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ANTIOXIDANT AND ANTIHYPERLIPIDEMIC EFFECT OF *Arthrospira jenneri* (Spirulina) IN MICE MODEL

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Abstract

The present study was developed with the objective of determining if *Arthrospira jenneri* has an antihyperlipidemic effect. *Arthrospira jenneri* or spirulina, blue-green planktonic algae, is gaining increasing attention due to its nutritional and medicinal properties, since it has essential amino acids and vitamins, because of this it's used in the production of nutritional supplements. To verify if it has a lipid-lowering effect, were made in vitro studies such as total phenolic compounds and DPPH and in vivo tests using mice model, who took a blood sample after treatment with *Arthrospira jenneri* at two doses (0.05g/100g and 0.3g/100g), determined total cholesterol, triglycerides, and catalase enzyme activity. The content in phenolic compounds was 9.81 ± 0.197 (EAG / g) and antioxidant capacity of *Arthrospira jenneri* was determined which was 32.86 ± 3.702 (ET / g). At the dose 0.05g / 100g, it presented a significant decrease in cholesterol levels and a significant decrease in triglyceride levels, and at dose 0.3g / 100g, it generates a significant increase in catalase activity. It`s concluded that *Arthrospira jenneri* was shown to have a lipid lowering effect.

Keywords: *Arthrospira jenneri, catalase, hyperlipidemia, mice*

Introduction

Currently, natural products have played an important role worldwide, as their use for the treatment and prevention of diseases has increased. These natural products have as main source plants of terrestrial or marine origin [1,2].

Coming of marine origin, those that are gaining the most attention are blue-green algae, due to their nutritional and medicinal properties, in this category we find Spirulina. Spirulina is a blue-green microalgae cyanophyte, present on the planet more than three thousand five hundred years ago, the best known genera are Spirulina and Arthrospira for example A. *platensis*, A. *jenneri*, S. *maxima* and S. *Platensis* [3].

This microalgae has a high nutritional value, because it contains 65% protein, it has essential amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine; it also contains betacarotene, thiamine, riboflavin, niacin, pyridoxine, cobalamin and alpha-tocopherol [4].

The contribution obtained if 15g of *A. jenneri* is consumed daily is protein (digestibility 90%), carbohydrates 0.003%, beta-carotene, Vitamin B12, B1, B6, E and linoleic acid [5].

The main registered uses for spirulina are as a food supplement and as a dye, but research work has been carried out on the pharmacology and toxicology of Spirulina. Some research has reported that Arthrospira is a viable alternative to treat malnutrition problems, attributing this to the bioactive compounds that its consumption provides. Studies carried out both in vivo and in vitro have contributed to increasing information on the benefits of consuming this food under different health conditions. In this case, benefits found in individuals with lipid-lowering problems, antiviral diabetes, hypertension, cancer, chronic obstructive pulmonary disease and metabolic syndrome [6,7].

There is no doubt that spirulina is a highly recognized nutritional food. Beyond the nutritional value for their compounds, many spirulina species possess specific therapeutic properties. Certain spirulina species have been shown to be immunomodulatory and biomodulatory properties. *S. platensis* has a positive and regulatory effect on the immune system [8].

Piñero and collaborators have shown that a protein extract of Spirulina platensis is a powerful free radical scavenger (hydroxyl and peroxyl radicals) and inhibits microsomal lipid peroxidation [9].

This microalgae can become a promising medicine for the treatment of diseases such as diabetes and hyperlipidemia, but more research is still needed. Thus, the objective of this investigation was determine if spirulina have a lipid lowering effect, in a mice model.

Methods

Obtaining and conditioning the sample (Spirulina)

The sample used in the development of this research was acquired in a natural products store, it is preparation in lyophilized powder whose main component is *Arthrospira jenneri*, this product is called spirulina. Furthermore, its solubility was assessed and found to be soluble in water.

Selection of specimens

The selection of the specimens was random, 30 specimens of *Mus musculus* var. *swiss* (Balb/c), males acquired from the bioterium of the National Institute of Health (INS) – Lima (Perú), weighing 30-50 g and 3 months old on average [10, 11].

