International Journal of Modern Agriculture, Volume 10, No.2, 2021
ISSN: 2305 - 7246

Anti Hyperlipidemic and Anti-Oxidant Activities of *Spirulina Platensis* Microalgae in Heat-Stressed Rats

Khashayar Bahramsari[1], Melika Tavakoli[1], Akram Pasandideh Tashakori[2], Hamed Nikpourian[1], Yasaman Allahgholi[1], Farhad Jazideh[3*]

[1]Student Research Committee, Semnan University of Medical Sciences, Semnan, Iran
[2]Former M.Sc student of Department of Plant Breeding and Biotechnology, Gorgan University of Agricultural Sciences and Natural Resources, Iran
[3]International Center for Neuroscience Research, Institute for Intelligent Research, Tbilisi, Georgia

Corresponding author: Farhad Jazideh, International Center for Neuroscience Research, Institute for Intelligent Research, Tbilisi, Georgia

Email: farhad.jazideh@gmail.com

Abstract

Objective: The current research was performed to investigate the effect of oral supplementing of *Spirulina platensis* microalgae (SPM) on some blood biochemical parameters, antioxidant status and growth performance of Wistar rats kept under heat stress (HS) condition (32°C for 6 h). **Methods:** Forty Wistar male rats were randomly grouped into five groups. The animals in control group received distilled water and other groups received 5, 10, 15 and 20 mg of SPM/kg of body weight (BW), for 35 days. BW was recorded at the beginning and at the end study to determine the changes in the BW and feed intake (FI) rats was daily measured. The blood samples were collected for measurement of glucose, cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), malondialdehyde (MDA) or activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX). **Results:** Our results showed that the serum concentrations of cholesterol, triglycerides and MDA were significantly lower in rats supplemented with 15 and 20 mg of SPM/kg of BW compared to other animals ($P<0.05$). Activities of GPX and SOD were elevated in animals treated with SPM at 20 mg of SPM/kg of BW when compared with other rats ($P<0.05$). The serum concentration of HDL-C, glucose, BW and FI were not influenced in treated animals ($P>0.05$). **Conclusion:** It can be concluded that oral supplementing the SPM at high levels, especially 20 mg of SPM/kg of BW, was optimum way to alleviate the negative effect of HS on blood parameters or antioxidant status in rats maintained under HS condition.

Key words: body weight, lipid profile, malondialdehyde, rat, superoxide dismutase, stress

Introduction

Stress has been defined as any environmental factors, such as temperature, relative humidity, immobility, and solar radiation, which can shift body temperature from neutral zone to higher temperatures. Stress is an important reason for depression (Leonard, 2001). There is evidence showing that antioxidant status could be influenced by stress or on the other words, stress could be increased lipid peroxides and decreased antioxidants status in liver and kidney (Oarada et al., 2008). On the basis studies on birds, stress, through changes in nutrient metabolism, influenced body weight and food consumption (Geraert et al., 1996). Regarding heat stress (HS), there are several ways to alleviate the HS on performance and antioxidant

status, such as nutrition strategic. *Spirulina microalgae* (SPM), a blue green alga, a feed natural supplement, has been applied in animal nutrition. The SPM has two different species; *Spirulina maxima* and *Spirulina platensis* (Oliveira et al., 1999). It has been shown that microalgae's have different components, such as 40% lipopeptides, 5.6% amino acids, 4.2% fatty acids, 4.2% macrolides and 9% amides (Mostafa et al., 2012). It has been well documented that microalgae lipopeptides have major role in physiological function, *i.e.* cytotoxic, antitumor, antiviral, antibiotics, antimalarial, antimycotics, multi-drug resistance reversers, antifeedant, herbicides, immunosuppressive factors (Burja et al., 2001) and also cholesterol lowering in animals and humans (Iwata et al., 1990). Microalgae is a nutritional valuable source, because in can supply some fatty acids that human body cannot synthesis them, i.e. linolenic acids (Valeem and Shameel 2005). Microalgae's also contain antioxidant pigments (Karawita et al., 2007; Lee et al., 2008) and thus protect cells against oxidative damage (Abd El-Baky et al., 2004). On basis animal studies, SPM increased body weight (Kharde et al., 2012) and reduced the plasma concentration of cholesterol, triglycerides, total lipids (Mariey et al., 2014) and phospholipids in plasma (Hosoyamada et al., 1991) and hepatic (De Rivera et al,. 1993). We hypothesized that SPM, because of its components, can alleviate negative effects of stress on performance, blood parameters and antioxidant status. Thus, this study was performed to investigate the effect of different levels of SPM on some blood biochemical parameters, antioxidant status and performance in Wistar rats kept under heat stress (HS) condition.

