ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(1): 3841-3848
doi: 10.25083/rbl/28.1/3841.3848

Received for publication, July, 27, 2023
Accepted, August, 14, 2023

Original article

Investigation of the antitumoral activity of *Arthrospira platensis* (*Spirulina platensis*) in mice

HÜSEYIN TEMEL[1], *SIBEL BAYIL OĞUZKAN[2], İZZETTIN GÜLER[3], ÖMER ERONAT[4], MEHMET ÖZASLAN[5]

[1-5] Faculty of Biology, University of Gaziantep, Turkey
[2] School of Health Sciences, University of Gaziantep, Turkey
[3] İslahiye M.Y.O. Medical Services And Techniques, University of Gaziantep, Turkey
[4] Faculty Of Medicine Department Of Surgical Medical Sciences, University of Gaziantep, Turkey

Abstract

In this study, the anti-tumoral effect of *S. platensis* was investigated in Balb-c mice with EAT (Ehrlich Acid Tumor). *S. platensis* was applied in the form of 200 mg/kg and 800 mg/kg concentrate. Blood taken from mice was separated into their serum and TAL (Total Antioxidant Level), TOL (Total Oxidant Level), ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) parameters were studied. Kidney, stomach, small intestine and large intestine tissues of the sacrificed subjects were removed and evaluated pathologically. As a result, when the biochemical values of *S. platensis* activity and control groups were compared statistically there was no statistically significant difference, excepting TOL. Also, in the pathological evaluation, the spread of the tumor in organs such as the large intestine and stomach was statistically significantly different.

Keywords

S. platensis, EAT, TAL, TOL, ALT, AST

*Corresponding author: Sibel Bayıl Oğuzkan. email: bayil@gantep.edu.tr

Introduction

Microalgae are phototrophic microorganisms with anaerobic metabolism [1] algae; Since they are the primary producers of watery environments, they form an organic food source for heterotrophic organisms, and they also provide oxygen to the environment by photosynthesis. S. platensis is a prokaryotic organism consisting of filamentous (helical), a few millimeters long, 3-12 μm thick cylindrical cells from the Cyanophyceae (Blue-green Algae) class [2]. S. platensis is an economically important algae containing 60-70% protein. It is also very rich in terms of vitamins (provitamin A, B12) and minerals (Fe, Ca, Mg) and organic coloring substances (green chlorophyll, phycocyanin, carotenoids). Due to the absence of cellulose membrane, it is easily digestible and has no toxic effects. S. platensis is the most widely used cyanobacteria and has been extensively studied in the medical and food industries [5]. Due to its high protein, minerals and vitamins, it can be used as a supplement in the treatment of many diseases [3]. In addition to its rich nutritional content, it also exhibits anti-inflammatory, anti-oxidative stress and immune-enhancing properties [6] and [7]. In fact, several studies have concluded that dietary Spirulina is helpful in the treatment and prevention of diabetes, diabetic nephropathy, hypercholesterolemia, and cancer [8] and [9]. Spirulina is widely researched in the pharmacological field due to its anti-inflammatory, antioxidant and anticancer effects [10] and [11]. It has been reported that Spirulina has an effect on the humoral and cellular immune system, stimulates lymphocytes in the blood [12], and increases the production of IgM antibodies in the spleen [13].

Hayashi & al. (1994 [23] reported that Spirulina protected from viral infections in cultured human and monkey cells. The antitumoral activity of marine algae was first studied by Nakazawa in aqueous extracts. It has been reported that the polysaccharide content of aqueous extracts is associated with antitumor activity [14]. Spirulina contains water-soluble photosynthetic protein-pigment complex with high antioxidant effect such as C-phycocyanin. In our study, the antitumoral activity of spirulinas produced in shössler medium in summer season conditions in Yalova University Armutlu Vocational School was evaluated.

