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RESEARCH ARTICLE

Therapeutic effect of the alkaloid extract of the cyanobacterium *Spirulina platensis* on the lipid profile of hypercholesterolemic male rabbits

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Abstract
The authors' objectives are to investigate the therapeutic effect of alkaloid extract of cyanobacteria *Spirulina platensis* on the lipid profile of hypercholesterolemic male rabbits and to identify the active compounds in the alkaloid extract. Male rabbits were divided into four groups of six animals. The intact rabbits in the first group served as a negative control. The second group served as a positive control (hypercholesterolemic rabbits). Over a 4-week period, hypercholesterolemic rabbits in the third group received a low dose of alkaloid extract (33 mg/kg), and the hypercholesterolemic rabbits in the fourth group received a high dose (66 mg/kg). The results revealed that both doses of alkaloid extract significantly decreased levels of cholesterol, triglycerides, low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) when compared to the control group, whereas the high-density lipoproteins (HDL) increased significantly compared to the control group. The active compounds in the alkaloid extract were identified using GC-mass. The most abundant compounds found in the extract were 1-(+)-ascorbic acid 2,6-dihexadecanoate, 9,12-octadecadienoic acid (Z, Z)-, hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ethyl ester, and gamolenic acid.

Keywords *Spirulina platensis* · Cholesterol · LDL · HDL · Rabbit

Introduction

Hyperlipidemia is high or abnormal levels of lipids or lipoproteins in the blood, a condition closely associated with heart diseases (Shattat et al. 2010). Hypercholesterolemia is caused by both environmental and genetic factors, with diet being the most prominent cause of its increase. Some scientists have expressed concern that the prevalence of animal protein and low fiber levels in the diet may increase the risk of cardiovascular disease and hypercholesterolemia. A balanced diet of plant proteins, unsaturated fatty acids, and dietary fiber may therefore lead to lower cholesterol levels in the blood (Iwamoto et al. 2000; Perugini et al. 2000; Schinella et al. 2000). Researchers and scientists around the world have undertaken research and published studies in an effort to find methods to treat hypercholesterolemia and avoid its risks. They have also turned their attention to finding alternative natural products and treatments to cure this disease so as to avoid the side effects caused by chemical drugs which may adversely affect functions of the body's organs.

In recent years, cyanobacteria have received widespread attention in this field due to their bioactive compounds (Abarzua et al. 1999; Dahms et al. 2006 Shimizu 2003; Trabelsi et al. 2016). Cyanobacteria produce these biologically active compounds as a strategy to survive in extreme and competitive environments, developing a significant level of diversity in the different metabolic pathways and thus constructing effective compounds (Barros et al. 2005;