Determination of total polyphenols

Determined according to the Singleton and Rossi method, a calibration curve was prepared using Gallic Acid as the standard, to obtain the equation of the line. Then 0.006 g of the lyophilized powder was weighed and placed in an eppendorf of 2 mL capacity, 2 mL of the solvent (distilled water) was added (cc=3 mg / mL), then it was taken to the sonicator for 40 minutes, three dilutions were prepared from this sample at concentrations 1, 0.5 and 0.3 mg / mL.

25 µL of each of the dilutions (3; 1; 0.5 and 0.3 mg / mL) and 100 µL of Folin-CioCalteu reagent 10% were taken, then stirred for 10 minutes at 50 ° C, 125 µL of 7% sodium carbonate was subsequently added and it was placed with agitation for 10 minutes at room temperature, subsequently the absorbance were

recorded at 760 nm, this test is performed in triplicate for each dilution [12].

Values were expressed in gallic acid equivalents (GAE) per gram of lyophilized powder of *Arthrospira jenneri* sample.

Determination of the antioxidant capacity 2,2-difenil-1-picrylhydrazyl (DPPH)

The Brand – Willians method was followed with modifications, first, a calibration curve is made, using Trolox as a standard, to obtain the equation of the corresponding line.

0.002 g of the lyophilized powder supplement whose active ingredient is *Arthrospira jenneri* was weighed and placed in an eppendorf with a capacity of 2 mL, 2 mL of the solvent (distilled water) were added, then it was taken to the sonicator for 40 minutes, from this sample was prepared two dilutions at concentrations 0.5 and 0.3 mg / mL.

In a microplate 12 µL of each dilution was placed (cc = 1 mg / mL, cc = 0.5 mg / mL and cc = 0.3 mg / mL), then 300 µL of the DPPH reagent was added in each well, it was placed in agitation during 15 minutes and immediately the absorbance was recorded at 517 nm, this test was performed in triplicate for each dilution [13].

Group distribution

Worked with 35 experimental specimens (*Mus musculus* var. *swiss* Balb/c), which distributed in five experimental groups: white group, control, spirulina 0.05, spirulina 0.3 and atorvastatin.

The white group received carboxymethyl cellulose (CMC) orally 10mL / kg and physiological saline 5 mL / kg intraperitoneally, the control group received CMC orally and triton intraperitoneally, and problem group spirulina 0.05 and spirulina 0.3 received the natural supplement of *Arthrospira jenneri* at doses of 0.05g / 100g and 0.3g / 100g respectively orally and triton intraperitoneally, the atorvastatin group received Atorvastatin (Lipitor®) 10mg / kg orally and triton intraperitoneally. The administration of CMC, spirulina or atorvastatin was carried out for a period of ten days.

Use a lyophilized powder of Spirulina (*Arthrospira jenneri*), which was suspended in CMC for its subsequent administration according to the body weight of the experimental specimens.

For the preparation of Atorvastatin, Lipitor® 10 mg tablets were used, one tablet was crushed and dissolved in 10 mL of CMC, obtaining a final concentration of 1mg / 1mL [14].

Hyperlipidemia induction

Induction was carried out by administering a nonionic surfactant, which was Triton X-305 intraperitoneally, this reagent was prepared at a concentration of 10% and the solvent used was 0.9% sodium chloride. The dose used was 400 mg / kg, to the groups: control, spirulina 0.05, spirulina 0.3 and atorvastatin, on day 9 of treatment, the administration was after fasting for at least 3 hours [15].

Cholesterol and Triglyceride Quantification

In order to determine the cholesterol and triglycerides, exsanguination of the specimens was performed; the blood sample taken from the submandibular venous sinus after anesthesia (ketamine 15 mg/kg; xylazine 2 mg/kg) and was collected in eppendorf and subsequently centrifuged, to obtain blood serum. Cholesterol and triglyceride quantification were carried out enzymatically using kits of determination by Spinreact [16,17]

Catalase Activity Assessment

Hemolysate Preparation

Blood was collected in blue-capped vacutainer tubes with 3.2% sodium citrate anticoagulant, then centrifuged, managing to separate the plasma and erythrocyte sediment. The latter is the one used to prepare the hemolysate, for which a third of the volume of sediment was diluted with distilled water, obtaining a final concentration of 5gHb / 100mL, and then a dilution of 1/500 is made from of the hemolysate [18,19].

