In Vitro Proof of Antiproliferative Effect of Soy Total Extract Via Mytochondrial Dehidrogenase Activity

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Introduction: Soy, Glycine max, Fam. Fabaceae is a species of vegetables originary from East Asia. The vegetal product used both in the pharmaceutical and nutritional area are the beans, Sojae semen. Vegetable soybean is rich in phytochemicals beneficial to the human being and is therefore considered a nutraceutical or a functional food crop. The aim of this study is to test the antiproliferative effect of soy total extract, employing different concentrations, on B164A5 murinic melanoma cell line, using the MTT proliferation assay.

Material and method: Soybean seeds were grounded and a solvent formed of DMSO-ethanol-water in 5-70-25 v/v/v ratio was prepared. The extraction was made at room temperature using an ultrasonic bath (Falc LCD Series) for 30 minutes, at 59 kHz. The solvent was evaporated with a rotary evaporator at 50°C. B164A5 cells were incubated 24 h with 100 µM, 50 µM, 30 µM, 15 µM, 1 µM, 0 µM soy total extract. The extraction was made at room temperature using an ultrasonic bath (Falc LCD Series) for 30 minutes, 59 kHz. The solvent was evaporated with a rotary evaporator at 50°C.

Results: MTT analysis showed an inhibition of all chosen concentrations of soy total extract. The inhibition was directly proportional with the concentration of soy total extract. Additionally, a change in the number and morphology of B164A5 cells was noticed starting from the concentration of 15 µM.

Conclusion: Our results suggest that soy total extract presents an antiproliferative effect in vitro on B164A5 murinic melanoma cell line.

Keywords: soy, total extract, B164A5 cells, MTT

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Introduction

Soy, Glycine max, Fam. Fabaceae is a species of vegetables, originary from East Asia. The vegetal product used both in the pharmaceutical and nutritional area are the beans, Sojae semen. Raw seeds of Glycine max have been reported to contain 139 calories, 68.2% moisture, 13.0 g protein, 5.7 g fat, 11.4 g carbohydrate, 1.9 g fiber, 1.7 g ash, 78 mg Ca, 158 mg P, 3.8 mg Fe, 0.40 mg thiamine, 0.17 mg riboflavin, 1.5 mg niacin, and 27 mg ascorbic acid per 100 g [1,2,3]. Vegetable soybean is rich in phytochemicals beneficial to the human being and is therefore considered a neutraceutical or a functional food crop. Soybean as a "functional food" that reduces the risk of a range of hazardous diseases like atherosclerosis, osteoporosis, various types of cancer (breast, uterus cancer, and prostrate) has attracted people’s attention across the globe [4]. Soybeans are the only common plant food that contain complete protein [5]. Beside these important constituents, a class of compounds called phytoestrogens – having as representative structures genistein and daidzein – were brought into light in the scientific world for their potentially beneficial health effects as anticarcinogens, cardioprotective agents and as alternative treatment to hormone replacement therapy in menopause [6,7].

The aim of this study is to test the antiproliferative effect of soy total extract, employing different concentrations, on B164A5 murinic melanoma cell line, using the MTT proliferation assay. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) is cleaved to formazan by enzymes of the endoplasmic reticulum-mytochondrial dehidrogenase (reductase). This bioreduction occurs intracellularly in viable cells only, and is related to NAD(P)H production through glycolysis. Therefore, the amount of formazan dye formed directly correlates to the number of metabolically active cells in the culture [8,9].

Material and method

Soy seeds were kindly provided by the Department of Plant Culture of the University of Agricultural Sciences and Veterinary Medicine, Timișoara, Romania. In a previous study, seeds were considered for quantitative analysis of total lipids (Soxhlet), proteins (Kjeldahl), polyphenols (Folin –Ciocâlteu) and isoflavones – daidzin, genistin, daidzein and genistein (HPLC).

Soybean seeds were grounded and a solvent formed of DMSO-ethanol-water in 5-70-25 v/v/v ratio was prepared [10]. The extraction was made at room temperature using an ultrasonic bath (Falc LCD Series) for 30 minutes, at 59 kHz. The solvent was evaporated with a rotary evaporator at 50°C.