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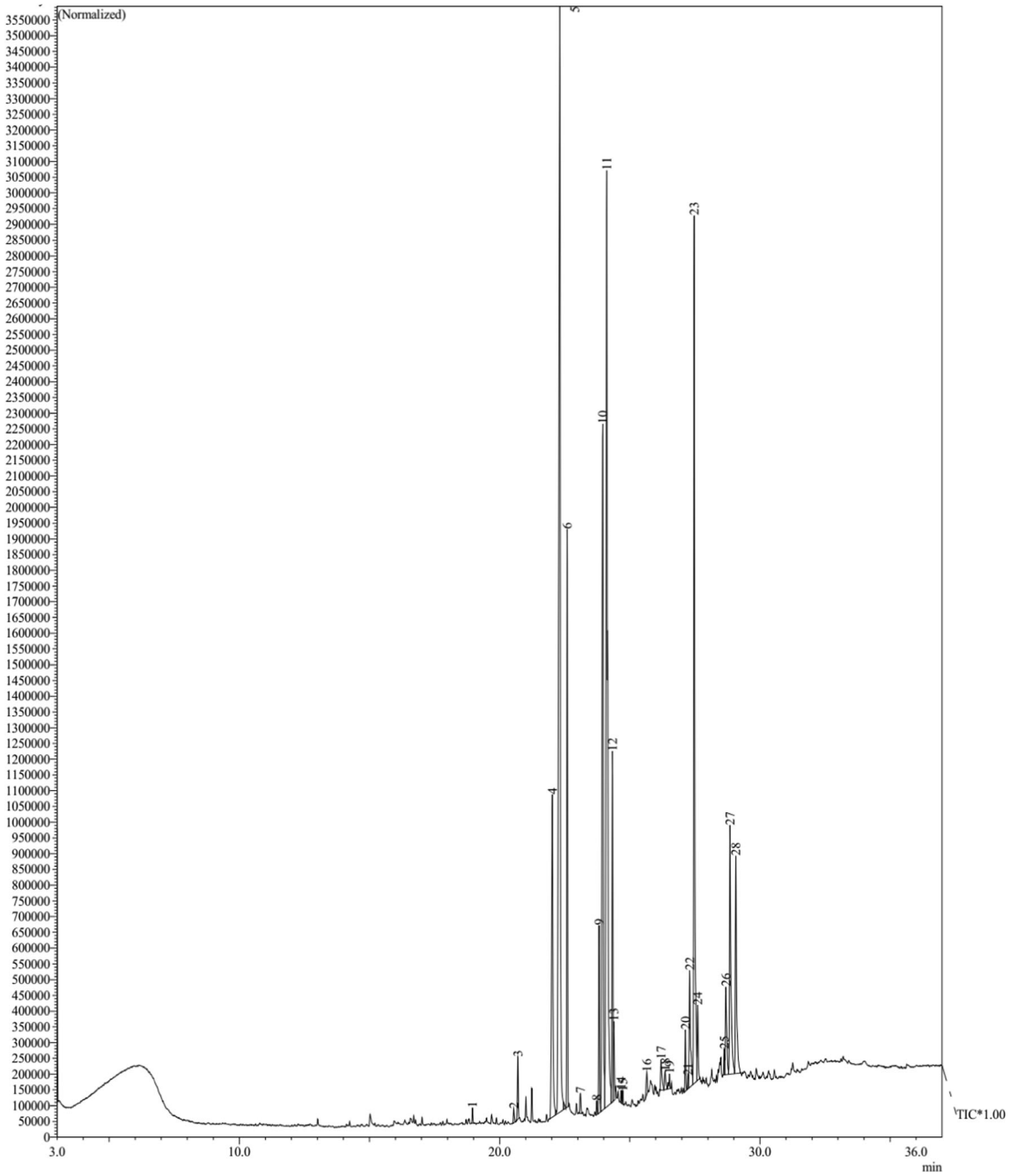

Fig. 1 Chromatogram of GC-mass analysis

Puglisi et al. 2004; Trabelsi et al. 2016). Studies have shown that they are an important source of many bioactive compounds as they synthesize them in their secondary metabolites. Cyanobacteria play an important antibacterial

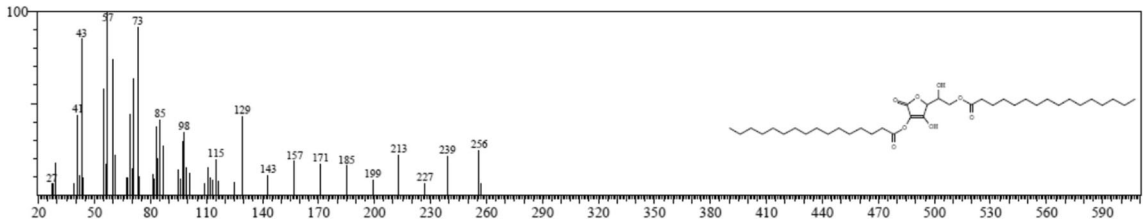

Fig. 2 Mass spectrum of 1-(+)-ascorbic acid 2,6-dihexadecanoate with chemical structure