Materials and methods

Balb – c Procurement of mice

In our study, 46 male Balb-c mice weighing 20-35 g were used. Experimental animals were provided by Gaziantep University Experimental Animals Research Center and were fed with standard pellet feed and water, providing a 12-hour day/night period at 25 ºC room temperature. After the approval of our study by Gaziantep University Experimental Animals Ethics Committee dated 24/04/2019 and numbered 95, all of the studies on animals were carried out at Gaziantep University Experimental Animals Research Center

EAT Tumor model

The EAT cell was obtained from Istanbul University Aziz Sancar Experimental Medicine Research Institute Laboratory Animals Department and brought to our laboratory by maintaining the cold chain. The EAT cells obtained from the tumor-formed stock animal were taken from the mouse and transferred to the mice in the Spirulina Treatment (200 mg/kg), Spirulina Treatment (800 mg/kg), 5-fluorouracil (20 mg/kg) and Control (EAT+H_2O) groups to be used in the study. .05 ml was injected i.p.

In the study, 7 groups were formed, each of which included 6 animals.

Assessment of blood samples

At the end of the application, cardiac blood was collected from all animals with a heparinized syringe. Serum was obtained from the blood taken, and TOL, TAL, ALT and AST levels were studied in order to determine the oxidant level in the serum. TAL and TOL levels were studied using the Rel Assay Diagnostics-TAL-TOL Assay Kit [21] and [22]. ALT and AST parameters were also studied using a kit from serum obtained to determine the blood value of liver damage.

The 7 groups formed in the study

	Groups	Way of delivery	Amount of delivery	Time
1	Spirulina platensis (200 mg/kg)	hesitation	0,03 ml (in distilled water)	10 days
2	Spirulina platensis (800 mg/kg)	hesitation	0,03 ml (with distilled water)	10 days
3	Spirulina platensis (200 mg/kg) + EAT	hesitation	0,03 ml (with distilled water)	1. day EAT- from 7 to 4 days Spirulina platensis
4	Spirulina platensis (800 mg/kg) + EAT	hesitation	0,03 ml (with distilled water)	1. gün EAT- 7 days from day 4Spirulina platensis
5	5-fluorouracil (200 mg/kg) + EAT	i.p	20 mg/kg	1. day EAT- 7 days from day 4 5-fluorouracil
6	Control d(H_2O) + EAT	hesitation	0,03 ml	1. day EAT- 7 days from day 4 distilled water
7	Control d(H_2O)	hesitation	0,03 ml	10 days

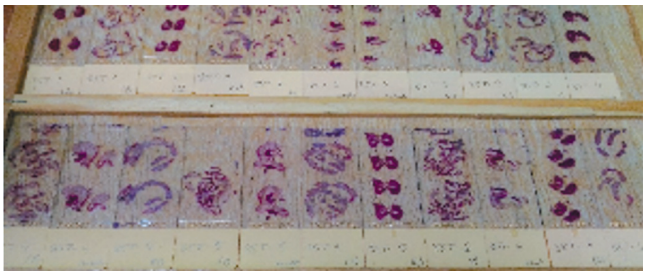


Figure 1: A frame from an image of tissues prepared for examination under a light microscope.

Histopathology

After undergoing routine tissue follow-up procedures with ethanol and xylene solutions at the Laboratory of the Department of Pathology of Gaziantep University Research Hospital, the blocking process was performed in paraffin. The prepared blocks were divided into sections with a thickness of 5µm and taken on lams as picture 1. Subsequently, the xylene evaporation process and the hydration process with ethanol were performed.

At the end of the study, small intestine, large intestine, stomach and kidney tissues were removed from the animals and placed in 10% formaldehyde. Then, the tissue was followed and the sections were taken and the preparation was prepared by staining with the "hematoxylin-eosin" method. The prepared preparations were evaluated by the pathologist with the help of a light microscope to determine whether there was tumor development in the tissues and the metastasis status of the tumor. Histopathological studies were carried out in Gaziantep University Research Hospital Pathology Department Laboratory.

Statistical Analysis

Statistical analyzes were performed according to the SPSS 21 program. Results were expressed as standard deviation or as a percentage. The t-test was used to measure the effect values on the groups, and the ANOVA test was used to reveal the differences between the groups. The p values of all statistical tests were two-sided and $p<0.05$ was considered statistically significant.

