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Short Communication

An alternative organic diet in preventing hyperlipidemia in cholesterol-fed Sprague-Dawley rats by *Arthrospira* (*Spirulina) platensis*

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Abstract

This study aimed to investigate the effects of *Arthrospira* (*Spirulina) platensis* in high cholesterol-fed Sprague-Dawley rats from a blood biochemistry analysis by evaluating the total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), and kidney and liver function parameters (total creatinine, blood urea nitrogen, aspartate aminotransferase, and alanine aminotransferase. The highest levels of TC, LDL, and TG recorded in rats fed the high cholesterol diet were 5.85±0.16 mmol/L, 2.93±0.65 mmol/L, and 3.32±0.14 mmol/L ($P<0.05$), respectively. However, these cholesterol levels were significantly reduced ($P<0.05$) after *Arthrospira* supplements (150 mg/kg and 300 mg/kg) for 27 days. There were no significant changes in HDL levels for all of the treated rats. Throughout the study, the kidney and liver functions of the rats remained unaffected. In conclusion, both dosages of *Arthrospira* displayed tremendous potential as an anti-hyperlipidemic agent.

Keywords: *Spirulina* sp, total cholesterol, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase

1. Introduction

Annually, about 17.5 million of people die of cardiovascular disease which represents 31% of global mortality (World Health Organization, 2016). Hyperlipidemia, which is also known as dyslipidemia, is a state of a high level of cholesterol in the blood which can significantly contribute towards the development of cardiovascular diseases such as atherosclerosis and coronary heart disease in humans (Nelson, 2013).

Algae have existed on earth from about 3.5 billion years ago. The sizes range from 0.2 to 2.0 μm in diameter (microalgae) and up to 60 m in length (macroalgae) (Christaki, 2014). Algae is one of the food sources or alternative supplements and are not new to people. It has probably been used for centuries according to Ciferri and Tiboni (1985). Over 1000 years ago, the Aztec civilizations, namely Toltecs, Mayas, and Kanembu, consumed alga specifically *Spirulina* sp. in their routine diet (Ciferri & Tiboni, 1985). It has also been recognized by the people of Chad in Central Africa as one of their edible food sources (Abdulqader, Barsanti, & Tredici, 2000). *Spirulina* sp. is an unbranched, free floating filamentous blue-green algae or cyanobacterium with spiral filaments (Deng & Chow, 2010). As a reference, *Arthrospira* or *Spirulina* sp. is known for its high essential proteins, vitamins, β-carotene, phycocyanin, and γ-linolenic acid (Costa, Linde, Atala, Mibieli, & Kruger, 2004). All of these nutrients have made this alga a reliable agent for anti-cholesterol (Kim & Kim, 2005), anti-inflammatory, antipyretic (Muhammad Nazrul *et al.*, 2014), anti-cancer (Konícková *et al.*, 2014), antioxidant (El-Sabagh, Eldaim, Mahboub, & Abdel-Daim, 2014), and antibacterial activities (Ozdemir, Karabay, Dalay, & Pazarbasi, 2004).

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In 1983, Devi and Venkataranam performed the first preclinical test of *Spirulina* sp. extract on albino rat on its potential in controlling hyperlipidemia (Devi & Venkata ranam, 1983). Since then, several researchers have confirmed these findings in bigger animal models (e.g., lamb, calves, broilers, and rabbits) and in humans. Rats are not typically viewed as good models for lipoprotein metabolism because of several dissimilarities between rats (mostly the wild type) and humans (Conn, 2008). However, rats were used in great studies related to hepatic cholesterol metabolism and plasma lipoprotein responses. In rats, cholesterol is transported primarily as a high density lipoprotein (HDL) cholesterol, small amounts of low density lipoprotein (LDL) cholesterol, trace amounts of very low density lipoprotein (VLDL), and very high activity of cholesterol 7α-hydroxylase (CYP7) (Norlin, Andersson, Björkhem, & Wikvall, 2000). These conditions cause rats to readily eradicate excess body cholesterol and resist developing atherosclerosis which is the hallmark event for coronary artery disease. Rats respond to a dietary cholesterol challenge by down-regulating cholesterol synthesis and up-regulating bile acid synthesis which results in changes in their total plasma levels (Alberti *et al.*, 2001). Thus, preliminary research related to hyperlipidemia in rats is significantly important to improve the health in humans.

