



Inflammatory factors and immunoglobulins alterations in subjects with type 2 diabetes mellitus treated with squalene

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ABSTRACT

Background: Squalene is a 30-carbon (as a polyunsaturated triterpene) compound that has been suggested to have several benefits. In recent years, its anti-diabetic, anti-inflammatory and antioxidant effects have been studied.

Objective: The aim of this study was to investigate the effect of different doses of squalene on different days on the changes in the levels of inflammatory cytokines and antibodies in people with type 2 diabetes and then compare it.

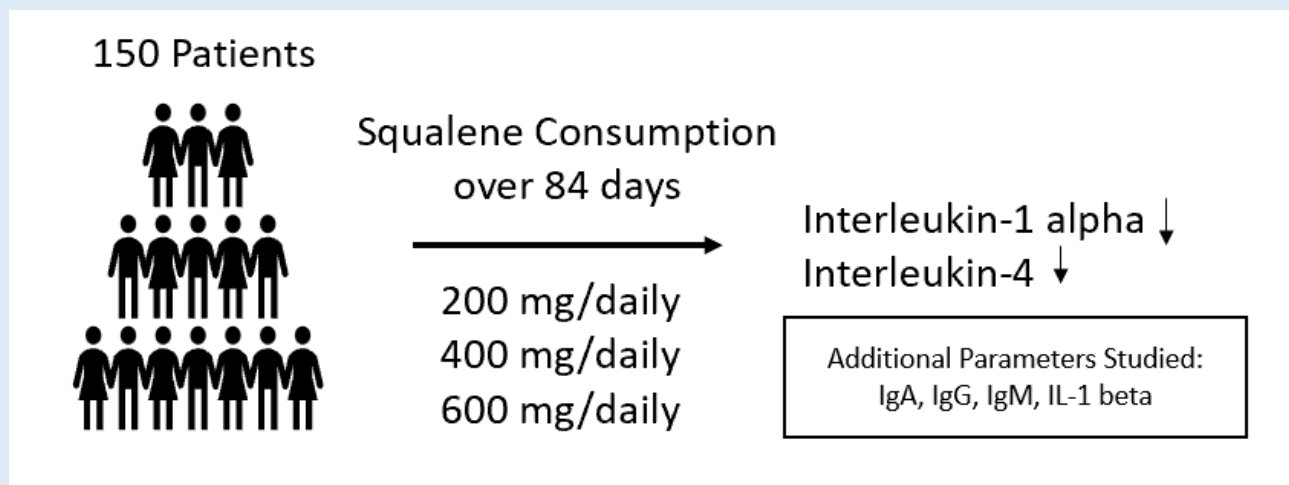
Methods: In this study, 4 groups with type 2 diabetes mellitus and 1 control group were selected. 4 groups with type 2 diabetes mellitus were divided into 1 diabetic group without squalene consumption for 84 days and 3 diabetic groups treated with squalene in doses of 200, 400 and 600 mg, respectively for 84 days. The levels of Interleukin-1 alpha, Interleukin-1 beta, Interleukin-4, immunoglobulin A, immunoglobulin G, immunoglobulin M, and as well as the glucose of all participants were measured by Enzyme-linked immunosorbent assay method.

Results: On days 14 and 28, a statistically significant difference (P value < 0.05) was observed in the level of Interleukin-1 alpha and Interleukin-4 in groups 3, 4 and 5 compared to group 2. This significant difference in the levels of interleukin-1 beta and immunoglobulin A was observed only on days 56 and 84. No statistically significant difference (P value > 0.05)

was observed in the levels of immunoglobulins G, M, and glucose during the consumption of squalene between the groups.

Conclusions: According to the time and dose, squalene can be effective in reducing inflammatory factors and increasing immunoglobulin A. However, additional studies are needed for the action mechanism and the effect of squalene.

Keywords: squalene, triterpene, interleukin, immunoglobulin, diabetes mellitus



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INTRODUCTION

Squalene is a 30-carbon isoprenoid compound belonging to the triterpene class and is commonly found in shark liver oil [1]. It is known to contribute to the synthesis of cholesterol and has been identified in human sebum, which has led to many research studies observing the health benefits that squalene has on humans [2]. Such benefits may include antioxidant properties, anticancer effects, high preferability in skin care, greater success rate as a drug carrier, the ability to detoxify, and uses as an anti-infective [3]. These conclusions have been reached through multiple research studies of squalene. To start, squalene has been linked to improve cardiovascular health. Antioxidant activity of squalene is accomplished by a transcription factor called nuclear factor E2-related factor 2 (Nrf2), which acts as a master regulator of antioxidant responses to cellular stresses. It

has been shown that this transcription factor in squalene reduces oxidative stress, offering a protective effect in cardiovascular health [4]. In addition, squalene has been connected to anti-cancer activities. There have been reports that a diet rich, like the Mediterranean diet, in squalene has been connected to a decline in mortality rates regarding cancer [5]. In a study where squalene was given as a treatment along with antitumor drugs, tumor growth was arrested, but it was not clear whether squalene exhibits antitumor activity [3]. More research must be done to determine the anticancer effects of squalene. Squalene has also been the center of discussion in the skin care industry. There were studies that showed squalene’s role in protecting the human skin from harmful UV rays and other sources of oxidative damage; it has also shown promising signs of skin

hydration [6]. Furthermore, squalene has been reported to be well tolerated whether injected intravenously or consumed orally and therefore, squalene is seen as a potential biomolecule to be used in disease management and therapy [7]. Finally, squalene has potential effects in type 2 diabetes mellitus (T2DM). T2DM is when the body is not making enough insulin or the insulin that is being produced is not working properly [8]. In patients with T2DM, their cholesterol metabolism is perturbed, but there have been studies that showed that the presence of squalene could reveal reliable information of cholesterol synthesis and absorption [9].

Immunoglobulins (Ig), also known as antibodies, are glycoproteins that are produced by plasma cells [10]. These immunoglobulins are crucial to the human immune and inflammatory systems because they participate in immune responses against bacteria, viruses, fungi, parasites, cellular antigens, chemicals, and synthetic substances. Immunoglobulins function by interacting with a B-cell receptor (BCR) on the cell surface of B lymphocytes which produces a signal to activate transcription factors to create antibodies against a foreign substance [11]. There are five main isotypes of immunoglobulins: A, D, E, G, and M [12]. Immunoglobulin G (IgG) is abundantly found in newborns because it is the only immunoglobulin that crosses the placenta through Fc-receptor interactions [13]. Immunoglobulin M (IgM) can be present without external antigens during prenatal development, and it possess anti-bacterial, anti-viral, anti-tumor activities to build immunological tolerance; they are also responsible for the transformation of B2-lymphocytes into memory cells [14]. Immunoglobulin A (IgA) interacts with the complement system [15]. They work by preventing its enzymatic digestion in order to protect the epithelial surfaces of the respiratory, digestive, and the genitourinary system [10]. Cytokines play a role in inflammatory responses. Interleukin-4 (IL-4) regulates antibody production, hematopoiesis,

inflammation, and the development of effector T-cell responses [16]. Interleukin-4 can improve insulin sensitivity and glucose tolerance by reducing lipid accumulation [17]. Interleukin-1 alpha (IL-1 α) is expressed in healthy tissues, hematopoietic, non-hematopoietic cells, and stimulates sterile and pathogen-induced inflammation [18]. Its counterpart, Interleukin-1 beta (IL-1 β), is important for host-defense responses to infection and injury [19]. We aimed to evaluate the effect of squalene on changes in the levels of IL-1 α , IL-1 β , IL-4 and immunoglobulins A, G and M in people with T2DM.