Results

When the analysis results are examined, the differences in biochemical parameters between the tumor-free groups given different doses of spirulina (200 mg/kg and 800 mg/kg) are as shown in Table 1. When the values were compared, it was determined that there was no statistically significant difference. ($p>0.05$)

When the groups given only different doses of spirulina (200 mg/kg and 800 mg/kg) were compared with the control group (Table 2), it was determined that there was no statistically significant difference in terms of TAL, TOL, OSI, ALT, AST values. ($p>0.05$)

It was determined that there was no statistically significant difference between TAL, ALT, AST ($p>0.05$) values in the treatment groups given different doses of EAT + spirulina (200 mg/kg and 800 mg/kg) (Table 3). However, it was determined that there was a statistically significant difference between these two groups in terms of TOL values (Table 3) ($p<0.05$).

There was no statistically significant difference in terms of TAL, TOL, OSI, ALT, AST values (Table 4) between the tumor and the treatment groups given different doses of spirulina (200 mg/kg and 800 mg/kg) and the tumor animals given 5-fluorouracil. determined ($p>0.05$).

When EAT+ Spirulina 200 mg/kg, EAT + Spirulina 800 mg/kg, EAT+ 5-fluorouracil and tumor-free healthy animals were compared, it was determined that there was no significant difference ($p>0.05$).

Histopathological Examination of EAT

At the end of the experiment, animals in all groups were sacrificed and the stomach, kidney, large intestine and small intestine tissues were removed by removing the ambuloc. All tissues were kept in 10% formaldehyde for 24 hours, then passed through different concentrations of alcohols and xylol and fixed within 24 hours. Then, tissue sections of 4 micron thickness were taken from the tissue samples prepared with paraffin blocks and deparaffinization process was applied. Afterwards, the samples stained with hematoxylin were cleared in xylol and evaluated under Nikon brand light microscope.

Preparations prepared from tissues were examined histopathologically. At the end of these examinations, tumor invasion was found in the kidney, stomach, large intestine and small intestine tissues in the groups given the tumor, and the tumor was evaluated by scoring method. The percentage of presence in the organs is accepted as 0, since there is no presence of tumor in the groups in which no tumors are formed. The extent of spread in organs of all groups is shown in Table 6.

According to the results of our analysis, the spread of EAT on the large intestine and stomach tissue was found to be statistically significant. ($p<0.05$). As a result of the Dunnett test, which was performed to determine which group the EAT spreads were in favor of, it was determined that the EAT spreads in the animals treated with Treatment and 5-fluorouracil were significantly lower than the control group (EAT+H2O).

The spread of EAT on the small intestine and kidney tissues was not statistically significant. ($p>0.05$). However, the

Table 1 - TAL– TOL, OSI and ALT – AST values of the groups given only spirulina (1st and 2nd Group)

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidatif stres index	ALT (U/L)	AST (U/L)
1. Group	0,47 ± 0,37	0,0132 ± 0	3,98 ± 2,03	43,83 ± 10,83	389,67 ± 128,9
2. Group	0,26 ± 0,07	0,0116 ± 0	4,66 ± 1,09	48,25 ± 23,13	458,25 ± 299,3
p value	0,321	0,172	0,562	0,69	0,626

Table 2 - TAL – TOL, OSI and ALT – AST values of only spirulina given (1st and 2nd Group) and Control (6th Group) groups

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidative stres ndex	ALT (U/L)	AST (U/L)
1. Group	0,47 ± 0,37	0,0132 ± 0	3,98 ± 2,03	43,83 ± 10,83	389,67 ± 128,9
2. Group	0,26 ± 0,07	0,0116 ± 0	4,66 ± 1,09	48,25 ± 23,13	458,25 ± 299,3
6. Group	0,47 ±0,19	0,01 ± 0,01	2,06 ± 1,58	46,5 ± 10,21	412,25 ± 186,95
p value	0,407	0,456	0,066	0,888	0,867

Table 3- Group 3 and 4 TAL – TOL, OSI and ALT – AST values

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidative stres index	ALT (U/L)	AST (U/L)
3. Grup	0,655 ± 0,75	0,0132 ± 0,001	3,47 ± 1,77	36,67 ± 14,39	449,83 ± 204,42
4. Grup	0,3131 ± 0,08	0,0116 ±0	3,88 ± 0,093	38,2 ± 6,72	473,2 ± 73
p değeri	0,34	0,043	0,656	0,832	0,815