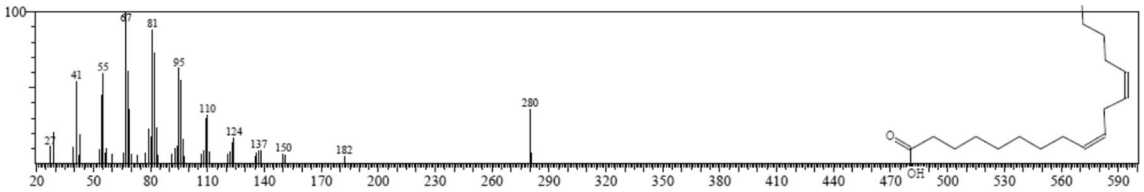

Fig. 3 Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-with chemical structure

(Jaki et al. 2000), antifungal (Kajiyama et al. 1998), antiviral (Patterson et al. 1994), anticancer (Gerwick et al. 1994), and anti-parasitic (Al-Jaber 2016) role and are effective in reducing fat levels (Yang et al. 2014).

Spirulina is a free-floating filamentous cyanobacteria, found in alkaline and highly saline water bodies, with high nutritional value, located in tropical and subtropical regions of the Americas, Asia, and Central Africa (Gershwin and Belay 2008).

The main morphological feature of *Spirulina* is the typical arrangement of its multicellular cylindrical trichomes in an open helix, usually of relatively large diameter, sometimes attenuated at the ends, and with evident cross-walls which can be clearly seen under the microscope. In addition, this genus has been used as food for centuries and is currently consumed worldwide as a dietary supplement (Deng and Chow 2010). It contains large amounts of protein (70% dry weight), carotenoid (4000 mg/kg), omega-3 and omega-6 polyunsaturated fatty acids, gamma linolenic acid (GLA), sulfolipids, glycolipids, polysaccharides, provitamins (vitamin A, vitamin E, various B vitamins), and minerals, including calcium, iron, magnesium, manganese, potassium, zinc, and selenium (Huang et al. 2007; Tokusoglu and Unal 2003). The World Health Organization (WHO) emphasized that this alga is not toxic and can be safely used as food (Salmean et al. 2015; WHO 1998). *Spirulina* has many pharmacological activities including antioxidant and anti-inflammatory, and could be used in many animal species as protective supplement against drug and pesticide toxicity (Abdel-Daim et al. 2013; Abdel-Daim 2014, Abdel-Daim et al. 2015; Abdelkhalek et al. 2015; Ibrahim and Abdel-Daim 2015; Abdel-Daim et al. 2016; Abdelkhalek et al. 2017). The present study was designed to investigate the effect of the alkaloid extract of *Spirulina platensis* on the lipid profile of hypercholesterolemic male rabbits.

Materials and methods

Preparation of alkaloid extract

The alkaloid extract was prepared according to Reichelt and Borowitzka (1984) by weighing 10 g of lyophilized moss mixed with 250 ml of ethyl alcohol, which were then mixed with 10% acetic acid. The extraction process was conducted using the continuous re-escalation Soxhlet device and filtered by grade 1 Whatman filter papers (0.2-mm-diameter slots). The filtrate was concentrated using a rotary evaporator at 50 °C and its pH modified to 9 by adding 25% of ammonia. After that, the solution was transferred to the separation funnel, 250 ml of chloroform was added, and the solution was shaken well and left for a period of time to isolate the alkaloid components from the water. The process was repeated five times at room temperature, and a material of a sticky texture was obtained then stored at laboratory temperature (25 °C).

The sticky substance weighed 1.5 g of the initial moss dark green. The samples were refrigerated at − 20 °C until used. Some of the active chemical compounds were isolated and identified from the alkaloid extract using GC-mass type GCMS-QP2010 Ultra.

Experimental animals

In this experiment, 24 male laboratory rabbits brought from the College of Medicine at the University of Basra were used. Their weights ranged from 1500 to 1600 g. They were housed in prepared cages and fed with bread and vegetables for a 10-day adjustment period. Temperature was maintained at 25 °C and humidity at 60%.

