

ORIGINAL RESEARCH

Cytotoxicity of Some Retail Food Supplements in the Market

Pinar Agyar Yoldas^{1*}  Taner Yoldas²  Nisa Sipahi¹ 

¹ Traditional and Complementary Medicine Application and Research Center, Duzce University, Duzce, Turkey

² The Scientific and Technological Research Application and Research Center, Duzce University, Duzce, Turkey

*Corresponding Author: Pinar Agyar Yoldas, e-mail: pinaragyaryoldas@duzce.edu.tr

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Abstract

Objective: Medicinal plants and products obtained from medicinal plants are widely used in the treatment of various diseases in our country as well as all over the world. However, scientific data on the biological effects and mechanisms of action of most extracts from medicinal plants are still inadequate. Because of that, interest in scientific research of the biological effects of medicinal plants and products obtained from medicinal plants is increasing day by day. In addition, the reliability of these products in terms of use apart from their effectiveness is very important for public health. In this study, we aimed to investigate the cytotoxicity of commercially marketed natural herbal food supplements in L929 (mouse fibroblast) cells.

Material-Method: In this study, the WST-1 cell proliferation test protocol was applied to examine the cytotoxicity of 19 different products on the market in L929 cell lines and the results were evaluated according to Elisa microplate reading data. The products were also tested at concentrations of 0.02, 0.03, 0.04, 0.05, 0.06 µg / mL, taking into account the amounts of daily use.

Results: When the results were evaluated, 19 different herbal food supplement products sold on the market were cytotoxic in the L929 cell line, in the concentration range of 0.02-0.06 µg/mL, it was observed that the products were not toxic at these doses.

Conclusion: Herbal food supplement products in the market consist of many components. Therefore, the first thing to look at is the safety of the product. In this study, the cytotoxicity of 19 different products sold in the market was examined by considering the daily usage amounts in terms of reliability. Further studies are needed to determine their effectiveness.

Keywords: L929, Cell Culture, Cytotoxicity, Food Supplement, Herbal Mixture

INTRODUCTION

Plants had been used in medical treatments until synthetic drugs were discovered in all cultures and all over the world since the existence of humanity. The first humans invented the method of herbal therapy by trial and error, by observing animals and plants. They have cured many diseases by transferring the information from generation to generation.

As civilization progressed, with the industrial revolution and the development of the pharmaceutical industry, people moved away from nature and synthetic drugs replaced the drugs in nature. Although medical science has developed tremendously, today the drugs are expensive and some drawbacks of synthetic products have emerged, as an alternative to the drugs produced by

the pharmaceutical industry has started to return to nature.

Today, herbs and natural herbal products are used in industry, food industry, cosmetics and perfumery, and many industrial areas. In addition, essential oil and its components obtained from plants are muscle relaxants, antibacterial, antiviral, antifungal, etc. It is used as¹⁻⁴.

Looking at the world in general, the idea and practice of returning to nature, most people prefer at least one of the alternative supportive treatment methods such as clothing, cosmetics, nutrition or treatment. According to the data of the World Health Organization (WHO), 80% of people (approximately 3.3 billion people) use traditional treatment methods for reasons such as the high cost



of synthetic drugs, frequent side effects, and the fact that medicinal plants can be easily obtained from nature⁵⁻⁶.

The most important issue in returning to the use of these traditional products is reliability. Herbal extracts or mixtures must first be proven to be safe for health. Then their effectiveness should be looked at. In this study, reliability was taken into consideration. Purpose of the research; Cytotoxicity in the L929 (mouse fibroblast) cell line was evaluated in vitro, depending on the daily use of the tested products.

MATERIALS AND METHODS

Materials

Food supplement products used in the study were supplied from AYM-net[®] Company (AYM-net Herbal and Cleaning Products Import Export Consulting Trade Limited Company). The products used in the experiment and their contents are shown in Table 1. Dosage ranges for cytotoxicity analysis were preferred over the dose ranges these herbal mixtures were known to be effective. The doses used in cytotoxicity analysis are 0.02, 0.03, 0.04, 0.05 and 0.06 µg/ml.

Methods

L929 mouse fibroblast cell line was used in the study. Cells were cultured in serum containing medium (10% inactivated fetal bovine serum and RPMI-1640 medium containing 1% penicillin + streptomycin as antibiotic) at 37 ° C in T25 cm² flasks in a medium containing 5% CO₂ and 95% moisture. Cells were used for cytotoxicity test when 70% of the cells were confluent in density.

