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Effects of *Spirulina platensis* on serum markers in rats fed with hydrogenated vegetable oil and/or cholesterol

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ABSTRACT

Aim: The aim of this study was to investigate changes in serum levels of glucose, insulin, lipoprotein (a), apolipoprotein A1, apolipoprotein B, C-reactive protein and total protein in rats fed with *Spirulina platensis* and/or hydrogenated vegetable oil and/or cholesterol added to the basal diet.

Method and Materials: Sixty-four Sprague-Dawley rats were used. The control group was fed with the basal diet. In addition to the basal diet, the experimental groups were fed with: Experiment 1, 43% hydrogenated vegetable oil; Experiment 2, 10% cholesterol; Experiment 3, 43% hydrogenated vegetable oil and 10% cholesterol; Experiment 4, 3% *Spirulina platensis*; Experiment 5, 43% hydrogenated vegetable oil and 3% *Spirulina platensis*; Experiment 6, 10% cholesterol and 3% *Spirulina platensis*; and Experiment 7, 43% hydrogenated vegetable oil and 10% cholesterol and 3% *Spirulina platensis*.

Results: Serum glucose, C-reactive protein and total protein concentrations were not significantly affected by the applications. *Spirulina* supplementation was not effective on the feeding with hydrogenated vegetable oil or cholesterol for serum insulin concentrations. *Spirulina* added to the diet significantly decreased serum lipoprotein (a) levels compared to the control group.Moreover, the improver effect of *Spirulina* on serum apolipoproteins A1 and B levels was not observed.

Conclusion: The dose of *Spirulina* used in this study may not be sufficient. Future studies with *Spirulina* will provide the basis for developing new drugs to prevent or treat hypercholesterolemia and cardiovascular diseases.

Keywords: Dietary cholesterol, Hydrogenated vegetable oil, Rat, *Spirulina platensis*.

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Introduction

Epidemiological and clinical studies have shown an inverse relationship between the intake of antioxidant compounds and the incidence of chronic diseases such as cardiovascular diseases (Deng and Chow, 2010). Appropriate metabolic control is achieved by appropriate dietary intervention (Parikh et al., 2001).Medicinal plants are used more or less successfully against life-threatening diseases such as diabetes (Gargouri et al., 2016). Some marine plants have recently attracted attention because of their ability to improve bone metabolism because they are rich in minerals and growth factors.

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In recent years, *Spirulina* has attracted great attention as a potentially valuable food source in the prevention and treatment of chronic diseases (Zeinalian et al., 2017). *Spirulina* is a microalgae found in the *Cyanophyceae* class and has an outstanding mixture of dynamic components such as minerals, vitamins, proteins, beta-carotene, tocopherols and phenolic acids (Mazokopakis et al., 2014; Aladaileh et al., 2020) (Table 1). *Spirulina* naturally grows in alkaline water basins with high salt in subtropical and tropical regions such as America, Mexico, Asia and Central Africa (Fig. 1). In addition to its antiviral, anticancer and anti-inflammatory effects, *Spirulina* has proven to have a variety of nutritional and pharmacological properties and also regulates lipid and carbohydrate metabolism (Sigamani et al., 2016).

Fig. 1. *Spirulina platensis* (http://www.hashmidawakhana.co.in/spirulina-platensis.html).

Previous studies suggest that *Spirulina* has beneficial effects on non-alcoholic fatty liver disease, oxidative stress, hyperglycemia, hypercholesterolemia and arterial hypertension (Fujimoto et al., 2012). The extract of *Spirulina* inhibited lipid peroxidation and immunostimulatory effect was observed in mice in which *Spirulina* was given diet (Sadeka et al., 2017). This antioxidant activity is mostly caused by phycocyanin. Phycocyanin is capable of cleansing free radicals, reducing nitrite production, suppressing the expression of inducible nitric oxide synthesis and inhibiting microsomal lipid peroxidation in the liver (Hozayen et al., 2016). Allophycocyanin in *Spirulina platensis* is an important phycobiliprotein agent because of its ability to transfer energy to chlorophyll molecules (Cherdkiatikul and Suwanwong, 2014), and has antidiabetic, antioxidant, antihypertensive, antiviral, immunomodulatory, liver protective, renal protective, neuroprotective, anticancer and antigenotoxic properties (Liu et al., 2016).