Inducing hypercholesterolemia

All males were randomly divided into two groups of 18 and 6 animals, respectively. The first group was injected orally with

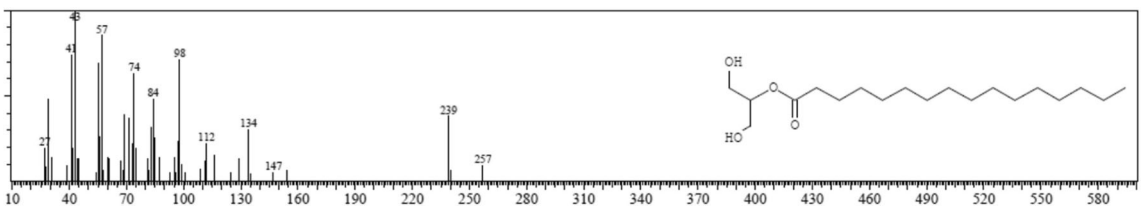

Fig. 4 Mass spectrum of hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester with chemical structure

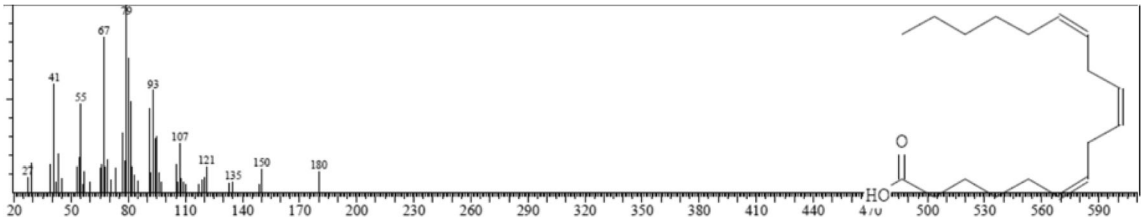

Fig. 5 Mass spectrum of gamolenic Acid with chemical structure

500 mg of soluble cholesterol (BDH product) for 2 weeks to induce hypercholesterolemia. The second group was injected orally with a physiological solution (normal saline) 0.9% to serve as a negative control group (Xu et al. 2000).

Experiment design

The rabbits were divided into four different treatment groups of six rabbits each:

- First group: a negative control, intact rabbits
- Second group: a positive control, hypercholesterolemic rabbits
- Third group: hypercholesterolemic rabbits treated orally with a low dose of 1 ml alkaloid extract of *S. platensis* (33 mg /kg), and
- Fourth group: hypercholesterolemic rabbits treated orally with a high dose of 1 ml alkaloid extract of *S. platensis* (66 mg /kg)

Doses were determined experimentally depending on LD50 which was 5000 mg/kg (Altug 2003). The rabbits were administered with doses daily for 4 weeks.

Blood samples were taken from the rabbits weekly from the bare inguinal vein using a 3-ml syringe. The blood was transferred to normal tubes and left 10–15 min to clot and then precipitated by centrifuge at 3500 rpm for 10 min. The serum was then separated from the blood and kept at − 10 °C (Thavasu et al. 1992).

Laboratory experiments

The present study included the measurement of the lipid profile of the rabbits including total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) using kits from Biolabo, France (Tietz 1999).

Statistical analysis

Statistical analysis was conducted with SPSS version-19. Data normality was tested using Kolmogorov-Smirnov. One-way ANOVA was performed to evaluate the significant difference between treatments under a probability of $P \leq 0.05$.

