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The effect of squalene on lipid profile and some oxidative biomarkers in patients with type 2 diabetes mellitus

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ABSTRACT

Background: Squalene, as an isoprene, is one of the components of amaranth oil and makes up about 8% of this oil. Squalene has anti-inflammatory and antioxidant effects that have been considered in many studies. In people with uncontrolled diabetes, there is an increase in total cholesterol and low-density lipoprotein (LDL) alongside a decrease in high-density lipoproteins (HDL). According to studies, squalene can be useful in lowering total cholesterol and triacylglycerol.

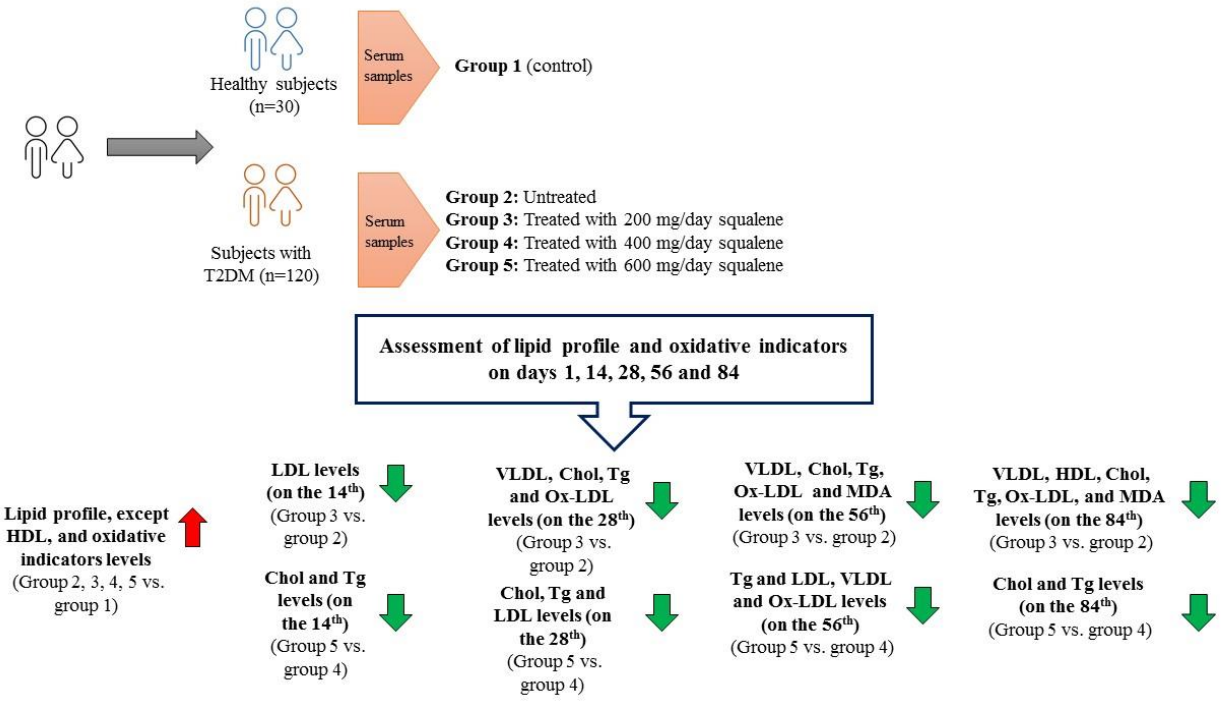
Objective: The aim of this study was to investigate whether squalene has an effect on lipid profile and a number of oxidative biomarkers in patients with type 2 diabetes.

Methods: In the present study, 30 healthy volunteers were selected as the control group and 120 volunteers with type 2 diabetes mellitus (T2DM) were selected. Subjects with diabetes were randomly divided into 4 groups. Group 1 was untreated with squalene and groups 3, 4, and 5 were treated with different doses of squalene for 84 days. Lipid profiles and oxidant biomarkers were examined on days 1, 14, 28, 56, and 84 according to the relevant protocols in all groups.

Results: Significant differences (P < 0.05) were observed between control and diabetic groups in the study of cholesterol, triglycerides, LDL, HDL, very low-density lipoproteins (VLDL), oxidized low-density lipoprotein (Ox-LDL), and malondialdehyde. There was a significant difference between the studied groups in the levels of parameters expressed in some different doses and days. At the levels of the parameters expressed in some doses and different days, a significant difference was observed between the groups treated and untreated with squalene.

Conclusion: This study shows that squalene as a bioactive compound can be effective to manage certain symptoms of diabetics including HDL, LDL, total cholesterol, and VLDL. From our findings, we observed that squalene consumption over the duration of 84 days resulted in increased levels of HDL in diabetic patients. It also resulted in decreased levels of total cholesterol, LDL, and VLDL in diabetic patients.

Keywords: Squalene, lipid profile, oxidative biomarkers, diabetes mellitus



INTRODUCTION

In diabetes, as a metabolic disease, in addition to increasing blood glucose, lipid profile levels also increase. Without controlling diabetes, increasing these biochemical parameters can lead to cardiovascular disease (CVD) [1].