Determination of Catalase Activity

Aebi's method was followed, with some modifications, the following system was prepared, the blank (1mL of phosphate buffer pH 7.0 and 2mL of the dilution of the hemolysate) and unknown (2mL of the dilution of the hemolysate and 1mL of 30mM hydrogen peroxide). The absorbance of the sample was read immediately in 240nm, every 10 seconds for 1 minute.

Catalase activity (U / mL) was obtained by multiplying the change in absorbance / min by 41 5550.

U/mL = (ΔA) (27.7 U/mL) (cuvette volume) (final dilution in the bucket)

U/mL = (ΔA) (27.7 U/mL) (3) (500)

U/mL = (ΔA) (41 550 U/mL)

U = Units of activity (µmol / min)

ΔA = Change in absorbance of compound

To determine the specific activity, that is, the proportion of the enzyme with respect to the protein concentration, in this case the hemoglobin value is used, that was determinate by spectrophotometric assay [20,21].

$$Specific\ Activity = \frac{CAT\ Activity}{Hb\ Concentration}$$

$$Specific\ Activity = \frac{U\ /mL}{g\ /mL}$$

$$Specific\ Activity = \frac{U}{g\ Hb}$$

Statistical

The statistical package SPSS v.22.0 used for data processing. The variables were analyzed using the ANOVA test with a significance level of 95% ($p < 0.05$), to establish if the difference was made using the Tukey HSD test. Charts were made using GraphPad Prism 7 Demo.

Ethics

International ethical standards for handling animals, approved by the American Association of Veterinary Medicine (AVMA) and the Regulations of the Office or Research Ethics Committee of the National University of Trujillo were followed [22,23].

Results

In Table 1, the results obtained with reference to the phenolic compounds in the lyophilizate of *Arthrospira jenneri* (spirulina) are presented, we obtained 9.8173 ± 0.1965 mg expressed in gallic acid equivalents per gram of sample. The free radical trapping activity is shown in Table 2, we worked with the spirulina lyophilizate dissolved in distilled water at the concentration of 1 mg / mL, obtaining as a result 32.8649 ± 3.7023 ET / g.

In Figure 1, we can see the variations in cholesterol values expressed in mg / dL in the study groups, 83.52 ± 18.52 (white), 126.24 ± 8.77 (control), 92.92 ± 5.20 (spirulina 0.05), 97.80 ± 19.51 (spirulina 0.3), 73.42 ± 3.82 (atorvastatin) and the triglyceride values (mg / dL) are as follows 93.60 ± 4.99 (white), 125.89 ± 14.35 (control), 98.78 ± 0.071 (spirulina 0.05), 116.25 ± 29.14 (spirulina 0.3) and 82.12 ± 2.63 (atorvastatin). The control group received triton and did not receive any treatment, with an elevation of 65% and 38% in cholesterol and triglycerides, respectively. In the groups that received spirulin treatment, we observed that the 0.05 spirulin group showed a 27% ($p < 0.05$) reduction in cholesterol values and a 23% ($p < 0.05$) reduction in triglyceride values. In the spirulina 0.3 group, a 20% ($p < 0.05$) reduction in cholesterol values and a 15% ($p < 0.05$) reduction in triglyceride values are observed.

