

# Comparative Study of *Spirulina platensis* and Atorvastatin on the Serum Lipid Profile and Hepatica Enzyme in the Persian Shepherd Dogs

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## Abstract

**Introduction and aim:** This study was concentrated on the algae effects and compare it with the atorvastatin drug on the lowering of cholesterol and blood sugar.

**Methods:** In this research 24 native breed dogs were used for 30 days, the dogs were divided into three groups of eight, a group of control, a group orally receiving 2gr *Spirulina*, and receiving a group of 5mg/kg of Atorvastatin, and then blood samples were analyzed.

**Results:** We observed mainly reduction and increase in blood glucose in *Spirulina* and atorvastatin receiver groups respectively ( $P= 0.001$ ). Evaluation of lipid factors in dogs were declined in total cholesterol, Low-Density Lipoprotein levels, and Triglyceride factors ( $P<0.001$ ). This research illustrated that the use of *Spirulina* in dogs for a short time could improve lipid and liver factors.

**Conclusion:** Considering that the results of the present study are showed that using *Spirulina* can be concluded without worrying about allergic reactions and its side effects. It can also be used as a replacement for chemical drugs.

**Keywords:** Atorvastatin, Blood sugar, Hepatica enzyme, Lipid profiles, *Spirulina platensis*

## Introduction

Recently, natural compounds have received much attention in the treatment of chronic metabolic disorders like dyslipidemia and diabetes mellitus. Algae and vegetables have gainful influences on people and animals' health and basically, they have antidiabetic, anti-inflammatory, antioxidant, and anticancer features (Shahreza, 2017). Because of high protein percent, vitamin B<sub>12</sub>, essential fatty

acids ( $\gamma$ -linolenic acid), and pigments as phycocyanin and carotenoid and *Spirulina* microalgae have nutrient important (Mostolizadeh *et al.*, 2020; Nege *et al.*, 2020). In addition, *Spirulina* has some pharmaceutical usage including brain function boost, behavior, and learning, detoxification support, protection from radiation and attribute in anticancer (Miller & Ward, 1966; Mani *et al.*, 2005). Since the medicines available for the treatment of dyslipidemia, as well as chemical synthetic

lipid-lowering medicines, are not completely safe and devoid of side effects, scientists are always trying to find traditional herbal substitutions with lower toxicity (Palsamy and Subramanian, 2009; Tashakori-Sabzevar *et al.*, 2016). For a very long period, medicinal herbs and algae were traditionally and particularly used in eastern countries to numerous healthy troubles as diabetes, hypertension, liver illness, cardiovascular problems, and tumor. Although multiple studies have been done to consider diverse factors influencing diabetes management (Larijani, 2007; Tashakori-Sabzevar *et al.*, 2016), medical knowledge concerning herbal medicines is not sufficiently efficient. Although cholesterol is an essential substance in cell membrane formation and plays a basic role in making steroid hormones and vitamin D, high content can always play a role as a risk factor for cardiovascular complications in the body. However, in both clinical and experimental research, endocrine diseases are the reason for atherosclerosis, but in dogs seem to be tolerance into atherosclerosis because of discrepancy in lipoprotein contents and metabolism (Davis *et al.*, 2001; Bashandy *et al.*, 2016; Nogueira *et al.*, 2018; Ebrahim, 2020). Atorvastatin is important in lowering blood cholesterol by inhibiting cholesterol-producing enzymes and consequently the risk of disease. Antioxidant and cholesterol regulatory and immune effects have been proven. In addition, this complement by disorder in metabolism has been induced via decrease serum lipids and progress glucose resistance (Mani *et al.*, 2005; Ahsan *et al.*, 2015; Zaid *et al.*, 2015; Amer, 2016). Since 1919, scientists have been paying attention to identifying the composition and properties of algae. Also, its beneficial effects on patients with HIV, herpes and lowering cholesterol, regulating blood pressure, and treating anemia, iron deficiency and prevention of infectious diseases, weight loss, etc. have been previously recorded (Kulshreshtha *et al.*, 2008; Abdelkhalek *et al.*, 2017; Lauritano *et al.*, 2016). Karimi & Zia in 2016, showed that the use of *Spirulina* can lead to number