The discovery of squalene in 1906 by Mitsumaru Tsujimoto led to the proof of its existence in human plasma by Goodman in 1964 [2], thereby leading to early studies of squalene and its effects [3-4]. One hundred grams of amaranth oil contains about 6 grams of squalene [5-6]. Squalene is a triterpene with 6 isoprene units, and is an intermediate for the synthesis of sterols, hormones, and vitamins [5, 6] that are found in both animal sources (e.g., sharks) [7] and plant sources (e.g., nuts and olive/nut oils) [8]. Studies have shown the effects of Squalene on the improvement of lipid factors. Among them, we can mention the effect of Squalene on

LDL, cholesterol, triglycerides, and fatty acids in studies [9-13]. The result of this research indicates the positive effects of Squalene on the mentioned parameters.

CVD is known as the leading cause of death and is a complication of type 2 diabetes, and it is associated with these parameters, in addition to high-density cholesterol (HDL) and very low-density cholesterol (VLDL) [14]. Furthermore, the results of studies on the relationship between CVD and hypertension indicate a direct relationship between them [15, 16]. The same is true of obesity, which is on the rise today [17]. Obesity and hypertension are among the factors that cause kidney disease in diabetics [18]. Albuminuria and proteinuria, which can indicate kidney disease, are no exception to the above rule, meaning they are associated with a higher risk of developing CVD [19, 20]. Studies have also shown that high serum IgA levels also play a role in the complications of diabetes. Elevated serum IgA levels are common in diabetic patients [21, 22].

In addition to the mentioned parameters, reactive oxygen species (ROS) such as hydrogen peroxide and superoxide anion also play an important role in causing diabetes and its complications [23].

In fact, the presence of ROS in the body is essential [24], but its high levels are effective in causing diabetes [24]. ROS also causes CVD through lipid peroxidation [25]. Lipid peroxidation results in the formation of oxidized low-density lipoprotein (Ox-LDL) [26], which in turn contributes to CVD by producing foam cells [27]. On the other hand, oxidative stress, which is an imbalance between oxidants and antioxidants [28] and has been linked to diabetes and CVD as mentioned earlier, increases the production of advanced glycation end products (AGEs) [29]. Ox-LDL, AGEs, and oxidative stress can increase the expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α) and interleukins [27, 30].

Antioxidants are one of the important factors that counteract oxidative stress and its destructive effects. Antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) fight against free radicals, especially superoxide anion radicals, and in this way, they play a role in controlling oxidative stress, its effects on the body, and causing disease [31].

The study observed the effects of amaranth oil on coronary heart disease and hypertension, and measured parameters such as lipid, inflammatory, oxidant, and antioxidant factors [Martirosyan]. To answer the question of whether squalene as one of the main components of amaranth oil produced the observed effects or not, we have examined the effect of pure squalene on the parameters related to diabetes in this study.

MATERIALS AND METHODS

Materials: Squalene (S3626) was purchased from Sigma Company (USA). The human glucose assay kit was purchased from MyBioSource Inc (USA). The malondialdehyde (MDA) assay kit was procured from Cayman chemical company (USA). The concentration of oxidized-low density lipoprotein (Ox-LDL) was determined by the kit purchased from Mercodia Company (Sweden). The assay kits of lipid factors including LDL and VLDL were purchased from MyBioSource Inc (USA). The HDL assay kit was prepared from abcam Company (USA). The assay kits of cholesterol and triglyceride were procured from Sigma Company (USA) and Pars Azmoon Company (Iran), respectively.

Participants: In this randomized study, 150 volunteers participated based on the following grouping:

Group 1: 30 healthy people (as control)

Group 2: 30 patients with T2DM, without consumption of squalene

Group 3: 30 patients with T2DM treated with 200 mg/day squalene

Group 4: 30 patients with T2DM treated with 400 mg/day squalene

Group 5: 30 patients with T2DM treated with 600 mg/day squalene

Patients in groups 3, 4, and 5 consumed squalene for 84 days. T2DM patients were selected among the people referred to Vali-Asr medical laboratory in Tehran, Iran. Concurring to the World Health Organization (WHO) criteria, individuals with fasting plasma glucose ≥ 126 mg/dL or glycated hemoglobin (HbA1c) ≥ 6.5% were considered as diagnosed T2DM. Informed consent was obtained from all patients.

General Features and Sampling: After grouping, blood samples were taken from all participants. Sampling was performed in five time periods on days 1, 14, 28, 56, and 84. In each of these time periods, anthropometric items including age, sex, weight, height, body mass index (BMI), systolic blood pressure, and diastolic blood pressure of all study participants were recorded. Blood samples were taken from all participants after 12 hours of nighttime fasting. After collecting blood samples, they were centrifuged (250 g for 10 min); following this, the serum was separated from the centrifuged samples. Then, isolated serum samples were used to assess the biochemical parameters.

Biochemical Measurement: In each of the above time periods, biochemical parameters were measured in all five groups. Two laboratory methods were used to measure these parameters: Enzyme–linked immunosorbent assay (ELISA) for glucose, Ox-LDL, VLDL, and colorimetric detection for cholesterol, triglyceride, HDL, LDL, MDA.

Statistical Analysis: Statistical analysis was done by Statistical Package for the Social Sciences (SPSS) (version 23, IBM, USA) software for Windows. All results were expressed as mean ± standard deviation (SD). An independent-sample T-test was used to compare the mean of general characteristics of the participants. Statistical significance was analyzed by a one-way ANOVA to compare the mean of the obtained data. 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-values < 0.05 were considered significant.