MATERIALS AND METHODS

Materials: Squalene (S3626, as a liquid with a purity of more than 98%) was purchased from Sigma-Aldrich Co (USA). The human glucose assay kit was purchased from MyBioSource Inc (USA). IgA, IgG and IgM assay kits were procured from Abcam Co (USA). The serum levels of IL-1 α and IL-1 β were assayed by kits procured from Diaclone Co (France). Assay kit of IL-4 was purchased from Abcam Co (USA).

METHODS

Participants: In this study, which is a randomized study, among the participants in this study, 30 healthy volunteers (as a control group) and 120 volunteers with T2DM were randomly selected. The control group was considered as group 1. Volunteers with T2DM were patients who were referred to Vali-Asr medical laboratory (Tehran, Iran) by an endocrinologist. The 120 volunteers with T2DM were randomly divided into 4 groups of 30 people. Group 2: patients with T2DM without squalene treatment. Group 3: patients with T2DM who were treated at a dose of 200 mg/day for 84 days. Group 4: patients with T2DM who were treated at a dose of 400 mg/day for 84 days. Group 5: patients with T2DM who were treated at a dose of 600 mg/day for 84 days. Groups 3, 4 and 5 received squalene as an oral

capsule (liquid filled oral). In this study, inclusion criteria according to World Health Organization (WHO), included the following: glycated hemoglobin (HbA1c) \geq 6.5%, fasting plasma glucose \geq 126 mg/dl and not taking corticosteroids. Patients with type 1 diabetes mellitus (T1DM) and other diseases and a history of surgery as well as young patients with T2DM were excluded from the study. Male and female volunteers participated in this study. All volunteers participating in this study were informed during the study and informed consent was obtained from them.

General features and sampling: Before studying the groups treated with squalene, anthropometric data including age, sex, weight, height, and body mass index (BMI) were recorded from all volunteers. After grouping the volunteers, blood samples were obtained from all participants under sterile conditions and after 12 hours of nighttime fasting, in four time periods on days 14, 28, 56 and 84. Blood samples were centrifuged (250 g for 10 min) to obtain serum and evaluate biochemical parameters. An enzyme-linked immunosorbent assay (ELISA) method was used to assay glucose, cytokines, and immunoglobulins of the studied groups according to the instructions of the suppliers.

Statistical Analysis: Statistical analysis was done by SPSS (version 23, IBM, USA) software for Windows. All results were expressed as mean \pm standard deviation (SD). The Kolmogorov-Smirnov test was used to analyze the normal distribution of data. P-values $<$ 0.05 were considered significant. An independent-sample T-test was used to compare the mean of general characteristics of the participants. The independent-sample T-test was also used to compare the mean of biochemical parameters between diabetic and control groups. 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.

RESULTS

BMI was calculated in the control group, untreated diabetic group, and in the diabetic group treated with squalene. The mean of this index was compared in different groups. In comparing the BMI of the untreated diabetic group and the diabetic groups treated with squalene with the control group (group 1), significant differences were observed on different days (P value $<$ 0.05). For the comparison of the BMI between the groups treated with squalene with each other and on different days, a very little decrease was observed, which was not significant (table 1). For example, as shown in table 1, on the 84th day in group 5, a decrease in BMI was observed compared to the 14th day, which is not significant (P value $>$ 0.05).

Table 1: Comparison of BMI mean in different groups

Group Days	Group 1 (n = 30)	Group 2 (n = 30)	Group 3 (n = 30)	Group 4 (n = 30)	Group 5 (n = 30)	P value*
14	30.6 \pm 3.63	35.83 \pm 3.60	36 \pm 2.86	35.46 \pm 3.01	34.4 \pm 3.78	< 0.05
28	30.6 \pm 3.63	35.83 \pm 3.60	35.73 \pm 2.74	35.1 \pm 2.78	34.03 \pm 3.68	
56	30.6 \pm 3.63	35.83 \pm 3.60	35.5 \pm 2.76	34.93 \pm 2.83	33.6 \pm 3.65	
84	30.6 \pm 3.63	35.83 \pm 3.60	35.3 \pm 2.65	34.6 \pm 2.83	33.4 \pm 3.58	
P value** $>$ 0.05						

Data are given as mean \pm SD. Group 1 represents the healthy (control) group. Group 2 represents the untreated diabetic group. Group 3 represents the diabetic group which consumed 200 mg of squalene daily. Group 4 represents the diabetic group which consumed 400 mg of squalene daily. Finally, group 5 represents the diabetic group which consumed 600 mg of squalene daily.

* Comparison of BMI mean in diabetic groups with control group

** Comparison of BMI mean within groups with each other on different days

Considering the importance of the immune system in the prevention and protection of infections in people with diabetes, in this study, the effect of squalene in different

doses and days on the alterations in the levels of some cytokines and immunoglobulins was investigated. The investigated cytokines were IL-1 α , IL-1 β and IL-4