In this study, emphasis was on the beneficial effects of *Arthrospira* (*Spirulina) platensis* on cardiovascular disease using the Sprague-Dawley rat model with highlights on hyperlipidemia and the safety profiles of enzyme activities of the kidney and liver.

2. Materials and Methods

2.1 Source of *A. platensis* and maintenance

A sample of *A. platensis* was isolated at Tasik Dayang Bunting, Langkawi, Malaysia. The strain was maintained and grown in modified Zarrouk's medium at an ambient temperature of 30±2 °C, pH 9.0, and under a light source from a cool white fluorescent lamp (30 µmol photon $m^{-2}s^{-2}$) with a photoperiod cycle of 12 hours light and 12 hours dark.

2.2 Animals and diets

A total of 16 male Sprague-Dawley rats (size range 125-185 g) were used in this investigation. The rats were randomly assigned into four experimental groups of four rats each: 1) negative control (NC) group fed commercial rat pellet, 2) positive control (PC) fed commercial rat pellet plus 3% cholesterol, 3) 150ASP group fed commercial rat pellet plus 3% cholesterol and treated with 150 mg/kg body weight of *A. (Spirulina) platensis*, and 4) 300ASP group fed commercial rat pellet plus 3% cholesterol and treated with 300 mg/kg body weight *A. (Spirulina) platensis*. To prepare the 3% cholesterol-enriched diet, pure cholesterol powder was dissolved in chloroform. Three grams of cholesterol was then mixed with 100 g of intact pellet. Any excess chloroform was evaporated in a fume hood until no chloroform odor was detected. The *A. platensis* treatments were done up to 27 days and water was given *ad libitum* to all animals throughout the period. *A. platensis* employed in this experiment was in a concentrated form. Hence, it was added to 1 mL of distilled water prior to the daily oral gavage feeding to the rats.

2.3 Blood collection and analysis

Under isoflurane anesthesia procedure, blood (1 mL) was drawn from a cardiac puncture using a 26-gauge needle (Terumo, Japan). Blood was collected accordingly on days 0, 9, and 27 prior to centrifugation at 5000 rpm for 10 min. The blood samples were analyzed for cholesterol levels (total cholesterol [TC], LDL, HDL, and triglycerides [TG]), kidney function parameters (total creatinine and blood urea nitrogen [BUN]), and liver enzymes (aspartate amino-transferase [AST] and alanine aminotransferase [ALT]) using auto-analyzer systems (Roche COBAS MIRA and Hitachi 902 Machine).

2.4 Statistical analysis

All data are expressed as mean±standard deviation (SD). One-way ANOVA was used to compare the statistical differences between treatment groups and the control group. Significance was accepted at $P<0.05$.

3. Results and Discussion

3.1 Total cholesterol

The γ-linolenic acid (GLA) is the most important fatty acid in *Arthrospira* sp. which can prevent the accumulation of fats and cholesterol in the body. Apart from mother's milk (Belay, Yochimichi, Miyakawa, & Shima matsu, 1993), there are several plant-based oils that contain this essential fatty acid which include evening primrose oil, borage oil, black current seed oil (University of Maryland Medical Center, 2016), and hempseed oil (Prociuk *et al.*, 2008). The hyperlipidemic effect of *Arthrospira platensis* accordingly depends on the availability of GLA (Belay *et al.*, 1993). Prociuk *et al.* (2008) reported that a hempseed-diet introduced in New Zealand white rabbits successfully returned the cholesterol-induced platelet aggregation to normal levels in line with the GLA increase.