RESULTS

General Features: The results for the general features of control and diabetic groups are given in Table 1. As shown in the table, BMI of control individuals were compared with those of diabetics. Statistically, there was a significant difference in comparison of BMI between the diabetic and control groups.

We also evaluated some important biomedical parameters (cholesterol, triglycerides, HDL, LDL, VLDL, Ox-LDL, MDA) five times in the serum sample of the control group and diabetics along with four doses of squalene (the first day, fourteenth day, the twenty-eighth day, the fifty-sixth day, the eighty-fourth day in 0 mg/day, 200 mg/day, 400mg/day and 600 mg/day doses). A significant difference was detected in the comparison of ox-LDL (28^{th}, 56^{th}, 84^{th} day in 200 mg/day), cholesterol (14^{th} day/ in 400 and 600 mg/day, 28^{th} day/ in 400 and 600 mg/day, 56^{th} day in 200 mg/day, 84^{th} day in 200, 400, 600 mg/day), triglycerides (14^{th} day in 400 and 600 mg/day, 28^{th} day in 200 mg/day, 56^{th} in 200 mg/day, 84^{th} day in 200 mg/day), HDL (84^{th} day in 200 mg/day), LDL (14^{th} day in 200mg/day, 56^{th} day in 200 mg/day and 84^{th} day in 200 mg/ day), VLDL (56^{th} day in 200, 400, 600 mg/day, 84^{th} day in 200 mg/day), and MDA (56^{th} in 200 mg/day and 84^{th} day in 200 mg/day) (Table1, Table2). There was a statistically significant difference (P value < 0.05) in the results of Diabetic groups compared to the control group.

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

Parameters / Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mmol/l)	HDL (µg/ml)	Ox-LDL (mU/l)	VLDL (µg/ml)	MDA (µmol/l)	BMI (kg/m^2)
Healthy 1 th day	159.63±5.1	101.63±5.6	5.83±0.9	193.83±19.7	12.46±0.8	27.37±3.9	2.77±0.1	30.60±3.6
Diabetic control (No squalene) 1 th day	192.12±6.8	142.36±4.4	10.72±0.8	121.16±10.5	20.61±1.1	41.42±3.9	3.60±0.2	35.83±3.6
Healthy 14 th day	159.63±5.1	101.63±5.6	5.83±0.9	193.83±19.7	12.46±0.8	27.37±3.9	2.77±0.1	36.13±2.9
Diabetic control (no squalene) 14 th day	192.03±6.9	142.36±4.4	10.72±0.8	121.16±10.5	20.61±1.1	41.42±3.9	3.60±0.2	35.90±3.0
Diabetic /200 squ/14 th day	191.06±7.0	141.36±4.4	9.81±0.8	122.83±9.3	20.25±1.1	40.53±3.9	3.59±0.2	34.80±3.8
Diabetic /400 squ/14 th day	190.03±6.9	140.36±4.4	9.80±0.8	123.26±10.5	20.09±1.2	40.18±3.9	3.58±0.2	30.60±3.6
Diabetic /600 squ/14 th day	186.06±6.9	137.36±4.4	9.77±0.8	124.93±10.1	19.82±1.1	39.24±3.9	3.54±0.2	35.83±3.6
Healthy 28 th day	159.63±5.1	101.63±5.6	5.83±0.9	193.83±19.8	12.46±0.8	27.37±3.9	2.77±0.1	36±2.9
Diabetic control (no squalene) 28 th day	192.03±6.9	142.36±4.4	10.72±0.8	121.16±10.6	20.61±1.1	41.42±3.9	3.60±0.2	35.46±3.0
Diabetic /200 squ/28 th day	189.03±6.9	140.03±3.5	10.72±0.8	122.53±9.9	19.71±1.1	39.75±3.9	3.58±0.2	34.40±3.8
Diabetic /400 squ/28 th day	187.03±6.9	138.13±3.4	9.68±0.7	125.53±9.3	19.61±1.2	38.56±3.7	3.56±0.2	30.60±3.6
Diabetic /600 squ/28 th day	181.03±6.9	136.16±4.5	9.47±0.8	127.23±9.9	19.13±1.2	37.44±3.7	3.54±0.2	35.83±3.6
Healthy 56 th day	159.63±5.1	101.63±5.6	5.83±0.9	193.83±19.8	12.46±0.8	27.37±3.9	2.77±0.1	35.73±2.7
Diabetic control (no squalene) 56 th day	192.03±6.9	142.36±4.4	10.72±0.8	121.16±10.6	20.61±1.1	41.42±3.9	3.60±0.2	35.10±2.8
Diabetic /200 squ/56 th day	185.36±13	132.86±4.5	9.38±0.8	126.26±8.6	19.44±1.5	38.37±3.9	3.46±0.2	34.03±3.7
Diabetic /400 squ/56 th day	180.03±6.9	132.86±4.5	8.86±1.1	130.76±10.8	19.08±1.1	35.31±3.9	3.44±0.2	30.60±3.6
Diabetic /600 squ/56 th day	178.03±6.9	130.73±3.7	8.37±0.8	133.16±10.5	18.31±1.2	33.29±3.9	3.42±0.2	35.83±3.6
Healthy 84 th day	159.63±5.1	101.63±5.6	5.83±0.9	193.83±19.7	12.46±0.8	27.37±3.9	2.77±0.1	35.50±2.8
Diabetic control (no squalene) 84 th day	192.03±6.9	142.36±4.4	10.72±0.8	121.16±10.5	20.61±1.1	41.42±3.9	3.60±0.2	34.93±2.8
Diabetic /200 squ/84 th day	181.03±6.9	134.36±4.4	9.62±0.8	130.16±10.5	19.04±1.1	38.38±3.9	3.45±0.2	33.60±3.6
Diabetic /400 squ/84 th day	180.23±4.7	133.36±4.4	9.55±0.8	131.16±10.5	18.81±1.1	37.40±3.9	3.43±0.2	30.60±3.6
Diabetic /600 squ/84 th day	177.03±6.9	131.36±4.4	9.54±0.8	134.16±10.5	18.61±1.1	36.37±3.9	3.41±0.2	35.83±3.6

