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

Danciu (Țiulea) Corina[1], Peev Camelia[1], Feflea Ștefana[1], Dehelean Cristina[2], Motoc A[3]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Victor Babeș University of Medicine and Pharmacy, Timișoara, Romania
[2] Department of Toxicology, Faculty of Pharmacy, Victor Babeș University of Medicine and Pharmacy, Timișoara, Romania
[3] Department of Anatomy, Faculty of Medicine, Victor Babeș University of Medicine and Pharmacy, Timișoara, Romania

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, 59 kHz. The solvent was evaporated with a rotary evaporator at 50ºC. B164A5 cells were incubated 24 h with 100 µM, 50 µM, 30 µM, 15 µM, 1 µM, 0 µM soy total extract.
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

Received: 18 March 2012

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, Timişoara, 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

Correspondence to: Corina Danciu
E-mail: corina_tiulea@yahoo.com

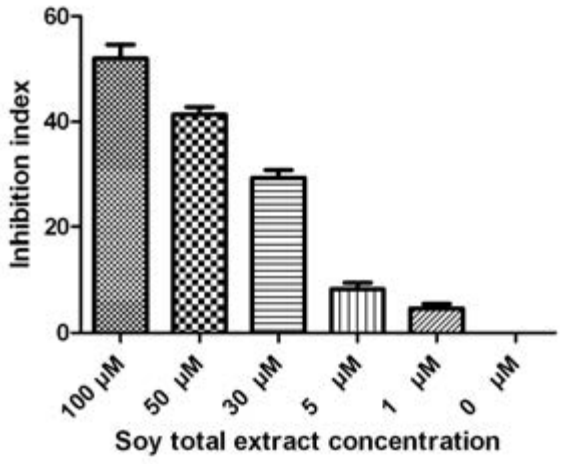

Fig. 1. Inhibition index for soy total extract

5% CO_2 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

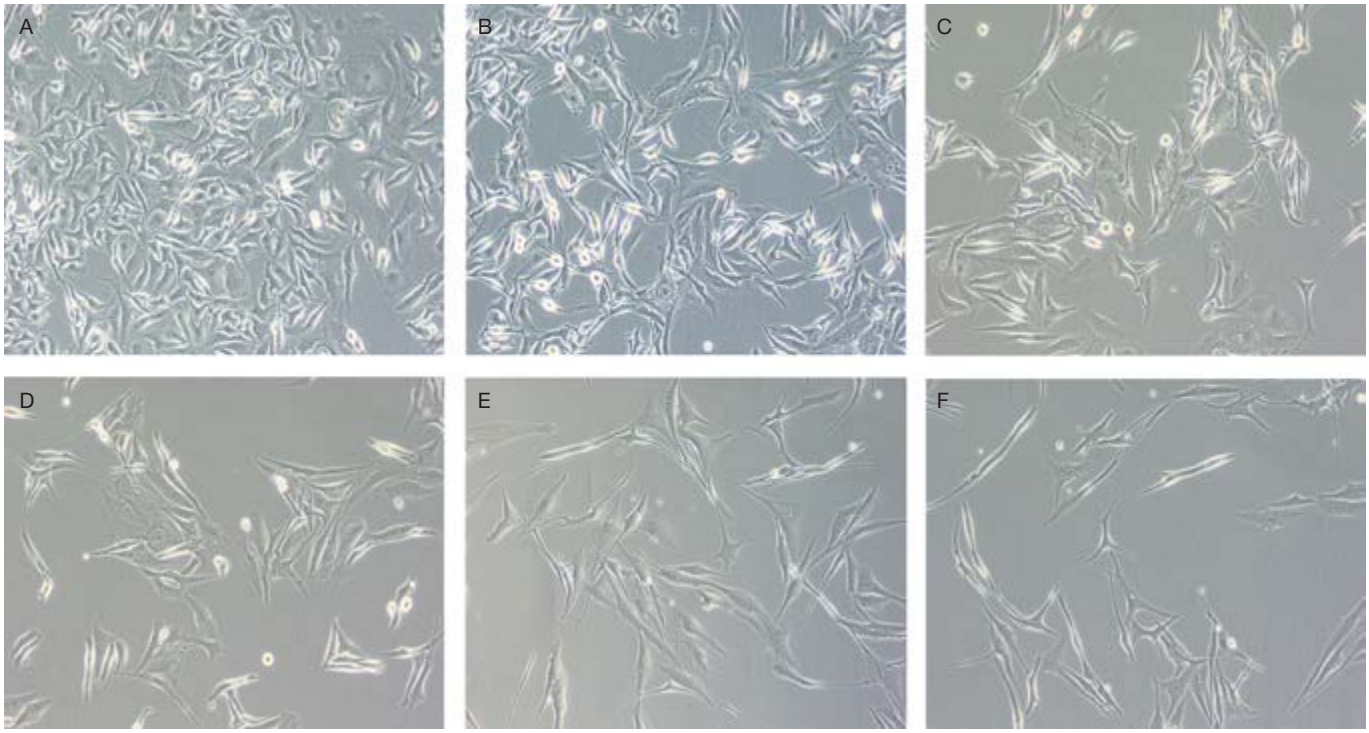

Fig. 2. The antiproliferative effect of soy total extract

sample, 39.40% proteins, 303 mg total polyphenols/100 g sample, heterosides: 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-Dimethylthiazol-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 reductaze, 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 poses 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

1. Duke JA. Handbook of legumes of world economic importance. Plenum Press. NewYork. 1981;256-278.
2. Bruneton J. Pharmacognosie.Phytochimie.Plantes medicinales. In: Composes du metabolisme primaire. Lavoisier Tec & Doc. Paris/France.1999;123-183.
3. Messina M. A brief historical overview of the past two decades of soy and isoflavone research. J Nutr. 2010;140(7):1350S-1354S.
4. Ponnusha BS, Subramaniyam S, Pasupathi P, Subramaniyam B, Virumandy R. Antioxidant and Antimicrobial properties of Glycine Max. A review. International Journal of Current Biological and Medical Science. 2011;1(2):49-62.
5. Endres GE. Soy Protein Products. AOCS press, Champaign, Illinois, USA, 2001, 345-371
