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Research Article

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Preventive effects of Spirulina platensis on exercise-induced muscle damage, oxidative stress and inflammation in taekwondo athletes: a randomized cross-over trial

Running title: Spirulina platensis & muscle damage

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Abstract

Background: Spirulina is an interesting nutritional supplement that has attracted a lot of attention. The aim of the present study was to examine the effect of spirulina supplementation on oxidative stress, inflammatory factors and plasma markers of exercise-induced muscle damage in male taekwondo athletes.

Results: A total of 18 trained taekwondo male athletes took part in a double-blind, placebo-controlled crossover study. Each subject received either spirulina (8 g/day) or placebo for 3 weeks. The study had two periods separated by a 14-day washout. Blood samples were taken after finishing a training checklist program (4 times in total).

There were no significant carryover effects; therefore, the two-week washout period was adequate. Compared to the placebo, a dose of 8 g / d of spirulina supplement over 21 days resulted in a significant decrease in plasma levels of lactate dehydrogenase (LDH), creatine kinase (CK) and interleukin 6 (IL6) and a significant increase in plasma levels of total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GPx) ($p<0.05$). There was not any statistically significant change in the plasma malondialdehyde (MDA) ($p>0.05$).

Conclusion: Due to the improvement of antioxidant and anti-inflammatory conditions as well as an appropriate protein content, spirulina supplementation can produce a preventive effect on exercise-induced muscle damage in taekwondo athletes.

Trial registration: Iranian Registry of Clinical Trials, IRCT20121110011421N4. Registered 12 July 2021 - Retrospectively registered, https://irct.ir/trial/11692.

Keywords: Anti-inflammatory; Antioxidant; Athletes; Muscle damage; Spirulina

Introduction

Today, the use of nutritional supplements in athletes with the aim of increasing exercise capacity or accelerating the process of muscle recovery has greatly increased. Various proteins and amino acids and their derivative metabolites, vitamins and minerals, and specific functional compounds are among the most popular nutritional supplements for athletes.[1,2]

Of these, spirulina (spirulina platensis) is a blue-green algae or photosynthetic cyanobacterium. It has been used in some cultures as a food source that grows in alkaline water; today it is chiefly known as a dietary supplement produced under controlled conditions to reduce the risk of contamination with heavy metals. Due to the lack of cellulose in the cell wall, spirulina is more digestible than other groups of algal species.[3] It is a rich source of protein (up to 65% dry weight) and is important for the composition of essential amino acids and the content of branched-chain amino acids (BCAAs).[4,5] In addition, spirulina has important antioxidant and anti-inflammatory properties and improves the immune system response due to its gamma linolenic acid (GLA) content as well as important functional compounds such as phycocyanins and high molecular weight polysaccharides (e.g., Immulina).[6,7] Studies of Spirulina platensis found no toxic effects on animals or humans.[8-10] However, usage above 15 grams per day is not recommended.[11] Due to the nutritional value of spirulina, preventive effects have been reported for diseases such as fatty liver, cardiovascular disease, diabetes, and cancer.[12] In fact, animal and human studies have confirmed spirulina's antioxidant and anti-inflammatory effects.[13-16]

Exercise-induced muscle damage is modulated by the severity and duration of the exercise. High tensile forces produced during muscle activity and oxidative stress caused by increased oxygen consumption cause disruption of muscle fibers, particularly at weakened z-lines.[17] High calcium concentrations in cytosol activate calcium-dependent proteolytic enzymes that then degrade the z-line of sarcomeres intensify this problem. Intracellular components and markers of muscle damage (e.g., lactate dehydrogenase and creatine kinase) diffuse into the plasma and these substances attract inflammatory factors. Such processes (inflammation and oxidative stress) lead to exercise-induced muscle damage.[17,18] The muscles of the body recover naturally over a period of time; however, in sports such as taekwondo, nutritional interventions have beneficial effects on accelerating the process of muscle recovery between competitive events.[17] Spirulina has antioxidant and anti-inflammatory properties.[19] In addition, the appropriate quantity and quality of protein found in spirulina can have a positive effect on accelerating recovery.

There have been few human studies that examined the effects of spirulina on muscle damage in examining various factors and results.[3,10,20] One study examined the effect of spirulina platensis supplementation on preventing skeletal muscle damage in humans who were not trained athletes. The results found antioxidant status improved but muscle damage indicators did not significantly change (daily dose 7.5 g for 3 weeks).[3] In another study, spirulina platensis (daily dose 7.5 g for 4 weeks) did not decrease muscle damage or oxidative stress in cycling athletes with adequate nutritional status.[20] Although several animal and human studies have reported the potential health benefits of spirulina due to its effective functional and nutritional compounds,[5,8,9,21] little information is available about the impacts of spirulina supplementation on injuries related to sport.

