Anti-Growth, Antioxidant, and Hepatoprotective Properties of *Spirulina Platensis* Extract

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Araştırma Makalesi/Research Article
DOI: *10.59312/ebshealth.1366847*
Geliş Tarihi / Received: *26.09.2023*
Kabul Tarihi / Accepted: *29.10.2023*

ABSTRACT

Spirulina platensis (S. platensis), a filamentous cyanobacterium often referred to as a blue-green alga, is recognized for its antioxidant, immunomodulatory, and anti-inflammatory properties. This study aimed to examine the growth-suppressing effects of S. platensis ethanolic extract on PANC-1 and MIA PaCa-2 human pancreatic cancer cells. Additionally, the in vivo hepatoprotective and antioxidant properties of S. platensis were explored. The ethanolic extract of S. platensis was lyophilized and dissolved in DMSO. Subsequently, PANC-1 and MIA PaCa-2 cell lines were exposed to concentrations ranging from 0.1-1000 µg/ml. Cell viability was assessed using sulforhodamine B viability assay. For in vivo evaluation of hepatoprotective and antioxidant properties, S. platensis was administered by gavage at a dose of 50 mg/kg/day over a four-week period. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured in the heart and liver of rats. The control group that received S. platensis showed a significant elevation in the activity of SOD and GSH-Px enzymes in the heart and liver tissues. There was a significant reduction in the ALT and AST enzyme levels. The extract notably hindered the growth of both the examined cell lines. Future research can focus on studying the effects of S. platensis extracts in conjunction with various chemotherapeutic agents or its effect on different cancer cell lines to better understand its anti-growth attributes.

Keywords: Spirulina platensis, Pancreatic cancer, PANC-1, MIA PaCa-2, SOD, GSH-Px.

Spirulina Platensis Ekstresinin Anti-Büyüme, Antioksidan ve Karaciğer Koruyucu Özellikleri

ÖZET

Spirulina platensis (S. platensis), sıklıkla mavi-yeşil alg olarak adlandırılan filamentöz bir siyanobakteri olup, antioksidan, bağışıklık düzenleyici, anti-inflamatuar özellikler dahil biyolojik etkinlikleriyle tanınır. Bu çalışma, S. platensis'in etanol ekstresinin PANC-1 ve MIA PaCa-2 insan pankreas kanseri hücre hatları üzerindeki büyümeyi engelleme etkilerini araştırmak amacıyla gerçekleştirildi. Ayrıca, S. platensis' in in vivo karaciğer koruyucu ve antioksidan özellikleri incelendi. S. platensis'in etanol ekstresi liyofilize edildi ve ardından DMSO'da çözüldü. Daha sonra PANC-1 ve MIA PaCa-2, 0.1 ile 1000 µg/ml arasında değişen konsantrasyonlarla muamele edildi. Hücre canlılığı, sülforhodamin B canlılık testi kullanılarak değerlendirildi. Karaciğer koruyucu ve antioksidan özelliklerin in vivo değerlendirmeleri için S. platensis, sıçanlara günde 50 mg/kg dozunda dört hafta boyunca gavaj yoluyla uygulandı. Sıçanların kalp ve karaciğer dokularındaki süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px), alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) aktivite seviyeleri ölçüldü. S. platensis alan kontrol grubunda kalp ve karaciğer dokularındaki SOD ve GSH-Px enzim aktivitesinde belirgin bir artış görüldü. ALT ve AST enzim seviyelerinde önemli bir azalma oldu. Ekstrakt, her iki incelenen hücre hattının büyümesini önemli ölçüde engelledi. Gelecekteki araştırmaların, S.platensis ekstrelerinin çeşitli kemoterapötik ajanlarla birlikte kullanımının veya farklı kanser hücre hatları üzerindeki etkilerinin daha kapsamlı bir şekilde anlaşılması için odaklanabileceğini düşündürmektedir.

Anahtar Kelimeler: Spirulina platensis, pankreas kanseri, PANC-1, MIA PaCa-2, SOD, GSH-Px

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This study was presented as an oral presentation at the 3rd International Multidisciplinary Cancer Research Congress (7-10 September 2023)

INTRODUCTION

Cancer represents a significant category of disease and remains a leading cause of death globally. Natural products play a crucial role as valuable reservoirs of potential anticancer compounds. While several plant-derived anticancer drugs have achieved clinical success, the emergence of drug resistance and the associated side effects have underscored the need for continued and extensive exploration of novel anticancer agents from natural sources for cancer treatment (Demain and Vaishnav, 2011, Mondal et al., 2012, World Health Organization, 2010).