Figure 2 shows the catalase activity expressed in U / mL, 1340.75 ± 280.538 (white), 627.079 ± 114.049 (control), 807.81 ± 144.11 (spirulina 0.05), 1188.25 ± 704.15 (spirulina 0.03) and 898.647 ± 326.204 (atorvastatin); this also shows the values of the specific activity of catalase expressed in U / Hb, 898,647 ± 326,204 (white), 3.94 ± 0.713 (control), 5.34 ± 1,053 (spirulina 0.05), 8.12 ± 4,759 (spirulina 0.3) and 5.62 ± 1,851 (atorvastatin). It should be noted that the control group, which only received newt and presented the highest lipid values in Figure 1, shows a significant reduction ($p < 0.05$) in catalase activity (53%) and in specific activity (56%). With the administration of spirulina, a reversal of these values has been achieved, approaching the values presented by the white group, which did not receive newt. In the spirulina 0.05 group, the catalase activity values (U / mL) increases 29% ($p < 0.05$) and the specific activity (U / Hb) increases 35% ($p < 0.05$). The values observed in the spirulina 0.5 group show a significant increase ($p < 0.05$) of 51% and 63% in catalase activity and specific activity respectively.

Discussion

Since currently the treatments commonly used in the treatment of acute and chronic degenerative

diseases do not provide the expected results, and this due to several factors, the need has arisen to seek new pharmacological alternatives that allow preventing and counteracting the development of these diseases. Therefore, in the present study, we aimed to evaluate the effect of Spirulina on one of the diseases with the highest incidence, dyslipidemia.

Hyperlipidemia and inflammation of the vascular endothelium are the most frequent and high-mortality initial pathologies in arterial pathology, atherosclerosis. There is great evidence that refers to the causal relationship of dyslipidemia and the risk of developing coronary heart disease. Cardiovascular diseases are the main cause of morbidity and mortality in the world, both in developed and developing countries, the latter being where the highest incidence and prevalence is observed. [24].

In order to induce hyperlipidemia in experimental animals, Triton X-305 was used, this agent acts by destabilizing the receptors that facilitate the absorption of lipids in the blood by peripheral tissues, and also prevents the entry of cholesterol into the hepatocyte, which leads to interference in the feedback inhibitory mechanisms of endogenous cholesterol synthesis, thus increasing cholesterol levels in the blood, also inhibits the enzyme lipoprotein lipase; causing blood lipid levels to rise [25].

When evaluating cholesterol levels, a significant difference ($p < 0.05$) was observed between the levels of the control group compared to the white group, spirulina 0.05, spirulina 0.3 and atorvastatin, observing a reduction percentage of 42% in the atorvastatin group, followed by 27% in the spirulina 0.05 group and a 20% reduction in the spirulina 0.3 group. Triglyceride values show a significant difference ($p < 0.05$) between the levels of the control group compared to the white group, spirulina 0.05, spirulina 0.3 and atorvastatin, and the highest percentage of reduction was for the atorvastatin group 36%, followed by spirulina 0.05 group 23 % and 15% in the spirulina 0.3 group.

As observed in the results obtained, the group in which the highest reduction in both cholesterol and triglycerides was obtained was the Atorvastatin group. This is because the efficacy of this drug to decrease atherogenic lipids is proven, however it has a special feature, it is a pleiotropic drug, that is, it has other therapeutic effects, such as modulation of endothelial function, anti-inflammatory and antioxidant effects; these effects are related to the inhibition of one of the first stages of cholesterol formation, the passage of HMG-CoA to mevalonic acid, as well as the inhibition of RHO and RHO kinase [26,27].

In two systematic reviews, the results presented show that the efficacy of spirulina supplementation to reduce triglyceride and cholesterol levels is significant, in these studies the genus *Arthrospira platensis* was used, and a significant reduction in the lipid and carbohydrate profile was observed. Thus, registering the lipid-lowering effects in one of the best-known genres of spirulina. However, in these two studies, the antioxidant activity of Spirulina is not determined [28,29].

In previous investigations, the immunomodulatory and antioxidant effects of spirulina have been verified, because it contains Phycocyanobilin, a homologous derivative of biliverdin, this component is a potent inhibitor of NAOH oxidase, in addition to showing a protective anti-teratogenic effect [30].

High levels of cholesterol and triglycerides generate oxidative stress and to counteract the harmful effect of the free radicals produced, the body has an antioxidant defense system that includes molecules and enzymes. The enzymes that participate in this process are superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The activity of these enzymes must be in balance to maintain intracellular oxide-reduction (REDOX) balance [31,32].