Therefore, the aim of the present study was to examine the effect of spirulina supplementation on oxidative stress, inflammatory factors, and plasma markers in exercise-induced muscle damage in male taekwondo athletes. We hypothesized that spirulina supplementation would decrease muscle damage caused by exercise via declining oxidative stress and inflammation.

Materials and Methods

Subjects

Eighteen well-trained taekwondo athletes volunteered to participate in the study. The subjects who were professional taekwondo athletes had at least three years of experience in taekwondo and had practiced at least 6 hours every week for the past 6 months. All subjects were informed about the risks, the possible discomforts and the benefits of supplementation before signing a written informed consent. They were asked to complete a medical and supplementation history questionnaire to determine their eligibility. Exclusion criteria were those who smoked in the last year or who were taking dietary supplements or following special diets or taking anti-inflammatory drugs in the last 3 months. The procedures were in accordance with the Good Clinical Practices and the Declaration of Helsinki.

Study design

This clinical trial was a double-blind, placebo-controlled crossover trial. For randomization, the eligible participants were randomly assigned into two groups based on balanced block randomization procedure with block size of 4, developed by the “Random allocation software”.

Randomization concealed in sequentially numbered, sealed, opaque envelopes, and kept by the head of faculty laboratory and also due to the use of block randomization method with different blocks, concealment is guaranteed in this study. The study had two periods separated by a 14-day washout (Figure 1). All the data were collected based on double-blind experiments. The 18 participants were randomly divided into two groups of nine each: the SP group that took spirulina in period 1 and then after the washout took the placebo in period 2; and the PS group that took the placebo in period 1 and then after the washout took spirulina in period 2. The spirulina sachet contained 8 g of Spirulina platensis flavored with lemon and mint (Qeshm Island Science and Technology Park by Drotat Setareh Qeshm). The placebo sachet was 8 g starch flour with the same flavor and green food coloring. The daily dosage of spirulina was well below the normally recommended maximum of 15 g.[11] The major components of spirulina with nutritional importance are shown in Table 1. three weeks of supplementation (in each period) and two weeks of washout in the study were similar to other relevant studies in this area.[3,10,22] The participants visited one week before the first exercise trial to factor out the effect of diet on the outcomes: to establish that they had similar levels of nutrient intake during the period of data collection, they were asked to record their diet for three days, including one weekend day, and to repeat this diet three days before their next four visits. The diet records were analyzed using Nutritionist IV software adapted to Iranian foods. On the first day of the study, the same diet plan developed by the research team was used and the participants were taken to the gym four hours after lunch. The specific training program consisted of dynamic warm up (jogging and stretching; training duration: 30 min.), fundamental motor skills practice (basic gait technique, weight transfer technique; training duration: 20 min.), specific flexibility training (training duration: 20 min.), specific technical practice (punches, blocks, kicks, twists; training duration: 30 min., performed in 9 teams of 2 people each), specific strength training (front leg lift and side leg lift; training duration: 20 min.). The overall exercise period duration per each individual was 2 hours. All training procedures were supervised by coaches to ensure training quality and consistency. The diet and training program were repeated 21 days later at the end of the first period and then at the beginning and end of the second period (4 times all in).

Blood collection and biochemical assessments

Blood samples were taken thirty minutes after exercise using an anticoagulated EDTA tube from a forearm vein and centrifuged at 3000 rpm. The plasma samples were stored at -70°C until further analysis. Plasma levels of lactate dehydrogenase (LDH) and creatine kinase (CK) were measured by photometric assay (Pars Azmoon, Iran). The ELISA method was used to measure the plasma levels of oxidative stress and inflammatory markers including: 1. total antioxidant capacity (TAC) (ZellBio GmbH, Germany, CV <3.4%, assay range=0.78-50mM), 2. malondialdehyde (MDA) (ZellBio GmbH, Germany, CV=7.6%, assay range=0.78-50uM), 3. superoxide dismutase (SOD) (ZellBio GmbH, Germany, CV=7.2%, assay range=5-100 U/ml), 4. glutathione peroxidase (GPx) (ZellBio GmbH, Germany, CV=4.7%, assay range=20-500 U/ml), 5. Interleukin-6 (IL-6) (Diaclone, Farance, CV=7.7%, assay range < 2 pg/ml).