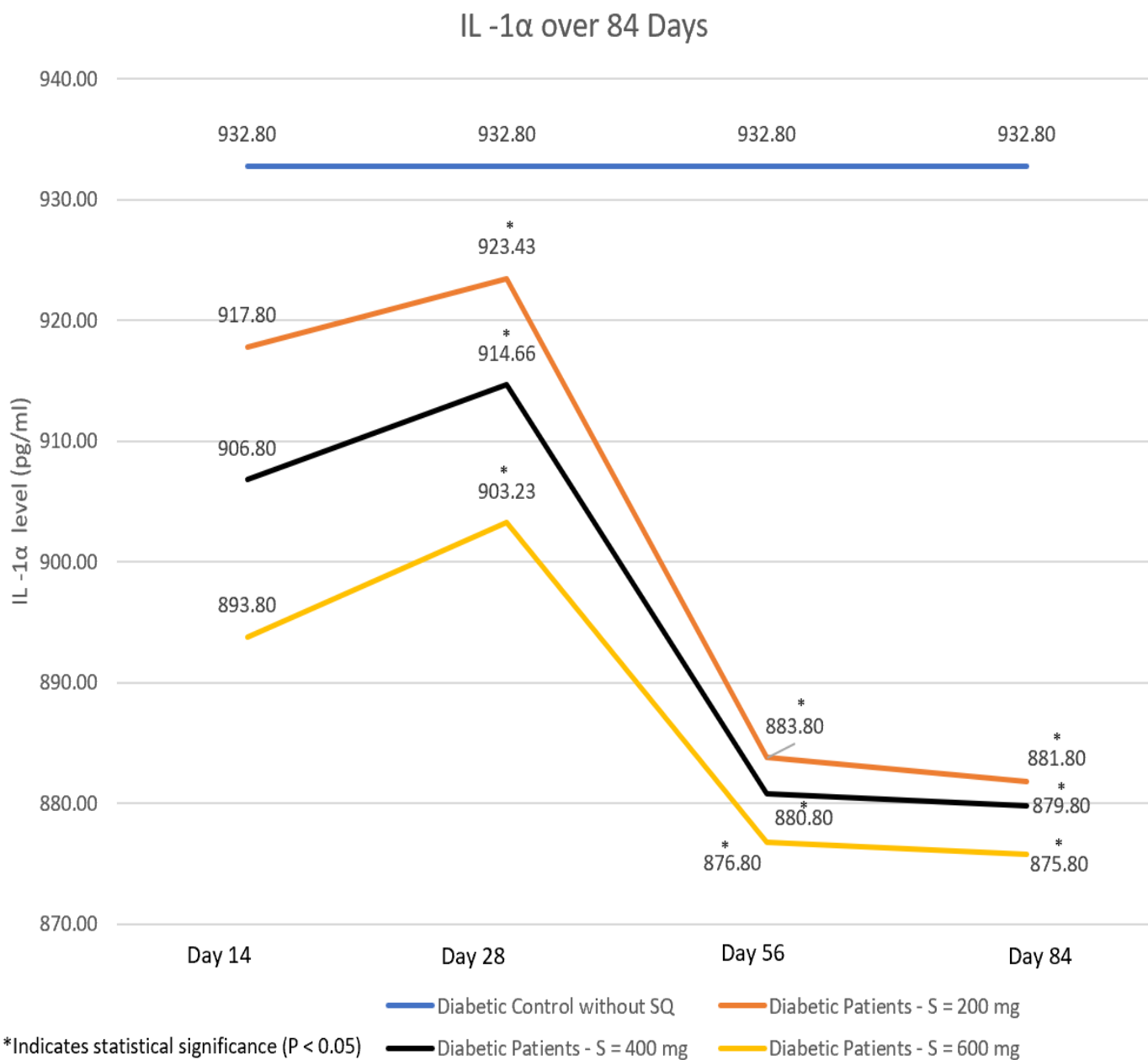


Figure 1. Graphical representation for data measurements collected for IL-1 α for the diabetic groups throughout the 84-day duration of the study. Data are given as mean.

Figure 1 illustrates the changes in IL-1 α over the study duration of 84 days. According to the results obtained from the data analysis, the levels of IL-1 α in groups 4 and 5, which received doses of 400 and 600 mg of squalene for 14 days, respectively, were significantly reduced in comparison with

group 2. This significant reduction was observed in the comparison of group 3, who received a dose of 200 mg of squalene daily, with group 5 (P value < 0.05). On the 56th day, comparing the levels of IL-1 α between groups 3, 4, and 5 with group 2, a significant difference was observed (P

value < 0.05). On the 84th day, comparing the levels of IL-1 α between groups 3, 4, and 5 with group 2, as on the 56th day, a significant difference was observed (P value < 0.05).

An interesting finding in this graph is that although there

had been a drop in IL-1 α values on days 56 and 84, there had been an initial increase in the IL-1 α values for all of the treated diabetic groups on day 28.

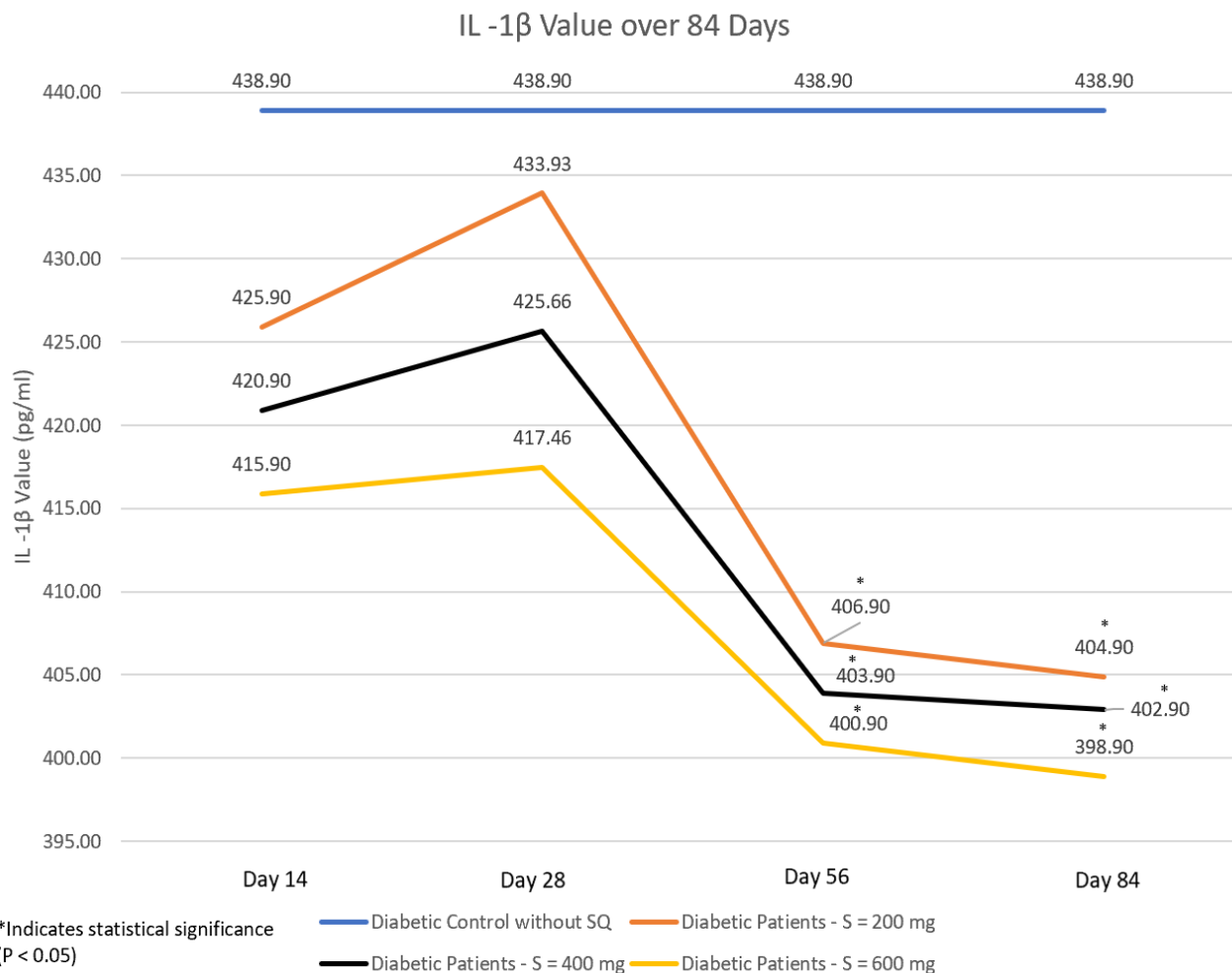


Figure 2. Graphical representation for data measurements collected for IL-1 β for the diabetic groups throughout the 84-day duration of the study. Data are given as mean.