Materials and Methods

Preparation of the Spirulina platensis microalgae

A commercial product, *Spirulina platensis* microalgae was prepared from Qheshm Sina Riz Jolbak Company, Tehran, Iran. The SPM was dissolved in water and presented to rats. The chemical composition of *Spirulina platensis* are shown in Table 1.

Table 1. Chemical composition of the SPM

Components	Amount
Dry matter (%)	95
Ether extract (%)	5.3
Crude protein (%)	61.8
Crude fiber (%)	9.5
Ash (%)	6.9
Ca (mg/100 g)	500
P (mg/100 g)	800
Fe (mg/100 g)	90
K (mg/100 g)	1235

Animals and experimental diets

Forty male Wistar rats, weighing 200±10g, were used in our study. Animals were individually grouped in cages with cycling temperature (daily: 21°C for 18 h and 32°C for 6 h) and a light cycling (12-h light and 12-h dark). Water and feed were *ad libitum* accessed. The rats were treated to five groups with 8 animals in per group. The control rats received the distilled water (without SPM). The animals in the other groups were orally treated with the SPM at rates of 5, 10, 15 and 20 mg of SPM/kg of body weight.

Sample collection and analytical procedures

The average daily feed intake (ADFI) was daily assessed. At 35 d of trial all the animals were weighed for final body weight (BW) and anesthetized and blood samples were taken of heart. The samples were centrifuged at 4000 r. min^{-1} for 5 min and stored at -20°C. Activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in serum were assessed by commercial kits as described by the kit manufacturers. Product of the oxidative degradation of polyunsaturated fatty acids malondialdehyde (MDA) was assessed as described by Mj *et al*. (1992). The serum concentrations of glucose, cholesterol, triglycerides and HDL-C were examined by commercial kit (Pars Azmoon, Tehran-Iran).

Statistical analysis

The blood samples were obtained from all animals but they did not pool. Each blood sample was considered as a data. Each blood sample was divided to different parts and investigated for different parameters. Our data were analyzed by Graph Pad Prism statistically software and the data were analyzed by one-way analysis of variance (ANOVA) with Dennett's multiple comparison post-test. The results are shown as mean ± standard deviation. A value of $P<0.05$ was considered to be statistically significant.

Results

Growth Performance

The growth performances of animals are shown in Table 2. We did not observe significant differences for BW and ADFI among groups ($P>0.05$).

Table 2. Body weight (BW) changes and average daily feed intake (ADFI) of rats fed with *Spirulina platensis* microalgae at levels 0 (SPM0), 5 (SPM5), 100 (SPM10), 15 (SPM 15), and 20 (SPM20)

Measurements	SPM0	SPM5	SPM10	SPM15	SPM20	SEM	*P*
BW (g)	20.81±0.9	21.55±0.87	20.81±0.9	21.37±0.85	20.76±0.13	0.08	NS
ADFI (g)	21.52±1.12	21.77±0.95	22.47±0.49	21.80±0.82	22.37±0.97	0.14	NS

SEM: standard error of means; *P*: P-value; NS: non-significant

Antioxidative status

Table 3 presents the serum antioxidative status in animals. Activities of GPX and SOD were significantly increased in rats supplemented with SPM at high levels compared to the control group ($P<0.05$; 15 and 20 mg/kg of BW). Animals treated with SPM at high levels, 15 and 20 mg/kg of BW, showed lower the serum concentration of MDA than other animals ($P<0.05$).