Table 1. General composition, essential fatty acids and pigments of *Spirulina platensis* (Vonshak 1997).

General composition	**(%)**	**Essential fatty acids**	**(g/kg)**
	3-7		8
Moisture	55-70	Linoleic acid	10
Protein	6-8	ɣ-linoleic acid	
Fat (Lipids)	15-25	**Pigments**	3.7
Carbohydrate	7-13	Carotenoids	10
Minerals (Ash)	8-10	Chlorophyll	140
Fiber		Phycocyanin	

The normalization of blood glucose levels and the development of dyslipidemia are the cornerstones of effective treatment for diabetes (Parikh et al., 2001). Many herbal drugs have been proposed for the treatment of diabetes mellitus (Gupta et al., 2010). Considering that dyslipidemia and oxidative and inflammatory stress contribute to diabetes mellitus, *Spirulina* may be the most important functional food for the treatment of diabetes (Jara et al., 2018). The addition of *Spirulina* may be useful in providing long-term glycemic control and improving lipid profile in diabetic patients (Gupta et al., 2010). The hypoglycemic effect of *Spirulina* is physiological and persistent (Parikh et al., 2001). Yousefi et al. (2019) reported that *Spirulina* can be applied as a safe and effective supplement in the case of metabolic syndrome components. Moreover, *Spirulina* preparations contain immunoactive insulin and can therefore potentially be useful in people with diabetes mellitus (Anwer et al., 2012).

Substances with antioxidant and/or anti-inflammatory properties may be useful in combating cardiovascular diseases. Some studies have shown decreases in atherogenic markers such as improvements in lipid profile. Some studies reporting *in vitro* and *in vivo* antioxidant and/or anti-inflammatory activities of *Spirulina* and extracts suggest that *Spirulina* may have a beneficial effect (Deng and Chow, 2010). For example, *Spirulina versicolor* has anti-atherogenic, cardioprotective and antihyperlipidemic effects in fructose-fed rats (Hozayen et al., 2016). The area of the aortic fat line in the hamsters receiving *Spirulina* supplementation is significantly reduced, suggesting that *Spirulina* has anti-atherogenic activity in patients with hypercholesterolemic and ischemic heart disease (Riss et al., 2007). The cardiovascular benefits of *Spirulina* are mainly due to anti-inflammatory, antioxidant and hypolipidemic effects (Deng and Chow, 2010).

The aim of this study was to investigate changes in serum levels of glucose, insulin, lipoprotein (a), apolipoprotein A1, apolipoprotein B, C-reactive protein and total protein in rats fed with *Spirulina platensis* and/or hydrogenated vegetable oil and/or cholesterol added to the basal diet.

Materials and Methods

Ethical approval

This study was approved by Istanbul University-Cerrahpaşa Animal Care and Use Committee (Approval number: 2011/20). All animal experiments were carried out in accordance with

the EU Directive 2010/63/EU for animal experiments.

Sampling

Sixty-four 280-300 g male Sprague-Dawley rats were placed in polypropylene cages in a 12 hour dark/12 hour light environment. The animals were randomly divided into eight groups, consisting of a control and seven experimental groups.

Feeding

The trial period was 60 days. Food and water were given to the rats as *ad libitum*. 100% pure *Spirulina platensis* in powder form was purchased from Alg BioTek (Feneryolu Mh., Kiziltoprak 34724, Istanbul, Turkey) and was administered to experimental animals at a dose of 3g/100g diet (Yigit et al., 2016).The control group was fed with the basal diet. In addition to the basal diet, the experimental groups were fed with: Experiment 1, 43% hydrogenated vegetable oil; Experiment 2, 10% cholesterol; Experiment 3, 43% hydrogenated vegetable oil and 10% cholesterol; Experiment 4, 3% *Spirulina platensis*; Experiment 5, 43% hydrogenated vegetable oil and 3% *Spirulina platensis*; Experiment 6, 10% cholesterol and 3% *Spirulina platensis*; and Experiment 7, 43% hydrogenated vegetable oil and 10% cholesterol and 3% *Spirulina platensis*. Composition of diets showed (Table 2).