enhancement of spermatogenesis cells and the content of testosterone and FSH in small laboratory mice. Research demonstrated that administration of atorvastatin increased the number of cells in the islets of Langerhans and increased cells also increased insulin secretion and as a result decreased blood sugar (Karimian *et al.*, 2013). Ramezani Motaghadeh *et al.* (2016), during this research atorvastatin influence on depression in mice, concluded that atorvastatin doubles depression in gonadectomy mice. During the research of Yousefi *et al.* (2014), it was observed that the use of *Spirulina* in carp enhancement of some components of serum proteins such as gamma globulin and total protein (Yousefi *et al.*, 2014). Cunningham *et al.* (2013) showed that atorvastatin was well effective in appropriate drug doses in different dogs. Akbari & Sandak Zehi (2016) well realized that adding 15g/kg of *Spirulina* alga powder to the diet of gray mullet to improve growth indices; nutrition; carcass quality and increase in fatty acids were effective in this fish (Akbari & Sandak Zehi, 2016). Otto & Malau, (2017) concluded that feeding cows with *Spirulina* powder increased the amount and quality of their milk (Otto & Malau, 2017). Akter *et al.* (2014) demonstrated that *Spirulina* extracts have in the treatment of diabetes an important effect (Akter *et al.*, 2014; Emami & Olfati, 2017). Mariey (2012) showed that the diet of laying hens using *Spirulina* affects the egg quality and their growth (Jamil *et al.*, 2015; Mariey, 2012). Zahroojian *et al.* (2013), were noticed that the consumption of 2.5% *Spirulina* in the diet leads to more yolk production and *Spirulina* had an effective role in protecting mice from statin-induced hyperlipidemia by reducing triglycerides and total fat in total hepatic lipids with a lower reduction. They had serum acylglycerols (Zahroojian *et al.*, 2013). This study was concentrated on the algae effects and compare it with the atorvastatin drug on the lowering of cholesterol and blood sugar.

## Materials and Methods

**Preparation of algae:** *Spirulina platensis* algae oral tablets were prepared from a human pharmacy made by Reyhan Naghshe Jahan Pharmaceutical Company in 2020.

**Animals:** Hypolipidemic and anti-inflammatory medical roles in *Spirulina cyanobacteria* are as important as its dietary value (Ali *et al.*, 2015; El-Tantawy, 2016). In the present study, 24 adult dogs from the Persian Shepherd breed with a weight range of 25-30 kg were used. During the experiment, the dogs were kept in separate cages under standard conditions in the 12-hour light-darkness cycle and at 25-30°C, so that they could easily access water and food. The dogs were fed with a fixed diet (chicken heads, cooked chicken, and paws) for 1 month. Also, they were given anti-parasitic medications of Mebendazole and Praziquantel (based on recommended dosage) for two weeks before the start of the study to remove any digestive parasitic disease, they have also been cleansed of skin parasites through being injected twice with Ivermectin hypodermic and their skin being washed with anti-parasitic shampoo. Then their health was thoroughly and carefully examined before the start of the study. All of their vital signs, such as heart rate, respiratory rate, body temperature, and blood pressure, were checked and recorded. All of the dogs were being examined at least twice a day and were kept in separate cages; the place they were kept, was rinsed every day; its floor and the cages were disinfected once every 4 days, and all of the reports were recorded. This study was carried out in the Veterinary Hospital of the Islamic Azad University of Shahrekord located in Kian city of Shahrekord in Iran and observed all of the ethical guidelines for manipulation of animals (Mosallanejad *et al.*, 2016). Animals were fed every day at 9 A.M., then after one hour they were given the specified dosage of the extract hence dogs were randomly divided into three groups of eight-member.

The first group was a control group that did not receive anything other than water and food.

The second group was the receiver of 2-grams of *Spirulina* algae tablets, equivalent to 4 tablets of 500-mg.

The third group was the receiver of oral administration of 5-mg/kg of atorvastatin.