Table 4 - Group 3,4 and 5 TAL – TOL, OSI and ALT – AST values

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidative stres index	ALT (U/L)	AST (U/L)
3. Group	0,655 ± 075	0,0132 ± 0	3,468 ± 1,77	36,667 ±14,4	449,83 ± 204,4
4. Group	0,313 ± 0,08	0,0116 ± 0	3,876 ± 0,93	38,2 ± 6,72	473,2 ±73
5. Group	0,377 ± 0,23	0,0138 ± 0	4,743 ±2,47	44,25 ± 13,9	468 ± 61,42
p value	0,502	0,061	0,55	0,627	0,96

Table 5: Group 3, 4.5 and 7 TAL – TOL, OSI and ALT – AST values

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidative stres index	ALT (U/L)	AST (U/L)
3. Group	0,655 ± 0,75	0,013 ± 0	3,4681 ± 1,77	36,67 ± 14,4	449,83 ± 204
4. Group	0,313 ± 0,08	0,012 ± 0	3,8758 ± 0,93	38,2 ± 6,72	473,2 ± 73
5. Group	0,377 ± 0,23	0,014 ± 0	4,7427 ± 2,47	44,25 ± 13,9	468 ± 61,42
7. Group	0,36 ± 0,06	0,01 ± 0	4,07 ± 0,3	33,5 ± 3	422 ± 62,76
p value	0,587	0,081	0,67	0,578	0,938

Table 6 - EAT tumor spread in the small intestine, large intestine, stomach, and kidneys

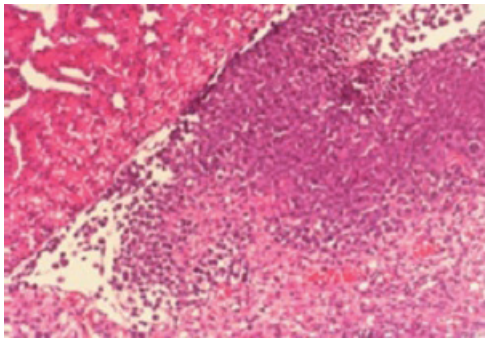
Name of group	Large intestine		Small intestine		Stomach		Kidney	
	Mean Organ Spread %	Result of pathology	Mean Organ Spread %	Result of pathology	Mean Organ Spread%	Result of pathology	Mean Organ Spread%	Result of Pathology
Spirulina (200 mg/kg)	0	(-)	0	(-)	0	(-)	0	(-)
Spirulina(800 mg/kg)	0	(-)	0	(-)	0	(-)	0	(-)
EAT + Spirulina (200 mg/kg)	7	(+)	8	(+)	21	(+)	13	(+)
EAT + Spirulina (800 mg/kg)	10	(+)	13	(+)	30	(+)	19	(+)
EAT + 5-Fluorouracil	6	(+)	8	(+)	14	(+)	14	(+)
Control (EAT+H_2O)	11	(+)	14	(+)	38	(+)	23	(+)
Control (H_2O)	0	(-)	0	(-)	0	(-)	0	(-)

Table 7 - Evaluation of EAT in Groups with Tumor (Group 3: EAT+ Spirulina 200 mg/kg, Group 4: EAT+Spirulina 800 mg/kg, Group 5 EAT+ 5-fluorouracil and Group 6: EAT+ H_2O)