Statistical analysis

Distribution of data was examined using the Kolmogorov–Smirnov test and was found not to differ significantly from normal distribution. Data were expressed as mean (SD) for quantitative data.

Differences in diet among the two groups (SP and PS) were examined using an independent t test. The method of generalized estimating equations was used to model the effect of the study agent, the effect of period, and the interaction of study agent and period, with subjects specified as the cluster.[23] There were no significant ($p > 0.05$) interactions, indicating no evidence of carryover effects, and there were no significant period effects for any of the end points. The point and interval estimate and *p*-values estimated from models with period effects were very similar to those obtained from raw means and results of paired *t* tests; we report the results of the latter. SPSS Version 21 (SPSS Inc., Chicago, IL) was used for statistical analysis. The level of statistical significance was considered to be $p<0.05$.

Results

As the baseline characteristics and dietary intake of participants presented in Table 2 show, there is no statistically significant difference between the two groups ($p > 0.05$). Supplementation compliance was completion of the placebo and spirulina confirmed by counting empty sachet packs. The only reported side effect of spirulina was slight bloating during the first few days. There were no significant carryover effects and period effects for any of end points ($p > 0.05$); therefore, the two-week washout period was considered to be adequate.

All of the metabolic variables, including LDH, CK, TAC, MDA, SOD, IL6 and GPx are depicted in Table 3. LDH and CK were significantly lower after spirulina intake than after the placebo ($p = .0001$). IL6, an important inflammatory indicator in the human body, was significantly lower after spirulina intake than after the placebo ($p = 0.003$). But, TAC, as an antioxidant indicator, was significantly higher after spirulina intake ($p = 0.0001$). There was no significant decrease of plasma

MDA after spirulina intake than after the placebo ($p = 0.18$). SOD ($p = 0.004$) and GPx ($p = 0.008$) were both significantly higher after spirulina intake than after the placebo.

Discussion

The aim of this study was to determine the effect of spirulina supplementation on reducing exercise-induced muscle damage in male taekwondo athletes. The results of this study indicated that taking 8 g / day of spirulina for 21 days, compared to taking the placebo, resulted in a significant decrease in plasma levels of LDH, CK and IL6 and a significant increase in plasma levels of TAC, SOD and GPx. Despite a decrease in plasma MDA, it was not statistically significant. Different mechanisms have been proposed for the cause of such muscle damage but an integration of models proposed by Armstrong and Smith is a more complete description in understanding this issue.[24,25] According to this model, high-intensity exercise disrupts skeletal proteins in muscle fibers, especially in the areas leading to Z-line. Accumulation of calcium released from the sarcoplasmic reticulum in the cytoplasm of muscle cells activates calcium-dependent proteolytic enzymes. On the other hand, increasing oxygen consumption in muscle cells during exercise causes free radicals. These two factors accelerate the process of destruction of muscle cell membranes and release enzymes (e.g., LDH and CK) from muscle cells into the blood. These enzymes cause chemotaxis of inflammatory agents to muscle fiber, and ultimately macrophages stimulate pain receptors by secreting prostaglandin E2. Therefore, the two factors in causing muscle damage are oxidative stress and inflammatory conditions in muscle fibers.[17] The physiological effects of spirulina supplementation are dependent on its nutritional and functional compounds. The effect of spirulina supplementation on IL-6 reduction has been examined in animal studies.[8,9] Human studies (daily dose 2 or 8 g for 12 weeks) have also shown the effect of spirulina supplementation on reducing IL6.[15,26,27] In this study, IL6, as an important inflammatory marker, was significantly reduced. The functional compounds of spirulina that can be effective in reducing IL6 are phycocyanin, immulina and gamma linolenic acid (GLA). Phycocyanin is the most important functional compound in spirulina that stimulated phosphorylation (caused inactive) inflammatory-related signaling molecules and inhibited pro-inflammatory cytokines formation in *in vitro* and animal studies.[6,28,29] Immulina is a high molecular weight polysaccharide extract from spirulina that has been shown to have anti-inflammatory effects.[7,30] GLA is the most abundant fatty acid in spirulina, and an increased ratio of GLA to arachidonic acid is capable of attenuating biosynthesis of arachidonic acid metabolites (i.e., prostaglandins series 2, leukotrienes series 4) and exerts an anti-inflammatory effect.[31-33] In this study, MDA, SOD, GPx, and TAC indices were used to evaluate the status of oxidative stress. Taking spirulina, compared with taking placebo, in taekwondo athletes significantly increased SOD, GPx, and TAC, and decreased MDA non-significantly. Animal studies have shown a decrease in MDA levels and an increase in the activity of antioxidant enzymes such as SOD and GPx after receiving spirulina.[14,16] Spirulina has also been found in human studies to increase the activity of antioxidant enzymes and to significantly increase the total antioxidant state.[3,15,26,27] In other human studies, taking spirulina reduced MDA significantly.[34,35] In studies with athletes, however, spirulina supplementation did not significantly alter MDA or TAC levels.[3,10,20] In human studies, different daily doses and durations have been used to check antioxidant indicators. In our study, the positive effect of spirulina on antioxidant status may be due to the high amount of phycocyanin, a main functional compound in spirulina that has the ability to scavenge free radicals, including alcoxyl, hydroxyl and peroxyl.[6,36] Carotenoids and tocopherols in spirulina can have antioxidant properties, but because of the smaller amounts, they are less important than phycocyanin.[21] LDH and CK are the main indicators in the evaluation of exercise-induced muscle damage. Lu et al. demonstrated positive changes in plasma concentrations of LDH and CK after receiving spirulina (but these changes were not significant; soy protein was the placebo). The daily dose and duration of spirulina supplementation in the study were similar to those in our study (7.5 g for three weeks), but the use of inappropriate placebo (Soy protein) was an important weakness of the study. Another study demonstrated that spirulina supplementation (daily dose 7.5 g for four weeks) did not alter the magnitude of LDH and CK levels in cycling athletes with adequate nutritional status.[20] In our study, LDH and CK significantly decreased after spirulina intake, compared to placebo intake, in taekwondo athletes. Improving stress oxidation and inflammation due to functional compounds in spirulina can reduce exercise-induced muscle damage and can decrease the release of LDH and CK into the serum.[3] An important factor in the effect of spirulina on reducing LDH and CK is its high protein content. The protein composition of spirulina has an abundance of branched-chain amino acids, especially leucine.[37] Branched-chain amino acids act as fuel in the exercised muscle, preventing or ameliorating the breakdown of cytoskeletal proteins.[2,38] Another possible factor is the effect of spirulina on increasing fat oxidation.[10] This factor can also help reduce amino acid catabolism and maintain muscle fiber structure. Maintaining muscle fiber structure can be effective in reducing secretion of LDH and CK into the serum.