Cell viability assay

Experimental studies were carried out in Duzce University Traditional and Complementary Medicine Application and Research Center, Cell Culture Laboratory. WST-1 (tetrazolium salt based test) proliferation test (Takara Bio Inc., Shiga, Japan) was used to evaluate the cytotoxicity of the food supplements to be tested⁷. When the cells reached the appropriate concentration, they were inoculated into 96-well culture dishes with 5x10⁴ cells per well. Solutions in the dose range of 0.02-0.06 µg / ml for each herbal food supplement product to be tested were prepared and added to the

nutrient medium. Each dose was studied in triplicate. A negative group was created with no product applied. Cells and products were incubated for 24 hours.

WST-1 test results the absorbance value (OD) of each well was read with a microplate reader (Biotek BT 800) at wavelength of 490 nm and reference range of 630 nm. Percentage of cell viability was calculated by dividing the optical density value measured in each well by the control optical density value and multiplying by hundred.

RESULTS

With this study, it was evaluated whether some food supplement products of, which are currently sold in the markets of our country, show toxic properties. The cytotoxicity of the products in different dose ranges was evaluated in vitro, considering the usage doses specified in the package insert. In this study where 19 different products were evaluated, L929 was applied to the mouse fibroblast cell line at doses of 0.02-0.06 µg / ml. According to the results of this research; it was found that the products for these doses did not show toxic activity. The results are grouped according to the portfolio of products and shown on the chart (Figure 1).

According to the analysis results, efficacy up to 90% cell viability was not taken into account in terms of data safety range. No toxic effects were seen at concentrations between the 0.02-0.06 µg / mL dose applied to the L929 cell line. In other words, it is possible to use safely at these concentrations and below.

DISCUSSION

Today, it is quite common to consume food supplements of various ingredients as a protective or auxiliary product for many diseases. But it should be noted that the toxicity that medicinal plants can create in cases of overdose and when mixed. The scientific accuracy of the plants to be used in all respects must be determined, discussed and proven by research. Some of the organic and inorganic compounds, mycotoxins and medicinal plants used for therapeutic purposes can cause liver and kidney damage⁸. Mensah et al (2019) demonstrated that plants have a toxic effect in their

Table 1. Food supplement products and their ingredients

Group	Product Code	Name of The Product	Product Content
Treatment support group / food supplements viability % graphics	A03	Black Cumin	Black cumin oil
	A06	Joint Copmlex	Glikosamine Sulfate
			Chondroitine Sulfate
			MSM (Methylsulfonylmetan)
			Hyaluronic Acid
	A08	Reishi Mushroom	Type 2 Collagen
			<i>Boswellia serrata</i>
			Reishi mushroom (<i>Ganoderma lucidum</i>)
	A09	Pilles Complex	<i>Aesculus hippocassaltium</i>
			<i>Asplenium ceterach</i>
			<i>Cassia sp.</i>
			<i>Urtica sp.</i>
A10	Cohosh Complex	Horse tail	
		<i>Ginko Biloba</i>	
		Green Tea	
		Valerian	
A11	Saccex Complex	<i>Melissa officinalis</i>	
		Black Cohosh	
		Olive Extrakt	
		Cinnamomum	
A12	Psa Complex	Citrus	
		<i>Urtica sp.</i>	
		<i>Berberis vulgaris</i>	
		<i>Epilobium angustifolium</i>	
A14	Curcuma Soft Gell	Cetreaht	
		<i>Equisetum arvense</i>	
		<i>Achillea millefolium</i>	
		<i>Urtica sp.</i>	
Weightening group / food supplements viability % graphics	A01	Active Form Herb Tea	Curcuma (Curcumin)
			<i>Prunus armeniaca</i> (%10)
			<i>Rosmarinus officinalis</i> (%30)
	A07	Form Complex	others (%5)
			Heather Leaf
			Erica Extrakt
			L-Carnitine
A16	Aloe Vera Energy Drink	Chitosan	
		Green Tea Extrakt	
		<i>Melissa officinalis</i>	
A17	Coffee Aktif Vitamin	Aleovera	
		L carnitin	
A24	Chitosan	<i>Camellia sinensis</i>	
		Reishi Mushroom (<i>Ganoderma lucidum</i>)	
Vitamin group / food supplements viability % graphics	A02	Omega 3	Vitamin A, B1, B2, B3, D3 (%2,17)
			Chitosan
	A04	Calcium Magnesium Zing	Calcium, Magnesium, Zinc, Vit D3
	A05	Red Ginseng	Omega 3
			Red Corean Ginseng Root
	A13	Multivitamin	Red Corean Ginseng Ekstrakt
			Panax Ginseng,Ginkgo biloba ve diğ erleri
	A15	C-Vit	Vit-C
A22	Vitamin 5	Royal Jelly, Pollen, Red Corean Ginseng, Propolis, Vit E, Vit A, Vit K2, Vit D3, Vit B12	