Methods

Blood samples were collected from all rats by coccygeal venipuncture on Day 30 and by cardiac puncture on Day 60. The samples were centrifuged at 2.500 x g for 15 minutes, then the sera were transferred to 1.5 ml microcentrifuge tubes and stored at -80 °C until use. Concentrations of serum glucose, lipoprotein (a), apolipoprotein A1, apolipoprotein B, C-reactive protein and total protein were measured spectrophotometrically using a biochemistry analyzer (Cobas 8000 Modular System, Roche Diagnostics, AG Industriestrasse 7 CH-6343 Rotkreuz, Switzerland) with commercial kits (Ben Biochemical Enterprise Srl, Via Pietro Toselli, 4, 20127 Milano, Italy). Serum insulin concentrations were measured using an ELISA equipment (µQuant, Bio-Tek, 100 Tigan St, Winooski, VT 05404, USA) with a commercial kit (Demeditec Diagnostics GmbH, Lise-Meitner-Straße 2, D-24145 Kiel, Germany).

Statistical analysis

The results are presented as mean±standard error of the mean. Data were compared by using analysis of variance (ANOVA coupled with Tukey's multiple range test) between groups within each blood sampling week for all blood indices at significance level of $p \leq 0.05$ (Ergun and Aktas, 2009). All statistical analyses were performed using software package program (SPSS for windows, Standard version 10.0, 1999, SPSS Inc., Headquarters, Chicago, IL, USA).

Results

On the 30th day of rats fed with basal diet and hydrogenated vegetable fat and/or cholesterol and/or *Spirulina platensis*; the serum glucose, insulin, lipoprotein (a), apolipoprotein A1, apolipoprotein B, C-reactive protein and total protein concentrations and statistical comparisons of the groups were showed (Table 3).

Serum glucose, lipoprotein (a), C-reactive protein and total protein concentrations were not significantly different between groups, on Day 30. Serum insulin concentration was significantly higher ($p \leq 0.05$) in Experiment 5 (43% hydrogenated vegetable oil and 3% *Spirulina platensis*) than in other all groups, on Day 30.

Serum apolipoprotein A1 concentrations on the 30th day were significantly higher in Experiment 6 (10% cholesterol and 3% *Spirulina platensis*) than in Experiments 1 (43% hydrogenated vegetable oil), 2 (10% cholesterol) and 3 (43% hydrogenated vegetable oil and 10% cholesterol) ($p \leq 0.05$). Serum apolipoprotein B levels were significantly lower ($p \leq 0.05$) in Experiments 1, 2 and 5 than in other groups, on Day 30.

On the 60th day of rats fed with basal diet and hydrogenated vegetable fat and/or cholesterol and/or *Spirulina platensis*; the serum glucose, insulin, lipoprotein (a), apolipoprotein A1, apolipoprotein B, C-reactive protein and total protein concentrations and statistical comparisons of the groups are shown in Table 4.

Serum glucose, C-reactive protein and total protein concentrations were not significantly different between groups, on Day 60. The serum insulin concentrations were the highest in Experiment 1 (43% hydrogenated vegetable oil) and the lowest in Experiment 6 (10% cholesterol and 3% *Spirulina platensis*), on Day 60. The levels on Day 60 were significantly higher ($p \leq 0.05$) in Experiments 1 and 5 (43% hydrogenated vegetable oil and 3% *Spirulina platensis*) than in Experiments 2 (10%

Table 2. Composition of diets (%).

Ingredients	Control	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Barley	23	5	16	-	23	5	16	-
Wheat	42	7	36	-	42	7	36	-
Rasmol	15	15	15	15	12	12	12	12
Soybean meal	15	15	15	15	15	15	15	15
Fish flour	2	12	5	14	2	12	5	14
Vegetable oil	2	2	2	2	2	2	2	2
Vitamin mineral premix	1	1	1	1	1	1	1	1
Hydrogenated vegetable oil	-	43	-	43	-	43	-	43
Cholesterol	-	-	10	10	-	-	10	10
Spirulina platensis	-	-	-	-	3	3	3	3

Control = Basal diet; Group 1 = Basal diet and 43% hydrogenated vegetable oil; Group 2 = Basal diet and 10% cholesterol; Group 3 = Basal diet and 43% hydrogenated vegetable oil and 10% cholesterol; Group 4 = Basal diet and 3% *Spirulina platensis*; Group 5 = Basal diet and 43% hydrogenated vegetable oil and 3% *Spirulina platensis*; Group 6 = Basal diet and 10% cholesterol and 3% *Spirulina platensis;* Group 7 = Basal diet and 43% hydrogenated vegetable oil and 10% cholesterol and 3% *Spirulina platensis*.