That is why in the present study, the enzymatic and specific activity of catalase was also determined, because the specific activity allows us to know the proportion of the enzyme with respect to the protein analyzed, that is, hemoglobin, thus obtaining the highest values low correspond to the control group (a reduction of 56%) because said group received the hyperlipidemia-inducing agent (Triton), we can observe significant difference when comparing these results with those shown by the

spirulina 0.3 group, which did receive Triton, but also, this group received the natural supplement of *Arthrospira jenneri* in a dose of 300 mg / kg we observed an increase of 63% and also with the spirulina group 0.05 with an increase of 35%. In the Atorvastatin group, the specific activity of catalase showed an increase of 42%, a value lower than that observed in the spirulina group 0.3.

Our findings coincide with what is exposed in the study carried out by Ismail, whose objective was to evaluate the effect of spirulina on oxidative stress, antioxidant status and lipid profile in patients with chronic obstructive pulmonary disease, obtaining as results with reference to antioxidant enzymes in especial catalase, a significant increase after the administration of spirulina, a supplement with spirulina for 30 days at 500 mg twice a day and the doses of 500 mg four times a day; before the treatment the catalasa levels was 31.53 kU/L, after 30 days of treatment 33.33 kU/L and after 60 days elevate the values ate 42.25 kU/L [33].

Likewise, in a systematic review focused on the pharmacology of Spirulina, they evaluated the administration of the oil extract of Spirulina in rats, generated a significant decrease in total liver lipids and triacylglycerol [34].

Finally, the results of *in vitro* test, such as total polyphenols and DPPH of the natural supplement of Arthrospira jenneri were carried out. Obtaining that the supplement contains 9.81 GAE / g and 32.86 TE / g respectively.

Studies carried out evaluating the antioxidant activity of aqueous and ethanolic extract of Arthrospira, the ethanolic extract shows greater antiradical and antioxidant activity (99.55%) with ABTS radical scavenging assay and while the extract with water registered slightly lower activities (95.3%) when tested with the DPPH Hydrogen Reaction Radicals. Another study shows that the aqueous extract of Spirulina plantensis has results 72.46% to 81.01% in the DPPH test. Showing in their results that the ethanol extract showed a greater number of anti-radical units, followed by the aqueous extract. It is inappropriate to compare these results with those of our research because they used the microalgae of a different genre, in addition to the fact that in the present investigation we worked with an already processed product of spirulina. But in both studies we can see that they have anti-radical activity [35,36].

In the present investigation, it has been found that the natural supplement of *Arthrospira jenneri* has a lipid lowering effect compared to the induced hyperlipidemia model, in both doses of 0.05 g / 100 g and 0.3 / 100g they showed a significant decrease in the cholesterol and triglyceride levels. In addition, it restores the antioxidant balance by increasing the activity of catalase; finding the highest values of catalase activity in the spirulina group 0.3.

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Table 1: Phenolic compounds present in *Arthrospira jenneri* (Spirulina)

		Lyophilized Spirulina
Phenolic compounds (mg GA/g)	1	9.5923
	2	9.9038
	3	9.9557
	X ± SD: 9.8173 ± 0.1965	

Lyophilized spirulina dissolved in distilled water (3mg / mL)
GA / g: equivalents expressed in gallic acid per gram of sample; X: average; SD: standard deviation

Table 2: *Arthrospira jenneri* (Spirulina) DPPH Radical Trapping Activity

		Lyophilized Spirulina
TE/g	1	32.3554
	2	29.4437
	3	36.7956
	X±SD: 32.8649 ± 3.7023	

Lyophilized spirulina dissolved in distilled water (1mg / mL)
TE / g: trolox equivalents per gram of sample; X: average; SD: standard deviation