**Sampling and tests:** The dosage assigned was based on the prescribed dose for lab rats (Mosallanejad *et al.*, 2016). To blood let the dogs, blood vessels of the hand (Cephalic veins) were used in this manner: the dogs were restrained; hairs covering the target place were shaved; then it was disinfected with some cotton and alcohol, and at last bloodletting was performed gently. Collecting blood specimens was carried out for each dog individually three times on 0, 15<sup>th</sup> and 30<sup>th</sup> days during the research by syringes of 5cc blood content. Afterward, the samples were transferred to the separate and marked test tubes. When collecting the samples were finished, they were immediately transferred to the specialty laboratory of clinical pathology inside Ayatollah Kashani hospital in Shahrekord. Later, the intended factors of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Triglyceride (TGL), Low-Density Lipoprotein (LDL), Cholesterol (CHOL), High-Density Lipoprotein (HDL), and fast blood sugar (FBS) were measured by enzymatic and photometric methods in the laboratory equipped with Spectrophotometric kits of Pars Azmoon Co. and by the Eco-Plast Auto Analyzer machine (made in Italy) having the 2006GD100139 serial number.

**Statistical analysis:** At the descriptive statistics level, indexes such as mean and standard deviation were used and data by SPSS software were analyzed. Results were shown with Mean  $\pm$ SD. In addition, the Tukey test was used to compare the mean of the two data groups. In all analyzes, a significance level of less than 0.05 will be considered.

## Results

Adding *Spirulina* to animal diets can treat some diabetic diseases by decreasing blood sugar;

help to improve insulin function and regulate hypercholesterolemia (Gupta *et al.*, 2010). Thus, the present research investigated the influence of *Spirulina* on the biochemical factors in the blood serum of the animal sort of samples, i.e., dogs.

**Fast blood sugar (FBS):** Comparing the FBS levels of the three groups indicated that according to Table 1, the mean blood glucose of dogs before the intervention did not differ significantly among the three groups ( $P > 0.05$ ). But on the 15<sup>th</sup> day after blood glucose intervention in the atorvastatin receiver group with a mean of  $100.37 \pm 2.72$  mg/dl significantly

more than the two control groups and *Spirulina* receiver group with an average of  $89.37 \pm 5.18$  mg/dl and  $80.75 \pm 3.84$  mg/dl, respectively. It was ( $P < 0.001$ ). As well, the lowest blood sugar means observed in *Spirulina* ( $P < 0.001$ ). On the 30<sup>th</sup> day, these differences were similarly significant ( $P < 0.001$ ). Evaluation of changes in mean blood sugar in each of groups over time using analysis of variance in repeated observations also showed that in the dog's control group the blood sugar level did not change significantly during 30<sup>th</sup> days but showed a decrease and increase in *Spirulina* and atorvastatin receiver groups respectively ( $P < 0.001$ ) (Table 1).

**Table 1.** Results of FBS variables in three groups

Variables	Time	Control	<i>Spirulina</i>	Atorvastatin	SL1	SL2	SL3
FBS mg/dl	Baseline	90.25±3/84	94.25±4.89	90.00±6.19	0/129	0.770	0.213
	15 days	89.37±5.18	80.75±3.84	100.37±2.72	<0.001	<0.001	<0.001
	30 days	91.75±4.52	72.88±3.23	107.13±3.48	<0.001	<0.001	<0.001
	L4	0.264	0.001	0.001			

Significance level 1 (SL1): Results of the mean of the variable between *Spirulina* receiver group and control  
 Significance level 2 (SL2): Results of mean variables between control and atorvastatin receiver groups  
 Significance level 3 (SL3): Results of mean variables between atorvastatin and *Spirulina* receiver groups  
 Significance level 4 (SL4): Results of the mean of the variable over time in each of the three groups

**Lipid profiles:** Hyperlipidemia is defined as the increase of lipid concentration (triglyceride or cholesterol) in blood. Hypertriglyceridemia (i.e., increased blood triglyceride concentration over 200mg/dl) is a cause of lipemia. Hyperlipidemia in dogs is divided into rudimentary and secondary; the latter is more general in dogs (Xenoulis & Steiner, 2010). In the present study, the evaluation of lipid profiles i.e., LDL, HDL, TG, and TCH among the three groups showed that, in general, all the intended lipid parameters at the beginning of the study were not significantly dissimilar ( $P > 0.05$ ). Mean LDL of dogs on the 15<sup>th</sup> and 30<sup>th</sup> days after LDL intervention in the atorvastatin receiver group with a mean of  $50.50 \pm 8.65$  and  $35.50 \pm 2.67$  mg/dl, respectively, then in the *Spirulina* receiver group with a mean of  $63.50 \pm 4.50$  mg/dl and  $60.50 \pm 7.19$  mg/dl were