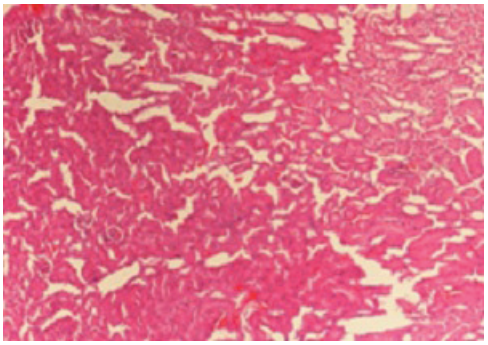
Group	Large intestine	Small intestine	Stomach	Kidney
	Organ involvement average %	Organ involvement average %	Organ involvement average %	Organ involvement average %
3. Group	6,67 ± 2,58	8,33 ± 4,08	20,83 ± 15,94	13,33 ± 5,16
4. Group	11 ± 4,18	14 ± 8,94	30 ± 6,12	19 ± 10,25
5. Group	6,25 ± 2,50	7,50 ± 2,89	13,75 ± 4,79	13,75 ± 6,29
6. Group	11,25 ± 2,50	13,75 ± 17,5	37,50 ± 10,41	22,50 ± 10,41
p	0,039	0,615	0,034	0,304

difference between the mean spreads of EAT in the kidney tissue was found to be statistically significant. For this reason, Dunnett's test was performed to determine which group favored the spread of EAT in the kidney tissue. As a result of the test, it was determined that the EAT spreads in the animals treated with treatment and 5-fluorouracil were significantly lower than the control group (EAT+H2O).

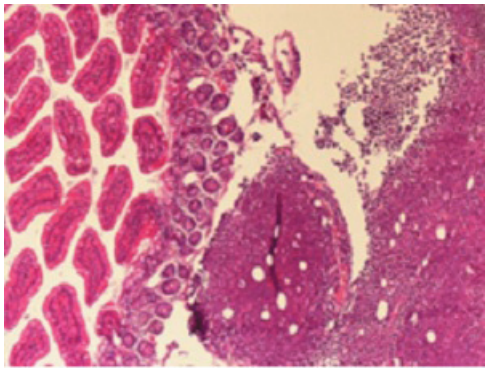
Kidney, small intestine, large intestine and stomach tissue sample sections of EAT and Control groups in Table 6 and Table 7 (H&E X 100)



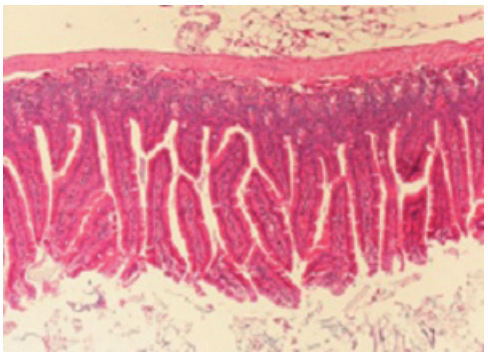
Picture 1: Kidney (with EAT)



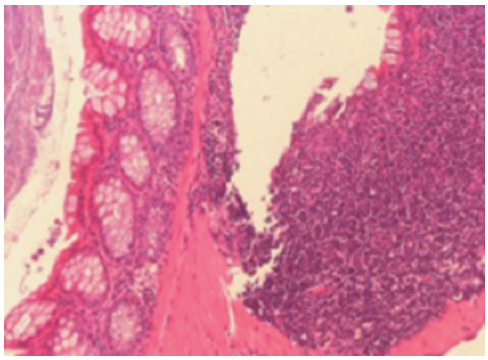
Picture 2: Kidney (Control)



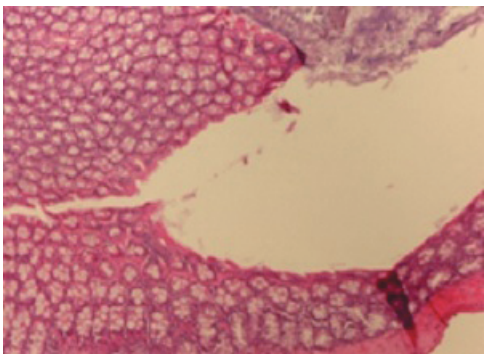
Picture 3:Small intestine (with EAT)



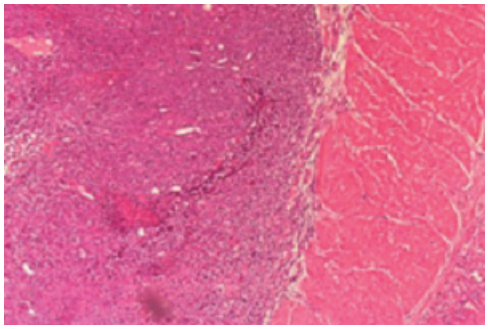
Picture 4: Small intestine (Control)