Data are given as mean ± SD. LDL, low density lipoprotein; HDL, high density lipoprotein; Ox-LDL, oxidized-low density lipoprotein; VLDL, very low-density lipoprotein; MDA, malondialdehyde; BMI, body mass index

Table 2: Multiple comparisons between the levels of biochemical parameters between the groups

Parameters / Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mmol/l)	HDL (µg/ml)	Ox-LDL (mU/l)	VLDL (µg/ml)	MDA (µmol/l)	BMI (kg/m²)
Diabetic14day vs. Diabetic200squ14day	0.55	0.42	0.000	0.50	0.20	0.35	0.89	0.70
Diabetic400squ14day vs. Diabetic600squ14day	0.02	0.001	0.88	0.54	0.42	0.34	0.26	0.31
Diabetic28day vs. Diabetic200squ28day	0.09	0.02	0.24	0.65	0.004	0.09	0.67	0.83
Diabetic400squ28day vs. Diabetic600squ28day	0.001	0.07	0.000	0.49	0.13	0.19	0.44	0.32
Diabetic56day vs. Diabetic200squ56day	0.003	0.000	0.06	0.07	0.002	0.003	0.003	0.89
Diabetic400squ56day vs. Diabetic600squ56day	0.33	0.09	0.000	0.14	0.010	0.01	0.74	0.29
Diabetic 84 day vs. Diabetic 200 squ84day	0.000	0.000	0.94	0.002	0.000	0.01	0.01	0.67
Diabetic400squ84day vs. Diabetic600squ84day	0.016	0.05	0.14	0.19	0.18	0.17	0.59	0.18
	P value							

LDL, low density lipoprotein; HDL, high density lipoprotein; Ox-LDL, oxidized-low density lipoprotein; VLDL, very low-density lipoprotein; MDA, malondialdehyde; BMI, body mass index