Figure 2 illustrates the alterations in IL-1 β over the 84-day duration of the study. During day 14, there was no significant difference between groups 3, 4 and 5, and comparing these groups with group 2 in the level of IL-1 β (P value > 0.05). As on day 14, no significant difference was observed in IL-1 β levels in comparison between groups (P value > 0.05). On the 56th day, comparing IL-1 β between groups 3, 4, and 5 with group 2, a significant difference was

observed (P value < 0.05). On the 84th day, comparing the levels of IL-1 β between groups 3, 4, and 5 with group 2, as on the 56th day, a significant difference was observed (P value < 0.05). An interesting finding in this graph is that although there had been a drop in IL-1 β values on days 56 and 84, there had been an initial increase in the IL-1 β values for all of the treated diabetic groups on day 28.

IL-4 Value over 84 Days

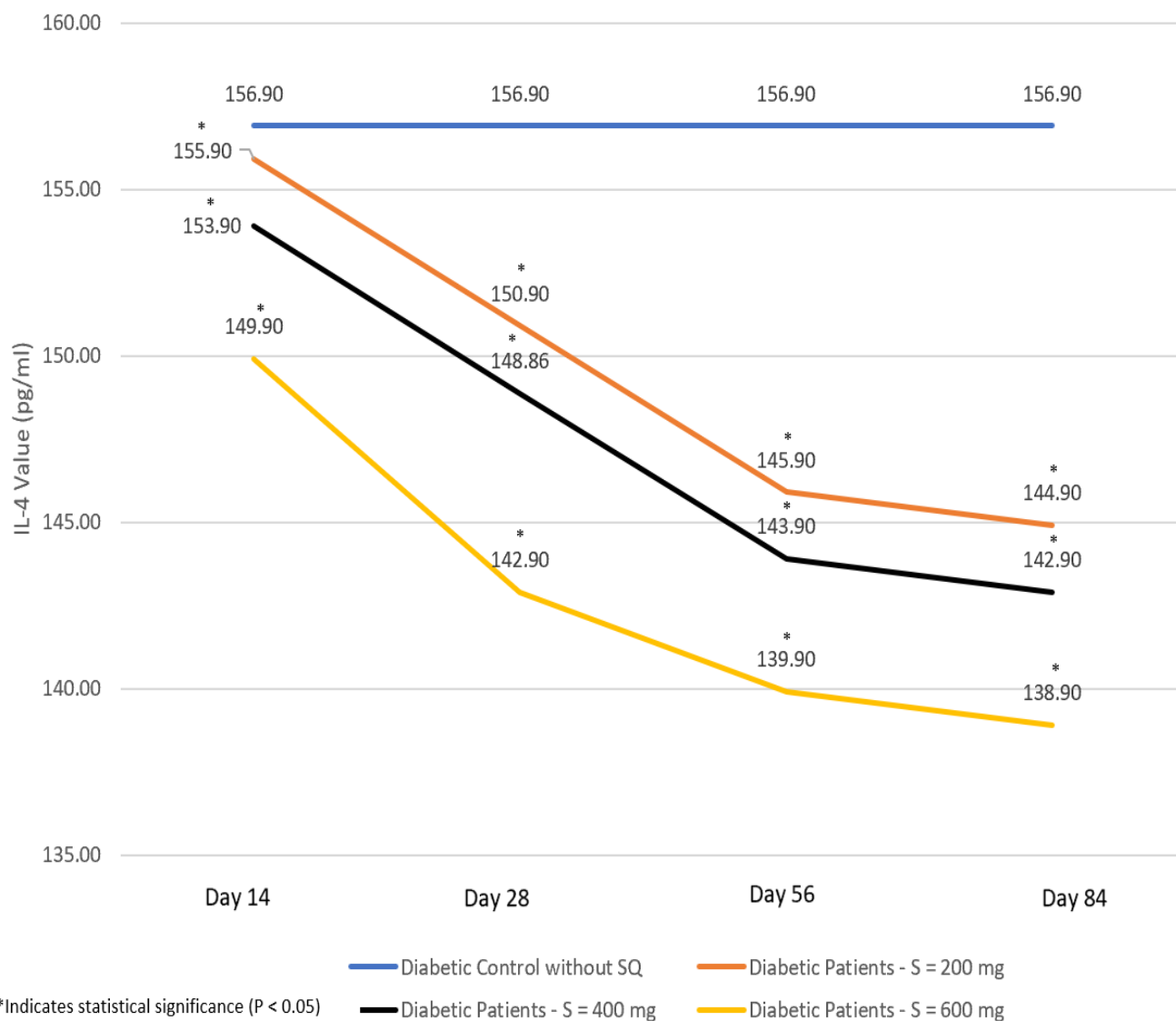


Figure 3. Graphical representation for data measurements collected for IL-4 for the diabetic groups throughout the 84-day duration of the study. Data are given as mean.

Figure 3 illustrates the alterations in IL-4 over the 84-day duration of the study. On day 14, in comparing the mean levels of IL-4 between group 5 and other groups, a significant difference was observed (P value < 0.05). Analysis of the data obtained on day 28 showed a significant difference in IL-4 levels between groups 3, 4 and 5 with group 2 and group 4 with 5 (P value < 0.05). On the 56th day, comparing the levels of IL-4 between

groups 3, 4, and 5 with group 2, a significant difference was observed (P value < 0.05). Finally, on day 84, a significant difference was observed in the comparison of interleukin-4 levels between group 5 with group 3, as well as between group 5 with group 4 (P value < 0.05). Additionally, between groups 3, 4, and 5 with group 2, as on the 56th day, a significant difference was observed.