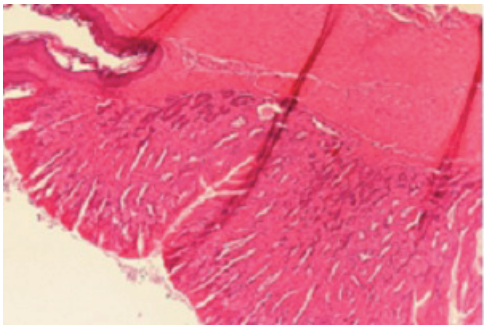
Picture 5: Large intestine (with EAT)



Picture 6: Large intestine (Control)



Picture 7: Stomach (with EAT)



Picture 8: Stomach (Control)

Discussion

Microalgae have been one of the main food and livelihood sources of people for many years in many countries due to their organic growth and development in aquatic environments (seas, lakes, fresh waters) and their rich food content [20] and J. Subhashini & al., 2004 [11]. While it is used as a nutritional supplement for humans, it is also actively used as animal feed in the poultry industry and aquaculture [20]. The use of microalgae in treatment began with the history of humanity. Thousands of years ago, people learned the therapeutic aspect of algae and benefited from them to lead a healthy life. As a result of studies on algae used in pharmacy and medicine, spirulina's enhancing effects on antimicrobial, cytotoxic, anti-mitogenic, anticancer and anti-tumoral activities were mentioned [1]. Noda et al. They examined the antitumor activity of polysaccharide and lipid parts of 24 different algae species against EAT and determined that some species showed significant effects.

Research on compounds with antitumor effects from algae continues [16] and [17]. In this study, EAT modeling of *S. Plantesis* algae produced in Yalova University Algae Production Unit was created and its anti-tumoral activity and biochemical effects were investigated in vivo on Balb-c mice. Barakat et al. In a study conducted in 2015, they determined the minimum and maximum dose (200 mg/kg, 800 mg/kg) for EAT to be similar to human breast tumor and for the anticancer activities of Spirulina platensis. When S. platensis or anti-tumoral activity studies in different species were examined, it was reported that spirulina was given by gavage method. When the group given 5-Fluorouracil was compared with the control group, it was observed that the tumor volume decreased. No significant decrease in tumor volume was observed in the groups given 200 mg/kg and 800 mg/kg S. platensis compared to the control group. has been observed to decrease significantly [18]. In our study, when the 5-Fluorouracil, *S.platensis*, 200 mg/kg and 800 mg/kg groups were compared, the EAT spread of the 5-Fluorouracil group and the 200 mg/kg S.platensis group gave significant results in the stomach and large intestine. The anti-tumoral activity of *S.platensis* is in parallel with this study.

Jiang et al. The effect of phycocyanin obtained from Spirulina on tumor progression and metastasis potential on rats with colon cancer by DMH was investigated. As a result of the study, it was observed that after the rats were induced with DMH, the number and size of tumors/lesions were reduced in those treated with phycocyanin. In our study, spirulina was given as a whole without being separated into its components. Also, Jiang et al. While colon cancer was formed on rats with DMH in our study, tumor formation was achieved with EAT in our study. As a result of the histopathological study, it was observed that the spread of EAT tumor in the large intestine was statistically significantly lower in the treatment groups given spirulina 200 mg/kg. Both studies show that spirulina is effective against cancer in the large intestine.