Table 3. The antioxidant status in serum of rats fed with *Spirulina platensis* microalgae at levels 0 (SPM0), 5 (SPM5), 100 (SPM10), 15 (SPM 15), and 20 (SPM20)

Antioxidant status	SPM0	SPM5	SPM10	SPM15	SPM20	SEM	*P*
MDA/nmol. mL^{-1}	$3.25±0.26^{a}$	$3.22±0.22^{a}$	$3.20±0.07^{a}$	$2.57±0.16^{b}$	$2.42±0.11^{b}$	0.05	*
SOD/U. mL^{-1}	$230±15.97^{b}$	$230±11.25^{b}$	$233±12.46^{b}$	$252±13.56^{a}$	$252±7.44^{a}$	2.02	*
GPX/U	$1550±32.26^{c}$	$1605±36.93^{bc}$	$1550±34.20^{c}$	$1630±55.29^{b}$	$1762±88.3^{a}$	13.13	**

SEM: standard error of means; *P*: P-value; NS: non-significant. ^{abc} Means in rows with different superscripts were significantly differ ($P<0.05$). NS: non-significant ($P>0.05$); $^{**}P<0.01$

Blood biochemical parameters

The serum concentration of blood biochemical parameters are presented in Table 4. SPM could not alter the serum concentration of glucose and HDL-C ($P>0.05$). The serum content of triglycerides and cholesterol was reduced in rats supplemented with SPM at high levels (15 and 20 mg/kg of BW) compared to other groups ($P<0.05$).

Table 4. The serum content of blood biochemical parameters (mg/dl) of rats fed with *Spirulina platensis* microalgae at levels 0 (SPM0), 5 (SPM5), 100 (SPM10), 15 (SPM 15), and 20 (SPM20)

Blood parameters	SPM0	SPM5	SPM10	SPM15	SPM20	SEM	*P*
Triglycerides	65±2.38[a]	61±6.57[a]	65±2.1[a]	50±6.01[b]	49±5.35[b]	0.86	*
Cholesterol	63±8.92[a]	78±14.91[a]	61±15.1[a]	45±14.37[b]	43±4.80[b]	3.74	*
HDL-C	42±1.50	43±1.50	44±3.20	43±0.92	43±1.85	0.30	NS
Glucose	202±18.46	203±19.28	205±14.46	207±19.20	205±15.52	8.97	NS

SEM: standard error of means; *P*: P-value; NS: non-significant. [abc] Means in rows with different superscripts were significantly differ ($P<0.05$). NS: non-significant ($P>0.05$); * $P<0.05$

Discussion

In the present study, oral supplementing with SPM at different levels not affected the ADFI and BW ($P>0.05$). Unfortunately, we cannot find any study in literature review showing that SPM can be improved growth performance in Wistar rats reared under HS condition. It is believed that thermal stress can influence productive performance, by changes in nutrient metabolism (Geraert et al., 1996) and nutrient digestibility in birds. Bird's studies showed that SPM can improve performance (Mariey et al., 2014; Kaoud, 2012; Kharde et al., 2012). The difference between our findings and other studies seems be related to animals' type or age and stress which may prevent efficiency the SPM on performance; resulting in SPM cannot show its effect on performance.

Our findings showed that SPM supplementing to diet, at high levels, increased activity of GPX and SOD. Stress increased lipid peroxides levels and lowered antioxidant status in liver and kidney (Oarada et al., 2008). It is well known that the glutathione peroxidase is one antioxidant defense system which may protect cell and tissues against oxidative stress, thus it plays major role in antioxidant system. SPM contains some antioxidant defense status for protection of cells against damage (Abd El-Baky et al., 2004). In one human study, Kalafati et al., (2010) found that SPM supplementing significantly reduced carbohydrate oxidation rate and elevated glutathione levels in comparison with placebo. Cphycocyanine, a derived protein from SPM, showed the antioxidant potential and its metabolites were capable to pass the blood-brain barrier; documenting that SPM showed protection impact against oxidative stress on rat's hippocampus (Rimbau et al., 1999). It is well shown that selenium is essential for GPX synthesis and copper for SOD synthesis. It seems that SPM can be provided copper and selenium for SOD and GPX synthesis. This idea would be needed more investigations. On the other hand, *Spirulina* contains a main enzyme, *i.e.* SOD, and it thus may increase SOD activity (Belay, 2002).