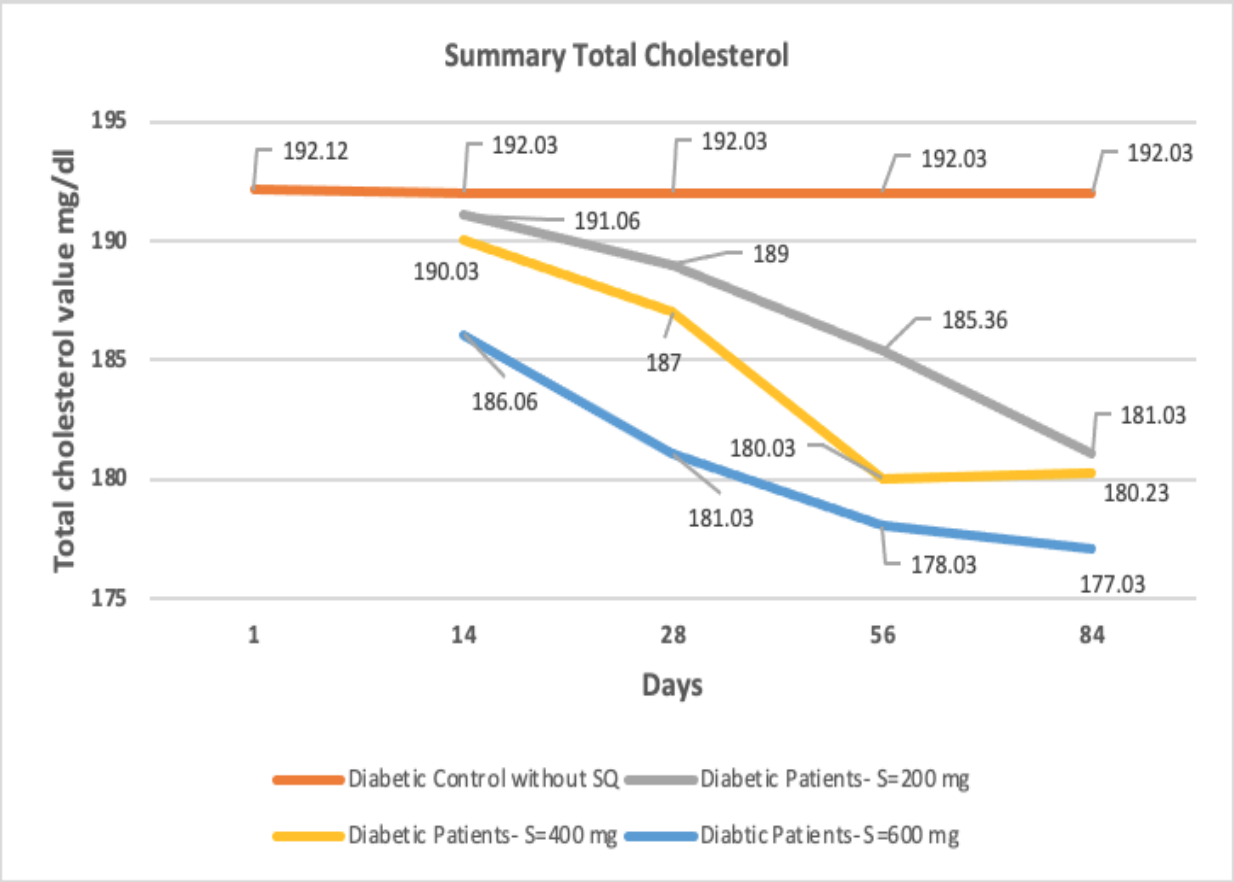


Figure 1: The effects of Squalene on total cholesterol over 84 days. Data are given as mean.

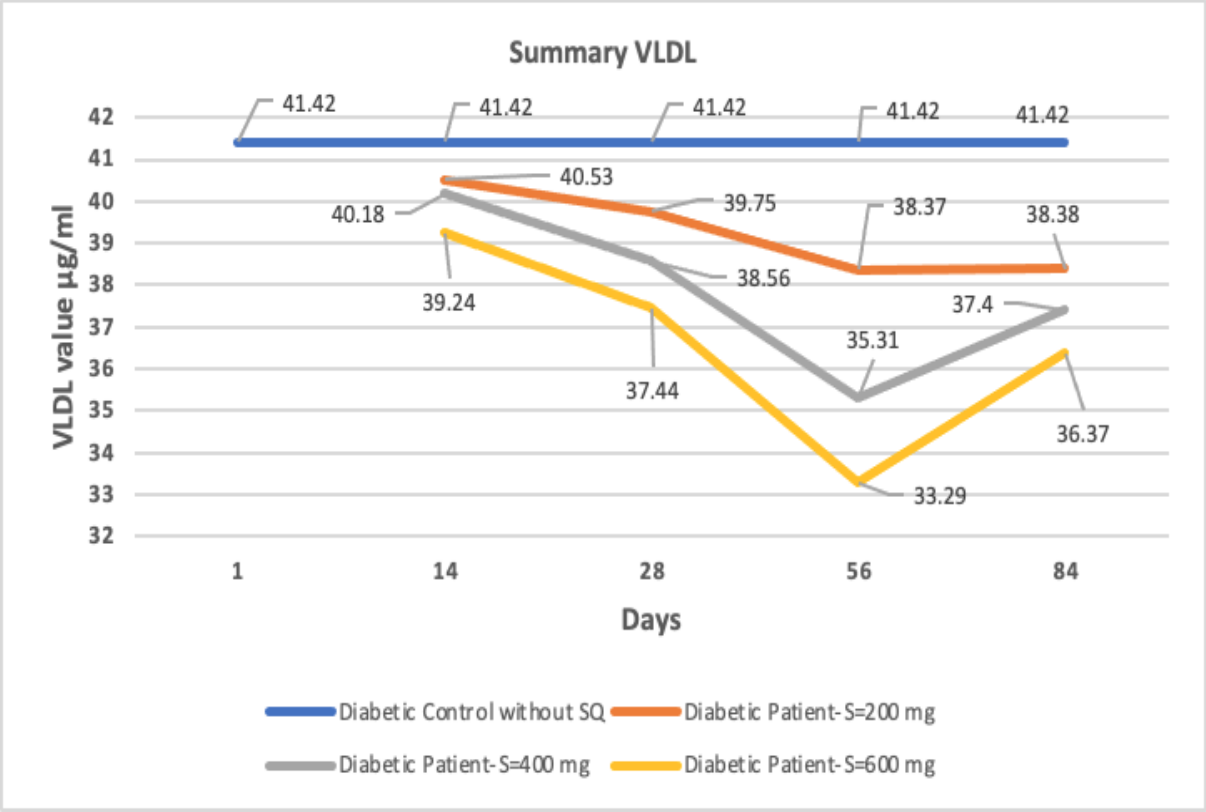


Figure 2: The effects of Squalene on VLDL over 84 days**.** Data are given as mean