Figure 1. % Viability Plot of food supplements. The control group was accepted as 100%.

essence, which varies depending on the dose and the solvent used in the extract⁹. Although aristolochic acid isolated from *Aristolochia* has a positive effect on kidney function, it leads to rapid renal failure in overdose¹⁰. *Glycyrrhiza* spp. (liquorice) and *Panax ginseng* are among the most preferred plants in food supplements. They are often used as antimicrobial, expectorant, effective in colds, therapeutic in respiratory functions and also, the active substances they contain have been reported to cause adverse reactions such as edema, hypertension and electrolyte imbalances, insomnia, fatigue in some formulations (like herbal mixtures)¹¹. In addition, there is a possibility that the beneficial effects of such medicinal plants and herbal products may turn into a harmful effect in long-term uses¹². Food supplements containing minerals or vitamins, such as zinc and magnesium, provide osmotic balance, are a catalyst in biochemical reactions, and are a stabilizer in some protein structures. But on the other hand, essential elements have an accumulative property and seriously threaten human health in high doses¹³.

In particular, the lack of a certain standardization and stable production techniques in the production of food supplements, which are often preferred in terms of their accessibility and easy use, suggests that the reliability of such products should be investigated. Even plants that have been involved in traditional medicine for centuries on the market today do not have standardized products that have been produced and analyzed with the exact concentration of substances known. So we tested the cytotoxicity of some food supplements, thinking that it is important to investigate the reliability of these products. In this study, it was found that the daily dose indicated in 19 different food supplements did not have a toxic effect. Albuz (2019) studied the determination of cytotoxic effects of ginger, turmeric and clove seeds on healthy cells, which are used as food supplements in daily life and have an important place in terms of Public Health. It was no found cytotoxicity at any concentration even the highest concentrations for ginger and turmeric extracts. On the other hand, the concentration of clove extract 1: 2 was reported

to be cytotoxic and the viability was 26.7 %¹⁴. In this study, no cytotoxic effect was found in the product containing turmeric. Yasar et al. (2020) found that the daily dose of a dietary supplement containing ginger and thyme does not cause acute and subacute toxicity in vivo¹⁵. The results in this study are in line with our findings. This has been found to be positive for the reliability and public health of products on the market.

A study using acute and subacute study models in Swiss albino mice showed that *Ganoderma lucidum* did not have significant toxicity and drew attention to the importance of its possible therapeutic uses¹⁶. Artiran et al. (2017), another study in sprague-Dawley type rats reported that daily supplementation of vitamin C in experimental animals did not pose a safety problem and even reduced the significant damage caused to the testes caused by gentamicin¹⁷. In this study, no toxic effects were observed in any of the reishi and vitamin-containing products.

One of the conditions in which food supplements are often used, which are predicted to be beneficial for health, is obesity. In cases of various diseases, such as obesity, the requirements of modern medicine should be met and licensed drugs should be used under the supervision of a doctor. But most of society resort to various natural ingredients to

weaken, as with some health problems. For this purpose, many herbal products find a place in the market. The most innocent danger in many supplements with the thought that it can aid weight loss is that they don't work. The number of cases indicating that supplements and herbal mixtures used for this purpose lead to serious liver damage is quite large¹⁸. On the other hand, no toxic effects were found in any of the weight loss product group included in this study. This indicates the reliability of the products at the specified doses, but it cannot be claimed that in vitro studies are literally clinical equivalent. Because cytotoxicity tests provide basic information about the behavior of a substance and create a source for subsequent clinical trials¹⁹.

CONCLUSION

In this study, the proliferative effectiveness of products used as a food supplement was studied in the L929 cell line at concentrations determined by taking into account the amount of daily use. According to the results of the study, products in the concentration range of 0.02-0.06 µg/mL do not show toxicity. This research supports that the tested food supplement products are non-toxic on the basis of concentrations created by taking into account the amounts of daily use. However, further studies are needed for their effectiveness.

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