significantly less than control with a mean of  $75.63 \pm 3.38$  mg/dl and  $78.50 \pm 4.69$ , respectively. In addition, evaluation of changes in mean LDL in each of the three groups over time using analysis of variance in repeated observations showed that the dog's LDL level did not change significantly during the 30<sup>th</sup> day in the control group ( $P = 0.085$ ), but both other groups had a significant reduction ( $P < 0.001$ ). Also, the mean of dog's HDL doesn't show mainly different among the groups ( $P > 0.05$ ). Although, HDL levels in the atorvastatin group with a mean of  $54.63 \pm 2.77$  mg/dl were significantly more than others ( $P < 0.001$ ). Likewise, on the 30<sup>th</sup> day, the highest HDL level was observed in the atorvastatin group ( $P < 0.001$ ).

In addition, investigating the changes HDL means in each other's overtime using analysis

of variance in repeated observations showed that the HDL level of dogs in the control group did not change significantly for 30 days ( $P=0.711$ ), but in both groups' others had an important increase ( $P<0.001$ ). In addition to before the intervention, the total cholesterol means of dogs was not significantly different among the three groups ( $P> 0.05$ ). On the 15<sup>th</sup> and 30<sup>th</sup> days, total cholesterol level has not displayed a difference between the *Spirulina* receiver group and the control ( $P=0.383$ ); but the lowest total cholesterol level was obtained in the atorvastatin group ( $P<0.001$ ). Also, assessment of total cholesterol means changes in each of groups over time using variance analysis demonstrated that total cholesterol level in dogs in the control group did not change significantly during 30 days ( $P= 0.506$ ), but in the other two groups had a remarkable decrease ( $P<0.001$ ). The result of the dog's triglyceride mean was not significantly varied among the three groups ( $P>0.05$ ). The maximum triglyceride level on the 15<sup>th</sup> day was observed in the control group with a mean of  $93/10\pm 25/143$  mg/dl ( $P<0.001$ ). Also, on the 30<sup>th</sup> day, triglyceride levels in the atorvastatin group with a mean of  $19/8\pm 63/124$  mg/dl were significantly lower than the control and *Spirulina* algae with an average of  $88/9\pm 25/144$  and  $14/5\pm 75/133$  mg/dl, respectively ( $P<0.001$ ). In addition, evaluation of mean changes in triglyceride in each of the three groups over time using analysis of variance in repeated observations also showed that the triglyceride level of dogs in the control group did not change significantly for 30 days ( $P=0.899$ ), but in the other two groups had a significant decrease (Table. 2).

**Liver enzymes:** According to the results, the mean AST of dogs before the intervention did not differ significantly among the three groups ( $P> 0.05$ ). But on the 15<sup>th</sup> day after AST intervention in the atorvastatin group with a mean of  $40.13\pm 1.46$  mg/dl significantly more than the two control groups and *Spirulina* algae with an average of  $37.38 \pm 1.41$  and  $30.13 \pm 2.26$  mg/dl, respectively ( $P<0.001$ ). Also, the least AST mean

to get in the *Spirulina* receiver group ( $P<0.001$ ). On the 30<sup>th</sup> day, these differences were similarly significant ( $P<0.001$ ). In addition, evaluation of changes in mean AST in each of the three groups over time using analysis of variance in repeated observations showed that the AST level of dogs in the control group did not change significantly for 30 days ( $P=0.491$ ) but decrease and increase dog's AST was seen in *Spirulina* and atorvastatin receiver groups respectively ( $P<0.001$ ). Also, mean dog's ALT before the intervention did not show significant differences among the three groups ( $P> 0.05$ ). On the 30<sup>th</sup> day after the intervention, the level of ALT was not shown between the control and *Spirulina* receiver groups significantly different ( $P= 0.903$ ); However, ALT level in the atorvastatin receiver group with a mean of  $49\pm 3.70$  mg/dl was significantly more than two other groups ( $P<0.05$ ). Also, on the 30<sup>th</sup> day, the ALT level in the atorvastatin receiver group with a mean of  $74.13\pm 5.69$  mg/dl was significantly more than the two other groups. In addition, evaluation of changes in mean ALT in each of the three groups over time using analysis of variance in repeated observations showed that in the control group ALT dog's level did not modify significantly for 30 days ( $P=0.662$ ), but dog's ALT in *Spirulina* receiver group significantly decreased ( $P=0.002$ ) and in the group, atorvastatin increased significantly.