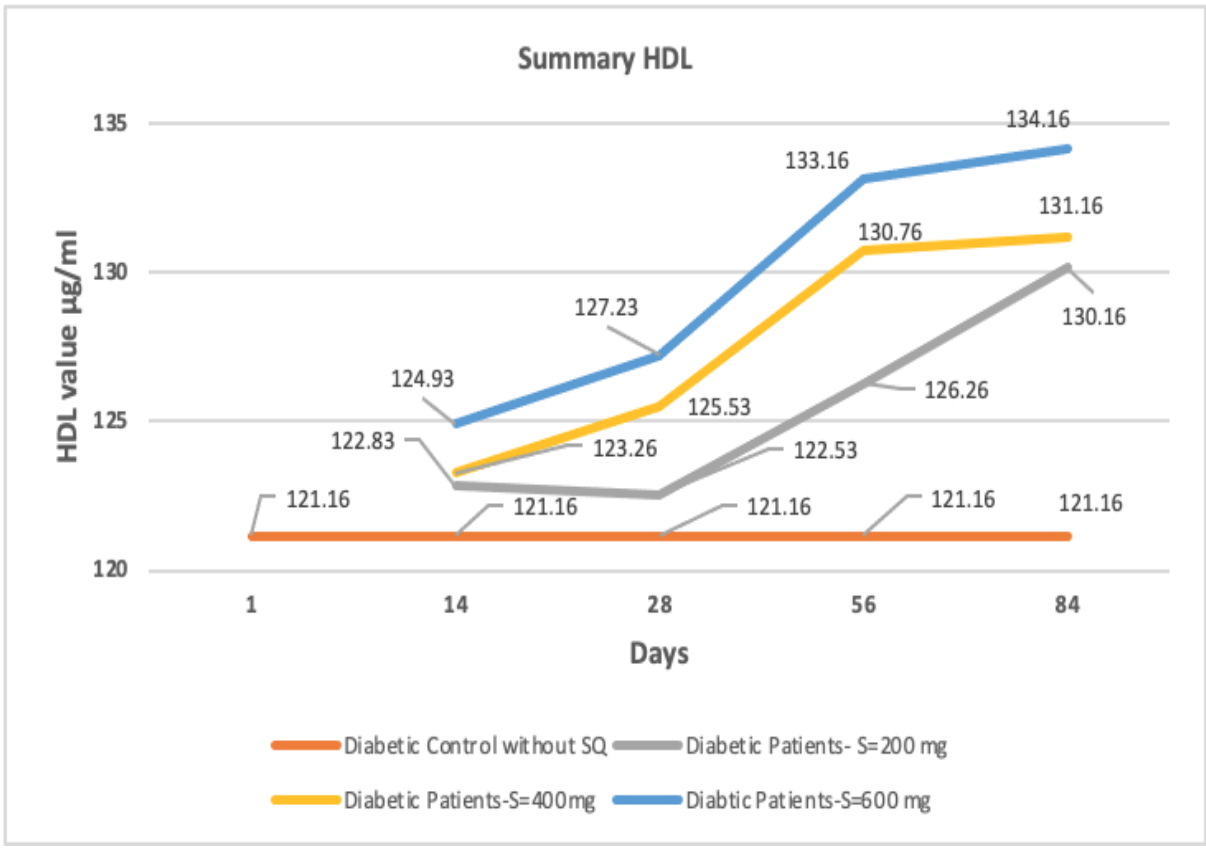


Figure 3: The effects of Squalene on HDL over 84 days. Data are given as mean.

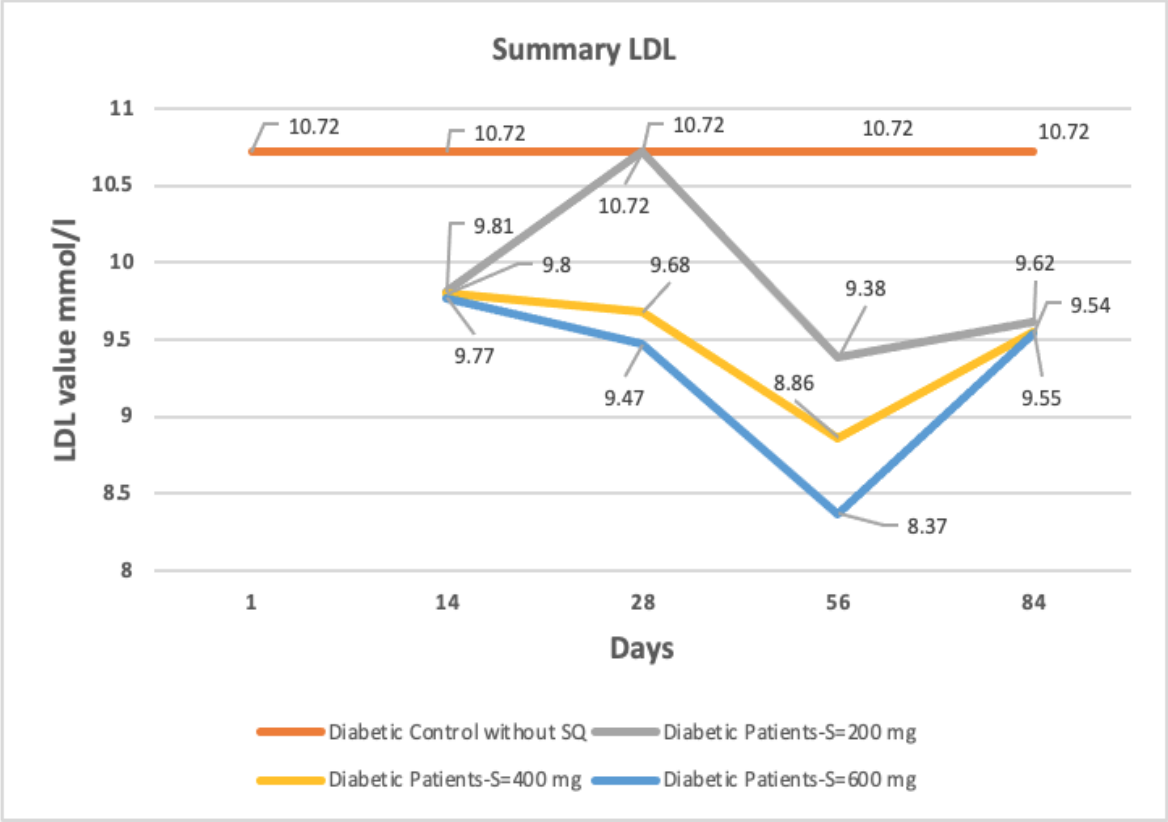


Figure 4: The effects of Squalene on LDL over 84 days. Data are given as mean.

