysis:*** Statistical analysis was done by SPSS (version 23, IBM, USA) software for Windows. All results were expressed as mean ± standard deviation (SD). Independent-sample T-test was used to compare the mean of general characteristics of the participants. Independent-sample T-test was also used to compare the obtained results between groups consuming squalene. Statistical significance, to compare the mean of the obtained data from diabetic groups with healthy control group, was analyzed by one-way ANOVA. After the one-way ANOVA test, Tukey post hoc was used. The

Kolmogorov-Smirnov test was used to analyze the normal distribution of data. $P < 0.05$ were considered significant.

**RESULTS:** The mean comparison of the results of measuring the levels of parameters related to energy production (ATP, NAD/NADH and CoQ10) as well as parameters related to inflammation producing mediators (NFκB, IκB-α, IκK-α and IκK-β) between the healthy control group and the diabetic groups is shown in Table 1. In the results comparison of the mentioned parameters between the healthy control group and the diabetic groups, a statistically significant relationship was observed. In this way, this significance was observed in reducing ATP, NAD/NADH and CoQ10 ($P < 0.05$) and increasing NFκB, IκB-α, IκK-α and IκK-β ($P < 0.05$) in diabetic groups compared to healthy control group. Table 2 shows the measurement of the levels of the mentioned parameters among the diabetic groups that were treated with different doses of squalene.

**Table 1:** Comparison between the levels of biochemical parameters of the control group with other groups

| Parameter / Group | ATP (nmol/well) | NAD/NADH (pmol/well) | CoQ10 (ng/ml) | NFκB (ng/ml) | IκB-α (ng/ml) | IκK-α (ng/ml) | IκK-β (ng/ml) |
|---|---|---|---|---|---|---|---|
| **Healthy control** | 6.93 ± 0.78 | 78.36 ± 6.14 | 39.03 ± 3.69 | 9.54 ± 1.31 | 75.33 ± 7.56 | 4.45 ± 0.83 | 56.06 ± 6.46 |
| **Diabetic control (No squalene)** | 3.66 ± 1.12 | 47.63 ± 6.51 | 22.07 ± 2.92 | 17.52 ± 0.97 | 128.06 ± 6.43 | 8.14 ± 0.65 | 81.13 ± 6.38 |
| | | | | P < 0.05 | | | |
| **Diabetic + 200 mg/day squ (14th day)** | 3.86 ± 1.00 | 48.06 ± 6.45 | 22.48 ± 2.92 | 17.21 ± 0.97 | 126.33 ± 6.41 | 8.00 ± 0.65 | 76.06 ± 5.56 |
| **Diabetic + 400 mg/day squ (14th day)** | 4.13 ± 0.82 | 48.83 ± 5.94 | 22.68 ± 2.92 | 16.90 ± 0.88 | 125.26 ± 6.34 | 7.93 ± 0.65 | 75.33 ± 6.14 |
| **Diabetic + 600 mg/day squ (14th day)** | 4.53 ± 1.10 | 49.36 ± 5.67 | 22.88 ± 2.92 | 16.72 ± 0.79 | 124.73 ± 6.08 | 7.83 ± 0.65 | 74.76 ± 6.36 |
| **Diabetic + 200 mg/day squ (28th day)** | 3.93 ± 1.01 | 48.53 ± 6.21 | 22.58 ± 2.92 | 17.14 ± 0.97 | 125.53 ± 6.20 | 7.96 ± 0.65 | 75.66 ± 5.59 |
| **Diabetic + 400 mg/day squ (28th day)** | 4.43 ± 0.97 | 49.43 ± 4.51 | 22.98 ± 2.92 | 16.85 ± 0.97 | 124.26 ± 5.96 | 7.82 ± 0.65 | 73.63 ± 5.61 |
| **Diabetic + 600 mg/day squ (28th day)** | 4.76 ± 1.19 | 50.26 ± 5.64 | 23.28 ± 2.92 | 16.64 ± 0.95 | 123.93 ± 6.29 | 7.79 ± 0.63 | 72.86 ± 5.61 |
| **Diabetic + 200 mg/day squ (56th day)** | 5.13 ± 1.19 | 50.53 ± 5.71 | 23.38 ± 2.92 | 16.60 ± 0.97 | 122.83 ± 6.02 | 7.71 ± 0.65 | 72.06 ± 6.86 |
| **Diabetic + 400 mg/day squ (56th day)** | 5.46 ± 1.13 | 50.86 ± 5.60 | 23.58 ± 2.92 | 16.57 ± 0.96 | 121.73 ± 6.09 | 7.64 ± 0.63 | 71.23 ± 5.51 |
| **Diabetic + 600 mg/day squ (56th day)** | 5.83 ± 1.01 | 51.13 ± 5.96 | 26.78 ± 2.92 | 16.54 ± 0.97 | 120.86 ± 6.31 | 7.57 ± 0.64 | 70.56 ± 5.28 |
| **Diabetic + 200 mg/day squ (84th day)** | 5.26 ± 1.08 | 50.73 ± 5.84 | 23.48 ± 2.92 | 16.58 ± 0.90 | 122.13 ± 6.24 | 7.70 ± 0.65 | 71.46 ± 5.14 |
| **Diabetic + 400 mg/day squ (84th day)** | 5.63 ± 1.12 | 51.06 ± 5.52 | 23.68 ± 2.92 | 16.55 ± 0.94 | 121.26 ± 6.37 | 7.62 ± 0.68 | 70.73 ± 4.46 |
| **Diabetic + 600 mg/day squ (84th day)** | 5.96 ± 0.89 | 51.33 ± 5.74 | 23.97 ± 3.11 | 16.53 ± 0.96 | 120.33 ± 6.23 | 7.55 ± 0.65 | 70.16 ± 4.60 |

Data are given as mean ± SD. Independent-sample T-test was used to compare between groups. ATP, Adenosine three phosphate; NAD, Nicotinamide adenine dinucleotide; CoQ10, Coenzyme Q10; NFκB, Nuclear factor kappa B; IκB-α, Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; IκK-α, IκB kinase α; IκK-β, IκB kinase β.