Our findings showed that SPM supplementing, at high levels, lowered the serum concentration of MDA. As mentioned before, stress elevated amount of lipid peroxides (Oarada et al., 2008) and subsequently increases MDA concentration. This result was in agreement with the other findings, which observed an improvement in serum lipid peroxidation in the rats fed with *S. platensis* (Kalafati et al., 2010). Mazzola et al. (2015) showed that rats supplemented with SPM showed significant decrease in brain thiobarbituric acid reactive substances than control animals. The *Spirulina* protected against free radicals and cell death, and increased antioxidant enzyme activities in plasma and liver (Ravi et al.,

2010; Chu et al., 2010) and prevented lipid peroxidation. Phycocyanin was capable to scavenge free radicals, and it also reduced nitrite production, inhibited inducible nitric oxide synthase expression, and prevented liver microsomal lipid peroxidation (Riss et al., 2007). On the other hand, *Spirulina* contains a main enzyme, *i.e.* SOD, showing that it can slow down rate of oxygen radical producing reactions (Belay, 2002). It can be stated that SPM prevented lipid peroxidation, or increase in MDA, through an increase in antioxidant enzymes. On the other, SPM supplementing also reduced the serum concentration of cholesterol in heat stressed rats. In addition, both clinical and animal studies have been documented that hypercholesterolemia elevated plasma lipid peroxidation and caused a major weak in antioxidant system (Mantha et al., 1993; Das et al., 2000; Nasar et al., 2009). Thus cholesterol lowering activity of SPM may indirectly improve MDA concentration.

In the current study, diet supplementing with SPM, 15 and 20 mg/kg of BW, reduced the serum concentration of triglycerides and cholesterol but it unaffected HDL-C and glucose. However, SPM supplementing reduced triglycerides and cholesterol content. Tawfeek et al. (2014) reported HS increased glucose and triglycerides concentrations in stressed birds. There is major relation between MDA content and some lipid parameters, so that a decrease in triglycerides was paralleled with decrease in MDA content. On basis our findings, SPM can show hypolipidemic effects and may involve in lipid metabolism. Colla et al. (2002) reported that SPM supplementing to diet decreased total cholesterol and triglycerides levels and increased HDL-cholesterol in hypercholesterolemic-induced rabbits. In clinical studies, researchers used Spirulina packed in capsule or caplets for treatment of hypercholesterolemia; hyperlipidemia and atherosclerosis (Eussen et al., 2010). Some studies showed a significant reduction in cholesterol and triglyceride contents of rats or mice fed with *spirulina* (Torres et al., 1998; Fong et al., 2000). It is believed that SPM supplementing may decrease absorption and/or synthesis of cholesterol in the gastro-intestinal tract (Mariey et al., 2012). These researcher's believed that SPM increases lactobacillus population (Mariey et al., 2012) and on the other hand lactobacillus reduces cholesterol concentration in blood through deconjugation of bile salts in the intestine (Surono, 2003). Nagaoka et al. (2005) showed that cphycocyanine protein derived of the *S. platensis* affected the serum content of cholesterol, showing hypocholesterolemic activity the SPM. MDA, however, is an indicator for lipid peroxidation and a decrease in MDA was related to antioxidant enzymes. Thus, a relation between MDA, antioxidant enzymes and cholesterol and triglycerides is reasonable. Thus, we believed SPM reduced lipid peroxidation and the serum content of some lipid profiles by increase in GPX and SOD activities.

The SPM not influenced the serum concentration of glucose. In contrast to our observations, Mariey et al. (2012) showed that SPM supplementation to laying hens diet lowered glucose concentration. In addition, Anitha and Chandralekha (2010) reported that supplementation of SPM reduced levels of fasting blood glucose in male non-insulin dependent diabetics. Animal types and physiological condition may be reason for difference between our findings and others.

Conclusion

In conclusion, results showed that supplementation with SPM did not influence growth performance, and the serum concentration of HDL-C and glucose. It was found efficacy the SPM at high levels (15 and 20 mg/kg of BW) in improving GPX and SOD activity and lipid parameters in rats reared under HS condition. It was cleared relation between lipid peroxidation and the serum concentration of some lipid profiles by increased GPX and SOD activities. It can be suggested high levels of SPM for heat stressed rats.

Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

References

1. Anitha L, Chandralekha K (2010) Effect of supplementation of Spirulina on blood glucose, glycosylated hemoglobin and lipid profile of male non-insulin dependent diabetics. Asian J Exp Biol Sci 1:36-46.
2. Abd El-Baky HH, El Baz FK, El-Baroty GS (2004) Production of antioxidant by the green alga *Dunaliella salina*. Int J Agric Biol 6(1): 49-57.
3. Belay A (2002). The potential application of spirulina (Arthrospira) as a nutritional and therapeutic supplement in health management, Review. J Am Nutraceutical Assoc 5(2): 27-48.
4. Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC (2001) Marine cyanobacteria- a prolific source of natural products. Tetrahedron. 57: 9347-9377.
5. Chu W-L, Lim Y-W, Radhakrishnan A, Lim P-E (2010) Protective effect of aqueous extract from Spirulina platensis against cell death induced by free radicals. BMC Complement Altern Med 10(1):53.
6. Colla LM, Alvarez J, Prato C, Baisch-Muccillo AL, Costa JAV (2002) Utilização da *Spirulina platensis* como inibidor da hipercolesterolemia experimental em coelhos. Dissertação Mestrado, Fundação Universidade de Rio Grande, Rio Grande
7. Das S, Vasisht S, Snehlata C, Das N, Srivastava LM (2000) Correlation between total antioxidant status and lipid peroxidantion in hypercholesterolemia. Curr Sci 78(4):486–487.
8. De Rivera C, Miranda-Zamora R, Diaz-Zagoya JC, Juarez-Oropeza M (1993) Preventive effect of *Spirulina maxima* on the fatty liver induced by a fructose-rich diet in the rat. Life Sci 53: 57-61
9. Eussen S, Klungel O, Garssen J, Verhagen H, van Kranen H, van Loveren H, Rompelberg C (2010) Support of drug therapy using functional foods and dietary supplements: focus on statin therapy. Br J Nutr 103(9): 1260-1277.
10. Fong B, Cheung, M, Lee, M (2000) Effect of dietary Spirulina on plasma cholesterol and triglyceride levels in mice. In: Abstracts. 4th Asia-Pacific Conference on Algal Biotechnol.pp.150.
11. Geraert PA, Padilha JCF, Guillaumin S (1996) Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. Br J Nutr 75: 195-204.
12. Hosoyamada Y, Takai T, Kato T (1991) Effects of water-soluble and insoluble fractions of *Spirulina* on serum lipid components and glucose tolerance in rats. J Jpn Soc Nutr Food Sci 44: 273-277
13. Iwata K, Inayama T and Katoh T (1990) Effect of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose induced hyperlipidemia in rats. J Nutr Sci Vitaminol 36:165-171.
14. Kalafati M, Jamurtas AZ, Nikolaidis MG, Paschalis V, Theodorou AA, Kouretas D, et al. (2010) Ergogenic and antioxidant effects of *Spirulina* supplementation in humans. Med Sci Sports Exerc 42:142-151.
15. Kaoud AH (2012) Effect of *spirulina platensis* as a dietary supplement on broiler performance in comparison with prebiotics. Sci J App Res 1 (2): 44-48.
16. Karawita R, Senevirathne M, Athukorala Y, Affan A, Lee YJ, Kim SK, Lee JB and Jeon YJ (2007) Protective effect of enzymatic extracts from microalgae against DNA damage induced by H2O2. Mar Biotech 9: 479-490.
17. Kharde SD, Shirbhate RN, Bahiram KB, Nipane SF (2012) Effect of Spirulina supplementation on growth performance of broilers. Indian J Vet Res 21: 66-69.