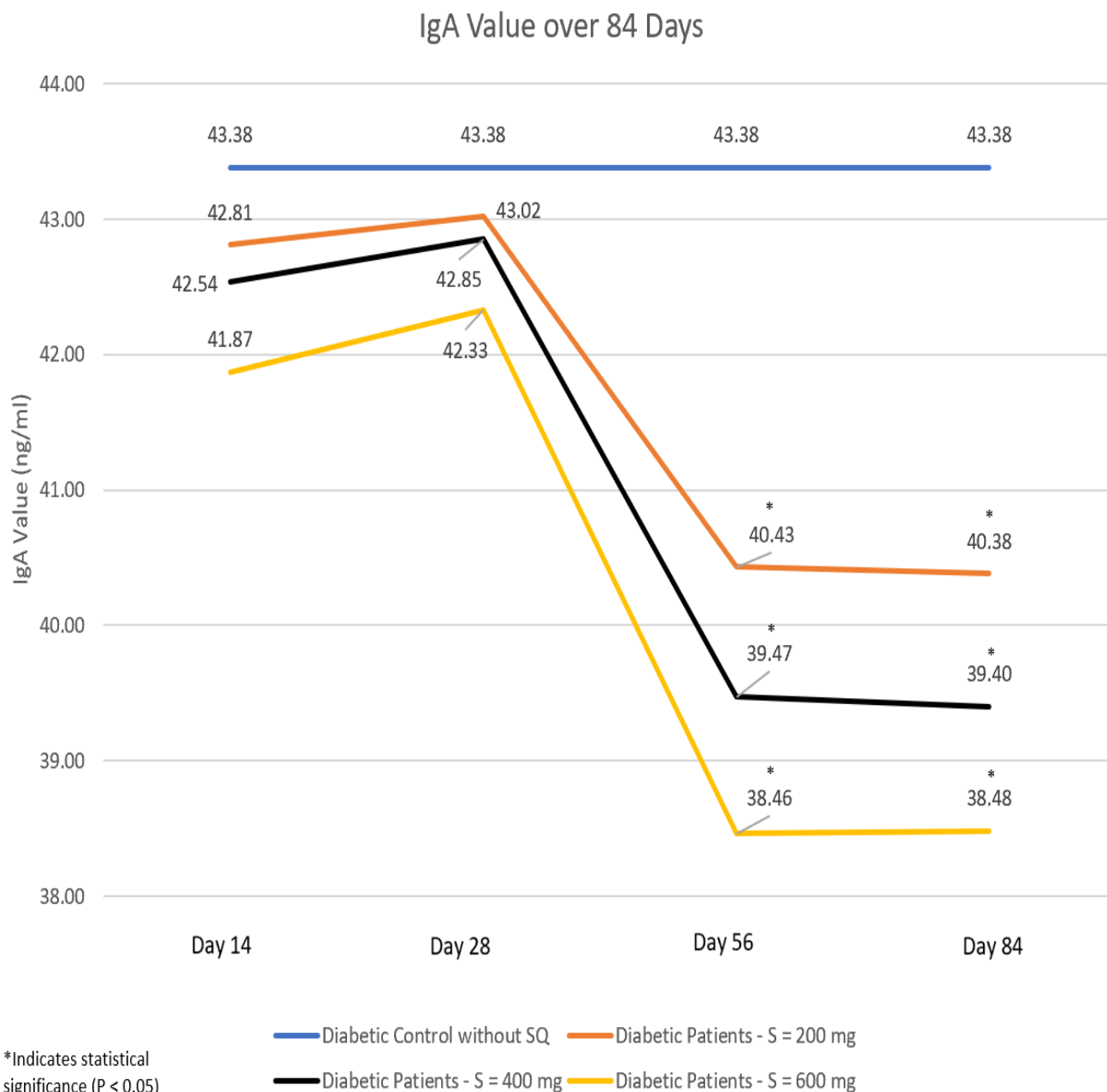


Figure 4. Graphical representation for data measurements collected for IgA for the diabetic groups throughout the 84-day duration of the study. Data are given as mean.

Figure 4 shows IgA measurements collected throughout the 84-day study. On the 14th and 28th days, no significant difference was observed in comparing the levels of IgA between different groups (P value > 0.05). Immunoglobulins levels were also examined on the 56th and 84th days. In these days, only in the level of IgA,

between groups 3, 4 and 5, with group 2, a significant difference was observed (P value < 0.05). An interesting finding in this graph is that although there had been a drop in IgA values on days 56 and 84, there had been an initial increase in the IgA values for all of the treated diabetic groups on day 28.

IgG Value over 84 Days

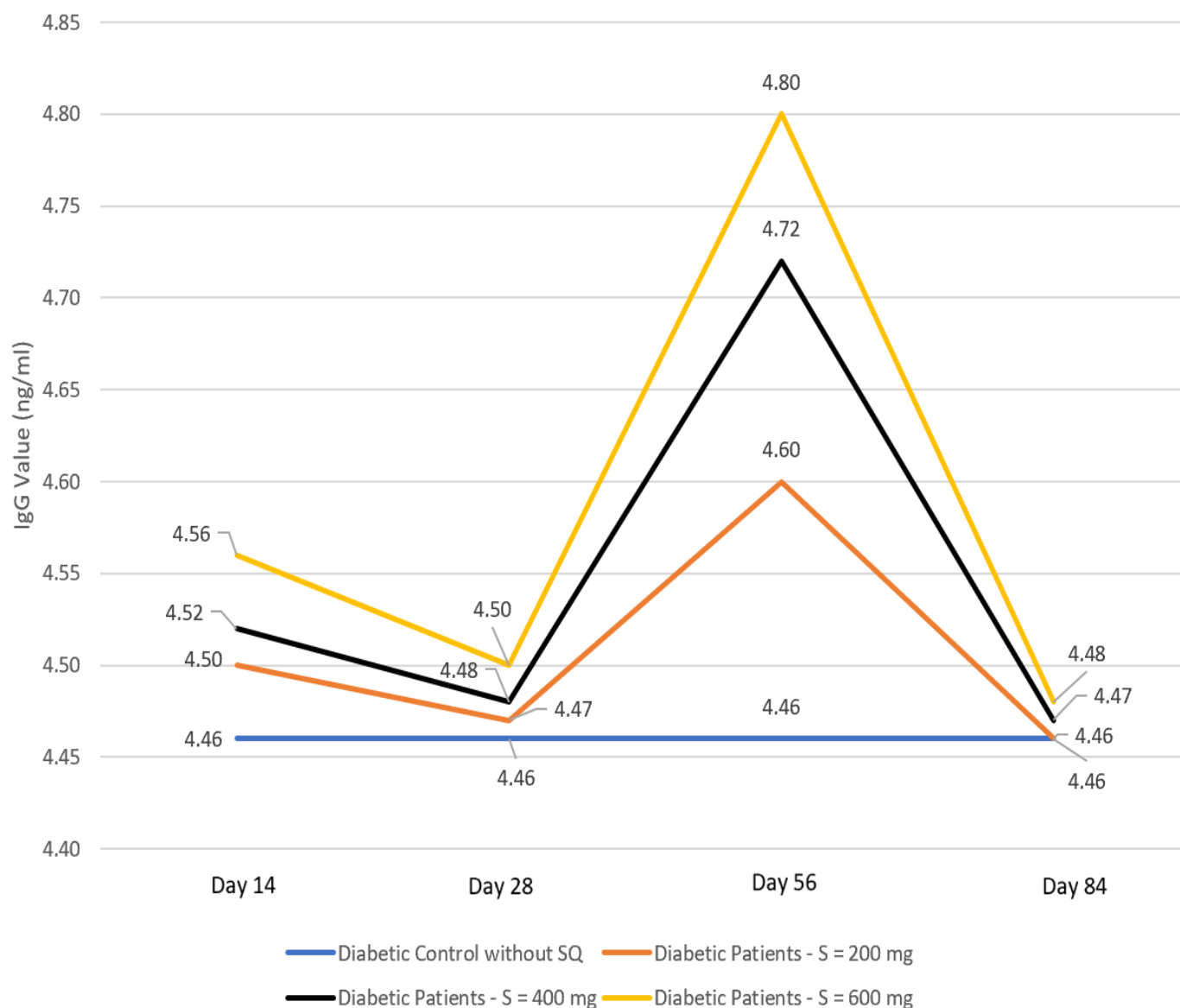


Figure 5. Graphical representation for data measurements collected for IgG for the diabetic groups throughout the 84-day duration of the study. Data are given as mean.