Table 1 denotes the serum lipid levels in all groups of Sprague-Dawley rats for 27 days of the experiment. A stable and notable interaction between the experimental time and the PC group (rats fed cholesterol) was observed in the TC, LDL, and TG levels. The serum lipid levels in both treatment groups showed no significant changes compared to the NC group (normal feed). At day 9, the difference of TC levels in rats fed cholesterol without *A.* (*Spirulina) platensis*) (PC group) and rats fed cholesterol with *A.* (*Spirulina) platensis*) (150ASP and 300ASP groups) were reduced by almost 2 fold ($P<0.05$). The levels decreased from 3.29±0.14 mmol/L in the PC group to 1.69±0.50 mmol/L and 1.69±0.26 mmol/L in the 150ASP and 300ASP groups, respectively. At day 27, the levels gradually decreased by almost 5 fold from 5.85±0.16 mmol/L in the PC group to 1.14±0.30 mmol/L and 1.15±0.23 mmol/L in the 150 ASP and 300ASP groups, respectively.

Cholesterol can be generated by almost of every cell in the body. Indeed, most cholesterol is manufactured in the

Table 1. Total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides (TG) in Sprague-Dawley rats in 0, 9, and 27 days of treatment.

	Time (day)	Negative control group (NC)	Positive control group (PC)	3% cholesterol plus 150 mg/kg body weight (150ASP)	3% cholesterol plus 300 mg/kg body weight (300 ASP)	Mean square between/ Mean square error (F)
TC (mmol/L)	0	1.82±0.41[a]	1.43±0.13[a]	1.43±0.17[a]	1.64±0.15[a]	F (2,30)=3.349 , P<0.05
	9	1.41±0.26[a]	3.29±0.14[b]	1.69±0.50[a]	1.69±0.26[a]	
	27	1.45±0.21[a]	5.85±0.16[b]	1.14±0.30[a]	1.15±0.23[a]	
LDL (mmol/L)	0	0.39±0.17[a]	0.46±0.23[a]	0.60±0.18[a]	0.41±0.14[a]	F (2,30)=12.00 7, P<0.05
	9	1.07±0.25[a]	0.66±0.18[ab]	0.50±0.13[ab]	0.62±0.10[ab]	
	27	0.95±0.64[a]	2.93±0.65[b]	1.05±0.26[a]	1.04±0.18[a]	
HDL (mmol/L)	0	1.15±0.40[a]	1.18±0.12[a]	1.16±0.14[a]	1.07±0.16[a]	F (2,30)=3.349 , P<0.05
	9	1.71±0.26[a]	1.43±0.19[a]	1.18±0.60[a]	1.19±0.15[a]	
	27	1.61±0.39[a]	1.42±0.35[a]	1.48±0.45[a]	1.14±0.28[a]	
TG (mmol/L)	0	1.10±0.56[a]	1.54±0.15[a]	1.13±0.12[a]	1.28±0.12[a]	F (2,30)=22.49 9, P<0.05
	9	1.68±0.19[a]	2.00±0.10[ab]	1.49±0.11[a]	1.54±0.13[a]	
	27	1.63±0.21[a]	3.32±0.14[b]	1.76±0.22[a]	1.68±0.22[a]	

*Values (mean ±SD) with different superscripts in the same row are significantly different at the 5% level

extra-hepatic tissues. Cholesterol is produced from acetyl-coenzyme A molecules by the enzyme hydroxymethyl-glutaryl-CoA reductase. When cholesterol levels in the plasma rise, this enzyme is inhibited (DeBose-Boyd, 2008). A diet rich in saturated fat causes the liver to increase fat storage, while highly unsaturated fatty acids decrease the cholesterol concentration. This event can be observed in the PC group as the TC augmented to about a 309% increase from day 0 to day 27. According to West and York (1998), high fat diets influenced the body fat in animals including rats, monkeys, swine, hamsters, dogs, and squirrels. It is consequently adapted from unclear mechanisms to link the correlation between dietary and body fat. Furthermore, these results were in line with Nagaoka *et al.* (2005) and also Zheng, Li, Zhang, Feng, and Zhang (2011). According to Nagaoka *et al.* (2005), casein successfully induced TC in rats only after 10 days of dietary implementation. The same case happened in Sprague-Dawley rats fed a diabetic diet. The TC level managed to increase to almost 39% compared to the normal group (Zheng *et al.*, 2011).

