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Antiproliferative Effect of IST-GLIO® Supplement Food Product on C6 Glioma Cells

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Abstract

Objective: Cancer is one of the leading causes of death worldwide and is characterized by the proliferation of abnormal cells. In recent years, as in the treatment of many diseases, there is also a return to nature in the treatment of cancer. In developing societies, the concepts such as prophylaxis of disease prevention rise to prominence in order to reduce the material and moral losses caused by disease treatment. Consequently, the use of supplementary food products occupies more and more space in daily life. In this study, it was aimed to investigate the antiproliferative properties of IST-GLIO® food supplement, which is one of these products and used in the Remember Regeneration Therapy Method (RTM), on C6 (rat glioma) cells.

Material-Method: WST-1 cell proliferation test protocol was applied to examine the antiproliferative effect of IST-GLIO® and the results were evaluated according to ELISA microplate reading data. The product was tested at concentrations ranging from 1 ng/mL to 100 ng/mL.

Results: When the results were evaluated, it was appeared to be effective in the concentration range of 6-20 ng/mL on the C6 glioma cell culture; it was found that the efficacy didn't occur through general toxicity, specifically reducing the reproduction of the cancer cells in question, since food supplement did not inhibit cell division further at higher doses.

Conclusion: As a result, non-specific toxicity is observed in many cultures that are similarly investigated in cell cultures, depending on concentration as high doses are obtained. The IST-GLIO® product does not show this feature is considered as promising.

Keywords: C6 Glioma, Antiproliferative, Cancer

INTRODUCTION

Cancer is used to describe neoplasia characterized by the uncontrolled proliferation of cells in a particular area of the organism due to the effect of epigenetic factors or a number of genetic changes[1]. According to the Turkish Cancer Research and War Agency, deaths from cancer rank second after cardiovascular diseases in the most common deaths list. Primary brain tumors account for 2% of all cancer-related deaths[2]. Although there are many kinds of treatment methods and application, the average life span of brain tumor patients is one year[3,4]. Due to prognosis of the tumor and the limited success of surgery and cytotoxic therapy in brain tumors, agents that are non-toxic and susceptible to brain tumor cells have been a source of hope for the development of new treatment methods. In the recent studies for this purpose, the anticancer effects of synthetic, herbal and fungal drugs against various types of cancer have been investigated.

Various herbal substances used in cancer treatments are used because they inhibit cell proliferation and in turn trigger apoptosis. Medicinal plant extracts and secondary metabolites obtained from these extracts; Investigation of antioxidant, free radical scavenging and anticancer activities is one of the widely studied topics today[5, 6, 7, 8]. Today, 20% of sentetic drugs used in Germany, more than 1/3 of the drugs used in Russia are herbal drugs[9]. Many medicinal plants have been studied extensively in cancer studies in vitro.

In the light of this information, the studies of plants are remarkable, but the combined studies are almost nonexistent. Therefore, in this study, IST-GLIO® food supplement with combined plant content and also used in the treatment of remember regeneration was used[10]. In our study, we investigated the in vitro effects of IST-GLIO® fortifying food product in rat C6 glioma cell line on cell proliferation in a dose-dependent manner.

MATERIALS AND METHODS

Materials

The food supplement used in the study were supplied from Naturin (Natural Products Pharmaceutical and Pharmaceutical Raw Materials Industry Trade Limited Company). The product content is shown in Table 1. The doses used are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppb.

Table 1. IST-GLIO® supplement food supplement content

No	Product content	Quantity in 1 capsule
1	*Urtica sp.* seed	237 mg
2	*Peganum harmala* (L.)	133 mg
3	*Silybum marianum* (L.)	89 mg
4	*Zingiber officinale*	74 mg
5	*Nigella sativa* (L.)	68 mg
6	*Curcuma longa* (L.)	67 mg
7	*Juniperus communis* fruit	44 mg
8	*Thymus sp.*	15 mg
9	*Foeniculum vulgare*	4 mg
10	*Pimpinella anisum*	3 mg
11	*Cassia acutifolia*	3 mg
12	*Syzygium aromaticum*	3 mg

Cell culture

Experimental studies were carried out at Duzce University, Traditional and Complementary Medicine Application and Research Center, Cell Culture Laboratory. In the study, C6 cell line was obtained from Nevsehir University. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma) with 10% Fetal Bovine Serum (Sigma) and 1% penicillin + streptomycin (Sigma) broth containing mixture, 37 °C in medium containing 5% CO2 and 95% humidity 25 cm^2 incubated in flasks.