## Discussion

The results of Gupta *et al.* (2010) demonstrated that some medical properties as antihyperglycemic activities by improving insulin, lowering blood glucose levels, and regulating triglyceride, cholesterol are made by *Spirulina* microalgae consumption. Mehrabianfard and co-worker in 2007 by administering atorvastatin to male rats found that administration of this drug increases the number of cells in the islets of Langerhans and also decreases blood sugar and consequently increases insulin secretion.

Data of Ma *et al.* in 2015 and even Pandey and colleagues in 2011 concluded that

*Spirulina* beta-carotene extract in diabetic rats showed hypoglycemic activity by lowering blood glucose levels and regulating increased absorption of water and streptozotocin-induced nutrients. Also, in 2017, Shams *et al.* concluded that *Spirulina* has anti-diabetic effects. Mosalanejad *et al.* (2016) concluded that taking atorvastatin with 5mg/kg dose once a day into 6 weeks led to a reduction in triglycerides, total cholesterol, and phospholipids in dogs. Muga & Chao showed that the simultaneous use of pyrolysis and fish oil to hamsters lowered cholesterol (Muga & Chao, 2014). In addition, Dadgar *et al.* (2011) referred to the affirmative effect of *Spirulina* on the mice's hyperlipidemia by reducing triglyceride and total fat along with lowering serum triglyceride. Also, Saeid and colleagues in 2013 observed that nourishment of piglet's duration 87 days with *Spirulina* led to decline the LDL and total cholesterol percent ineffective on weight gain. Evans *et al.* (2015) indicated that *Spirulina* affected the triglyceride, HDL, cholesterol, and LDL of broiler chicken. Briand *et al.* concluded that therapy of dogs by atorvastatin significantly reduced total cholesterol; Phospholipids are plasma triglycerides. At doses higher than 5 mg/kg, this drug increased HDL-C in Beagle dogs (Briand *et al.*, 2006), which in many cases is fully resemblance to the results of our project. Yousefi *et al.* (2014) by studying the effect of oral consumption of *Spirulina* algae on general protein content and electrophoretic pattern of carp serum proteins concluded that using *Spirulina* algae in carp rises some components of serum proteins as gamma globulin and total protein. Gharibi and co-workers recorded that white shrimp larvae treated with *Spirulina* mass index were higher and had a significant difference (Gharibi *et al.*, 2013). Being one of the major organs in the body, the liver is responsible for regulating many metabolic and physiological processes, producing bile, detoxifying, excreting waste materials, synthesizing and adjusting some essential hormones in the body. AST and ALT are the most important and the most functional liver diagnostic enzymes, whose rise in the blood are

symptoms of damages to the liver and are considered as specific criteria for the diagnosis of hepatocellular necrosis. Enzymes are entered into the bloodstream in the event of injury to the hepatocytes' cell membrane. Therefore, measuring the levels of ALT and AST enzymes is clinically and toxicologically important. Change in the activity level of these enzymes indicates tissue damages resulting from toxins or disease so that the increase in ALT and AST levels are considered hepatocellular damage indicators. Zahroojian & partners in 2013 as Otto in 2017 observed that using *Spirulina* in the diet of poultry and livestock led to the production of more yolk and improved milk quality in cows. Feeding the sheep for 35 days with *Spirulina* improved daily weight growth and food uptake. EL-Sabagh in 2014 was also observed an increase in hemoglobin; total leukocyte count; serum globulin while a decrease in amino aspartate transferase; cholesterol and glucose concentrations were observed in the comparison control group. Kharde and colleagues in 2012 recorded that nutrition broilers with diets containing *Spirulina* algae from 300 to 500 mg/kg along 6 weeks notable increased the body weight means versus to the control group, which was resembled results were observed by Raju in 2005 (Farag *et al.*, 2016). *Spirulina* C-phycocyanin pigment, a great antioxidant, was found in a study of broilers exposed in their diet to heat in 2% *Spirulina* algae, MDA levels were significantly less than in other birds exposed to heat. The effect of *Spirulina* extracts hypolipidemic has been investigated by several testing on the animal's laboratory (El-Sayed *et al.*, 2018).