**Table 2:** Multiple comparisons between the levels of biochemical parameters between the groups in different days

| Parameter / Group | ATP (nmol/well) | NAD/NADH (pmol/well) | CoQ10 (ng/ml) | NFκB (ng/ml) | IκB-α (ng/ml) | IκK-α (ng/ml) | IκK-β (ng/ml) |
|---|---|---|---|---|---|---|---|
| **Diabetic first day vs. Diabetic 14 day (200squ)** | 0.47 | 0.79 | 0.59 | 0.22 | 0.30 | 0.41 | 0.002 |
| **Diabetic first day vs. Diabetic 28 day (200squ)** | 0.34 | 0.58 | 0.50 | 0.13 | 0.12 | 0.29 | 0.001 |
| **Diabetic first day vs. Diabetic 56 day (200squ)** | 0.00 | 0.07 | 0.09 | 0.00 | 0.00 | 0.01 | 0.00 |
| **Diabetic first day vs. Diabetic 84 day (200squ)** | 0.00 | 0.05 | 0.06 | 0.00 | 0.00 | 0.01 | 0.00 |
| **Diabetic 14 day vs. Diabetic 28 day (200squ)** | 0.80 | 0.77 | 0.89 | 0.78 | 0.62 | 0.81 | 0.78 |
| **Diabetic 14 day vs. Diabetic 56 day (200squ)** | 0.00 | 0.12 | 0.23 | 0.01 | 0.03 | 0.09 | 0.01 |
| **Diabetic 14 day vs. Diabetic 84 day (200squ)** | 0.00 | 0.09 | 0.19 | 0.01 | 0.01 | 0.08 | 0.00 |
| **Diabetic 28 day vs. Diabetic 56 day (200squ)** | 0.00 | 0.20 | 0.29 | 0.03 | 0.09 | 0.14 | 0.03 |
| **Diabetic 28day vs. Diabetic 84 day (200squ)** | 0.00 | 0.16 | 0.23 | 0.02 | 0.04 | 0.12 | 0.00 |
| **Diabetic 56 day vs. Diabetic 84 day (200squ)** | 0.65 | 0.89 | 0.89 | 0.96 | 0.66 | 0.95 | 0.70 |
| **Diabetic first day vs. Diabetic 14 day (400squ)** | 0.07 | 0.46 | 0.42 | 0.01 | 0.09 | 0.21 | 0.00 |
| **Diabetic first day vs. Diabetic 28 day (400squ)** | 0.00 | 0.21 | 0.23 | 0.01 | 0.02 | 0.06 | 0.00 |
| **Diabetic first day vs. Diabetic 56 day (400squ)** | 0.00 | 0.04 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Diabetic first day vs. Diabetic 84 day (400squ)** | 0.00 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Diabetic 14 day vs. Diabetic 28 day (400squ)** | 0.20 | 0.66 | 0.69 | 0.81 | 0.53 | 0.51 | 0.26 |
| **Diabetic 14 day vs. Diabetic 56 day (400squ)** | 0.00 | 0.17 | 0.23 | 0.16 | 0.03 | 0.08 | 0.00 |
| **Diabetic 14 day vs. Diabetic 84 day (400squ)** | 0.00 | 0.13 | 0.18 | 0.14 | 0.01 | 0.08 | 0.00 |
| **Diabetic 28 day vs. Diabetic 56 day (400squ)** | 0.00 | 0.28 | 0.43 | 0.26 | 0.10 | 0.28 | 0.10 |
| **Diabetic 28 day vs. Diabetic 84 day (400squ)** | 0.00 | 0.21 | 0.35 | 0.23 | 0.06 | 0.25 | 0.03 |
| **Diabetic 56 day vs. Diabetic 84 day (400squ)** | 0.57 | 0.89 | 0.89 | 0.94 | 0.77 | 0.90 | 0.70 |

| Parameter / Group | ATP (nmol/well) | NAD/NADH (pmol/well) | CoQ10 (ng/ml) | NFκB (ng/ml) | IκB-α (ng/ml) | IκK-α (ng/ml) | IκK-β (ng/ml) |
|---|---|---|---|---|---|---|---|
| **Diabetic first day vs. Diabetic14 day (600squ)** | 0.00 | 0.27 | 0.29 | 0.00 | 0.04 | 0.07 | 0.00 |
| **Diabetic first day vs. Diabetic 28 day (600squ)** | 0.00 | 0.10 | 0.11 | 0.00 | 0.01 | 0.03 | 0.00 |
| **Diabetic first day vs. Diabetic 56 day (600squ)** | 0.00 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Diabetic first day vs. Diabetic 84 day (600squ)** | 0.00 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Diabetic 14 day vs. Diabetic 28 day (600squ)** | 0.43 | 0.54 | 0.60 | 0.72 | 0.61 | 0.80 | 0.22 |
| **Diabetic 14 day vs. Diabetic 56 day (600squ)** | 0.00 | 0.24 | 0.23 | 0.41 | 0.02 | 0.12 | 0.00 |
| **Diabetic 14 day vs. Diabetic 84 day (600squ)** | 0.00 | 0.18 | 0.17 | 0.40 | 0.00 | 0.10 | 0.00 |
| **Diabetic 28 day vs. Diabetic 56 day (600squ)** | 0.00 | 0.56 | 0.51 | 0.67 | 0.65 | 0.18 | 0.10 |
| **Diabetic 28 day vs. Diabetic 84 day (600squ)** | 0.00 | 0.47 | 0.38 | 0.65 | 0.03 | 0.15 | 0.04 |
| **Diabetic 56 day vs. Diabetic 84 day (600squ)** | 0.59 | 0.89 | 0.81 | 0.98 | 0.74 | 0.91 | 0.75 |
| P value | | | | | | | |