Ouhtit & al. (2014 [19] investigated the chemical inhibitory effect and underlying mechanisms of action of Spirulina against mammary carcinogenesis on 7,12-dimethylbenz[a] anthracene (DMBA)-induced female albino rats. It has been reported that Spirulina cleared DMBA-induced rat mammary tumors, which was clearly confirmed by morphological and histological methods, and Spirulina supplementation reduced the incidence of mammary tumors from 87% to 13%. In our study, tumor formation was achieved by administering EAT, which is similar to human mammary tumors, on Balb-c mice. As a result of the histopathological examination performed in the treatment group with Spirulina, which we gave at a dose of 200 mg/kg, it was observed that the spread of EAT tumor in the large intestine and stomach decreased, and Spirulina was found to be effective against the tumor. In our previous study, the effectiveness of phycocyanins obtained from spirulina produced in Yalova University Algae unit was investigated against EAT tumor cells, and it was found that antioxidant levels were higher in the treatment groups compared to the control [24]. In our study, significant differences were found between oxidant levels in the treatment group. Again, in our study with phycocyanin, at the end of the pathological evaluations (200 mg) phycocyanin was determined to be effective against EAT in both treatment and protection groups, and in our current study, a statistical difference was found in the spread of the tumor in the large intestine and stomach organs.

In this study, in which the antioxidant and hepatoprotective effects of Spirulina platensis were evaluated in determining the in-vivo anti-tumoral activity; The biochemical parameters of the spirulina groups created to measure the value of Spirulina depending on the dosage effect on normal mice were compared and it was found that it was not statistically significant. When we compared the biochemical parameters of the control group with spirulina in different dosages, differences were observed, but it was not found statistically significant. The biochemical parameters of the treatment groups at different dosages were compared and were not found to be statistically significant, but the differences in TOL values were found to be statistically significant. Differences in biochemical values (TAL, TOL) were found between Spirulina treatment groups, 5-fluorouracil and Control groups, but it was not found to be statistically significant.

In the histopathological examination (large intestine, small intestine, stomach, kidney) of Spirulina, treatment groups, 5-fluorouracil and control groups, it was observed that there were differences in the spread of EAT in the organs.

In the histopathological examination of the group in which we gave S. platensis at a dosage of 200mg/kg, EAT spreads were found to be statistically significantly lower in the stomach and large intestine. In the kidney, it was found statistically significant when the mean spread of EAT was taken.

The fact that spirulina causes a decrease in oxidant parameters and the spread of EAT in the stomach and intestines is less than the control group shows that spirulina has an inhibitory effect on EAT spreads.

Acknowledgment

S.Plantesis used in this study was produced in Yalova University Armutlu Vocational High School Algae Production Unit by Prof. Dr. It was produced with the supervision and contribution of Betül Güroy. Thank you for your contribution to the study.