Table 3. Some serum markers in rats fed with hydrogenated vegetable oil and/or cholesterol and/or *Spirulina platensis* on Day 30.

Markers	Control	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6	Experiment 7
Glucose (mg/dl)	111±6.3	110±3.0	108±4.6	120±4.9	107±3.5	119±4.5	102±4.8	118±3.1
Insulin (µg/l)	1.07±0.16^{b}	0.88±0.06^{b}	0.60±0.03^{b}	0.62±0.09^{b}	0.98±0.16^{b}	1.70±0.31^{a}	0.86±0.17^{b}	0.80±0.06^{b}
Lipoprotein (a) (mg/dl)	32.1±0.22	32.5±0.24	32.3±0.26	32.0±0.20	32.5±0.23	32.3±0.20	32.2±0.08	32.3±0.21
Apolipoprotein A1 (mg/dl)	1.98±0.27^{ab}	1.49±0.25^{b}	1.47±0.27^{b}	1.55±0.24^{b}	2.42±0.17^{ab}	1.94±0.11^{ab}	2.64±0.25^{a}	1.85±0.29^{ab}
Apolipoprotein B (mg/dl)	6.95±0.16^{a}	3.17±0.28^{b}	1.83±0.61^{b}	6.42±0.93^{a}	5.67±0.66^{a}	3.15±0.47^{b}	7.59±0.86^{a}	6.45±0.51^{a}
C-reactive protein (mg/l)	6.02±0.34	8.32±1.08	9.12±0.77	7.16±0.25	6.68±0.71	7.28±1.03	8.09±0.53	7.26±0.87
Total protein (g/dl)	5.05±0.20	5.47±0.26	5.61±0.20	5.01±0.25	5.23±0.28	5.40±0.15	4.92±0.20	5.19±0.21

n = 8; Mean±Standard error of mean; ^{a,b} = Different superscripts indicate significant differences between groups (p≤0.05); Control = Basal diet; Experiment 1 = Basal diet and 43% hydrogenated vegetable oil; Experiment 2 = Basal diet and 10% cholesterol; Experiment 3 = Basal diet and 43% hydrogenated vegetable oil and 10% cholesterol; Experiment 4 = Basal diet and 3% *Spirulina platensis*; Experiment 5 = Basal diet and 43% hydrogenated vegetable oil and 3% *Spirulina platensis*; Experiment 6 = Basal diet and 10% cholesterol and 3% *Spirulina platensis*; Experiment 7 = Basal diet and 43% hydrogenated vegetable oil and 10% cholesterol and 3% *Spirulina platensis*.

Table 4. Some serum markers in rats fed with hydrogenated vegetable oil and/or cholesterol and/or *Spirulina platensis* on Day 60.

Markers	Control	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6	Experiment 7
Glucose (mg/dl)	104±5.3	123±3.6	105±3.8	126±5.2	94±4.0	123±8.7	112±6.9	124±8.2
Insulin (µg/l)	0.90±0.08^{ab}	1.17±0.09^{a}	0.56±0.12^{bc}	0.93±0.17^{ab}	0.61±0.18^{bc}	1.10±0.16^{a}	0.48±0.06^{c}	0.59±0.08^{bc}
Lipoprotein (a) (mg/dl)	32.4±0.09^{ab}	32.6±0.27^{ab}	32.1±0.12^{ab}	31.9±0.23^{b}	31.9±0.15^{b}	32.5±0.21^{ab}	32.7±0.07^{a}	32.4±0.10^{ab}
Apolipoprotein A1 (mg/dl)	2.19±0.13^{ab}	1.19±0.27^{c}	1.90±0.19^{abc}	2.38±0.15^{a}	2.29±0.15^{a}	2.19±0.12^{ab}	1.41±0.24^{bc}	2.08±0.23^{ab}
Apolipoprotein B (mg/dl)	5.24±0.21^{a}	4.93±0.24^{ab}	4.52±0.44^{ab}	6.32±0.23^{a}	4.95±0.62^{ab}	5.71±0.51^{a}	3.34±0.49^{b}	5.26±0.58^{a}
C-reactive protein (mg/l)	7.25±0.92	6.64±0.31	8.48±1.03	7.35±1.22	6.82±0.91	9.32±1.07	8.36±0.82	7.42±0.55
Total protein (g/dl)	4.85±0.25	5.80±0.18	5.23±0.37	5.10±0.18	5.08±0.12	5.30±0.24	5.30±0.26	5.31±0.72