Data are given as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison posthoc test between the groups. ATP, Adenosine three phosphate; NAD, Nicotinamide adenine dinucleotide; CoQ10, Coenzyme Q10; NFκB, Nuclear factor kappa B; IκB-α, Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; IκK-α, IκB kinase α; IκK-β, IκB kinase β.

In this table, the comparison of the levels of measured parameters on different days among groups 3, 4 and 5 is shown. In group 3, which received a dose of 200 mg of squalene for 84 days, ATP, NAD/NADH and CoQ10 and NFκB, IκB-α, IκK-α and IκK-β were measured in the first day (before receiving squalene), 14, 28, 56 and 84. In the comparison of the results between days 1 and 14 and days 1 and 28, only the changes in IκK-β levels were statistically significant. In comparison of the results between days 1 and 56 and days 1 and 84, the changes in ATP, NFκB, IκB-α, IκK-α and IκK-β levels were statistically significant. In comparing the results between days 14 and 28, no significance was observed in any of the mentioned parameters ($P > 0.05$). In the comparison of the results between days 14 and 56, statistically significance was observed in ATP, NFκB, IκB-α and IκK-β levels. In the comparison of the results between days 14 and 84, statistically significance was observed in ATP and IκK-β levels. In comparing the results between days 28 and 56, statistically significance was observed in ATP, NFκB and IκK-β levels. In the comparison of the results between days 28 and 84, statistically significance was observed in

ATP, NFκB, IκB-α and IκK-β levels. Finally, in group 3, in comparing the results between days 56 and 84, no significance was observed in any of the mentioned parameters (P > 0.05).

In group 4, which received a dose of 400 mg of squalene for 84 days, ATP, NAD/NADH, CoQ10, NFκB, IκB-α, IκK-α and IκK-β levels were assayed in the first day (before receiving squalene), 14, 28, 56 and 84, same as group 3. In the comparison of the results between days 1 and 14, statistically significance was observed in NFκB and IκK-β levels. In the comparison of the results between days 1 and 28, statistically significance was observed in ATP, IκB-α, NFκB and IκK-β levels. In the comparison of the results between days 1 and 56, a statistically significant was observed in all the parameters except CoQ10. In the comparison of the results between days 1 and 84, statistically significant differences were observed in the levels of all measured parameters. According to this result, it can be stated that the changes in the parameters levels are time dependent. In comparing the results between days 14 and 28, same as group 3, no significance was observed in any of the mentioned parameters (P > 0.05). In the comparison of the results between days 14 and 56 and 14 and 84, statistically significance was observed in ATP, IκB-α and IκK-β levels. In the comparison of the results between days 28 and 56, a significant difference was observed only in ATP levels. In the comparison of the results between days 28 and 84, statistically significance was observed in ATP and IκK-β levels. Finally, in group 4 same as group 3, in comparing the results between days 56 and 84, no significance was observed in any of the mentioned parameters (P > 0.05).

In group 5, which received a dose of 600 mg of squalene for 84 days, ATP, NAD/NADH, CoQ10, NFκB, IκB-α, IκK-α and IκK-β levels were assayed in the first day (before receiving squalene), 14, 28, 56 and 84, same as group 3 and group 4. In the comparison of the results between days 1 and 14, statistically significance was observed in ATP, NFκB, IκB-α and IκK-β levels. In the comparison of the results between days 1 and 28, statistically significance was observed in ATP, NFκB, IκB-α, IκK-α and IκK-β levels. In the comparison of the results between days 1 and 56 and 1 and 84, statistically significance was observed at all levels of parameters. in comparing the results between days 14 and 28, no significance was observed in any of the mentioned parameters (P > 0.05). In the comparison of the results between days 14 and 56 and 14 and 84, statistically significance was observed in ATP, IκB-α and IκK-β levels. In the comparison of the results between days 28 and 56, a significant difference was observed only in ATP levels. In the comparison of the results between days 28 and 84 (like the comparison between days 14 and 56 and also days 14 and 84), statistically significance was observed in ATP, IκB-α and IκK-β levels. Finally, in this group same as group 3 and group 4, in comparing the results between days 56 and 84, no significance was observed in any of the mentioned parameters (P > 0.05). As the results show, the change in the levels of some parameters depends on the time of squalene consumption and the dose of squalene. For example, taking a dose of 600 mg of squalene for 84 days' causes changes in the levels of all parameters under investigation.

