Vol.52, n. 5: pp. 1253-1259, September-October 2009
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Effect of Microalga *Spirulina platensis* (*Arthrospira platensis*) on Hippocampus Lipoperoxidation and Lipid Profile in Rats with Induced Hypercholesterolemia

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ABSTRACT

Studies have been conducted on microalga Spirulina platensis *(*Arthrospira platensis*) due to its therapeutic potential in several areas, including the capacity for preventing and decreasing the damages caused by hyperlipidemia and the antioxidant activity. The aim of the study was to evaluate the effect of microalga* Spirulina platensis *on hippocampus lipoperoxidation and lipid profile in rats with induced hypercholesterolemia during 60 days. The measurement of hippocampus lipoperoxidation did not demonstrate significant difference ($p>0.05$) when* Spirulina platensis *was added to hypercholesterolemic diet. The evaluation of lipid profile showed that the administration of the microalga in therapeutic and preventive ways led to a significant protective effect ($p<0.05$) from hypercholesterolemia.*

Key words: *Spirulina*, *Arthrorspira*, lipids, cholesterol, antioxidant

INTRODUCTION

The alteration of plasma cholesterol and triglycerides levels may result in dyslipidemias and lead to the occurrence of cardiovascular diseases, mainly atherosclerosis. The oxidation of LDL (low-density lipoprotein) is indicated as a cause of atherosclerosis. Factors capable of avoiding this oxidation and modifying the quantities of lipoproteins constitute a way of decreasing the formation of the atherosclerotic plaque (Peruguini et al., 2000). Epidemiological and clinical studies established an inverse relation between the intake of compounds with anti-oxidant effects and the incidence of chronic diseases, like the cardiovascular ones (Belay, 2002).

The microalga *Spirulina* (*Arthrospira*) has been studied extensively due to its therapeutic potential in several areas, including the capacity of preventing and decreasing the damages caused by hyperlipidemia and the antioxidant activity (Belay, 2002). *S. platensis* is a photoautotrophic filamentous cyanobacterium used mainly as food supplement (Henrikson, 1994) because it has proteins (55-70%), sugars (12-25%), essential fatty acids (18%), vitamins, and minerals in its chemical constitution (Sanchez et al., 2003). *Spirulina*

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contains carotenoid pigments, especially beta-carotene and zeaxantine, besides phycocoanine (Estrada et al., 2006) and phenolic compounds (Colla et al., 2006), substances with well-known antioxidant activity. The absence of phycotoxins is an advantage of *Spirulina* when compared to other cyanobacteria and studies on chronic and subchronic toxicity did not reveal any toxic effect related to its intake (Sanchez et al., 2003; Salazar et al., 1998), as long as there was absence of toxigenic cyanobacteria in its culture (Costa et al., 2006).

Experiments conducted in different animal models demonstrated that a supplementary diet with *Spirulina* could promote a decrease in plasma (Iwata et al., 1990; Hosoyamada et al., 1991) and hepatic (De Rivera et al., 1993) total cholesterol, LDL, triglycerides, and phospholipids, besides increasing HDL (high-density lipoprotein) (Hosoyamada et al., 1991). In humans, studies have indicated a significant reduction in the total cholesterol, LDL, VLDL (very low-density lipoprotein), and triglycerides, elevation in HDL cholesterol (Ramamoorthy and Premakuri, 1996), and reduction of atherogenic effect (Nakaya et al., 1988). Nagaoka et al. (2005) identified the mechanism how *S. platensis* induced hypercholesterolemia and concluded that C-phycocyanine protein derived from the phycobiline pigment developed an essential role on such microalga`s capacity.

Miranda et al. (1998) attributed the antioxidant effect from the *Spirulina* extract to beta carotene, tocopherol and phenolic compounds present in the composition of the microalga. According to Estrada et al. (2001), the phycocyanine protein extracted from *S. platensis* has the capacity of interacting with the reactive species to oxygen generated during the oxidative process through the sequestration of free radicals. The results from several studies have indicated that the utilization of *Spirulina* as a diet supplement constituted a strategy for the prevention of health problems due to the injuries produced by the free radicals (Belay, 2002).