Antiproliferative assay

Anticancer activities of plant extracts were performed according to the sodium salt of 4-[3-(4 iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolium]-1,3-benzene disulfonate (WST-1) Cell Proliferation Assay System (Takara Bio Inc., Shiga, Japan). The WST-1 test used to determinacy to toxic effect and metabolic activity is a non-radioactive, spectrophotometric, colorimetric test based on the separation of tetrazolium salts from living cells. Tetrazolium salts are converted to formazan salts by cellular enzymes. An increase in the number of live cells leads to an increase in mitochondrial dehydrogenase activity in the samples. With the increase of enzyme activity, there is an increase in the form of cells stained with formazan in correlation with the amount of cells that are metabolically active in culture cells. Formazan dye is produced by cells that are metabolically active, and the absorbance of the cells stained at the wavelength specified by the spectrophotometer is measured[11].

According to this method, when the cells reached the appropriate concentration, the cells were seeded in a 96-well plate at a density of $5x10^4$ cells/well. Solutions of IST-GLIO® food supplement ranging from 1ng / ml to 100 ng/ml were prepared and added to the medium. All experiments were performed in triplicates. It was also created in the negative group with no products applied. The product was incubated with cells for 24 hours.

After incubation, WST-1 solution was added to each well. The plate was incubated at 37 °C for 4

hours. At the end of incubation, the absorbance value (OD) of each well was read in the plate ELISA (Cytation ™ Biotek, USA) at 490 nm wavelength and 630 nm reference range. Cell viability percentage was calculated by dividing the optical density value measured in each well by the control optical density value and multiplying by the face.

RESULTS

Cell viability on the C6 glioma cell line treated and untreated with the IST-GLIO® food supplement was shown in graphic 1. According to ELISA microplate reading results, the group classified as negative control without any product was evaluated as 100% alive and the results given in Table 1 were obtained by calculating the % viability in the test concentrations.

Table 1. Cell viability rates

Concentrations	% Viability
1 ppb	97
2 ppb	96
3 ppb	96
4 ppb	93
5 ppb	93
6 ppb	78
7 ppb	78
8 ppb	78
9 ppb	77
10 ppb	76
20 ppb	72
30 ppb	71
40 ppb	72
50 ppb	72
60 ppb	70
70 ppb	70
80 ppb	70
90 ppb	70
100 ppb	70

According to the results of the analysis, the effectiveness up to 90% cell viability was not considered in terms of data security interval. It was assumed that there was no efficacy accordingly at concentrations between 1-5 ng/mL doses applied to the C6 glioma cell line. It was understood that the cell viability rate in the concentrations between 6-20 ng/mL was in the range of 70-80%, thus inhibiting moderate to strong cell growth. Since the 70% viability rate was maintained between 20-100 ng/mL concentrations, the maximum effective concentration was thought to be achieved.

DISCUSSION

Plants have played an important health role since ancient times. Traditional plant-based medicines are still of great importance to people living in developing countries. It is also a resource for the discovery of new drug candidates. However, it needs to be scientifically evaluated to confirm the use of medicinal plants. Mortality rates in cancer patients are increasing day by day. It is an indication of the limited efficiency of current treatments used in cancer treatment. The search for cancer treatment for many years has focused on chemically synthesized compounds. Over the past few decades, research has focused on the use of natural products, such as raw plant extracts or a combination of different phytochemicals[12].

The IST-GLIO® food supplement used in the study contains different plant combinations. Anticancer properties of most of these plants have been proven by studies. The anticancer effect of *Urtica dioica* has been investigated in breast cancer cells and it has been revealed that its use with cancer drug paclitaxel may have therapeutic potential. In another study, it was revealed that the apoptotic effects of nettle extract of dichloromethane extract were examined, prostate cancer had positive results[13]. In another study, it showed the inhibitory effect of cell proliferation on prostate cancer cells (LNCaP and hPCPs) by the plant's aqueous and ethanol extracts[14]. Also in a report, the anticancer effects of this plant against esophageal cancer are mentioned. In another study, the anti-proliferative effect on human prostate cancer cells with nettle root extract has been proven[15]. They proved that the seed extract MDA-MB-231 (breast cancer) of *Peganum harmala* L. induces apoptosis and inhibits the growth of its cells[16].