References

1. S. Aktar, G.E. Cebe, Alglerin Genel Özellikleri, Kullanım Alanları Ve Eczacılıktaki Önemi, Ege University, Faculty Of Pharmacy, Department Of Pharmaceutical Botany, 35100 Bornova-İzmir, Turkey, Ankara Ecz. Fak. Derg. 2010, V.39 (3) Pp. 237-264;
2. S. Aksay, R. Arslan, Spirulina'dan (Spirulina Platensis) Klorofil-A Ve Fikosiyanin Pigment Ekstraksiyonuna Ultrasonikasyon Süresinin Etkisi, Akademik Gıda, 2018, 16(3) 307-312, Doi: 10.24323/Akademik-Gida.475362;
3. H. Yilmaz, M. Duru, Syanobakteri Spirulina Platensis'in Besin Kimyası Ve Mikrobiyolojisi, Türk Bilimsel Derlemeler Dergisi, 2011, 4(1): 31-43;
4. M. Qureshi, A. RA; Spirulina Platensis Exposure Enhances Macrophage Phagocytic Function In Cats, Immunopharmacol Immunotoxicol, 1996, 18(3):457-63;
5. R. Koníčková, K. Vaňková, J. Vaníková, et al. Anti-cancer effects of blue-green alga Spirulina platensis, a natural source of bilirubin-like tetrapyrrolic compounds. Ann 1072 Ge et al. The efficacy of Spirulina in tumor chemotherapy © Translational Cancer Research. All rights reserved. Transl Cancer Res 2019, 8(4):1065-1073;
6. I.P. Joventino, H.G. Alves, L.C. Neves, The microalga Spirulina platensis presents anti-inflammatory action as well as hypoglycemic and hypolipidemic properties in diabetic rats. J Complement Integr Med 2012, 9:Article 17;
7. C.H. Nielsen, P. Balachandran, O. Christensen, et al. Enhancement of natural killer cell activity in healthy subjects by Immulina(R), a Spirulina extract enriched for Braun-type lipoproteins, Planta Med, 2010, 76:1802-8;
8. S. Buono, A.L. Langellotti, A. Martello, et al. Functional ingredients from microalgae. Food Funct, 2014, 5:1669-85;
9. P.D. Karkos, S.C. Leong, C.D. Karkos, et al. Spirulina in clinical practice: evidence-based human applications. Evid Based Complement Alternat Med, 2011, 2011:531053;
10. V.B. Bhat, K.M. Madyastha, C-Phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro, Biochemical and Biophysical Research Communications, 2000, 275, 1, 20–25;
11. J. Subhashini, S. V. K. Mahipal, M.C. Reddy, M. M. Reddy, A. Rachamallu, P. Reddanna, Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562," Biochemical Pharmacology, 2004, 68,3; 453–462;
12. M. Qureshi, J. Garlich, M. Kidd, Dietary Spirulina Platensis Enhances Humoral And Cell-Mediated Immune Functions In Chickens, Immunopharmacol Immunotoxicol, 1996, 18(3):465-76;
13. K. Hayashi, T. Hayashi, I. A. Kojima, Natural Sulfated Polysaccharide, Calcium Spirulan, Isolated From Spirulina Platensis: In Vitro And Ex Vivo Evaluation Of Anti-Herpes Simplex Virus And Anti-Human Immunodeficiency Virus Activities, Aids Res Hum Retroviruses, 1996, 12(15):1463-71.
14. E. Taskin, Z. Caki, M. Ozturk, E. Taskin, O. Kurt, Antimicrobial and antitumoral activities of marine algae, International Review of Hydrobiology, 2010, 3 (1), 37-50;
15. N. Kaba, E. Çağlak, Deniz Alglerinin İnsan Beslenmesinde Kullanılması, E.U. Journal Of Fisheries & Aquatic Sciences, 2006, Volume 23, Suppl. (1/2): 243-246:
16. H. Güner, V. Aysel, A. Sukatar, M. Ozturk, The Aegean Sea Flora, Turkey I. Cyanophyta, Chlorophyta And Phaeophyta, Turkish Journal Of Botany, 1985, 9 (2), 277-282;
17. G. Özdemir, Z. Horzum, A. Sukatar, N. U. Karabay-Yavaşoğlu, Antimicrobial Activities Ofvolatile Components And Various Extracts Of Dictyopteris Membranaceae And Cystoseirabarbata From The Coast Of Izmir, Turkey, Pharmaceutical Biology, 2016, 44, 183-188;
18. W. Barakat, S. M. Elshazly, A. A. Mahmoud, Spirulina Platensis Lacks Antitumor Effect Against Solid Ehrlich Carcinoma In Female Mice, Advances In Pharmacological Sciences, 2015;

19. A. Quhtit, F.M. Ismail, A. Azim, A. Fernando, Chemoprevention of Rat Mammary Carcinogenesis by Spirulina. American Journal of Pathology, 2013, 184 (1);
20. N. Kaba, E. Çalak, Deniz Alglerinin İnsan Beslenmesinde Kullanılması, E.Ü. Su Ürünleri Dergisi, 2006, Volume 23:243-246;
21. M. M. TARPEY, D.A. WINK, M.B. GRISHAM, Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations, Am J Physiol, Regul Integr Comp Physiol, 2004, 286(3): R431–44;
22. B. Halliwell, J. M. C. Gutteridge, Free radicals in biology and medicine. Third ed. Oxford: Oxford Science Publications, 2000, p. 617–24;
23. O. Hayashi, T. Katoh, Y. Okuwaki, Enhancement of antibody production in mice by dietary S. platensis. J. Nutr. Sci. Vitamino, 1994, 40(5):431–441;
24. S. Bayil Oğuzkan, İ. güler, G. B. kut, K. bakir, M. özaslan, Investigation of Antitumor Activity of Phycocyanin Obtained from Spirulina (Arthrospira) platensis in Mice, International journal of pharmacology, 2023, 19: 114-121.