18. Lee SH, Karawita R, Affan A, Lee JB, Lee BJ and Jeon YJ (2008) Potential antioxidant activites of enzymatic digests from benthic diatoms *Achnanthes longipes*, *Amphora coffeaeformis*, and *Navicula* sp. (Bacillariophyceae). J Food Sci Nutr 13:166-175.
19. Leonard BE (2001) Stress nor-epinephrine and depression. J Psychiat Neuro Sci 26: 511-516.
20. Mantha SV, Prasad M, Kalra J, Prasad K (1993) Antioxidant enzymes in hypercholesterolemia and effects of vitamin E in rabbits. Atherosclerosis 101(2):135–144.
21. Mariey YA, Samak HR, Abou-Khashba HA, Sayed MAM, Abou-Zeid AE (2014) Effect of using spirulina platensis algae as feed additives for poultry diets: 2-productive performance of broiler. Egypt Poult Sci 34: 245-258
22. Mariey YA, Samak HR, Ibrahem MA (2012) Effect of using spirulina platensis algae as a feed additive for poultry diets: 1-productive and reproductive performances of local laying hens. Egypt Poult Sci (32) (I): 201-215.
23. Mostafa SSM (2012) Microalgal biotechnology: prospects and applications. Plant Sci 12: 275-314. doi: 10.5772/53649.
24. Mazzola D, Fornari F, Vigano G, Oro T, Vieira Costa JA, Bertolin TE (2015) *Spirulina platensis* enhances the beneficial effect of exercise on oxidative stress and the lipid profile in rats. Brazil Arch Biol Technol 6:961-969. doi: 10.1590/S1516-89132015060216
25. Mj R, Portal B, Meo J et al (1992) Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. Clin Chem 38: 704–9
26. Nagaoka S, Shimizu K, Kaneko H, Shibayama F, Morikawa K, Kanamaru Y, Otsuka A, Hirahashi T, Kato T (2005) A novel protein C-phycocyani plays a crucial role in the hypocholesterolemic action of Spirulina platensis concentrate in rats. J Nutr 135: 2425-2430.
27. Nasar M, Jarrari A, Naseer M, Subhani T, Shetty B, Shakeel F (2009) Antioxidant status of atorvastatin in hypercholesterolemic patients. J Serb Chem Soc 74(10):1063–1073.
28. Oarada M, Tsuzuki T, Gonoi T, Igarashi M, Kamei K, Nikawa T, Hirasaka K, Ogawa T, Miyazawa T, Nakagawa K, Kurita N (2008) Effects of dietary fish oil on lipid peroxidation and serum triacylglycerol levels in psychologically stressed mice. Nutr 24(1): 67–75.
29. Ravi M, De SL, Azharuddin S, Paul SFD (2010) The beneficial effects of spirulina focusing on its immunomodulatory and antioxidant properties. Nutr Diet Suppl 2: 73–83.
30. Rimbau V, Camins A, Romay C, González R, Pallàs M (1999) Protective effects of Cphycocyanin against kainic acid-induced neuronal damage in rat hippocampus. Neurosci Lett 276, 75-78
31. Riss J, Décordé K, Sutra T, Delage M, Baccou JC, Jouy N, Brune JP, Oréal H, Cristol JP, Rouanet JM (2007) Phycobiliprotein C-phycocyanin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. J Agric Food Chem 55:7962–7967.
32. Oliveira MACL, Monteiro MPC, Robbs PG, Leite SGF (1999) Growth and chemical composition of Spirulina maxima and Spirulina platensis biomass at different temperatures. Aquacult Int 7:261-275.
33. Surono IS (2003) In vitro probiotic properties of indigenous Dadih lactic acid bacteria. Asi–Aust J Anim Sci 16: 726–731.
34. Tawfeek SS, AbdAlla Hassanin KM, Ibrahim Youssef IM (2014) The effect of dietary supplementation of some antioxidants on performance, oxidative stress, and blood parameters in broilers under natural summer conditions. J World's Poult Res 4(1): 10-19
35. Torres-Duran PV, Miranda-Zamora R, Paredes-Carbajal MC, Mascher D, Diaz-Zagoya JC, Juarez-Oropeza M (1998) Spirulina maxima prevents induction of fatty liver by carbon tetrachloride in the rat. Biochem Mol Biol Int 44:787-793.
36. Valeem EE and Shameel M (2005) Fatty acid composition of blue-green algae of Sindh, Pakistan. Int J Phycol Phycochem 1:83-92.