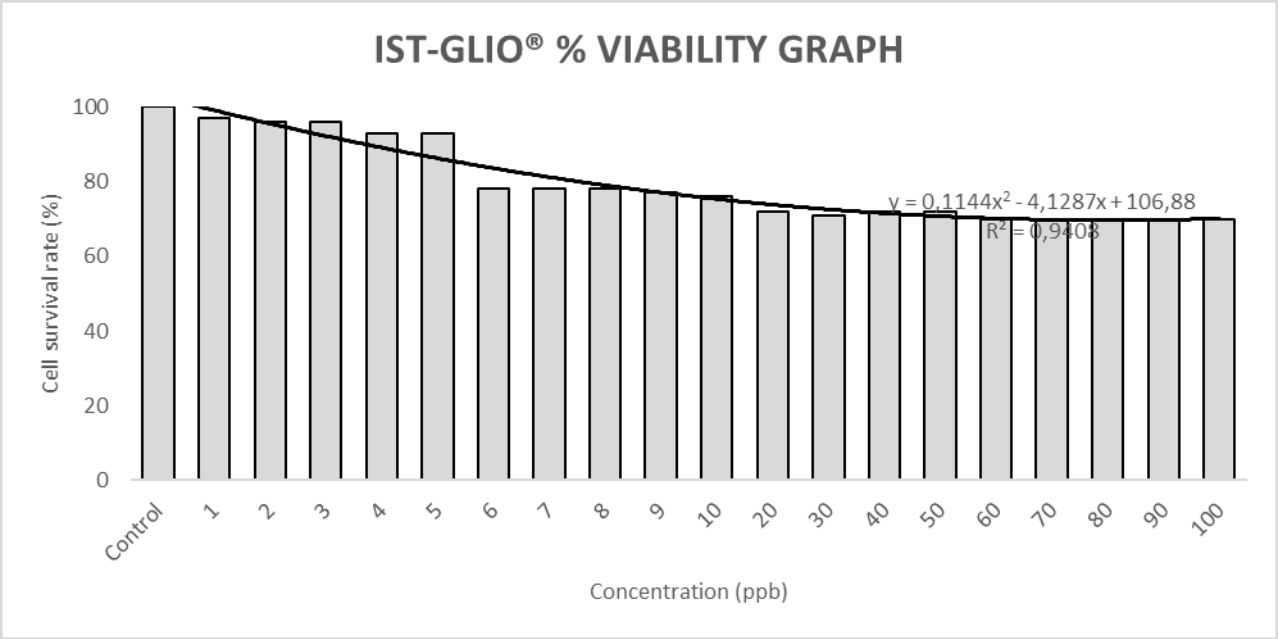

Figure 1. Effect of different doses of IST-GLIO® on C6 cell survival rates. The control group was accepted as 100%

Many studies on *Silybum marianum* are available in the literature. Some concentrations of silybin have been found to inhibit growth in human prostate cancer cells and reduce tumor volume by 53-64%. It has also been suggested that silymarin causes regression of skin tumors. In another study, it has been revealed that it decreases hyperplasia and proliferation index. In another study, it was revealed that Silymarin both inhibits cell growth and inhibits DNA synthesis in different breast and cervical human-carcinoma cells[17]. Aqueous extract of *Zingiber officinale* acts on breast cancer cells (MCF7 and MDA-MB-231). It has been demonstrated that it inhibits the growth of cancer cells and induces cell death[18]. *Nigella sativa* extracts have been evaluated for their anticancer properties on the MCF-7 cell line and have been shown to be a potential therapeutic agent for cancer[19]. In another study, when the effects on kidney cancer cells (ACHN) were evaluated, apoptotic effect was seen on the cells. In another study, it has been shown to inhibit cell growth, apoptosis and increased cell morphological changes on colorectal cancer cells. It has also been shown to induce programmed cell death and anticancer activity is observed in an alcoholic extract of *Nigella sativa*[20]. The anticancer effect of *Curcuma longa* aqueous extract on sarcoma and breast cancer has been demonstrated[21]. Curcumin has demonstrated significant anticancer effects against many different types of cancer, including in vitro and in vivo prostate cancer, MCF7 (human breast adenocarcinoma), colorectal cancer, pancreatic cancer, and head and neck cancer. Investigation of the cytotoxic properties of turmeric on liver cancer cells (HepG-2) showed that curcumin-mediated cytotoxicity caused apoptosis of cancer cells via mitochondrial route[22]. Cytotoxic activities were shown in *Juniperus communis* leaf extracts EJ138 (human bladder), HepG-2 (human liver hepatocellular carcinoma), A549 (human lung carcinoma) and MCF7 (human breast adenocarcinoma) cell lines[23]. *Thymus sp.* among its active ingredients, thymol and carvacrol are the most important plant phenol compounds that are useful in the treatment of breast cancer and colorectal cancer. In one study, *Thymus sp.* has been shown to inhibit the growth, proliferation of human colorectal cancer cell[24]. Cell cycle arrest, which can occur through synergistic effects