3.2 Low density lipoprotein

At day 9, there were no significant increases in the LDL levels in the PC and treatment groups. However, as time extended to day 27, the LDL levels of the rats fed cholesterol without *A. platensis* (PC group) decreased more than 2 fold compared to rats fed cholesterol with *A. platensis* (150ASP and 300ASP) and the values were significant at P<0.05. The same scenario happened in the TG levels. The levels increased over time in the PC group and in both treatment groups. The 3% cholesterol feeding successfully induced LDL levels in rats (except for NC group). However, the increase slowed down after two different treatment dosages of *A. platensis* (150 and 300 mg/kg body weight). It demonstrated that the bodies of the rats could adapt well to the supplements.

According to Nagaoka *et al.* (2005), after 10 days of observation, there were lower LDL levels in the Wistar rats fed casein with *Spirulina* compared to Wistar rats fed casein without *Spirulina*. Throughout the 60 days of the experiment, Bertolin *et al.* (2009) recorded a 231% LDL increase in the hypercholesterolemic diet compared to the hypercholesterolemic diet with *Spirulina* that demonstrated only a 61% increase.

3.3 High density lipoprotein

In general, there were no significant differences between the PC, 150ASP, and 300ASP groups and the NC group. The same observation was recorded by Bertolin *et al.* (2009) during the execution of a hypercholesterolemic diet with *Spirulina* in Wistar rats. The role of HDL is to transport cholesterol and its excess from cells and tissues to the liver which later becomes a part of the bile. Reduced HDL has been related to susceptibility enhancement in bad cholesterol. In rats, HDL carries the cholesterol to the liver, before the cholesterol conversion into bile acid by 7α-hydroxylase (CYP7) (Russell, 2003). As reported by Li *et al.* (2010), CYP7 plays a crucial and major role in eliminating cholesterol.

3.4 Triglycerides

Significant differences in the TG levels (P<0.05) were displayed between the PC and treatment groups only after 27 days of the experiment. The TG levels decreased by almost 1.8 fold (3.32±0.14 mmol/L [PC] to 1.76±0.22 mmol/L [150ASP] and 1.68±0.22 mmol/L [300ASP]). On the other hand, the TG levels in the treatment groups were nearly similar with the NC group.

Depletion of the bile acid pool could drive the TG levels to increase (Grundy, Ahrens, & Jr Salen, 1971; Li *et al.*, 2010) and this event is linked to the liver function itself. Healthy and normal livers of rats are known to influence TG homeostasis and the function is better with the addition of hepatic effect supplement. Watanabe *et al.* (2004) successfully

used cholic acid to prevent hepatic TG accumulation including its serum elevation and also VLDL secretion in hypertriglyceridemic mice. In this experiment, the addition of *Spirulina* as a supplement in the diet effectively controlled elevation of the TG levels. Based on Bertolin *et al.* (2009), there was only a 45% increase in the TG levels in rats fed cholesterol with *Spirulina* compared to rats fed cholesterol without *Spirulina* (159%).

3.5 Kidney and liver function tests

The normal ranges for BUN, total creatinine, AST, and ALT were 5.60-8.25 mmol/L, 32.74-44.87 μmol/L, 95.55-145.10 U/I, and 53.50-77.00 U/I, respectively (Table 2). Throughout the experiment, the BUN and total creatinine levels were within the normal ranges. On day 27, there were slight increases in the levels of AST and ALT, particularly in the 300ASP group (AST 150.20±1.15 U/I), PC group (ALT 78.50±5.50), and 150ASP group (ALT 100.50±15.50). However, there were no significant differences displayed compared to the control group ($P>0.05$). Overall, in terms of time points (day 0 to 27), it differed significantly on the levels of serum cholesterol and kidney and liver parameters ($P<0.05$).