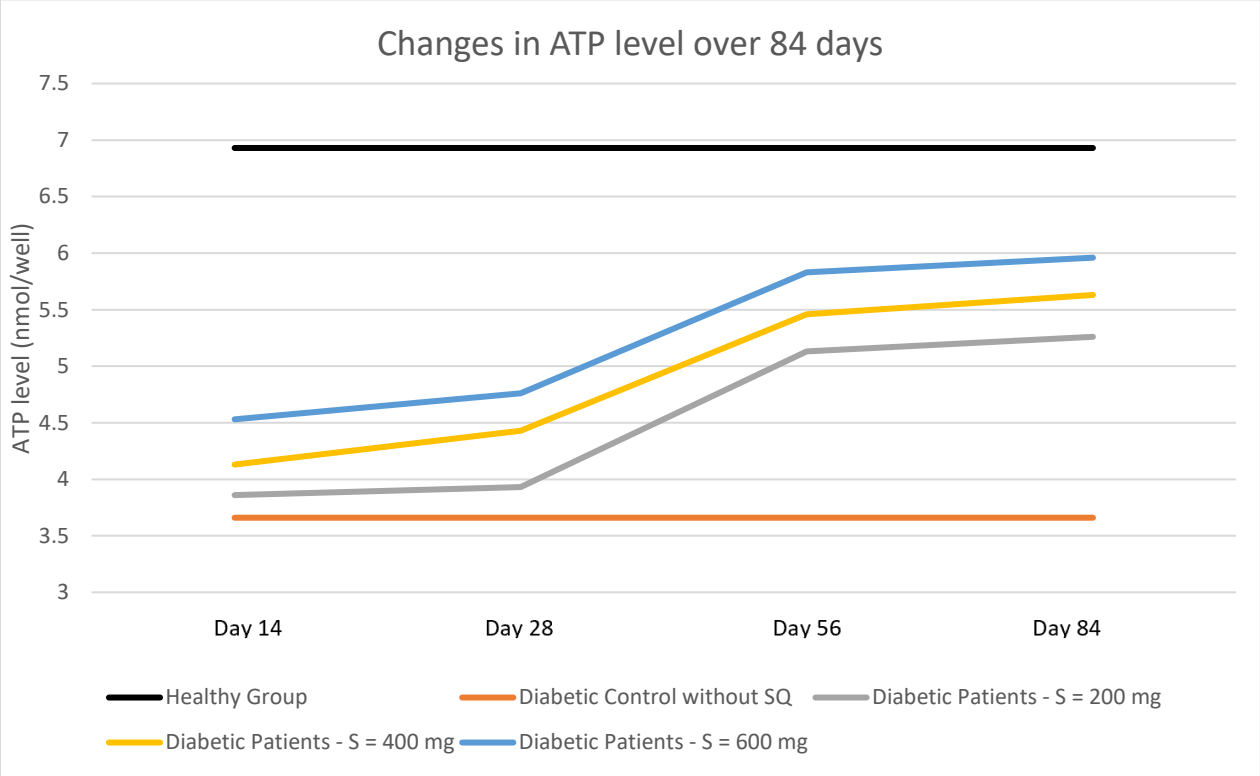



**Figure 1.** Changes in ATP level in all five experimental groups throughout 84 days

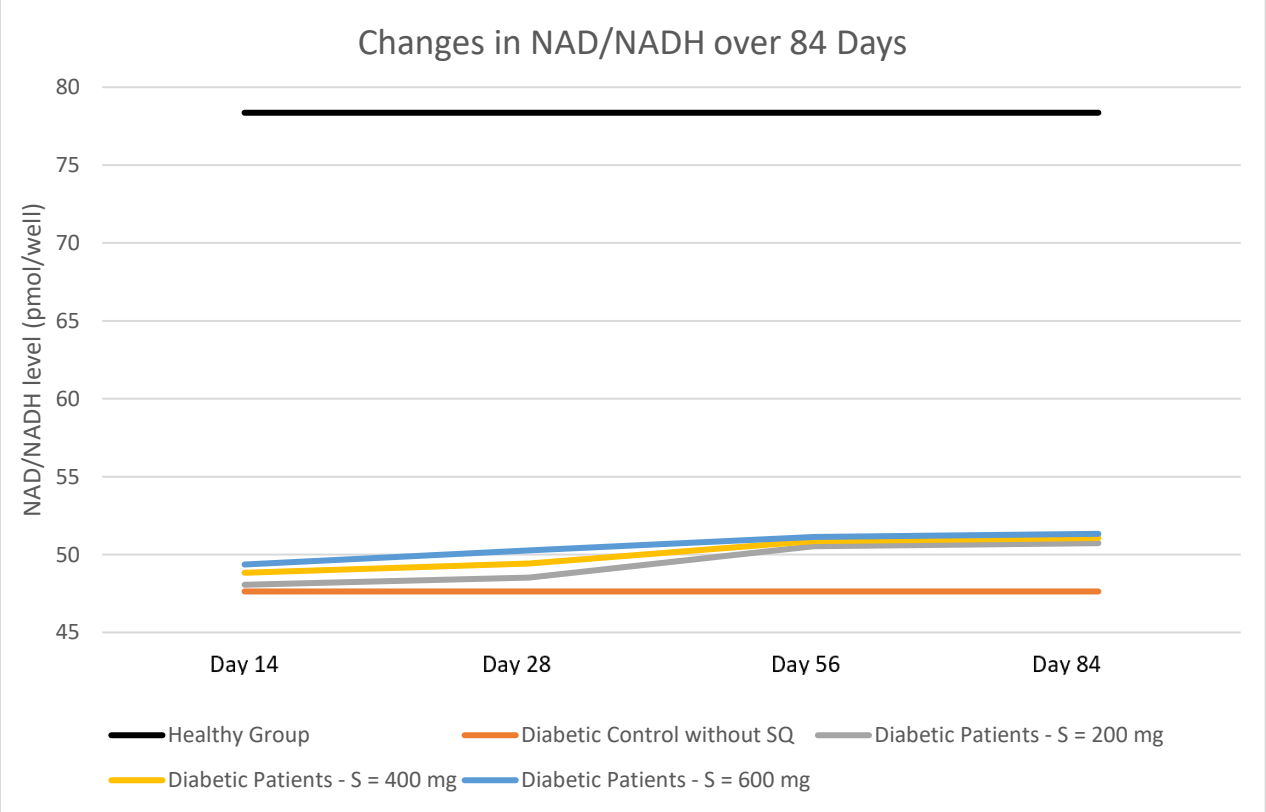



**Figure 2.** Changes in NAD/NADH level in all five experimental groups over 84 days

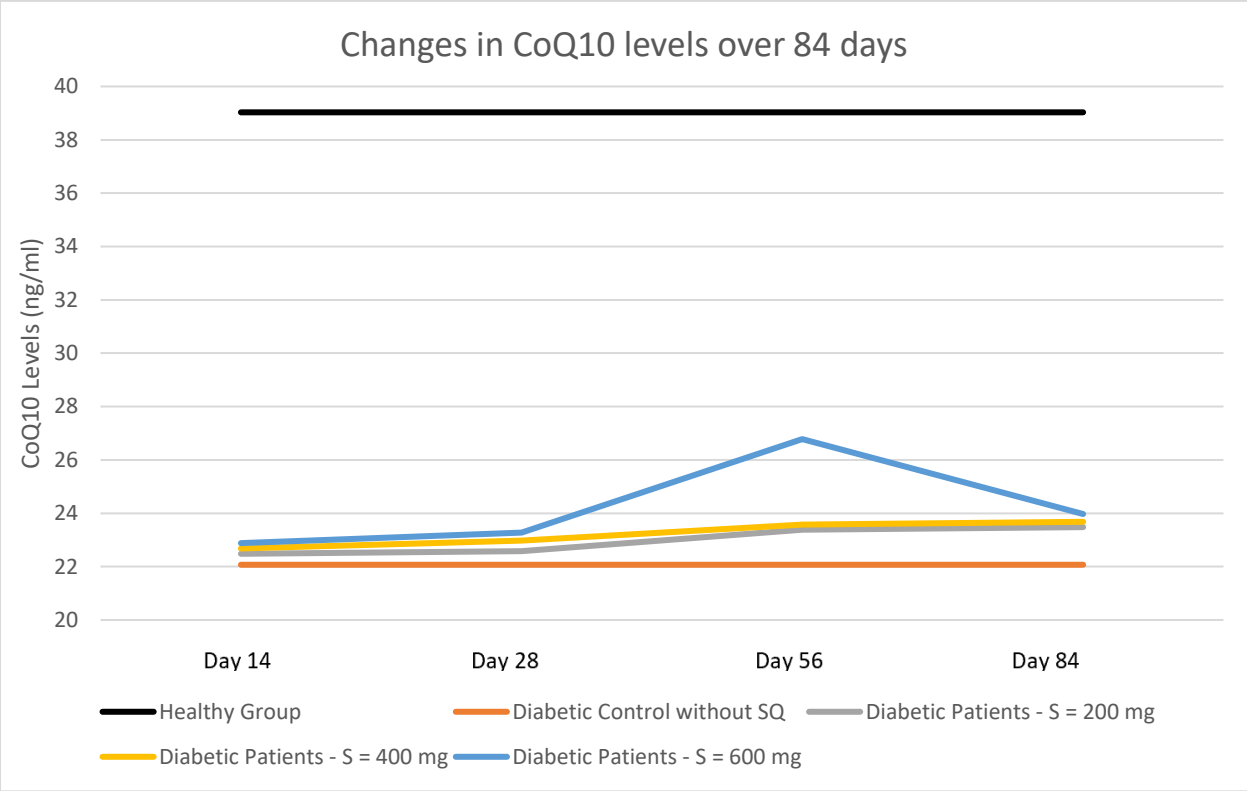



**Figure 3**. Changes in CoQ10 levels in all five experimental groups over 84 days

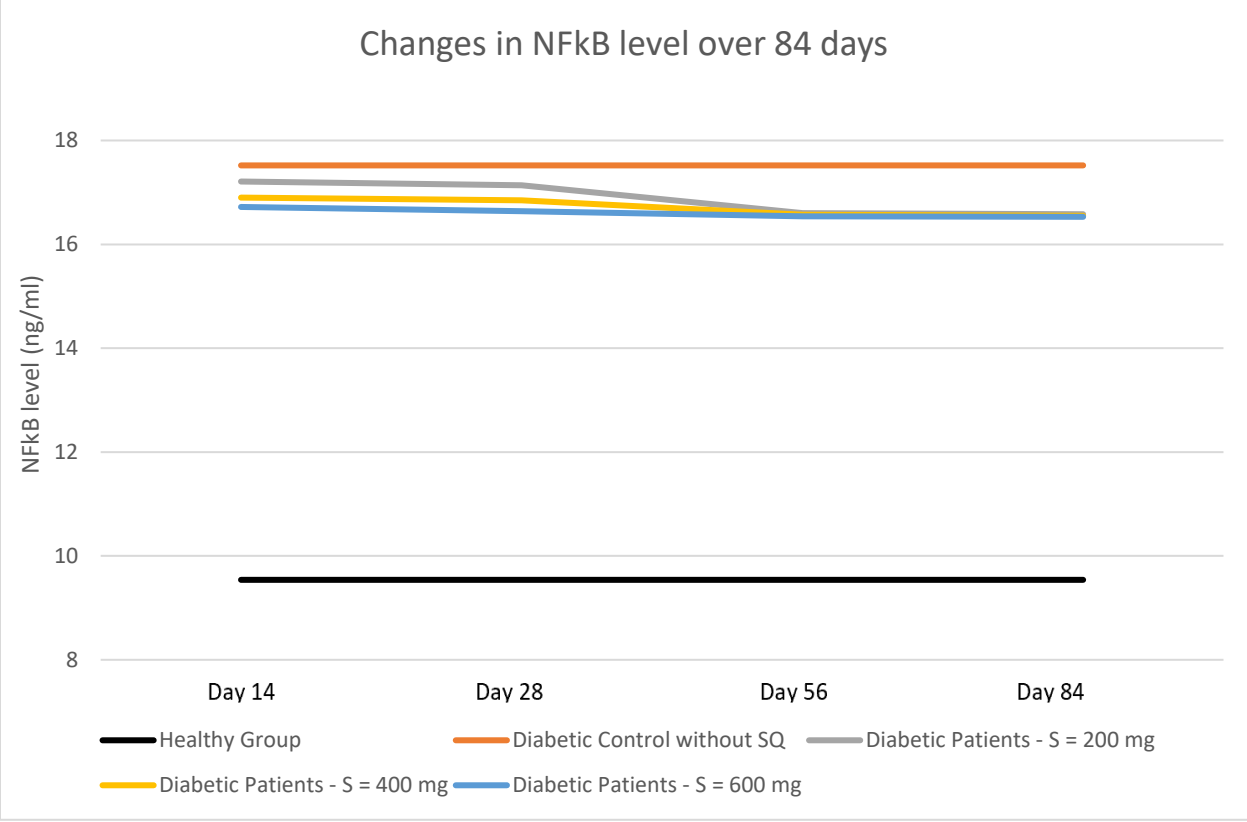



**Figure 4.** Changes in NFκB levels in all five experimental groups over 84 days

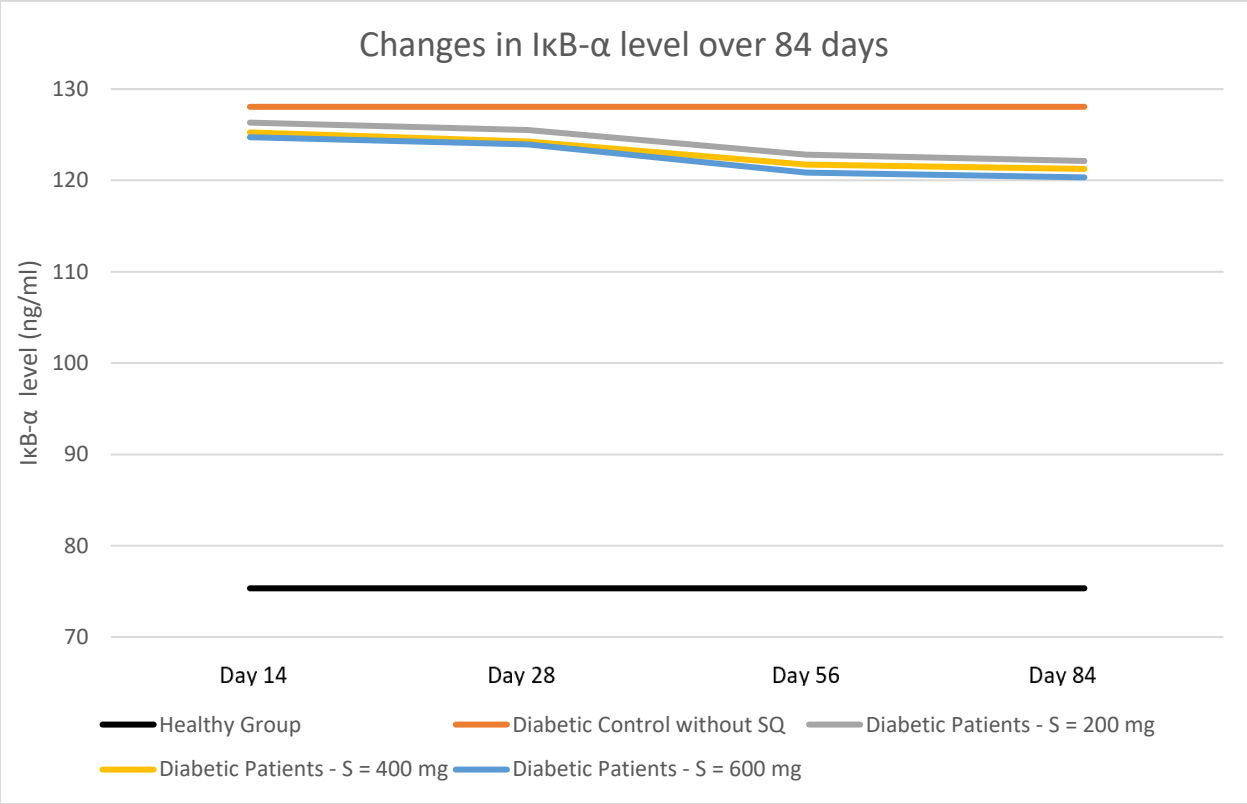



**Figure 5.** Changes in IκB-α levels in all five experimental groups over 84 days

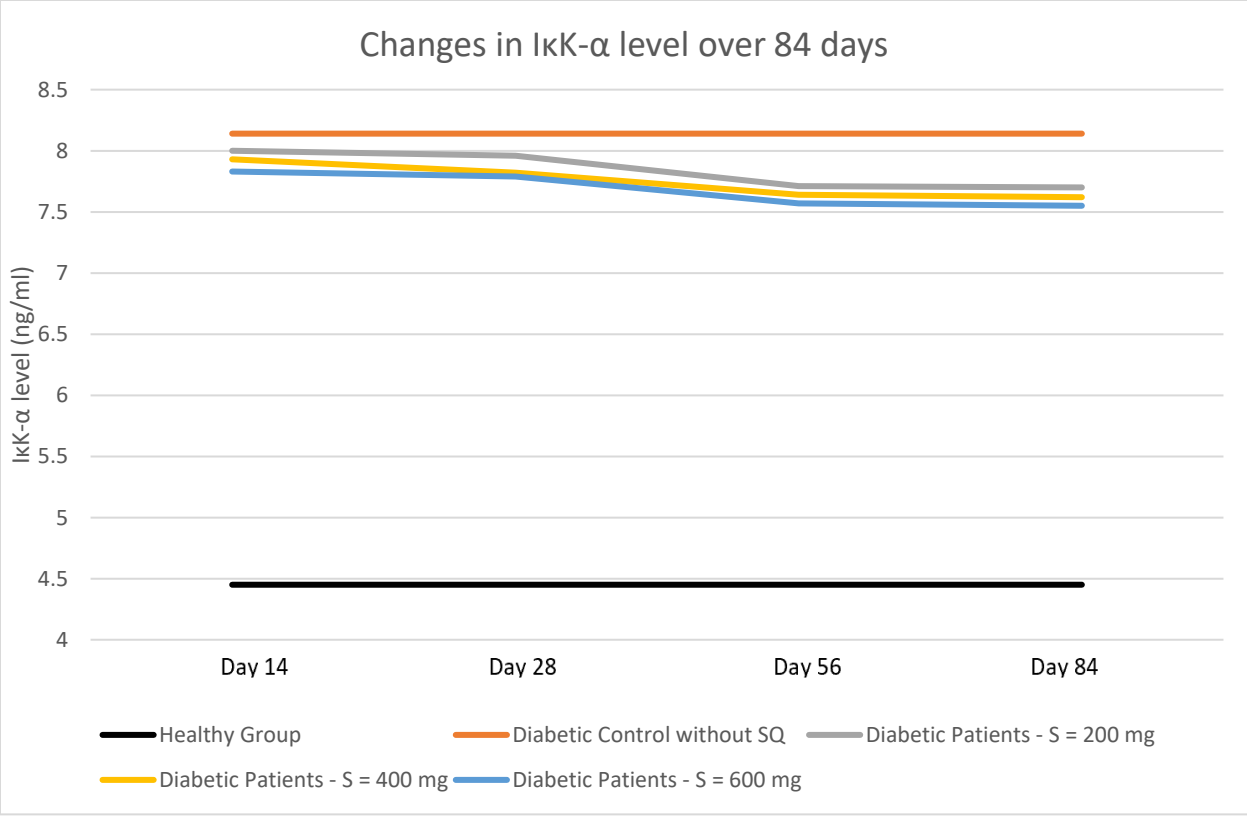



**Figure 6.** Changes in IκK-α level in all five experimental groups over 84 days

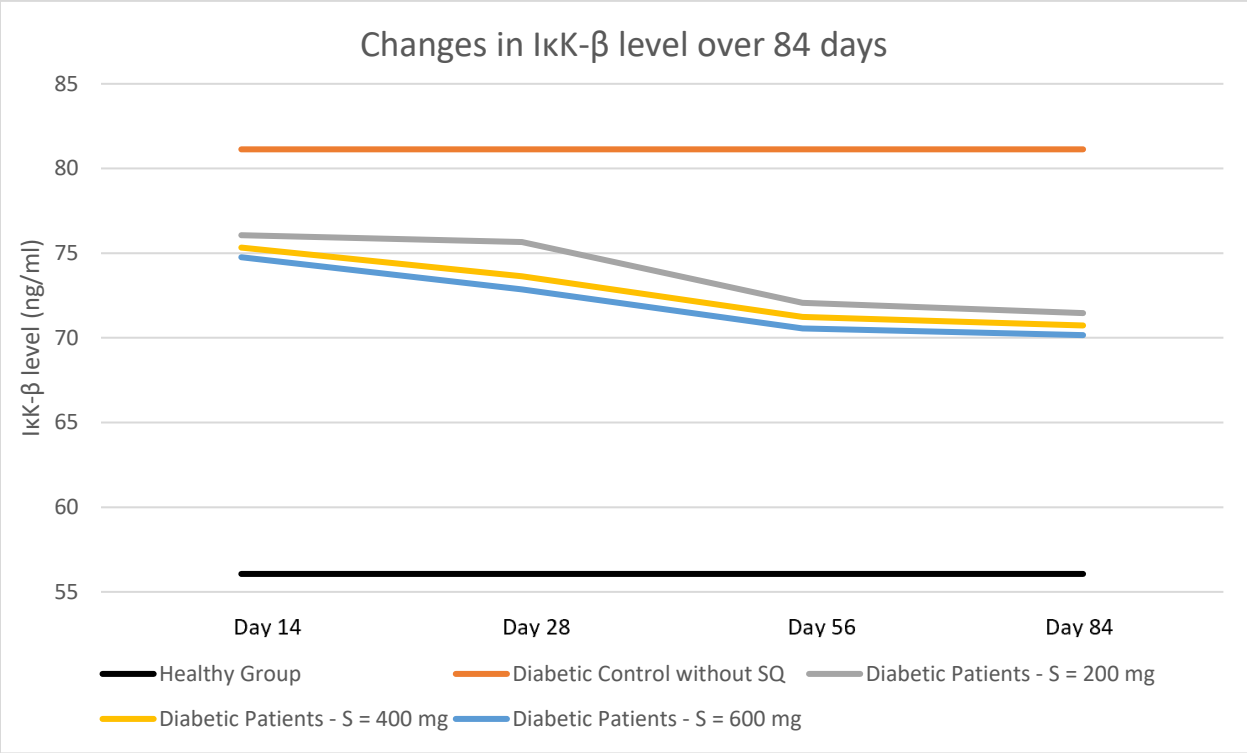



**Figure 7.** Changes in IκK-β level in all five experimental groups over 84 days

**DISCUSSION:** Squalene as a part of amaranth oil and a bioactive compound has been considered in many studies. The role of this natural triterpene as an antioxidant agent, anti-inflammatory factor, and other cases in diseases such as cancer and diabetes has been studied and discussed [8-11]. In this study, a number of factors related to energy production and cellular energy, such as ATP, NAD/NADH and CoQ10, as well as factors related to inflammation, such as NFκB, IκB-α, IκK-α and IκK-β, was investigated in patients with T2DM who received various doses of