DISCUSSION

In this study, the effect of squalene on lipid profile and oxidative indices was investigated. Increased levels of total cholesterol, LDL, as well as the ratio of LDL to HDL in the blood, seem to play a role in the development of cardiovascular disease. In a way, it can be said that these parameters are important risk factors in the development of cardiovascular disease and other diseases [32]. Squalene, along with intake of some vitamins and polyunsaturated fatty acids, can regulate lipid metabolism [33]. It was reported that squalene has antioxidant properties and thus can have a protective effect on the heart [34]. Due to the antioxidant effects of squalene, a decrease in oxidative indices and ROS is expected.

Few studies have been performed on the effects of squalene on biochemical parameters in human models. Gabas-Rivera et al. [12] investigated the effect of squalene on lipid profiles in animal models. They reported that dietary squalene increased levels of HDL-cholesterol and paraoxonase-1, and decreased total cholesterol at a 1g/kg dose in Apoe-deficient mice. They also showed that treating mice with the same dose of squalene significantly reduced MDA levels. They additionally reported that 1g/kg of squalene significantly increased HDL in wild-type mice. In the present study, treatment of diabetics with a dose of 600 mg of squalene on the 56th day in comparison to untreated diabetics showed a significant decrease in LDL levels, as shown in figure 4. This study did not show a significant decrease in MDA levels but still showed slightly positive effects. HDL levels showed a significant increase in diabetics receiving squalene (at a dose of 200, 400 and 600 mg on the 84th day) compared to diabetics without squalene treatment.

In our study, we observed HDL levels to be maximized in diabetic patients consuming 600 mg of squalene daily. As shown in figure 3, it is observed that HDL levels increased by 7.38% in this group from day 14

to day 84. The highest increase, however, was observed specifically between days 28 to 56, where there was a 4.66% increase of HDL within this group in these two data measurements alone. Therefore, from days 14 to 56, there was an overall increase of 6.58% HDL in this group. By analyzing these percentage changes, we see the importance of longevity in prescribing squalene as a potential agent in increasing HDL levels. This is because the highest percentage increase in HDL was observed between days 28 and 56 (4.66%). We see that the increase between days 28 and 56 specifically makes up 63.14% of the total increase in HDL in group 5 (diabetic patients who consumed 600 mg of squalene daily). Taking 600 mg of squalene daily for less than 56 days, consequently, would not adequately provide an optimal increase in HDL level (as there was only a 1.84% increase from days 14 to 28 in this group).

This idea of importance in longevity in prescription of squalene to increase HDL levels is also shown in group 4 (diabetic patients that consumed 400 mg of squalene daily). As shown in figure 3, the highest percentage increase of HDL in this group was observed between days 28 and 56. In this group, HDL increased by 4.16% between these days. However, from days 56 to 84, there was only a 0.3% increase. Therefore, if 400 mg of squalene is used to increase HDL, optimal results occur until at least 56 days. Prior to this (from days 14 to 28), there was only a 1.84% increase of HDL in this group.

Continuing this trend, we see that group 3 (diabetic patients that consumed 200 mg of squalene daily) showed the greatest increase in HDL levels after 28 days (figure 3). However, in this group, HDL levels actually decreased by 0.24% from days 14 to 28. From these findings, if 200 mg of squalene was prescribed to diabetics to increase HDL, it would be ineffective to prescribe for less than 28 days. From days 28 to 56, we observed a 3.04% increase in HDL in this group, and from days 56 to 84, we observed a 3.08% increase in HDL. These results indicate that if 200 mg of squalene was prescribed to diabetics to increase HDL, the most effective increase would be shown after a duration of 84 days. As described in the previous two groups mentioned, the duration of the prescription of squalene intake plays an important role in the effectiveness of increasing HDL.

In a study by Scolastici et al., [35] squalene was given to Wistar rats hepatocarcinogenesis model at doses of 100 and 150 mg per 100 g (for 56 days). They suggested that squalene has no protective effect against carcinogens and also increases the level of total cholesterol. However, in our study, squalene significantly reduced LDL at doses of 400 and 600 mg (at 56 days) in squalene-treated diabetic patients as compared to the untreated diabetic patients.

Zhang et al. [36] studied the effect of squalene and shark liver oil on serum lipid levels in hamsters. They treated the hamsters with doses of 0.05%, 0.5%, and 0.1% squalene for 4 weeks. They also studied cholesterol levels in adipose, liver, and heart tissue. They reported that the serum total cholesterol levels increased significantly in the treated-squalene group with doses of 0.5% and 0.05% compared to the control group. In the group treated with a 0.5% squalene dose, their serum triglyceride and HDL levels showed a significant increase compared to the control group. However, the increase in these lipid parameters were not significant at other doses of squalene. They found that the effect of squalene at doses of 0.1%, 0.5%, and 0.05% on cholesterol concentration in liver tissue was significantly higher compared to the control group. Squalene at a dose of 0.1% had a significant reduction in adipose tissue cholesterol. Squalene at a dose of 0.5%, compared to other doses, was significantly stored in the liver and adipose tissue.