Antioxidant enzyme levels are key indicators used to assess the antioxidant effects of certain dietary components within the body. From a molecular biology standpoint, the stimulation of antioxidant enzymes in the body is intimately linked to the consumption of antioxidant-rich compounds in our diet. In physiological systems, GSH-Px and SOD are primary enzymes used to gauge oxidative stress. Biochemically, intracellular antioxidant activities and the balance of oxidizing agents and antioxidants are intricately connected to the consumption of antioxidant-rich food substances. Two of the most notable enzymes for evaluating oxidative stress within an organism are GSH-Px and SOD (Yegin and Mert, 2013).

The liver is the body's largest and most essential metabolic organ, and consists of various functional and anatomical structures. The liver enzymes that have come to mind are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Aminotransferases are involved in the interconversion of amino and keto acids during carbohydrate and nitrogen metabolism. ALT is a liver-specific cytosolic enzyme.

AST is an enzyme found in the liver, heart, pancreas, and muscles among other parts of the body. Although this enzyme is present throughout the body, it is commonly associated with liver health. Elevated serum aminotransferase levels can occur not only as a result of hepatocellular necrosis, where intracellular enzymes are released into the bloodstream, but also due to increased membrane permeability in cases of non-necrotic cellular injury (Ersoy, 2012).

Recent studies have suggested that amplification of free radicals and lipid peroxidation may contribute to the onset of various diseases. The connection between oxidative stress and several ailments, such as diabetes, cancer, cardiovascular and neurological issues, asthma, and aging, has been established. Oxidative stress can lead to changes in antioxidant enzyme levels. Interest in plant-based products with antioxidant properties is growing daily, both in metabolic diseases and in healthy individuals.

Microalgae are important organisms owing to their ability to produce various chemical and biological compounds. Vitamins, pigments, proteins, minerals, lipids, and polysaccharides are the main products obtained. Compared to other living sources, algae are particularly rich in compounds such as polyunsaturated fatty acids (PUFA), gamma-linolenic acid (GLA), allophycocyanin, zeaxanthin, and mixoxanthophyll among their pigments. Microalgae are being studied for their rich contents of proteins,

fatty acids, minerals, vitamin pigments, and many other valuable cellular metabolites. In this context, the production and harvesting of the blue-green algal species *S. platensis* has become more popular, mainly because of its ease of production and harvest (Alavi and Golmakani, 2017; Andrade et al., 2018; Chen et al., 2019; Choopani et al., 2016).

S. platensis is a planktonic organism characterized by high carbonate and bicarbonate levels and a high pH in aquatic environments. It is known for its high protein, gamma-linolenic acid, B12, E, and C vitamins, as well as elements such as zinc, iron, calcium, manganese, and selenium. The low fat content also contributes to its commercial significance. Studies have shown that Spirulina platensis exhibits antiviral, anticancer, antibiotic, antioxidant, immune-boosting, cardiovascular protective, hypocholesterolemic, and anti-allergic effects. S. platensis is suggested to be a dietary supplement that can lower blood glucose levels and counteract oxidative damage owing to its antioxidant properties (Tokusoglu and Unal, 2003).

Aim

The objective of this study was to examine the inhibitory effect of an ethanol-derived extract from *S. platensis* on PANC-1 and MIA PaCa-2 cells. Additionally, the in vivo hepatoprotective-antioxidant properties of *S. platensis* were explored.

METHODS

Preparation of extracts

Spirulina platensis powder was sourced from Muğla, Turkey. The ethanolic extract of *S. platensis* was lyophilized and then dissolved in dimethyl sulfoxide (DMSO)(50 mg/kg).

Cell culture

PANC-1 and MIA PaCa-2 human pancreas carcinoma cells were kindly provided by (Bursa Uludag University, Turkey). PANC-1 and MIA PaCa-2 cells were cultured in RPMI 1640 medium supplemented with penicillin G (100 U/mL), streptomycin (100 mg/mL), L-glutamine, and 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO2.