### Conclusion

Finally, due to the results, dogs' blood glucose in the *Spirulina* algae group decreased significantly and in the atorvastatin group increased significantly. In general, observation of this essay is resembling with most like studies. Therefore, attention can be directed toward its anti-diabetic, anti-atherosclerotic, antioxidant effects. This research illustrated that the use of *Spirulina* microalgae in dogs for

a short time could improve lipid and liver factors. Thus, after considering the point of view of this project, we achieved that probably oral consumption of *Spirulina* algae reduces blood sugar and some parameters related to blood lipids. Therefore, consumption of this

algae is recommended for the treatment of animals suffering from diabetes, high cholesterol, and hepatically diseases. It should be noted that the main active ingredient in algae is Spirulina according to research on C-phycoocyanin.

**Table 2. The lipid profiles and liver enzymes variables at three times**

Variables	Time	Control	<i>Spirulina</i>	Atorvastatin	SL1	SL2	SL3
LDL (mg/dl)	Baseline	74.75±2.43	79.00±9.02	73.62±3.29	0.152	0.698	0.074
	15 days	75.63±3.38	63.50±4.50	50.50±8.65	0.001	<0.001	<0.001
	30 days	78.50±4.69	60.50±7.19	35.50±2.67	<0.001	<0.001	<0.001
	SL4	0/085	<0.001	<0.001			
HDL mg/dl	Baseline	34.37±2.50	36.50±4.57	33.75±3.28	0.245	0.730	0.137
	15 days	33.13±4.22	36.25±3.41	54.63±2.77	0/090	<0.001	<0.001
	30 days	34.00±2.62	52.63±2.77	53.00±3.82	<0.001	<0.001	<0.001
	SL4	0.711	<0.001	<0.001			
TG mg/dl	Baseline	142.75±4.71	147.37±4.84	144.00±4.14	0.056	0.590	0.155
	15 days	143.25±10.93	132.63±6.72	131.25±5.70	0.016	0.007	0.738
	30 days	144.25±9.88	133.75±5.14	124.63±8.19	0.016	<0.001	0.033
	SL4	0.899	<0.001	<0.001			
Total Cholesterol mg/dl	Baseline	278.62±9.05	275.62±9.93	0.557	0.557	0.246	278.62±9.05
	15 days	276.00±7.05	272.13±3.36	0.383	<0.001	<0.001	276.00±7.05
	30 days	279.88±6.45	264.13±3.98	<0.001	<0.001	<0.001	279.88±6.45
	SL4	0.506	<0.001				0.506
AST IU/L	Baseline	36.50±2.56	39.62±4.93	37.50±3.34	0.110	0.599	0.269
	15 days	37.38±1.41	30.13±2.36	40.13±1.46	<0.001	0.006	<0.001
	30 days	36.25±2.12	26.50±1.60	53.25±7.89	<0.001	<0.001	<0.001
	SL4	0.491	<0.001	0.001			
ALT IU/L	Baseline	42.00±4.31	44.25±4.53	44.12±3.72	0.296	0.323	0.953
	15 days	44.25±5.34	44.00±2.72	49.00±3.70	0.903	0.030	0.023
	30 days	42.87±4.45	37.88±1.89	74.13±5.96	0.035	<0.001	<0.001
	SL4	0.662	0.002	<0.001			

Significance level 1 (SL1): Results of the mean of the variable between the control and Spirulina receiver groups

Significance level 2 (SL2): Results of mean variables between control and atorvastatin receiver groups

Significance level 3 (SL3): Results of mean variables between atorvastatin and Spirulina receiver groups

Significance level 4 (SL4): Results of the mean of the variable over time in each of the three groups

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