The present study aimed to evaluate the influence of the administration of microalga *S. platensis* biomass on the hippocampus lipoperoxidation and the serum lipid profile in rats with induced hypercholesterolemia.

MATERIALS AND METHODS

Male Wistar rats (Ratus norvegicus) weighing 226.6±25.6 g were used. The experiments with lipid profile were accomplished with 40 rats distributed in five experimental groups (n=8). Four rats from each group were also used in the experiments for evaluating the hippocampus lipoxidation. The animals were submitted to the treatments during a 60-day period. Daily individual diet was 20g and water ad libitum.

The treatments consisted in the administration of four diets to the experimental groups: control diet (standard diet for rodents), hypercholesterolemic diet (addition of 3.5 g of cholesterol for 1.000 g of standard diet), diet with *Spirulina* (addition of 5.0 g of *S. platensis* biomass for 1,000 g of standard nourishment), hypercholesterolemic diet with *Spirulina* (addition of 5.0 g of *S. platensis* biomass for 1,000 g of hypercholesterolemic diet). This way, the mean daily intake of cholesterol and *Spirulina* biomass, according to each group, was 70 g of cholesterol/day and 100 mg of *Spirulina*/day, corresponding to 310 to 442 mg/kg of body mass, respectively.

The groups named Control Diet (CD), ypercholesterolemic Diet (HD), and Hypercholesterolemic Diet with *Spirulina* (HD+*S*) received the same diet (for each group) during the whole period of the experiment. The Hypercholesterolemic Diet and *Spirulina* Diet group (HD/*S*) received hypercholesterolemic diet during the first 30 days of treatment, and then changed to *Spirulina* diet, constituting the group in which the microalga`s therapeutical potential was tested for hypercholesterolemia. The *Spirulina* Diet and Hypercholesterolemic Diet group (*S*D/HD) received diet with *Spirulina* during the first 30 days and in the remaining treatment period, changed to hypercholesterolemic diet, representing the group where the microalga`s preventive effect was evaluated for hypercholesterolemia.

The experimental groups and their respective treatments are shown in Table 1.

Table 1 - Experimental groups and treatments.

Groups	Treatments
CD	Control Diet
HD	Hypercholesterolemic Diet
HD+*S*	Hypercholesterolemic Diet with *Spirulina*
HD/*S*	Hypercholesterolemic Diet and *Spirulina* Diet
*S*D/H	*Spirulina* Diet and Hypercholesterolemic Diet

In the beginning of treatment (time 0), after a 12-h, the animals were anaesthetized with ethylic ether for weighing and collecting the blood samples through ocular puncture. At the end of the treatment (60 days), the animals were decapitated by guillotine for obtaining the blood samples and removal of the hippocampus in those rats used for lipoperoxidation measurement. From the collected blood serum, the measurements of total cholesterol (TC), HDL, LDL, VLDL, and triglycerides (TG) were determined through the enzymatic method (Labtest Diagnostica). The samples from hippocampus were homogenized for the lipoperoxidase measurement through the TBARS technique (Thiobarbiturate Acid Reactive Substances) described by Esterbauer & Cheeseman (1990).

The results were presented as mean ± standard deviation and compared through the Variance Analysis and Tukey`s Test for comparing the means with a significance of 5%.

RESULTS AND DISCUSSION

The administration of *S. platensis* biomass to the rats did not influence the body weight. In all the animals, a significant increase ($p<0.05$) in the final weight (time 60) took place when compared to the beginning (time 0), but without any significant difference between the groups (Table 2). Similar result was obtained by Araujo et al. (2003), where the supplementary diet with *Spirulina* biomass also did not influence the animals` food intake and body weight.