between the active ingredients of fennel (*Foeniculum vulgare*) and clove (*Syzygium aromaticum*) oils, and its effect on Caco-2 cells by apoptosis has been demonstrated[25]. In addition, the acute toxicity of the IST-GLIO® food supplement product in experimental animals was examined and no toxicity was found[26].

Looking at the literature, almost every plant in the IST-GLIO® food supplement product has been studied in different cancer cell lines alone. As a result of the studies, it has been revealed that these plants inhibit growth and proliferation in different cancer cell lines.

In our study, these plants were combined and studied in different cancer lines (C6-glioma) by making use of the synergistic effects of herbal mixtures. In our study, 19 different concentrations were applied and in the cell culture of the product; It appears to be effective in the concentration range of 6-20 ng/mL. It appears to be effective by blocking cell reproduction. It is understood that the efficacy is not through general toxicity, specifically reducing the reproduction of the cancer cells in question, since higher doses do not inhibit cell division more. Many products that are similarly investigated in cell cultures show non-specific toxicity depending on concentration as high doses are obtained. The fact that the IST-GLIO® product does not show this feature has been considered as promising. Although successful results are obtained in cell culture, any product must be supported by clinical research in order to talk about anti-carcinogenic activity.

CONCLUSION

In this study, the antiproliferative efficacy of the IST-GLIO® product, which is used as a food supplement product, in the concentrations determined by considering the daily usage amount, was studied in the C6 glioma cell line. According to the results of the study, it is understood that the product is effective in the concentration range of 6-20 ng/mL. It is thought to show non-specific toxicity as the product retains its effectiveness as higher doses are reached. This research supports the effectiveness of IST-GLIO® food supplement product in glioma cell culture and the level of evidence should be increased by clinical research.

REFERENCES

1. Nussbaum LR, McInnes RR, Willard FH. Genetik ve Kanser. *Thompson&Thompson Genetics in Medicine.* İstanbul, Güneş Kitabevi, 2005.
2. Derici E. Rho kinaz ve siklooksijenaz inhibitörleri ile siklofosfamid kombinasyonlarının çeşitli tümör hücre serilerine etkileri. *Mersin Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi.* 2007.
3. Mikkelsen T. Cytostatic agents in the management of malignant gliomas. *Cancer Control:JMCC* 1998; 5(2):150-162.
4. Salford LG, Siesjö P, Skagerberg G, Persson BRR, Larsson EM, Lindvall M, Visse E, Widegren B. Search for effective therapy against glioblastoma multiforme clinical immunisation with autologous glioma cells transduced with the human interferon- γ gene. *International Congress Series.* 2002; 1247:211-220.
5. Hsu CL, Huang SL, Yen GC. Inhibitory effect of phenolic acids on the proliferation of 3T3-L1 preadipocytes in relation to their antioxidant activity. *Journal of Agricultural and Food Chemistry*. 2006;54(12): 4191-4197.
6. Granado-Serrano AB, Martın MA, Izquıerdo-Pulıdo M, Goya L, Bravo L, Ramos S. Molecular mechanisms of (-)-Epicatechin and Chlorogenic acid on the regulation of the apoptotic and survival/proliferation pathways in a human hepatoma cell line. *Journal of Agricultural and Food Chemistry*. 2007;55(5):2020-2027.
7. Marinova EM, Toneva A, Yanishlieva N. Comparison of the antioxidative properties of caffeic and chlorogenic acids. *Food Chemistry*. 2009;114(4): 1498–1502.
8. Vizotto M, Porter W, Byrne D, Cisneros-Zevallos L. Polyphenols of selected peach and plum genotypes reduce cell viability and inhibit proliferation of breast cancer cells while not affecting normal cells. *Food Chemistry.* 2014;1:64: 363–370.
9. Uçar T. Bazı bitkisel çayların mineral madde içeriği üzerine farklı demleme ve kaynatma sürelerinin etkisi. *Konya, Selçuk Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi*. 2006.
10. Yasar M. The remember regeneration therapy method: An overview of new therapy protocol to approach diseases. *Journal of Complementary Medicine Research*. 2019;10(1):68-80.
11. Doyle A, Griffiths JB. Cell and tissue culture: Laboratory procedures in biotechnology. *Scientific Consultancy & Publishing*. 1998;62-64.