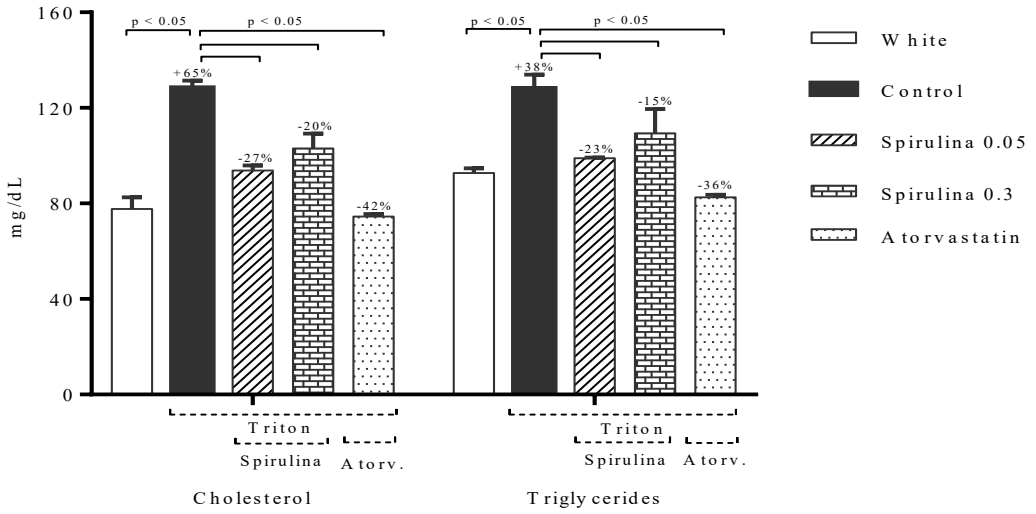

Figure 1. Effect of spirulina on cholesterol and serum triglycerides (mg / dL) in mice receiving Triton

Values are expressed as mean ± SD. p <0.05 is considered a significant difference. Percentages of variation with triton and spirulina are shown.

Control, Spirulina 0.05 and Spirulina 0.3 groups received Sol. Triton 10% intraperitoneally. Spirulina 0.05: *Arthrospira jenneri* (0.05g / 100g), Spirulina 0.3: *Arthrospira jenneri* (0.3g / 100g).

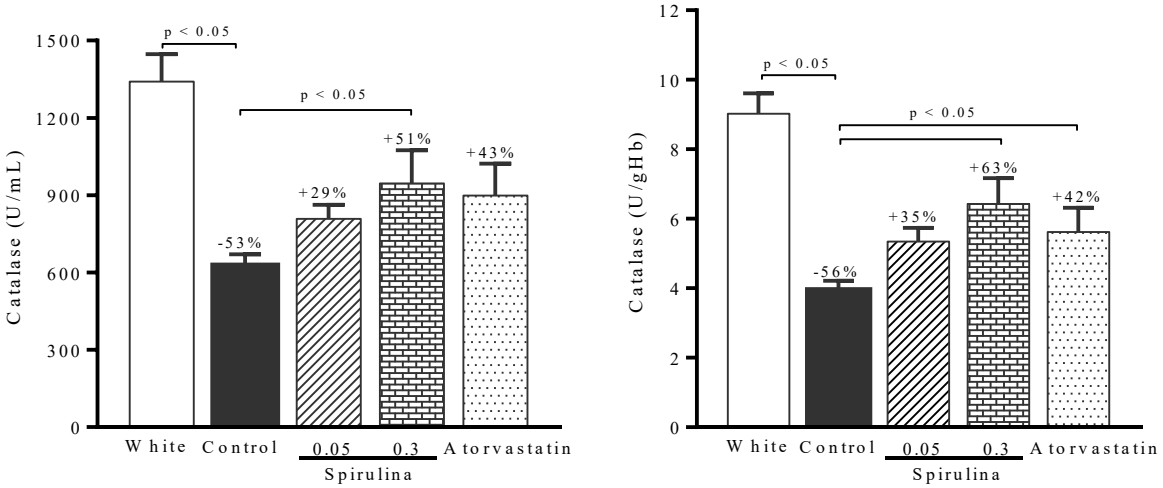

Figure 2. Effect of spirulina on Activity (U / mL) and Specific Activity (U / g Hb) of catalase in mice receiving Triton.

Values are expressed as mean ± SD. p <0.05 is considered a significant difference. Percentages of variation with triton and spirulina are shown.