The lipoperoxidation measurement in the rats` hippocampus did not differ significantly ($p>0.05$) between the groups, and there was only a trend to a decrease of the oxidative stress caused by the induction of hypercholesterolemia in the preventive and therapeutic treatments, represented by the *S*D/H and HD/*S* groups, respectively (Fig. 1). However, Rimbau et al. (1999), who used C-phycocyanine, protein derived from *Spirulina platensis*, for testing the antioxidant potential, demonstrated that metabolites from that protein had the ability to pass the blood-brain barrier, offering protective effect against oxidative stress on rats` hippocampus. Miranda et al. (1998) also described an inhibition of lipoperoxidation by *Spirulina* in experiments *in vitro* with brain tissue and *in vivo* with rat plasma.

Table 2 - Variation of rats` body weight during the experimental period.

Treatments	Initial weight (g)	Final weight (g)
CD	236,8 ± 12,4 [bc]	334,0 ± 22,4 [e]
HD	249,9 ± 6,3 [c]	330,9 ± 9,8 [de]
HD+*S*	237,0 ± 22,1 [bc]	326,8 ± 24,0 [de]
HD/*S*	212,3 ± 21,1 [ab]	308,8 ± 22,3 [de]
*S*D/H	197,3 ± 21,5 [a]	300,4 ± 15,7 [d]

* Different letters represent significant difference (p=0.05), n=8.

CD: Control diet; HD: Hypercholesterolemic diet; HD+*S*: Hypercholesterolemic diet with *Spirulina*; HD/*S*: Hypercholesterolemic diet and *Spirulina* diet; *S*D/H: *Spirulina* diet and hypercholesterolemic diet.

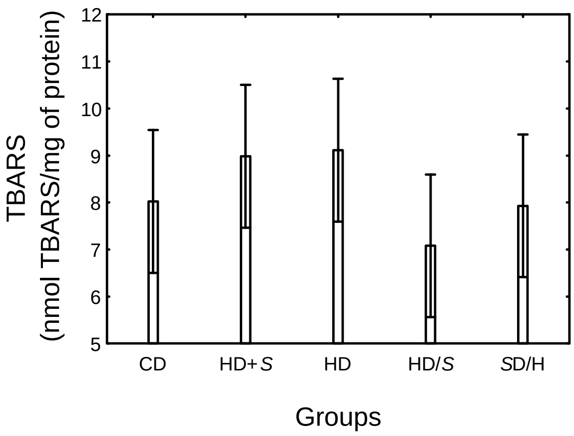


Figure 1 - Lipoperoxidation measurement in hippocampus. ($p<0.05$); n=4. CD: Control diet; HD: Hypercholesterolemic diet; HD+*S*: Hypercholesterolemic diet with *Spirulina*; HD/*S*: Hypercholesterolemic diet and *Spirulina* diet; *S*D/H: *Spirulina* diet and hypercholesterolemic diet.

Regarding the lipid profile, analysis of variance demonstrated a significant difference in the results of TC and TAG obtained from the different treatments ($p<0.05$) and in the experiment duration (time) ($p<0.05$). The interaction between the treatment and duration was significant ($p<0.05$), and it should be considered instead of the individual factors (Table 3).

Table 3 - Significance levels (p) for Variance Analysis of the experiment duration and treatment on the total cholesterol (TC), HDL, LDL+VLDL, and triglycerides (TG).

	p			
	TC	**HDL**	**LDL+VLDL**	**TAG**
Mean	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment	< 0.0001	< 0.0001	0.0004	< 0.0001
Time	0.3034	< 0.0001	0.0004	< 0.0001
Treatment x Time	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 4 presents the comparison of the means from the variables time and treatment using Tukey`s Test with 5% of significance for the responses TC, HDL, LDL+VLDL, and TAG. Figures 2a and 2b represent the interaction graph of the means between the factors treatment and time for TC and TAG levels.

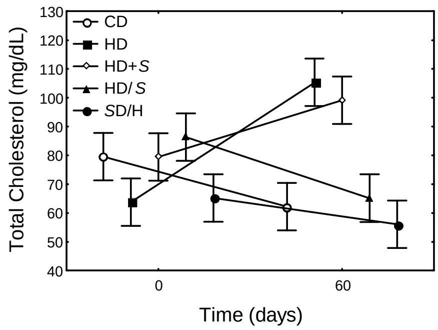
CD (control diet) group did not show significant difference ($p>0.05$) in the TC (Fig. 2a), LDL+VLDL, and TAG levels (Fig.2b), while the HDL levels decreased significantly ($p<0.05$) during the treatment.