12. Alawode TT. An overview of the anti-cancer properties of some plants used in traditional medicine in Nigeria. *International Research Journal of Biochemistry and Bioinformatics*. 2013;3(1):7-14.
13. Mohammadi A, Mansoori B, Aghapour M, Shirjang S, Nami S, Baradaran B. The *Urtica dioica* extract enhances sensitivity of paclitaxel drug to MDA-MB-468 breast cancer cells. *Biomedicine Pharmacotherapy*. 2016; 83:835-842.
14. Durak I, Biri H, Devrim E, Sözen S, Avcı A. Aqueous extract of *Urtica dioica* makes significant inhibition on adenosine deaminase activity in prostate tissue from patients with prostate cancer. *Cancer Biology Therapy*. 2004;3(9):855–857.
15. Konrad L, Müller HH, Lenz C, Laubinger H, Aumüller G, Lichius JJ. Antiproliferative effect on human prostate cancer cells by a stinging nettle root (*Urtica dioica*) extract. *Planta Medicine.* 2000;66(1):44–47.
16. Shabani SHS, Tehrani SSH, Rabiei Z, Enferadi ST, Vanoozzi GP. *Peganum harmala* L.'s anti growth effect on a breast cancer cell line. *Biotechnology Reports.* 2015;(8):138-143.
17. Abascal K, Yarnell E. The many faces of *Silybum marianum* (milk thistle). *Alternative and Complementary Therapies*. 2003;9(5):251–256.
18. Moheghi N, Tavakkol Afshari J, Brook A. The cytotoxic effect of *Zingiber officinale* in breast cancer (MCF7) cell line. *The Horizon of Medical Sciences.* 2011;17(3):28–34.
19. Khurshid Y, Syed B, Simjee SU, Beg O, Ahmed A. Antiproliferative and apoptotic effects of proteins from black seeds (*Nigella sativa*) on human breast MCF-7 cancer cell line. *Complementary Medicine and Therapies.* 2020;13;20(1):5.
20. Ait Mbarek L, Ait Mouse H, Elabbadi N, Bensalah M, Gamouh A, Aboufatima R, Benharref A, Chait A, Kamal M, Dalal A, Zyad A. Anti-tumor properties of blackseed (*Nigella sativa* L.) extracts. *Brazilian Journal of Medical and Biological Research.* 2007;40:839–847.
21. Ranjbari J, Alibakhshi A, Arezumand R. Pourhassan MM, Rahmatı M, Zarghamı N, Namvaran MM. Effects of *Curcuma longa* extract on telomerase activity in lung and breast cancer cells. *Zahedan Journal of Research in Medical Sciences.* 2014;16(10):1-6.
22. Tomeh MA, Hadianamrei R, Zhao X. A review of curcumin and its derivatives as anticancer agents. *International Journal of Molecular Sciences*. 2019;20(5).
23. Groshi AA, Evans AR, Ismail FMD, Nahar L. Sarker SD. Cytotoxicity of Libyan *Juniperus phoenicea* against human cancer cell lines A549, EJ138, Hepg2 and MCF7. *Pharmaceutical Sciences* 2018;24(1):3-7.
24. Abaza MS, Orabi KY, Al-Quattan E, Al-Attiyah RJ. Growth inhibitory and chemo-sensitization effects of naringenin, a natural flavanone purified from *Thymus vulgaris*, on human breast and colorectal cancer. *Cancer Cell Int.* 2015;24:15.
25. El-Garawani IM, El-Nabi SH, Dawoud GT, Esmail SM, Abdel Moneim AE. Triggering of apoptosis and cell cycle arrest by fennel and clove oils in Caco-2 cells: the role of combination. *Toxicology Mechanisms and Methods.* 2019;29(9):710-722.
26. Yasar M, Senogul O, Gok A, Agan K, Agan AF, Beyazcicek E, Coskun SK. Systemic Investigation of Acute Toxicity of Some Food Supplements on the Market in Turkey. International Journal of Traditional and Complementary Medicine Research. 2020(1):1:1-11.