Cytotoxicity assay

PANC-1 and MIA PaCa-2 cell lines were treated with concentrations ranging from 0.1 to 1000 µg/ml. Cell viability was evaluated using a sulforhodamine B assay. Then, PANC-1 and MIA PaCa-2 cells were seeded at a density of 5 x103 cells per well of 96-well plates. Cells were then treated with different concentrations of the extracts and incubated for 48 h. The assay was terminated by the addition of ice-cold 50% (w/v) trichloroacetic acid. SRB 0.4% (w/v) in 1% (v/v) acetic acid staining was then performed. The bound dye was extracted using 10 mM unbuffered Tris and optical density was measured at 564 nm with an ELISA reader. The viability of the cells that were treated was compared to that of the

control cells that were not treated, utilizing the following equation.

Cell viability (%) = [100 × (Sample Abs) / (Control Abs)].

Animals

Eighteen mature male Wistar rats were obtained from Bursa Uludag University, Turkey. The rats weighed 350-400 g each. Animals divided into two groups as the healthy rats (control group) "C", the healthy rats fed with *Spirulina platensis* "C+SPE". *Spirulina platensis* extract was administered to rats via gavage four weeks (50 mg/kg/day). The experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals at Bursa Uludag University, Turkey,

Sampling and Measuring of Blood Parameters

Blood samples were collected from the hearts under anesthesia. The blood taken into the dry tube was centrifuged for 10 min in a Nuve NF 200 centrifuge (Turkey) to isolate the serum, whereas the blood taken into the heparin tube was centrifuged to isolate the plasma. Once separated, the samples were preserved at -20°C. After collecting blood samples, tissues from organs such as the heart and liver were collected, washed with saline solution, and kept at -20°C for future evaluation. Before the study, the tissues were homogenized in a 1.15% KCl solution at a 1:10 ratio at 14,000 rpm for 15-30 min. Subsequently, the homogenate was centrifuged at 10,000 × g for 30 min and the supernatant was used for analysis. The parameters including SOD and GSH-Px levels were determined using an ELISA kit (Sunlong Biotech, China). The samples were placed in tubes containing EDTA. After centrifugation, the plasma layer was isolated and analyzed. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were measured in the autoanalyzer (TCHO-P 238608, Fuji Dri-Chem, Japan).

Statistical Analysis

The mean values and standard deviations of the data obtained from biochemical tests were statistically evaluated using one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA). Differences between groups were determined at a significance level of $p<0.05$, using Tukey's honestly significant difference (HSD) test. The statistical analyses were conducted using the SPSS 23.0 software package."

FINDINGS AND DISCUSSIONS

It was found that the extracts inhibited Figure 1. The cytotoxic effects of varying concentrations of *S. platensis* extract on the PANC-1 and MIA PaCa-2 cell lines were examined.

The cytotoxic effects with varying concentrations of *S. platensis* extract on PANC-1and MIA PaCa-2 cell lines growth of cells in a dose-dependent manner and prominently reduced the cell viability at the 1000 ug/mL. The toxic effects of the extract on human pancreatic adenocarcinoma cell lines are illustrated in Figure 1. Due to its antioxidant properties, *S. platensis* can neutralize free radicals, thereby reducing cellular damage. This could potentially help in reducing the free radical damage that

contributes to the development of cancer. Furthermore, our findings in the study have indicated that *S. platensis* may inhibit the proliferation of cancer cells. and promote their apoptosis. Our study serves as an initial assessment of the cytotoxic effects of *S. platensis* on human pancreatic cancer cell lines. Various studies have corroborated synergistic or antagonistic interactions with chemotherapeutic drugs (Pezzani et al., 2019).

It was found that the extracts inhibited Table 1. Antioxidant enzyme levels were significantly increased compared with the control rats. The GSH-Px and SOD levels in the liver and heart are shown in Table 1. Animals were fed a diet containing *S. platensis*; in the liver, GSH-Px and SOD levels increased by 28% and 80%, respectively, whereas in the heart of the rats, GSH-Px and SOD increased by 19% and 33%, respectively. In addition, AST and ALT levels were significantly lower in the C+SPE group compared with the control group (Table 1). AST and The ALT enzyme levels were reduced by 15% in the C+SPE group. Upon metabolism in the body, PUFA's broken down into constituents that possess antioxidant properties. As a result, they help stimulate antioxidant enzymes. These data suggest that the antioxidant compounds present in *S.platensis* significantly bolster antioxidant activity in rat systems ($p \leq 0.05$). This might be attributed to the synergistic action of the phenolic compounds and polyunsaturated fatty acids found in *S. platensis*. As mentioned earlier, *Spirulina platensis* is a natural food supplement known for its significant antioxidant capacity. Food components with antioxidant potential typically encompass bioactive compounds characterized by numerous double bonds, including flavonoids, vitamins and minerals, phenolic compounds, as well as ω-3 and ω-6 polyunsaturated fatty acids. Our findings also substantiate these pieces of information. In the given control group receiving *S. platensis*, a decrease in AST and ALT levels was observed. Considering that the levels of AST and ALT enzymes tend to increase because of conditions such as chronic illness, medication usage, intense exercise, or even in the early stages of a chronic liver disease that may not produce significant symptoms, the observed reduction in these enzymes in healthy groups receiving *S. platensis* extract is significant. *S. platensis* may contain components that can support the detoxification processes of the liver. However, further clinical research is needed to better understand the full effects of *Spirulina platensis* on liver health.