There are few human studies on the effect of spirulina on exercise-induced muscle damage. The use of appropriate dosage and adequate supplementation duration were among the strengths of this study; however, relying on an itemized exercise program to induce muscle damage without an external control index was a limitation.

Conclusion

In conclusion, the long-term use of Spirulina as a natural food may decrease side effects of exercise-induced muscle damage. Because of its effect on improvement of antioxidant and anti-inflammatory conditions as well as having an appropriate protein content, spirulina as a supplement can be effective in the recovery of athletes. The reasons behind these effects are not fully understood. Direct evaluation of skeletal muscle behavior during exercise-induced muscle damage can provide a better understanding and, thus, requires further investigation.

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Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Tehran University of Medical Sciences (No: IR. TUMS.VCR. REC. 1396.3464). The trial was registered in the Iranian Registry of Clinical Trials (Registration No. IRCT20121110011421N4; https://irct.ir/trial/11692). A consent form was completed by each participant before conducting the study and any details that might disclose the identity of the subjects in the study have been omitted.

Data Sharing

The dataset is available from the corresponding author on reasonable request.

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Table 1- Major components of spirulina with nutritional importance [5,36]

Components	Nutritional properties
Protein	65 % weight of spirulina, supply 10 nonessential and 8 essential amino acids contains branched chain amino acids
Phycocyanin	Main functional compounds that has anti-oxidant and anti-inflammatory effects
Gamma linolenic asid (GLA)	Most abundant fatty acids that has anti-inflammatory effects
Immulina	High molecular weight polysaccharide that has anti-inflammatory effects
Vitamin and minerals	Supply small amounts of vitamins and minerals

Table 2- Baseline characteristics and dietary intake differences between SP and PS groups

Variable	SP(n=9)	PS(n=9)	P-value*
Age (years)	26.6 (2.3)	26.7 (1.6)	0.900
Weight (kg)	67.7 (3.1)	68.1 (3.6)	0.790
BMI (kg/m 2)	21.3 (1.0)	21.8 (0.9)	0.070
Energy (kcal)	2258 (61.02)	2346 (83.01)	0.400
Carbohydrate (g)	336 (11.07)	364 (19.21)	0.190
Fat (g)	76.4 (3.03)	70.35 (3.68)	0.220
Protein (g)	58.88 (1.95)	64.22 (3.39)	0.200
Vit E (mg)	8.13 (1.46)	9.1 (1.47)	0.630
Vit C (mg)	66.4 (7.30)	72.66 (7.07)	0.530
Selenium (mcg)	182.22 (20.66)	157.78 (17.06)	0.370
Valine (mg)	2548 (178.06)	2342 (347.06)	0.340
Leucine (mg)	5761 (235.04)	5590 (239.05)	0.600
Isoleucine(mg)	3151 (242.02)	2824 (120.03)	0.240