n = 8; Mean±Standard error of mean; ^{a,b,c} = Different superscripts indicate significant differences between groups (p≤0.05); Control = Basal diet; Experiment 1 = Basal diet and 43% hydrogenated vegetable oil; Experiment 2 = Basal diet and 10% cholesterol; Experiment 3 = Basal diet and 43% hydrogenated vegetable oil and 10% cholesterol; Experiment 4 = Basal diet and 3% *Spirulina platensis*; Experiment 5 = Basal diet and 43% hydrogenated vegetable oil and 3% *Spirulina platensis*; Experiment 6 = Basal diet and 10% cholesterol and 3% *Spirulina platensis*; Experiment 7 = Basal diet and 43% hydrogenated vegetable oil and 10% cholesterol and 3% *Spirulina platensis*.

cholesterol), 4 (3% *Spirulina platensis*), 6 and 7 (43% hydrogenated vegetable oil and 10% cholesterol and 3% *Spirulina platensis*). Serum insulin levels were significantly higher ($p \leq 0.05$) in Control and Experiment 3 (43% hydrogenated vegetable oil and 10% cholesterol) than in Experiment 6, on Day 60.

Serum lipoprotein (a) concentration was significantly higher ($p \leq 0.05$) in Experiment 6 than in Experiments 3 and 4 on Day 60. Serum apolipoprotein A1 concentrations on Day 60 were the highest in Experiment 3 and the lowest in Experiment 1. The concentrations were significantly higher ($p \leq 0.05$) in Experiments 3 and 4 than in Experiments 1 and 6, on Day 60. Serum apolipoprotein A1 levels were significantly higher ($p \leq 0.05$) in Control and Experiments 5 and 7 than in Experiment 1. Serum apolipoprotein B concentrations were significantly lower ($p \leq 0.05$) in Experiment 6 than in Control and Experiments 3, 5 and 7, on Day 60.

Discussion

Mani et al. (2000) found a significant reduction in fasting blood glucose levels in patients receiving *Spirulina* at a dose of 2 g/day for 21 days. Similarly, Layam et al. (2006) found the same effect in diabetic rats treated with *Spirulina* at 15 mg/kg for 45 days. Abouzid et al. (2014) determined that *Spirulina versicolor* had anti-hyperglycemic activity in diabetic mice. Fasting and postprandial blood glucose levels were significantly reduced in two experimental groups receiving 1 or 2 g of *Spirulina* daily for two months in accordance with the decrease in carbohydrate and protein intake (Kaur et al., 2008).In contrast, 2 g/day *Spirulina* supplementation for 2 months caused a minor reduction in both fasting and postprandial glucose levels (Parikh et al., 2001). Lee et al. (2008) reported that *Spirulina* supplementation in patients with type 2 diabetes did not cause a decrease in plasma levels of fasting blood glucose and that *Spirulina* could not have a beneficial effect on blood glucose concentration in diabetic patients. Moura et al. (2012) found that addition of *Spirulina* could not improve serum glucose homeostasis in diabetic rats. Vide et al. (2015) determined that *Spirulina* enriched with silicone did not inhibit hyperglycemia.