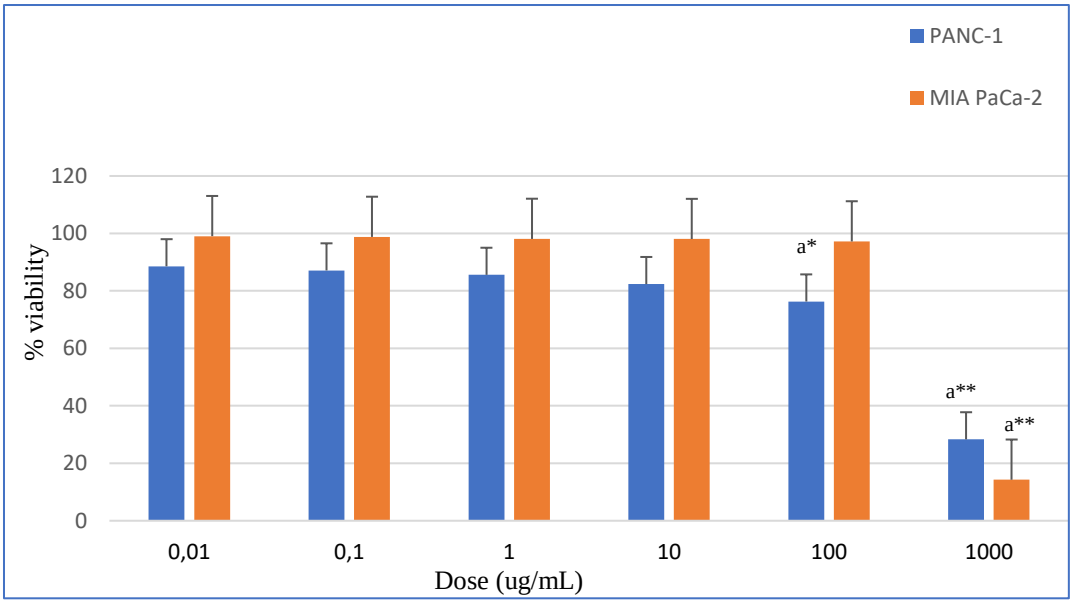

Figure 1. The cytotoxic effects using various concentrations of *S. platensis* extract.extract on PANC-1 and MIA PaCa-2 cell lines.

The viability of the control group was 100%, a; compared to the control group. p<0.05*, p<0.01*

Table 1. GSH-Px and SOD levels in heart and liver tissue and AST and ALT levels in blood.

	Liver GSH-Px (ng/mL)	Heart GSH-Px (ng/mL)	Liver SOD (ng/mL)	Heart SOD (ng/mL)	AST (IU/L)	ALT (IU/L)
Control	14.3 ± 2	15.4 ± 1	1.0 ± 0.1	1.5 ± 0.2	131.3 ± 4.8	70.7 ± 6.8
Control+SPE	18.5 ± 1^{a*}	18.2 ± 1^{a*}	1.8 ± 0.1^{a*}	2.0 ± 0.2^{a*}	111.8 ± 2^{a*}	60.2 ± 5.9^{a*}

a; compared to the control group. p<0.05*
GSH-Px: Glutathione peroxidase, SOD: Superoxide dismutase, AST: Aspartate aminotransferase: ALT: alanine aminotransferase.

CONCLUSION

In summary, our study has yielded findings indicating that *Spirulina platensis* suppresses the growth of pancreatic cancer cell lines while increasing antioxidant enzyme levels in the liver and heart tissues. This information emphasizes the belief that this plant-based form, rich in antioxidants, may play a significant role in assisting treatment not only in healthy individuals but also in metabolic and chronic diseases.

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