In the HD and HD+*S* groups, the TC (Fig. 2a), LDL+VLDL, and TAG levels enhanced significantly ($p<0.05$). On the other hand, HDL did not show significant alteration ($p>0.05$) during the treatment. These results demonstrated that the cholesterol addition to the diet induced hypercholesterolemia in the animals. This effect was also observed in the HD+*S* group, for which the hypercholesterolemic diet was supplemented with *S. platensis*, suggesting that the microalga intake associated with a cholesterol-rich diet did not modify the lipid levels.

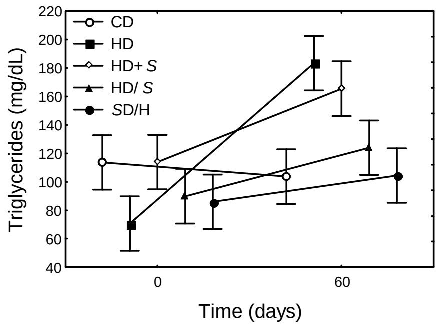
Tabela 4 - Comparison of means between the variables time and treatment through the Tukey`s Test with 5% of significance for the total cholesterol (TC), HDL, LDL+VLDL and triglycerides (TAG).

		Treatments				
	Time (days)	**CD**	**HD**	**HD+*S***	**HD/*S***	**SD/H**
TC (mg/dL)*	0	79,5 ± 8,4 [bc]	63,8 ± 6,8 [ab]	79,4 ± 9,5 [bc]	86,4 ± 10,2 [cd]	65,2 ± 7,2 [ab]
	60	62,2 ± 10,1 [ab]	105,3 ± 22,8 [d]	99,1 ± 17,0 [d]	65,1 ± 5,4 [ab]	56,0 ± 8,4 [a]
HDL (mg/dL)*	0	31,0 ± 6,4 [bc]	36,2 ± 2,9 [bc]	33,2 ± 4,6 [bc]	39,0 ± 7,0 [c]	28,1 ± 7,5 [b]
	60	12,3 ± 5,1 [a]	29,0 ± 4,4 [b]	32,2 ± 6,9 [bc]	32,3 ± 3,3 [bc]	28,4 ± 6,8 [b]
LDL+VLDL (mg/dL)*	0	48,9 ± 13,7 [cd]	22,1 ± 3,5 [a]	37,2 ± 9,7 [abc]	45,6 ± 16,0 [bcd]	36,3 ± 8,4 [abc]
	60	47,7 ± 9,1 [cd]	73,2 ± 18,8 [e]	60,1 ± 20,4 [de]	33,3 ± 5,0 [abc]	26,9 ± 5,0 [ab]
TAG mg/dL)*	0	113,6 ± 13,8 [ab]	70,8 ± 21,5 [a]	113,9 ± 26,8 [ab]	89,9 ± 26,5 [ab]	85,9 ± 16,8 [ab]
	60	103,6 ± 24,5 [ab]	183,4 ± 46,0 [d]	165,5 ± 36,5 [cd]	124,1 ± 24,7 [bc]	104,3 ± 19,4 [ab]

*Different letters represent significant difference (p=0.05), n=8.
CD: Control diet; HD: Hypercholesterolemic diet; HD+*S*: Hypercholesterolemic diet with *Spirulina*; HD/*S*: Hypercholesterolemic diet and *Spirulina* diet; *S*D/H: *Spirulina* diet and hypercholesterolemic diet.



(a)



(b)

Figure 2 - (a) Total cholesterol and (b) triglycerides in times 0 and 60. (p<0.05), n=8. CD: Control diet; HD: Hypercholesterolemic diet; HD+*S*: Hypercholesterolemic diet with *Spirulina*; HD/*S*: Hypercholesterolemic diet and *Spirulina* diet; *S*D/H: *Spirulina* diet and hypercholesterolemic diet.