Figure 5 shows the data collection for IgG levels over the course of the 84-day study. On the 14th and 28th days, no significant difference was observed in comparing the IgG levels between different groups (P value > 0.05). Immunoglobulins levels were also examined on the 56th and 84th days. No significant difference was observed in the levels of IgG in different groups and on days 56 and 84 either (P value > 0.05). An interesting finding of this figure is the changes in IgG values throughout the study. On day 28, we can observe a drop in the IgG values for all

the treated diabetic groups. However, these values spiked on day 56, with IgG values at their highest for all of the treated diabetic groups. Again, this showed an inverse effect on day 84, with IgG values plummeting from day 56. The overall IgG, however, did show an overall decrease from the initial amount by the conclusion of the study. Although these fluctuations in data did not show to be statistically significant, future studies may focus on how squalene intake may cause continuous rises and drops in IgG levels of diabetics.

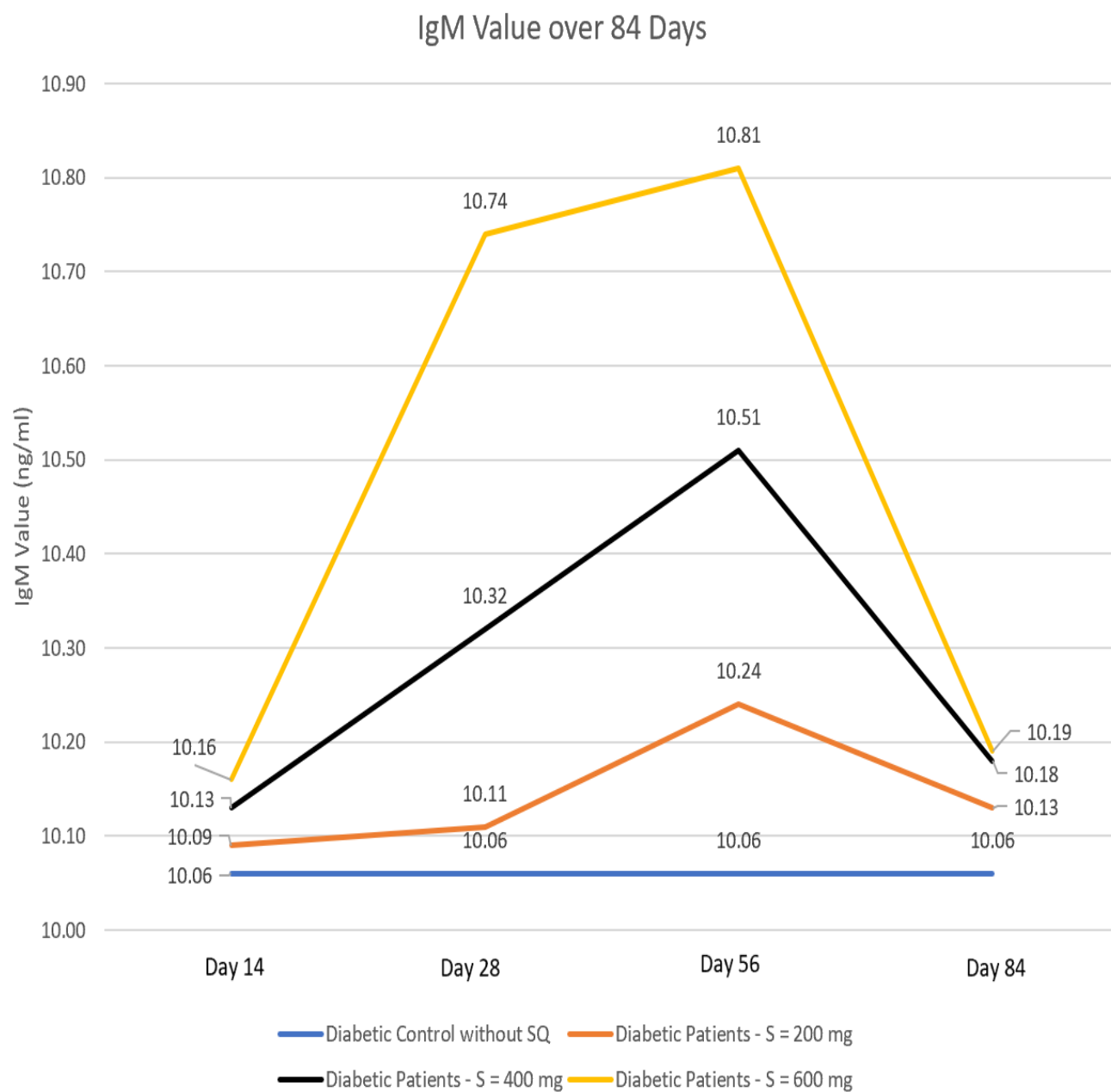


Figure 6. Graphical representation for data measurements collected for IgM for the diabetic groups throughout the 84-day duration of the study. Data are given as mean.

Figure 6 shows the data collection for IgM levels over the course of the 84-day study. On the 14th and 28th days, no significant difference was observed in comparing the IgM levels between different groups (P value > 0.05). Immunoglobulins levels were also examined on the 56th and 84th days. No significant difference was observed in the levels of IgM in different groups and on either days

56 and 84 (P value > 0.05). One noticeable finding in this graph is that although IgM levels showed an increase at the end of the study as compared to initial recordings, days 28 and 56 were identified as days of major peaks in IgM levels. Therefore, the highest IgM levels were observed to be on day 56, where each treated diabetic group exhibited the maximum IgM value throughout the study.

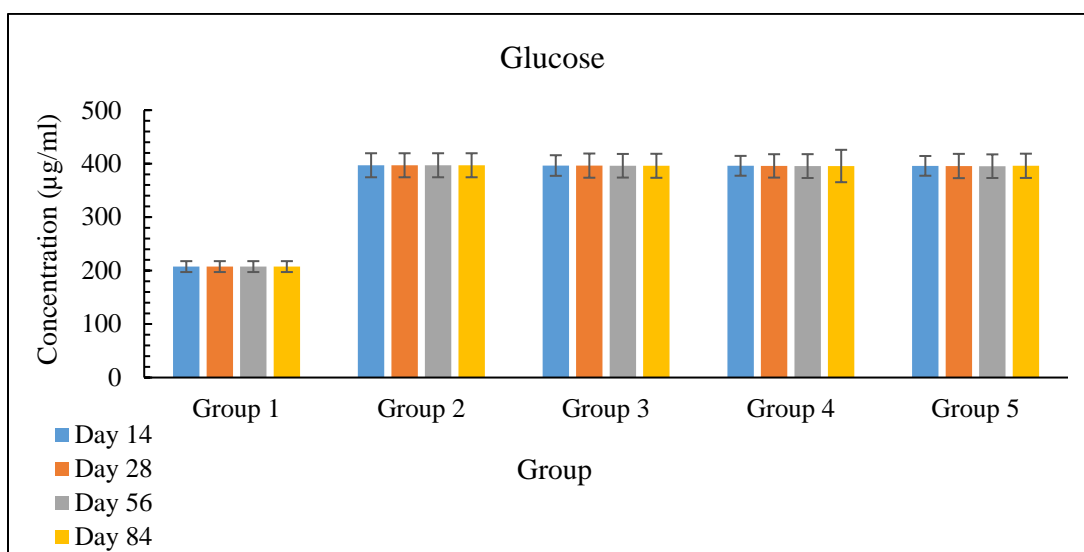


Figure 7. Graphical representation of changes in glucose levels in various groups on different days. Data are given as mean ± SD.