B164A5 cells were acquired from Sigma Aldrich (ECACC and Sigma Aldrich, origin Japan stored UK), were adherent cells and had a spindle shaped morphology, showing fibroblast-like characteristics. The B164A5 cell line is a melanin pigment producing mouse melanoma. The complete growth medium for these cells was Dulbecco’s Modified Eagle’s Medium (DMEM), supplemented with 10% fetal calf serum (FCS) and 1% Penicillin/Streptomycin mixture (Pen/Strep, 10,000 IU/ml) and 2% HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. The cells were cultured by incubation at 37°C in
5% CO₂ atmosphere. When the confluence was 70–80% (every two or three days) the cells were passed using 0.25% Trypsin – 1 mM EDTA solution followed by centrifugation (5 minutes, 1200 rpm) and replated in T75 culture flasks at a subcultivation ratio of 1:10 to ensure optimal proliferation.

For the test of soy total extract, 100 μL cell suspension containing 6000 cells of B16 melanoma cells were seeded onto a 96-well microplate and attached to the bottom of the well overnight. After 24 hours, 100 μL of new medium containing 10% FCS and different concentrations of the total soy extract: 100 μM, 50 μM, 30 μM, 15 μM, 1 μM were added. DMSO was used to prepare stock solutions of the tested samples and the highest DMSO concentration (0.1%) of the medium did not have any significant effect on the cell proliferation. On day three, 10 μL MTT reagent of 5 mg/mL was added. The intact mitochondrial reductase converted and precipitated MTT as purple crystals during a 4 h contact period. After four hours, the precipitated crystals were dissolved in 100 μL of solubilisation solution. Finally, the reduced MTT was spectrophotometrically analysed at 570 nm, using a reference of 656 nm with an ELISA reader.

**Results**

The inhibition index of soy total extract was directly proportional with the concentration, as it can be seen in Figure 1. At the highest tested concentration, the inhibition index of 100 μM soy total extract was 52%, while at a 10 times lower concentration the inhibition index was 4.6%. Increasing this last concentration five times was translated by an inhibition index of 8.33%. The highest inhibition index was recorded for 30 μM and 50 μM with values of 29.3% and 41.3% respectively.

The antiproliferative effect of soy total extract can also be seen in the pictures taken after incubation with different tested compounds (Figure 2a–f). We can observe that between Figure 2a, corresponding to blank (medium in the cell culture with no treatment) and Figure 2f, corresponding to cells incubated with 100 μM soy total extract in the medium, there is a remarkable difference between the cell number and morphology. This observation supports the idea of an antiproliferative effect of soy total extract.

**Discussions**

Previous analysis showed that the chemical composition of soy total extract is formed of 21.4% total lipids/100 g
sample, 39.40% proteins, 303 mg total polyphenols/100 g sample, heteroses: daidzin 819 mg/g and genistin 905.6 mg/g, and aglycones: daidzein 91 mg/g and genistein 119 mg/g. Values fit literature data [10,11,12].

The in vitro tests regarding the antiproliferative potency of soy total extract was done using the MTT proliferation assay on B164A5 murinic mouse melanoma cell line. MTT is a colorimetric assay for the determination of cell viability and cell proliferation. MTT (3-(4,5-Dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) is a tetrazolium salt that is converted into a purple formazan product after reduction by mitochondrial enzymes of the endoplasmic reticulum-mytochondrial dehidrogenase (reductase). This bioreduction occurs intracellularly in viable cells only, and is related to NAD(P)H production through glycolysis [13,14,15]. The inhibition index was calculated as 1-(absorbance sample X/absorbance sample blank). One can observe an inhibition index of soy total extract which is directly proportional with the concentration. In the scientific literature genistein and daidzein are known for their antiproliferative action. Genistein (4,5,7-trihydroxyisoflavone) is the aglicon of genistin, it was identified as the predominant isoflavone in soybean and presents phytoestrogenic properties. The anticancerous mecanism of genistein also involves inhibition of protein tyrosine kinases, an important group of enzymes in signal transduction for regulation of cellular activity, topoisomerase II, 5 α reductase, protein histidine kinase and other enzymes involved in phosphatidylinositol turnover, all of which may contribute to the antiproliferative or pro-apoptotic effects of genistein [16,17,18]. Daidzein is the aglycone of daidzin, the second most plentiful isoflavone in soy- Glycine max Fam. Fabaceae after genistein and possesses the same properties. The effect of soy total extract was tested also in prostate cancer cells and the research concluded that food products that bear a combination of active compounds may be more efficient and safer as chemo-preventive agents than individual compounds [19].

Conclusions
Our results suggest that soy total extract presents an antiproliferative effect in vitro on B164A5 murinic melanoma cell line.

References