6. Klein BC, King AA. Genistein genotoxicity: Critical considerations of in vitro exposure dose. Toxicology and Applied Pharmacology. 2007;224(1):1-11.
7. Ball ER, Caniglia MK, Wilcox JL, Overton KA, Burr MJ, Wolfe BD, Sanders BJ, Wisniewski AB, Wrenn CC. Effects of genistein in the maternal diet on reproductive development and spatial learning in male rats. Horm Behav. 2010;7(3):313-322.
8. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;65(1-2):55-63
9. Qarawi MA, Carrington S, Blagbrough IS, Moss SH, Pouton CW. Optimization of the MTT Assay for B16 Murine Melanoma Cells and its Application in Assessing Growth Inhibition by Polyamines and Novel Polyamine Conjugates. Pharmacy and Pharmacology Communications. 1997;3(5-6):235-239.
10. Luthria DL, Biswas R, Natarajan S. Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean. Food Chemistry. 2007;105:325-333.
11. Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales. 4e édition revue et augmentée. Lavoisier Tec & Doc. Paris. 2009;171-174, 414-417.
12. Tiulea C, Peev C, Feflea S, Vlase L, Dehelean C, Pop G. A screening for the most representative classes of compounds from two varieties of soy. Journal of Agroalimentary Processes and Technologies. 2011;17(1):15-19.
13. Liu Y, Peterson DA, Kimura H, Schubert D. Mechanism of cellular 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) reduction.J Neurochem. 1997;69:581-593.
14. Berridge MV, Tan AS. Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. Arch Biochem Biophys. 1993;303:474-482.
15. Vistica DT, Skehan P, Scudiero D, Monks A, Pittman A, Boyd M. Tetrazolium-based Assays for Cellular Viability: A Critical Examination of Selected Parameters Affecting Formazan Production. Cancer Res. 1991;51:2515-2520.
16. Banerjee S, Li Y, Wang Z, Sarkar FH. Multi-targeted therapy of cancer by genistein. Cancer Lett. 2008;269(2):226-234.
17. Pavese JM, Farmer RL , Bergan CR. Inhibition of cancer cell invasion and metastasis by genistein. Cancer Metastasis Rev. 2010, 29(3):465-482.
18. Lee JS, Son KH, Sung MK et al. Anticarcinogenic properties of a daidzein-rich fraction isolated from soybean. J.Med.Food. 2003;6(3):175-181.
19. Hsu A, Bray TM, Helferich WG, Doerge DR, Ho E. Differential effects of whole soy extract and soy isoflavones on apoptosis in prostate cancer cells. Exp Biol Med, 2010, 235(1):90-97.