The administration of *S. platensis* biomass in a therapeutic way to the HD/*S* group, which changed to *Spirulina* diet after hypercholesterolemia induction, led to a significant reduction (p<0.05) in the final TC levels (Fig. 2a) when compared to the initial ones. *Spirulina* diet also maintained the LDL+VLDL and TAG levels (Fig. 2b), preventing the enhancement in these parameters observed in HD group. HDL levels remained statistically unchanged (p>0.05). In a study conducted by

Colla et al. (2002), *S. platensis* addition to the diet of hypercholesterolemic-induced rabbits caused a decrease in the total cholesterol and triglycerides levels and an increase in the HDL cholesterol. In the studies performed by Torres-Duran et al. (1999) and Huang et al. (2005), rats were submitted to the induction of fatty liver by carbon tetrachloride and of diabetes by alloxane, respectively. The triglycerides and total cholesterol levels were significantly decreased in the animals that received a diet added with *Spirulina*. *Spirulina* supplementation to patients with type II diabetes mellitus (Parikh et al., 2001) and hyperlipidemic nephrotic syndrome (Samuels et al., 2002) improved the patients` lipid profile through a reduction in the total cholesterol, LDL and triglycerides levels.

The *S*D/H group (*Spirulina* diet and hypercholesterolemic diet), in which the preventive effect of the microalga *Spirulina* was tested against the development of hypercholesterolemia, all parameters remained unchanged ($p>0.05$) at the end of the treatment, indicating a preventive potential for the elevation of TC (Fig. 2a), LDL+VLDL, and TAG levels (Fig. 2b) observed in HD group, besides the prevention of the decrease in HDL observed in the CD group. This preventive effect of *Spirulina* also was observed by Ble-Castillo et al. (2002) and Rodriguez-Hernandez et al. (2001) in the induction of fatty liver and on hepatic and serum lipid levels.

In a study about the hypocholesterolemic action of *Spirulina platensis* in rats, Nagaoka et al. (2005) demonstrated that the *S. platensis*-derived phycocyanine protein influence the serum cholesterol concentration, suggesting a hypocholesterolemic activity of the microalga in animals.

One of the first studies about the reduction in serum cholesterol due to the administration of microalga *Spirulina* was conducted by Devi and Venkataraman in 1983 and, since then, several works have confirmed such action of *Spirulina* in studies involving animals and humans (Belay, 2002).

Concerning the lipid profile, the therapeutic and preventive groups exhibited a decrease in TC levels from 86.4 ± 10.2 mg/dl to 65.1 ± 5.4 mg/dl, and from 65.2 ± 7.2 mg/dl to 56.0 ± 8.4 mg/dl, respectively, and in the LDL+VLDL levels from 45.6 ± 16.0 mg/dl to 33.3 ± 5.5 mg/dl, and from 36.3 ± 8.4 mg/dl to 26.9 ± 5.5 mg/dl, respectively.

There was also maintenance of TAG and HDL levels. These results pointed to a potential usefulness for microalga *S. platensis* in the treatment and prevention of hypercholesterolemia.

RESUMO

A microalga *Spirulina platensis* (*Arthrospira platensis*) vem sendo fonte de pesquisas devido a evidências de seu potencial terapêutico em diversas áreas, dentre elas a capacidade de prevenção e diminuição dos danos causados por dislipidemias e sua atividade antioxidante. Objetivou-se avaliar o efeito da microalga *Spirulina platensis* sobre a lipoperoxidação no hipocampo e perfil lipídico sérico em ratos com hipercolesterolemia induzida durante 60 dias. A dosagem da lipoperoxidação no hipocampo não demonstrou diferença significativa ($p>0,05$) quando *Spirulina platensis* foi adicionada na dieta hipercolêsterolemica. A avaliação do perfil lipídico demonstrou que a administração da microlaga de forma terapêutica e preventiva demonstrou efeito significativo ($p<0,05$) na proteção do desenvolvimento de hipercolesterolemia.

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Received: May 15, 2007;
Revised: October 23, 2007;
Accepted: November 10, 2008.