In the present study, serum glucose concentrations were not significantly different between groups on Days 30 and 60. These findings are similar to the findings of Parikh et al. (2001), Lee et al. (2008), Moura et al. (2012), and Vide et al. (2015); however they are contrary to the findings of Mani et al. (2000), Layam et al. (2006), Abouzid et al. (2014), and Kaur et al. (2008). The reasons for this difference may be the absence of diabetes mellitus in rats of the present study, or differences in the dose of *Spirulina platensis* between this study and other studies.

Vázquez-Velasco et al. (2015) suggested that the addition of *Spirulina* increased the bioavailability of insulin. Oral administration of *Spirulina versicolor* improved insulin sensitivity and serum insulin levels in fructose-induced diabetic rats (Hozayen et al., 2016). Gupta et al. (2010) reported that insulin levels increased in diabetic rats treated with *Spirulina*. Ou et al. (2016) noted that the addition of *Spirulina platensis* increased insulin levels in diabetic mice. Moura et al. (2012) found that the diabetic *Spirulina* group had lower concentrations of serum insulin than those of diabetic exercise and diabetic exercise-*Spirulina* groups. However, Lee et al. (2008) found that *Spirulina* supplementation in patients with type 2 diabetes did not cause a decrease in fasting levels of plasma insulin. Similarly, Parikh et al. (2001) and Muthuraman et al. (2009) found that *Spirulina* did not significantly reduce insulinemia.

In the current study serum insulin concentration was significantly increased by *Spirulina platensis* supplementation in the hydrogenated vegetable oil added group on Day 30; but the concentrations did not significantly change by *Spirulina platensis* supplementation in the hydrogenated vegetable oil added group or the cholesterol added group on Day 60. The increase on the 30th day is similar to the findings of Gupta et al. (2010). The reason for this increase can be explained as follows. *Spirulina* is a rich protein source and provides good quality protein. Protein and amino acid uptake are known to induce insulin secretion (Parikh et al., 2001). However, this increase was not observed on the 60th day, probably due to the adaptation of rats. The findings of the 60th day of this study were consistent with the findings of Lee et al. (2008), Parikh et al. (2001), and Muthuraman et al. (2009). Since the addition of *Spirulina platensis* to the diet did not alter serum glucose levels (Table 3), it is not expected to affect serum insulin levels.

Vide et al. (2015) reported that the high-fat fed group showed a 194% increase in the insulin level of the blood compared to the standard-fed group and the high-fat diet caused insulin resistance.

Similar to Vide et al. (2015), in the present study, the hydrogenated vegetable oil added group had higher serum insulin concentrations than the others. According to Romain et al. (2012), insulin resistance caused by a high-fat diet is often associated with disorders such as oxidative stress and low-grade inflammation, both of which may cause atherosclerosis.

High-fat diet increases vascular dysfunction (Suh et al., 2011). Vide et al. (2015) reported that *Spirulina*, which is rich in silicon, may reduce atherosclerosis formation. Hozayen et al. (2016) noted that the treatment of diabetic rats with *Spirulina versicolor* significantly improved the atherogenic determinants. Reductions in atherogenic markers in rats treated with processed fructose and *Spirulina versicolor* support the idea that the addition of *Spirulina versicolor* to the diet may reduce the risk of heart disease (Hozayen et al., 2016). A recent study has shown that consuming 2 g/day of *Spirulina* for three months normalizes blood pressure in hypertensive and overweight individuals (Miczke et al., 2016).

Lipoprotein (a) is a cholesterol-rich low density lipoprotein particle containing 1 molecule apolipoprotein B100 and apolipoprotein (a) bound by disulfide bond. Cholesterol in the structure of lipoprotein (a) contributes to the expansion of atherosclerotic plaques more than cholesterol in the structure of low-density lipoprotein because lipoprotein (a) is bound to the extracellular matrix through apolipoprotein (a) and apolipoprotein (b) constituents of the structure after switching from plasma to arterial intima (Nielsen, 1999). High levels of lipoprotein (a) may increase the risk of cardiovascular disease. The reasons for this are the atherogenesis accelerated as a result of the intimal storage of lipoprotein (a) cholesterol, and/or the prothrombotic or anti-fibrinolytic effects of apolipoprotein (a) having structural homology with plasmin and plasminogen but not fibrinolytic activity. Patients with high or low risk of cardiovascular disease/coronary heart disease are advised to screen for high lipoprotein (a) (Nordestgaard et al., 2010). In the current study, the addition of cholesterol to *Spirulina* diet significantly increased serum lipoprotein (a) concentrations on Day 60. Comprehensive literature reviews have been conducted to discuss serum lipoprotein (a) concentrations, but no studies have been found similar to the subject of this study.