squalene on different days. The results of the study of the mentioned parameters showed that the changes in the levels of these parameters in diabetic patients receiving squalene are different in comparison with patients who did not receive squalene. In some cases, these changes depend on time and dose of squalene. Unlike other studies that have been conducted on animal and cellular models [12, 13], our study was conducted on patients with T2DM. In a study conducted in 2018 by Sánchez-Quesada et al., the effect of squalene on immune system stimulation and wound and tissue repair was studied [14]. They reported that the effect of squalene extracted from olive oil induces an increase in the synthesis of anti-inflammatory cytokines such as interleukins 10 and 13 in THP-1 macrophage cells. Squalene reduces pro-inflammatory signals such as TNF-α and NFκB in these cells. Skin damage is one of the consequences of uncontrolled diabetes that squalene can be effective in reducing and preventing. In our study, the changes in the levels of the pro-inflammatory signal NFκB in the diabetic groups were significant compared to the control group (table 1). In diabetic patients who received a dose of 600 mg of squalene on days 14, 28, 56 and 84, a significant decrease in NFκB was observed in comparison with the first day (table 2). Regarding the reduction of NFκB levels, our study agreed with the study of Sánchez-Quesada et al.

In a study conducted by Sanchez-Fidalgo et al., the effect of squalene at a concentration of 0.02% and 0.1%