Serum concentrations of leakage enzymes present in high activity in the liver serve as biomarkers of hepatocellular injury, specifically ALT and AST (Reuben, 2004). While the BUN and total creatinine levels are traditionally the most widely used screening tests to evaluate renal function in all series. Phycocyanin in *Spirulina* sp. may be responsible for the suppression of renal toxicity (Belay *et al.*, 1993). Fukino, Takagi, and Yamane (1990) reported that phycocyanin extract of *Spirulina* sp. could ease renal toxicity induced by *p*-aminophenol (pain reliever) and cisplatin (anticancer drug). The phycocyanin contained protective effects against renal failure commonly caused by mercury and pharmaceutical drugs (Fukino *et al.*, 1990). Results for this experiment were in line with Zheng *et al.* (2011) and Ozkol, Tuluce, Dilsiz, and Koyuncu (2013). According to Zheng *et al.* (2011), there were no significant changes in ALT, AST, BUN or creatinine levels during the implementation of *Selaginella tamariscina* as an additional supplement in treating hyperglycaemic Sprague-Dawley rats. The ranges of ALT, AST, BUN, and creatinine levels were about 73.80-140.57 U/I, 166.50-245.43 U/I, 7.13-14.20 mmol/L, and 40.28-44.88 μmol/L, respectively. Ozkol *et al.* (2013) reported that natural supplement sources such as *Urtica dioica*, *Thymus vulgaris*, *Myrtus communis*, *Scolymus hispanicus*, and *Cinnamomun zeylanicum* also showed positive effects towards diabetes with no significant changes in kidney and liver parameters (Ozkol *et al.*, 2013).

4. Conclusions

The experimental amount of 3% cholesterol-rich diet successfully induced a hyperlipidemic event demonstrated by the TC, LDL, and TG levels in Sprague-Dawley rats. At the end of the study period, both dosage levels of the local *Arthrospira* (*Spirulina) platensis* had proven its potential in reducing the serum lipids from the bodies of the rats without adverse effects on the kidney or liver.

Table 2. Blood urea nitrogen, total creatinine, aspartate aminotransferase and alanine aminotransferase of rats for each group for 27 days.

	Time (day)		
	0	9	27
BUN (mmol/L)			
NC	8.25±2.65^{a}	6.60±0.30^{c}	5.60±0.40^{a}
PC	7.15±0.85^{a}	5.20±1.30^{ab}	6.25±0.15^{a}
150ASP	7.90±0.40^{a}	4.80±0.40^{b}	5.80±0.50^{a}
300ASP	8.00±0.50^{a}	4.40±0.40^{b}	5.70±0.90^{a}
F (2,30)=27.832, P<0.05			
Total creatinine (μmol/L)			
NC			
PC	32.74±2.26^{a}	35.84±0.29^{cd}	44.87±1.98^{a}
150ASP	33.02±4.24^{a}	37.25±1.13^{d}	33.02±3.11^{a}
300ASP	28.22±2.26^{a}	31.32±0.28^{a}	36.69±12.42^{a}
	28.78±2.82^{a}	33.86±1.69^{ab}	39.50±2.83^{a}
F (2,30)=12.800, P<0.05			
AST (U/I)			
NC	95.55±3.35^{a}	116.65±10.85^{b}	145.10±34.20^{a}
PC	101.65±17.35^{a}	114.85±3.15^{b}	144.05±3.55^{a}
150ASP	104.41±4.70^{a}	103.50±2.80^{ab}	131.45±32.05^{a}
300ASP	98.70±4.55^{a}	93.90±4.20^{a}	150.20±1.15^{a}
F (2,30)=26.941, P<0.05			
ALT (U/I)			
NC	54.50±7.50^{ab}	53.50±7.50^{a}	77.00±6.00^{ab}
PC	50.50±8.50^{a}	59.50±0.50^{ab}	78.50±5.50^{ab}
150ASP	69.50±2.50^{b}	65.00±1.00^{c}	100.50±15.50^{b}
300ASP	63.50±0.50^{ab}	58.5±0.50^{ab}	64.00±7.00^{a}
F (2,30)=25.715, P<0.05			

*Values (mean±SD) with different superscripts in the same column are significantly different at the 5% level

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