Since apolipoproteins A1 and B are the main protein components of high-density lipoprotein and low-density lipoprotein, respectively, they are frequently investigated as quantitative risk factors of coronary heart disease. Apolipoproteins A1 and B measurements can be used to assess the risk of coronary heart disease (Parikh et al., 2001). Medium chain fatty acids negatively affect the ratio of apolipoprotein A1 to apolipoprotein B. A diet rich in monounsaturated and polyunsaturated fatty acids along with restriction of certain carbohydrates has a good effect on apolipoprotein A1 levels. Cell cultures have been shown to have a decrease in the activity of apolipoprotein A1 gene promoter in the presence of trans fatty acids. The promoter of apolipoprotein A1 is sensitive to the oxidation of the cell and some antioxidants present in high concentrations may suppress the activity of the apolipoprotein A1 promoter (Mooradian and Haas, 2014).

Niebauer et al. (1996) showed that changes observed with addition of *Spirulina* (a significant decrease in apolipoprotein B and a significant increase in apolipoprotein B, leading to a marked increase in A1:B) correlated well with the low incidence of coronary heart disease. Parikh et al. (2001) examined patients with type 2 diabetes mellitus who received *Spirulina* 2 g per day for 2 months. At the end of the study, they found a significant decrease in atherogenic markers. The level of apolipoprotein B showed a significant decrease with a significant increase in apolipoprotein A1 level. Young and Hyun (2005) examined 26 women with hypercholesterolemia over 60 years of age. *Spirulina* consumption at 7.5 mg/day for 8 weeks resulted in a significant decrease in serum apolipoprotein B levels. Parikh et al. (2001) reported that 16.1 mg reduction of apolipoprotein B induced by *Spirulina* was remarkable because apolipoprotein B was independently associated with cardiovascular diseases and described high-risk phenotypes in patients with normocholesterolemic type 2 diabetes.

In the present study, while the addition of *Spirulina platensis* to cholesterol diet significantly elevated serum apolipoprotein A1 level on Day 30, the addition of *Spirulina platensis* to normal diet or cholesterol diet did not significantly alter serum apolipoprotein A1 levels on Day 60. Feeding with the hydrogenated vegetable oil diet on Day 60 significantly reduced the level of serum

apolipoprotein A1 and the addition of *Spirulina platensis* to the hydrogenated vegetable oil diet significantly increased the level of serum apolipoprotein A1. These findings are similar to the findings of Niebauer et al. (1996) and Parikh et al. (2001). Since apolipoprotein A1 is the major protein component of high-density lipoprotein, the high serum apolipoprotein Al level is desirable. The main mechanism of the atheroprotective effects of apolipoprotein A1 is the reverse transport of cholesterol via high-density lipoprotein cholesterol (Wang and Briggs, 2004). In the present study, the addition of *Spirulina platensis* to cholesterol diet or hydrogenated vegetable oil diet did not significantly reduce serum apolipoprotein B levels. This may be because the dose of *Spirulina platensis* added to the diet is not sufficient to reduce serum apolipoprotein B and hence low-density lipoprotein levels.

Conclusion

All levels were within normal ranges. Serum glucose, C-reactive protein and total protein concentrations were not significantly affected by the applications.The feeding with hydrogenated vegetable oil and/or cholesterol were not effective on serum insulin concentrations. *Spirulina* supplementation was not effective on feeding with hydrogenated vegetable oil or cholesterol in terms of serum insulin concentrations. The improver effect of *Spirulina* on serum apolipoproteins A1 and B levels was not observed. The dose of *Spirulina* used in this study may not be sufficient. Future studies, which will bring a mechanical perspective to the therapeutic effects of *Spirulina*, will provide the basis for the development of new drugs to prevent or treat hypercholesterolemia and cardiovascular diseases.

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Conflict of interest

Authors declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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