Results

Mass spectrometry of alkaloids extracted from alga *S. platensis*

The present study detected 28 peaks by the GC-mass as a result of algal extract component analysis (Fig. 1). One of the compounds was 1-(+)-ascorbic acid 2,6-dihexadecanoate with a retention time of 22.309 min and molecular weight of 652 KD (Fig. 2), 9,12-octadecadienoic acid (Z,Z)- with a retention time of 24.114 min and molecular weight of 266 KD (Fig. 3), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl with a retention time of

Table 1 Effect of the alkaloid extract of alga *Spirulina platensis* on the level of cholesterol in hypercholesterolemic rabbits (mg/dl, mean ± SD, $n = 6$)

Treatment	Time			
	First week	Second week	Third week	Fourth week
Negative control group (normal saline)	$105.200^{a} \pm 6.30$	$105.200^{a} \pm 6.30$	$105.200^{a} \pm 6.30$	$105.200^{a} \pm 6.30$
Positive control group (cholesterol)	$331.95^{b} \pm 65.26$	$265.58^{b} \pm 32.898$	$207.76^{b} \pm 13.92$	$201.25^{b} \pm 26.05$
First treatment group (33 mg/kg)	$231.53^{c} \pm 41.63$	$215.66^{c} \pm 24.66$	$151.82^{c} \pm 8.77$	$132.01^{c} \pm 10.93$
Second treatment group (66 mg/kg)	$243.92^{ac} \pm 61.31$	$210.40^{c} \pm 52.37$	$138.72^{c} \pm 4.87$	$118.54^{ac} \pm 20.66$

The different superscript letters a, b, and c indicate significant differences ($P \leq 0.05$) between the coefficients compared to the control groups

Table 2 Effect of the alkaloid extract of *S. platensis* on the level of triglyceride of hypercholesterolemic rabbits (mg/dl, mean ± SD, $n = 6$)

Treatment	Time			
	First week	Second week	Third week	Fourth week
Negative control group (normal saline)	$116.56^{a} \pm 38.84$	$116.56^{a} \pm 38.84$	$116.56^{a} \pm 38.84$	$116.56^{a} \pm 38.84$
Positive control group (cholesterol)	$383.70^{b} \pm 59.72$	$290.76^{b} \pm 39.00$	$261.16^{b} \pm 15.47$	$243.92^{b} \pm 66.61$
First treatment group (33 mg/kg)	$183.90^{a} \pm 54.71$	$144.60^{a} \pm 49.09$	$134.14^{a} \pm 20.60$	$122.53^{a} \pm 27.861$
Second treatment group (66 mg/kg)	$159.94^{a} \pm 52.90$	$111.81^{a} \pm 26.06$	$124.03^{a} \pm 20.88$	$95.994^{a} \pm 10.337$

The different superscript letters a, b, and c indicate significant differences ($P \leq 0.05$) between the coefficients compared to the control groups

27.473 min and molecular weight of 330 KD (Fig. 4), and gamolenic acid with a retention time of 23.963 min and molecular weight of 278 KD (Fig. 5).

Effect of the alkaloid extract of *S. platensis* on the cholesterol level of hypercholesterolemic rabbits

The results showed that the use of the alkaloid extract of *S. platensis* led to a significant decrease in cholesterol level ($P \leq 0.05$) when both alkaloid extract doses were administered orally and for all weeks compared to the positive control group (Table 1).

Effect of the alkaloid extract of *S. platensis* on the triglyceride level of hypercholesterolemic rabbits

Statistical analysis results showed a significant decrease in the triglyceride level ($P \leq 0.05$) after algal alkaloid extract treatment of laboratory animals during the treatment period when compared with the positive control group for all weeks and for both doses (Table 2).

Effect of the alkaloid extract of alga *S. platensis* on the HDL of hypercholesterolemic rabbits

Statistical analysis also showed a significant increase in HDL in the third week and for both doses and in the fourth week in the high dose only at a probability level of $P \leq 0.05$ when the rabbits were treated with alkaloid extract in comparison to the positive control group (Table 3).

Effect of alga *S. platensis* alkaloid extract on the low-density lipoprotein level of hypercholesterolemic rabbits

Results showed a significant decrease in the low-density lipoprotein level (LDL) ($P \leq 0.05$) when the rabbits were treated with algal alkaloid extract in all weeks and both doses in comparison to the positive control group (Table 4).