BMI: Body mass index - Values are expressed as mean (SD)

SP group mean spirulina first then placebo intake and PS group mean placebo first then spirulina intake

* Independent sample t-test, significant difference P <0.05

Table 3- Mean (SD) of metabolic end points after 21 days of spirulina or placebo intake

Variable	Spirulina	Placebo	MD (95% CI)	P-value*
LDH (u/l)	272.89 (50.18)	304.83 (55.8)	-31.94 (-42.01 to -21.87)	<0.001
CK (u/l)	135.06 (30.59)	154.78 (30.12)	-19.72 (-28.39 to -11.05)	<0.001
TAC (mM)	0.82 (0.03)	0.69 (0.03)	0.12 (0.11 to 0.14)	<0.001
MDA (uM)	1.73 (0.50)	1.82 (0.55)	-0.08 (-0.21 to 0.04)	0.18
IL6 (pg/ml)	1.19 (0.26)	1.31 (0.24)	-0.12 (-0.19 to -0.04)	0.003
SOD (U/ml)	53.44 (9.82)	51.11 (10.83)	2.33 (0.84 to 3.82)	0.004
GPx (U/ml)	188.33 (42.73)	173.89 (48.76)	14.44 (4.32 to 24.56)	0.008

The mean is based on the 18 observations from the spirulina phase and 18 observations from the placebo phase

*Paired *t* tests. There are no significant carryover effects

Significant difference p<.0.05

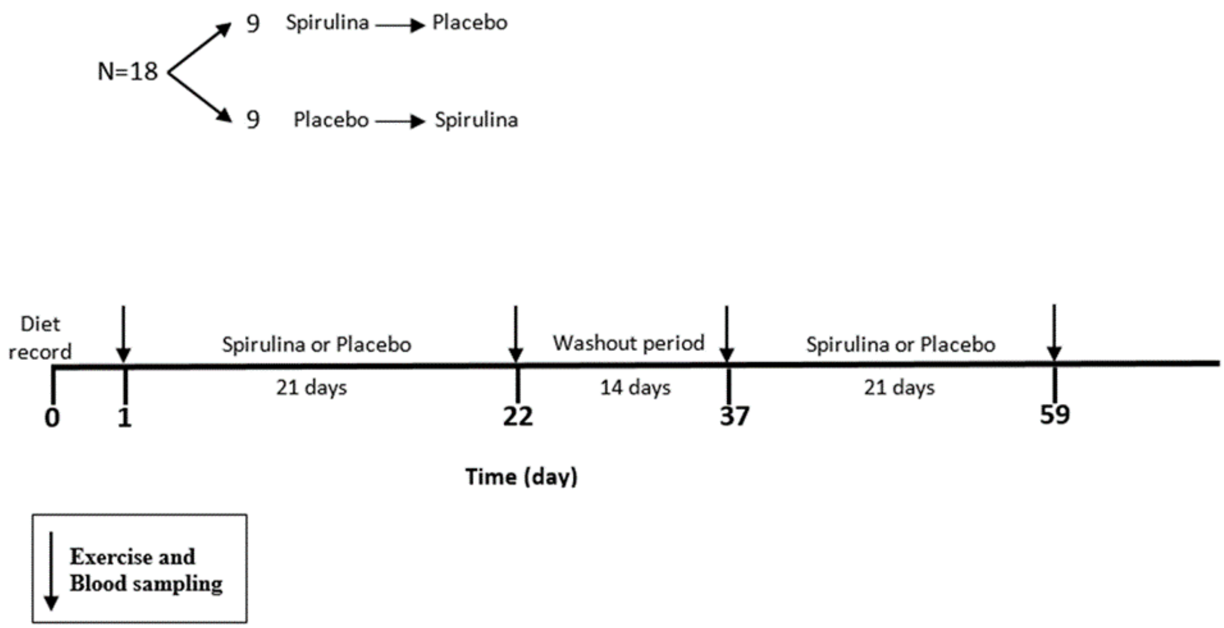

Figure 1- Study diagram