In the present study, glucose concentration was also measured in all groups (Figure 7). A significant difference was observed (P value < 0.05) in the comparison of glucose levels only between the diabetic groups, treated

and untreated with squalene, with the control group. There was no significant difference in glucose levels between treated groups with squalene compared to before treatment (P value > 0.05).

Table 2: Percentage changes in interleukin levels throughout the duration of the 84-day study

Group	Interleukin	IL-1α Value	IL-1α Percent Change	IL-1β Value	IL-1β Percent Change	IL-4 Value	IL-4 Percent Change
Group 2 (Diabetic Control)	Day 14	932.8		438.9		156.9	
Group 3 (Diabetic 200 mg/daily)	Day 14	917.8	1.61%	425.9	2.96%	155.9	0.64%
Group 4 (Diabetic 400 mg/daily)	Day 14	906.8	2.79%	420.9	4.10%	153.9	1.91%
Group 5 (Diabetic 600 mg/daily)	Day 14	893.8	4.18%	415.9	5.24%	149.9	4.46%
Group 2 (Diabetic Control)	Day 28	932.80		438.9		156.9	
Group 3 (Diabetic 200 mg/daily)	Day 28	923.43	1.00%	433.93	1.13%	150.9	3.82%
Group 4 (Diabetic 400 mg/daily)	Day 28	914.66	1.94%	425.66	3.02%	148.86	5.12%
Group 5 (Diabetic 600 mg/daily)	Day 28	903.23	3.17%	417.46	4.88%	142.9	8.92%
Group 2 (Diabetic Control)	Day 56	932.8		438.9		156.9	
Group 3 (Diabetic 200 mg/daily)	Day 56	883.8	5.25%	406.9	7.29%	145.9	7.01%
Group 4 (Diabetic 400 mg/daily)	Day 56	880.8	5.57%	403.9	7.97%	143.9	8.29%
Group 5 (Diabetic 600 mg/daily)	Day 56	876.8	6.00%	400.9	8.66%	139.9	10.83%
Group 2 (Diabetic Control)	Day 84	932.8		438.9		156.9	
Group 3 (Diabetic 200 mg/daily)	Day 84	881.8	5.47%	404.9	7.75%	144.9	7.65%
Group 4 (Diabetic 400 mg/daily)	Day 84	879.8	5.68%	402.9	8.20%	142.9	8.92%
Group 5 (Diabetic 600 mg/daily)	Day 84	875.8	6.11%	398.9	9.11%	138.9	11.47%

Table 2 provides a table for calculation of percentage differences in interleukin levels throughout the duration of the study. These percentage differences were calculated by comparing the values of the treated diabetic groups (groups 3, 4, and 5) as compared to the untreated, diabetic control group (group 2). As illustrated, noticeable changes in data collection occur

around day 56 for IL-1 α and IL-1 β . For example, on day 28, the percentage change IL-1 α for group 3 (diabetic 200 mg/daily) was 1.00%. However, this percentage change jumped from 1.00% to 5.25% on day 56. Another example of this is that on day 28, the percentage change of IL-1 β for group 3 (diabetic 200 mg/daily) was 7.29%. However, this percentage change jumped from 1.13 to 7.29% on day 56.

Table 3: Percentage changes in immunoglobulin levels throughout the duration of the 84-day study

Group	Immunoglobulin	IgA Value	IgA Percent Change	IgG Value	IgG Percent Change	IgM Value	IgM Percent Change
Group 2 (Diabetic Control)	Day 14	43.38		4.46		10.06	
Group 3 (Diabetic 200 mg/daily)	Day 14	42.81	1.31%	4.50	0.90%	10.09	0.30%
Group 4 (Diabetic 400 mg/daily)	Day 14	42.54	1.94%	4.52	1.35%	10.13	0.70%
Group 5 (Diabetic 600 mg/daily)	Day 14	41.87	3.48%	4.56	2.24%	10.16	0.99%
Group 2 (Diabetic Control)	Day 28	43.38		4.46		10.06	
Group 3 (Diabetic 200 mg/daily)	Day 28	43.02	0.83%	4.47	0.22%	10.11	0.50%
Group 4 (Diabetic 400 mg/daily)	Day 28	42.85	1.22%	4.48	0.45%	10.32	2.58%
Group 5 (Diabetic 600 mg/daily)	Day 28	42.33	2.42%	4.50	0.90%	10.74	6.76%
Group 2 (Diabetic Control)	Day 56	43.38		4.46		10.06	
Group 3 (Diabetic 200 mg/daily)	Day 56	40.43	6.80%	4.60	3.14%	10.24	1.79%
Group 4 (Diabetic 400 mg/daily)	Day 56	39.47	9.01%	4.72	5.83%	10.51	4.47%
Group 5 (Diabetic 600 mg/daily)	Day 56	38.46	11.34%	4.80	7.62%	10.81	7.46%
Group 2 (Diabetic Control)	Day 84	43.38		4.46		10.06	
Group 3 (Diabetic 200 mg/daily)	Day 84	40.38	6.92%	4.46	0.00%	10.13	0.70%
Group 4 (Diabetic 400 mg/daily)	Day 84	39.4	9.17%	4.47	0.22%	10.18	1.19%
Group 5 (Diabetic 600 mg/daily)	Day 84	38.48	11.30%	4.48	0.45%	10.19	1.29%

Table 3 provides a table for calculation of percentage differences in immunoglobulin levels throughout the duration of the study. These percentage differences were also calculated by comparing the values of the treated diabetic groups (groups 3, 4, and 5) as compared to the untreated, diabetic control group (group 2). Table 3 provides interesting findings on days 28, 56, and 84. IgA

showed a large jump in percentage change from days 28 to 56. For example, group 5 (diabetic 600 mg/daily) had an IgA percentage change of 2.42% on day 28. However, this jumped to 11.34% on day 56. Interestingly, IgG showed a similar trend. This immunoglobulin, however, proves to be unique when treated with squalene past 56 days. As shown on table 3, day 28 showed very little

percentage changes for all the treated diabetic groups in terms of IgG. However, these percentage changes increased dramatically on day 56. On day 84, however, there showed to be an inverse effect, with the IgG value dropping dramatically to values similar to those of day 28. For example, on day 84, group 3 (diabetic group which consumed 200 mg of squalene daily) had a percentage change of 0.00% as compared to the diabetic control. This indicates that after 84 days of intaking 200 mg of squalene daily, this diabetic group had the same immunoglobulin level of the control without any percentage difference. Prior to this, however, the same group had a percentage change of 3.14% on day 56.