Effect of the alkaloid extract of alga *S. platensis* on the very low-density lipid level of hypercholesterolemic rabbits

Results showed a significant decrease in the very low-density lipid (VLDL) level with a probability of $P \leq 0.05$ when the

Table 3 Effect of the alkaloid extract of *S. platensis* on the level of high-density lipoprotein (HDL) of hypercholesterolemic rabbits (mg/dl, mean ± SD, $n = 6$)

Treatment	Time			
	First week	Second week	Third week	Fourth week
Negative control group (normal saline)	$31.39^{a} \pm 4.31$	$31.39^{a} \pm 4.31$	$31.39^{a} \pm 4.31$	$31.39^{a} \pm 4.31$
Positive control group (cholesterol)	$39.01^{b} \pm 1.45$	$38.75^{b} \pm 1.660$	$36.27^{b} \pm 2.13$	$38.40^{b} \pm 1.21$
First treatment group (33 mg/kg)	$38.38^{b} \pm 3.20$	$39.15^{b} \pm 1.383$	$39.74^{c} \pm 0.73$	$40.19^{b} \pm 1.13$
Second treatment group (66 mg/kg)	$40.30^{b} \pm 2.15$	$40.90^{b} \pm 0.57$	$41.07^{c} \pm 0.78$	$42.18^{c} \pm 0.59$

The different superscript letters a, b, and c indicate significant differences ($P \leq 0.05$) between the coefficients compared to the control groups

Table 4 Effect of the alkaloid extract of *S. platensis* on the level of low-density lipoprotein (LDL) of hypercholesterolemic rabbits (mg/dl, mean ± SD, $n = 6$)

Treatment	Time			
	First week	Second week	Third week	Fourth week
Negative control group (normal saline)	$50.49^{a} \pm 9.79$	$50.49^{a} \pm 9.79$	$50.49^{a} \pm 9.79$	$50.49^{a} \pm 9.79$
Positive control group (cholesterol)	$217.83^{b} \pm 11.77$	$170.00^{b} \pm 14.05$	$123.75^{b} \pm 13.67$	$117.05^{b} \pm 7.34$
First treatment group (33 mg/kg)	$156.36^{c} \pm 24.48$	$144.20^{c} \pm 19.05$	$85.667^{c} \pm 6.89$	$68.404^{c} \pm 4.18$
Second treatment group (66 mg/kg)	$171.64^{c} \pm 20.84$	$147.46^{c} \pm 22.57$	$72.837^{c} \pm 3.22$	$57.166^{a} \pm 3.19$

The different superscript letters a, b, and c indicate significant differences ($P \leq 0.05$) between the coefficients compared to the control groups

rabbits were treated with algal alkaloid extract for all weeks and both doses in comparison to the positive control group (Table 5).

Discussion

The results of the present study showed a decrease in the level of total cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoproteins (VLDL), as well as an increase in the level of high-density lipoproteins in serum of hypercholesterolemic rabbits, induced by oral gavage with the alkaloid extract of *S. platensis* for each week compared with the positive control group, thus corroborating other findings (Bertolin et al. 2009; Deng and Chow 2010; Elsheekh et al. 2014; Torres-Duran et al. 2014).

Reduction in the serum cholesterol level may be due to the fact that the alkaloid extract of *S. platensis* acted similarly to statins. The alkaloid extract can inhibit the enzyme -3-hydroxy-3-methyl glutaryl Co-A reductase (HMG-Co-A reductase) responsible for the process of cholesterol synthesis, acting as a competitive inhibitor by linking with it. This led to a reduction in cholesterol production in the liver and increased production of the fat-breaking enzyme lipoprotein lipase (LPL) (Moor et al. 2017). Another reason for cholesterol increasing is its conversion to bile acids in the liver by binding alkaloid extract to the bile salts and inhibiting their reabsorption from the small intestine and to be used again in the liver, and then their increased excretion through the waste, resulting in inhibition of the enterohepatic circulation of bile salts (Nagaoka et al. 2005).