Kim et al. [37] studied the effect of amaranth on the lipid profile of diabetic rats. They found that both

amaranth grain and amaranth oil consumption by diabetic animals decreased total cholesterol, triglyceride, and VLDL concentrations. In our study, treatment of the diabetic group with squalene at doses of 200, 400, and 600 mg and on the 28, 56, and 84 days showed a significant reduction in serum cholesterol compared to the diabetic group without treatment with squalene. There was a significant decrease in triglyceride levels in the diabetic group treated with 600 mg for 56 days when compared to the untreated group.

In a study done by Martirosyan et al. [6], 125 patients were studied with CVD, specifically coronary heart disease and hypertension accompanied with obesity. They found that amaranth oil, a food product with a significant amount of Squalene, produced a statistically significant decrease in total cholesterol from 6.60 to 5.29 mmol/L with the dose of 600 mg of squalene for 21 days. There was additionally a significant decrease in total cholesterol with the doses of 100, 200, and 400 mg of squalene for 21 days. They also found decreases in triglycerides from 2.49 to 1.58 mmol/L, LDL from 4.42 to 3.32 mmol/L, and VLDL from 1.25 to 0.79 mmol/L; and an increase of HDL from 1.15 to 1.19 mmol/L all with the dose of 600 mg for 21 days. However, the changes of these parameters were not statistically significant. In our study, we showed that there was a significant difference in total cholesterol levels between the diabetic group that received 600 mg and the diabetic group that received 400 mg on days 14 and 28. The study also showed a significant difference in HDL levels on day 56 between the groups that received 400 mg and 600 mg compared to the diabetic control group, as well as in the diabetic group that received 600 mg with the diabetic group that received 200 mg. Additionally, the diabetic control group and the diabetic group that received 200 mg showed a significant difference of HDL on day 84. The study also showed a significant difference in LDL levels on day 14, 28, and 56, but showed reverse effects after 56 days. This data shows that in some parameters, significant changes are directly related to the duration of treatment and the dose of squalene. In a study by Hoang et al., [38] Hep G2 cell lines were treated with squalene (at concentrations of 50 and 100 μM). At these squalene concentrations, PPARα transcription was induced more than 75% and 100% as compared to the control group. They mentioned that squalene is a peroxisome proliferator-activated receptor-alpha (PPARα) agonist. They also studied the effects of squalene on intracellular lipid levels. It was reported that squalene significantly reduced triglyceride and cholesterol content compared to the control group. It has also shown an increase in HDL levels. This reduction in lipid levels is dose dependent. In this case, our study agrees with the research of Hoang and colleagues in that squalene may play a protective role against CVD, atherosclerosis, and insulin resistance by absorbing blood fatty acids into hepatocytes. In our study, alterations in total cholesterol, triacylglycerol, and HDL levels were also significant at some doses and times (dose-dependent and time-dependent). For example, in figure 4, the lowest level of LDL was found to be in the group 5 (diabetic group which consumed 600 mg of squalene daily) on day 56 (8.37 mmol/l). This is a 14.32% decrease in levels from day 14, which had been 9.77 mmol/l. However, on day 84, the level of LDL spiked to 9.54 mmol/l, indicating a 13.97% increase in LDL.

Additionally, group 3 (diabetic group which consumed 200 mg of squalene daily) showed a 9.27% increase in LDL from days 14 to 28. From days 28 to 56, however, this dropped by 12.5%. Therefore, if patients were instructed to consume a 200 mg dosage of squalene, it would show maximum yield reduction in LDL if taken for at least 56 days. Past this point, we see a 2.55% increase in LDL in group 3, thereby nullifying the necessity to intake longer than 56 days. These key findings indicate the significance for future studies in quantifying maximum dosages and setting time frames

for the greatest yield benefit. Currently the Functional Food Center classifies functional foods as being, "natural or processed foods that contain biologically-active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms''[39]. Future investigations should be focused on the molecular mechanisms of squalene lipid managing properties, thereby choosing an appropriate food vehicle to bring to the market.

CONCLUSION

There were significant differences between the control group and the diabetic groups that consumed squalene. This is because squalene consumption worked in raising HDL levels and reducing total cholesterol, LDL, triglyceride, and VLDL levels as well as oxidizing biomarkers. A key finding of this study was the importance of dosage amounts and prescription duration, as certain results were found to be most effective with a certain dosage before or after a certain data recording day. The results of this study signify the importance of squalene as a potential management agent for reducing the symptoms in type 2 diabetic patients. Future studies focusing on dosage amounts, longer durations tested, and larger sample sizes can allow for advancement in understanding the effectiveness of squalene to manage the symptoms in patients with type 2 diabetes.

List of Abbreviations: LDL: AGEs, Advanced glycation end products; BMI, Body mass index; CVD, Cardiovascular disease; Catalase, CAT; ELISA, Enzyme–linked immunosorbent assay; GPX, Glutathione peroxidase; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; Malondialdehyde, MDA; Ox-LDL, Oxidized low-density lipoprotein; PPARα, Peroxisome proliferator-activated receptor-alpha; ROS, Reactive oxygen species; SD, Standard deviation; SPSS, Statistical Package for the Social Sciences; SOD, Superoxide dismutase; TNF-α, Tumor necrosis factor-alpha; T2DM, Type 2 Diabetes Mellitus; VLDL, Very low-density lipoprotein; WHO: World Health Organization;

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