DISCUSSION

Inflammatory factors can play an important role in causing T2DM and its consequences. T2DM can be considered as an inflammatory disease. Obesity, which is one of the risk factors for T2DM, can play a role in changing the components of the immune system. One of these immunological alterations is the change of special cytokines. It has been suggested that one of the cytokines responsible for the inflammatory state in T2DM is the activation of IL-1 β and nuclear factor- κ B (NF- κ B) [20]. Immunoglobulins, which are secreted against antigens and are also called antibodies, are specialized protein molecules that protect the body against infections [10]. Studies have reported the anti-inflammatory effects of squalene, but the anti-inflammatory properties of squalene are still unclear. In a study conducted by Cardeno et al., [21] the effects of squalene on the expression of genes of inflammatory factors were investigated. They stimulated human monocytes and neutrophils with lipopolysaccharide to induce an inflammatory response (LPS-mediated inflammatory response) and then exposed these cells to different concentrations for 18 hours of squalene. It was reported that the treatment of monocytes and neutrophils with a

dose of 50 μ M significantly decreases the expression of IL-1 β and TNF- α genes. Squalene reduces mRNA levels of NF- κ B downstream genes such as TNF- α and IL-1 β . In our study, the reduction of IL-1 β was not dose-dependent or time-dependent. So that this reduction was not significant on days 14 and 28 among the treated groups with each other and with group 2, but a significant reduction was observed on days 56 and 84 between groups 3, 4 and 5 and group 2 (Figure 1C and 1D). Therefore, with a dose of 200 mg and within 56 days, a significant decrease in IL-1 β levels can be observed.

In a study conducted by Sanchez-Fidalgo et al., [22] mice with colitis were treated with squalene (at a dose of 3 grams per day) for 4 weeks. They stated that the expression of IL-1 β and TNF- α genes in mice with colitis is reduced by squalene consumption. Squalene plays a role in reducing the expression of genes of inflammatory factors through p38 mitogen-activated protein kinase and NF- κ B pathways.

Dormont et al. [23] designed a medicinal nanoparticle based on squalene. The antioxidant and anti-inflammatory effects of this drug were studied in vivo and in vitro. By treating mice with this nanoparticle, they reported a decrease in the inflammatory factor TNF- α , and an increase in the anti-inflammatory factor (IL-10) compared to the control group. In the present study, the anti-inflammatory effects of squalene in the form of medicine were investigated in T2DM patients, and the reduction of inflammatory cytokines (IL-1 α , IL-1 β and IL-4) was significantly observed in some days and some doses (Figure 1). Type 2 cytokines such as IL-4 and IL-13 play an important role in causing some inflammations [24]. Squalene was studied as an influenza vaccine adjuvant in the study of Deng et al [25]. They found that the emulsion containing squalene, as an adjuvant, can enhance the effect of influenza vaccine by increasing IL-4 producing cells and increasing immunogenicity by producing more IgG. In our study, the consumption of

squalene by diabetics was associated with a decrease (not significant) in the levels of IL-4 compared to group 2 on different days, and an increase (significant) in the levels of this cytokine compared to group 1 (Figure 1). Before Deng's study, Shahiwala and Amiji [26] investigated immunogenicity in an animal model using an emulsion containing squalene. They reported that oral treatment significantly increased the systemic IgG and mucosal IgA responses compared to nasal treatment. In the present study, IgA levels decreased significantly in groups 3, 4, and 5 compared to group 2 on days 56 and 84. The levels of IgM and IgG decreased in these days, which was not significant (Figure 2).

Amaranth oil, which contains a high level of squalene, was found to reduce proteinuria levels [27]. Also, observed was a statistical significance in changes in albumin, proteinuria, cystatin C, and creatinine levels [28].

It has been reported that squalene has an antidiabetic activity. In a recent study conducted by Widawati et al., [29] the effect of squalene on blood glucose reduction and its comparison with metformin was investigated on rats with T2DM. They treated rats with squalene (at a dose of 160 mg/kg) for 12 days. They found that treating rats with squalene significantly reduced glucose levels in diabetic rats. In a study conducted by Valdes et al., [30] the effect of squalene on blood glucose levels in normal mice and mice with T2DM was investigated. They reported that squalene reduces glucose levels in normal and diabetic mice, but this reduction is not significant. In our study, glucose reduction was not observed in diabetics on different days of treatment with squalene (Figure 7).

In our current study, IL-4 levels were found to have shown an overall decrease when treated with squalene. This decrease was found to be more pronounced with a larger dosage of squalene intake. On day 84, there had been an 11.47% percentage decrease for group 5

(diabetic 600 mg/daily) as compared to the untreated diabetic control group. These findings seem to be opposite to what has previously been published. In a paper published in 2007, work had been conducted to investigate the effects of amaranth oil on coronary heart disease and hypertension with obesity. Amaranth oil is composed majorly of squalene [31]. In their study, it was found that with application of amaranth oil, IL-4 levels showed an overall increase. A reason for this occurrence may be due to the fact that the other components in amaranth oil exclusive to squalene may interact with the body to cause the increase in IL-4. Future studies may investigate the mechanisms for how amaranth oil as compared to squalene alone impact IL-4 levels. The other findings in this paper correlate to the findings of this study, with decreases both shown in IL-1 β and IgM and increases in IgG [32]. Although the findings for this study for IgG and IgM had not been statistically significant, these correlations may be an area for future research to investigate.

CONCLUSION

The anti-diabetic, antioxidant and anti-inflammatory properties of squalene have been investigated in many studies, and in this study, for the first time, we investigated the anti-inflammatory and immunogenic properties of squalene on people with type 2 diabetes. According to the obtained results and analysis of the data obtained from the results, we found that squalene in different doses and on different days can cause significant changes in the levels of cytokines such as IL-1 α , IL-1 β and IL-4. However, a significant decrease in the levels of IL-1 α was observed on the 14th day and in the dose of 600 mg of squalene (group 5) compared to groups 4, 3 and 2. The same result was obtained for IL-4. Regarding IL-1 β , a significant decrease was observed on days 56 and 84 and in groups 5, 4 and 3 compared to group 2. Regarding the role of squalene in

immunogenicity, only a significant decrease in IgA levels was observed on days 56 and 84 in groups 5, 4, and 3 compared to group 2. According to the results, it can be stated that squalene can play an important role in the changes of inflammatory and immune parameters in a time-dependent and dose-dependent manner. The role of squalene in the significant changes of blood glucose was not observed. Nevertheless, more research in this field seems necessary. Future studies may aim to refine specific dosages to a specific time in order to allow for the identification of the most effective use of squalene as a potential treatment option. By furthering research in this direction, squalene may potentially be identified as an effective bioactive compound for aiding the immune system.

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List of abbreviations: IL-1 α , Interleukin-1 alpha; IL-1 β , Interleukin-1 beta; IL-4, Interleukin-4; DM, diabetes mellitus; HbA1c, glycated hemoglobin; T2DM, type 2 diabetes mellitus; IgG, Immunoglobulin G; IgA, Immunoglobulin A; IgM, Immunoglobulin M; Nrf2, nuclear factor E2-related factor 2; NF- κ B, nuclear factor- κ B; TNF- α , tumor necrosis factor- α .

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