The decrease in the lipid level can be attributed to the alga antioxidant metabolites, especially ascorbic acid, extremely capable of preventing oxidation of low-density lipoprotein (LDL). When LDL is oxidized in the body, the deposition of cholesterol in the lining of the blood vessels increases, causing the occurrence of arteriosclerosis; antioxidants therefore play a significant role in preventing the accumulation of lipids in blood vessels by eliminating the lipid peroxides derived through lipid peroxidation and free radicals (Karadeniz et al. 2009). Sangeetha et al. (2016) stated the ability of *S. platensis* to inhibit lipid peroxidation in diclofenac-treated rats and scavenge free radicals by impairing the activity of catalase superoxide dismutase which catalyzes hydrogen peroxide (H_2O_2) and protects the body from the effect of hydroxyl radicals that lead to LDL oxidation and lipid peroxidation.

Among the most important unsaturated fatty acids identified in the alga *S. platensis* alkaloid extract are linoleic

Table 5 Effect of the alkaloid extract of *S. platensis* on the level of very low-density lipids (VLDL) in hypercholesterolemic rabbits (mg/dl, mean ± SD, $n = 6$)

Treatment	Time			
	First week	Second week	Third week	Fourth week
Negative control group (normal saline)	$23.312^{a} \pm 7.77$	$23.312^{a} \pm 7.77$	$23.312^{a} \pm 7.77$	$23.312^{a} \pm 7.77$
Positive control group (cholesterol)	$76.740^{b} \pm 6.76$	$58.150^{b} \pm 7.80$	$52.274^{b} \pm 3.02$	$48.785^{b} \pm 1.77$
First treatment group (33 mg/kg)	$36.781^{c} \pm 6.85$	$28.920^{a} \pm 6.58$	$26.779^{a} \pm 4.08$	$23.254^{a} \pm 5.59$
Second treatment group (66 mg/kg)	$31.989^{ac} \pm 7.97$	$29.862^{a} \pm 8.20$	$24.806^{a} \pm 4.177$	$19.198^{a} \pm 3.71$

The different superscript letters a, b, and c indicate significant differences ($P \leq 0.05$) between the coefficients compared to the control groups

and gamolenic (GLA) acids which belong to the omega group 6. These two unsaturated fatty acids play an important role as anti-atherosclerosis agents through their effect on blood dilution, regulation of heartbeat, and reduction of platelet aggregation (Kritchevsky 2003). They also play a role in regulation of lipid synthesis and accumulation in the body by inducing the activity of the enzyme lipoprotein lipase and then reducing the accumulation of fatty acids in adipose tissue and muscle; they also have the ability to reduce the cholesterol level in blood through preventing free fatty acid access to the liver (Botelho et al. 2005; Okwu and Emenike 2006).

The algal extracts can activate the enzyme lecithin cholesterol acyltransferase (LCAT) which inhibits the biosynthesis of cholesterol and also plays an important role in the transverse cholesterol pathway when cells are unable to metabolize cholesterol. The presence of the enzyme (LCAT) increases the ability of HDL to stimulate the flow of cholesterol from cells to their receptors in the liver to decompose (Aviram and Davies 2004; Moor et al. 2017; Okwu and Emenike 2006; Rozenberg et al. 2003). The reduction in the triglyceride (TG) level of hypercholesterolemic rabbits treated with algal extract may be due to the presence of active compounds that activate lipoprotein lipase (LPL), which hydrolyzes TG to fatty acid and glycerol in the liver (Mead et al. 2002). The algal extract may also activate the cholesterol-lowering protein, cholesterol ester transfer protein (CETP), which transfers triglycerides to the VLDL molecule and then is destroyed in the liver, leading to a decrease in the serum TG level (Howell et al. 1998).

Conclusion

The active chemical compounds in the alkaloid extract of *S. platensis* play an important role in treating hypercholesterolemic male rabbits. These compounds succeed in decreasing levels of total cholesterol, triglycerides, and low-density lipoprotein, and in increasing the level of high-density lipoprotein in the serum of rabbits treated with algal extract.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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