for 1 month was investigated on mice models of acute colitis. They suggested that squalene can improve oxidative events and restore the expression of pro-inflammatory proteins to the baseline state [15]. This role of squalene is probably done through p38 mitogen-activated protein kinase (MAPK) and NFκB signaling pathways. In the study of Sanchez-Fidalgo et al., the levels of pro-inflammatory cytokines TNF-α and interleukin-1 beta (IL-1β) were also investigated. Squalene in both concentrations decreased the expression percentage of TNF-α and IL-1β, compared to the control group. This reduction was more significant with higher squalene concentration (0.1%). Mice treated with squalene in the two mentioned concentrations prevents the destruction and decomposition of IκB-α and blocks the translocation of NFκB family transcription factor (p65) from the cytoplasm to the nucleus. It has been found that IκB-α inhibits NFκB by covering the nuclear localization signals (NLS) of NFκB proteins and keeps them inactive in the cytoplasm [16]. In our study, a decrease in NFκB and IκB-α was observed in the group 3, 4 and 5 in some doses of squalene and on some days compared to the first day of treatment and the group 2. The levels of both of these inflammation-related factors were increased in the diabetic group compared to the control group.

As a result of the presence of reactive oxygen species (ROS) in the skin, the formation of NFκB and stimulation of the expression of pro-inflammatory factors occurs which can lead to skin damage. In a study that was conducted by Horax et al. on rats exposed to Ultraviolet-B (UV-B) rays, the effect of treating the rats' skin with squalene, as a topical administration, was investigated [17]. It was reported that treating the damaged skin of mice with squalene for 4 weeks was effective in reducing NFκB expression in fibroblast cells (The expression of NFκB in the untreated control group was almost 3 times that of the treated group).

ATP production in mitochondria depends on the proton gradient, and if electron transfer and proton production in the electron transport chain are disrupted, ATP production will also be disrupted. It has already been shown that ATP production is lower in diabetic patients whose disease is not controlled and there is insulin resistance [18]. According to the results in Table 2, there was a significant difference in the levels of ATP and energy-related factors such as NAD/NADH and CoQ10 in some groups receiving squalene compared to group 2 and compared to the first day. In the comparison of the mean of these parameters in groups 2, 3, 4 and 5 compared to the healthy control group, a significant decrease was observed. This result of our study confirms that DM reduces energy production. It can be stated that hyperglycemia causes a decrease in energy production and insulin resistance in the long term.

**CONCLUSION:** The result of this study indicated that squalene in natural compounds, as a bioactive compound, plays an important role in reducing inflammatory mediators, as well as increasing energy production, and in this way, it can be effective and useful in reducing the consequences of DM. Further studies are needed to validate the findings of this research.

**Abbreviations:** type 2 diabetes mellitus (T2DM), Adenosine triphosphate (ATP), low density cholesterol (LDL), very low-density cholesterol (VLDL), dextran sulfate sodium (DSS), glycated hemoglobin (HbA1c), Nicotinamide adenine dinucleotide (NAD), Coenzyme Q10 (CoQ10), Nuclear factor kappa B (NFκB), Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (IκB-α), IκB kinase α (IκK-α); IκB kinase β (IκK-β).

**Competing interests:** The authors declare that they have no competing interests.

**Authors contributions:** HM and DM discussed the idea of Squalene effects to the cellular energy level for diabetic

patients. HM contributed to the selection of volunteers to participate in the study and doing the experimental and clinical work. MRA and ASM participated in data collection and analysis of the results. DM, IS, and HS participated in the manuscript writing and drawing the graphs and analyzing the results. All authors reviewed, commented on, and approved the final manuscript.

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