

Considerations Regarding the Anti-inflammatory Action of Soy Total Extract Vs. Soy Isoflavonoid Daidzein on Animal Model

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Introduction: The aim of this study is to investigate the anti-inflammatory action of daidzein on animal model a less studied isoflavone than genistein on this topic and to compare it's anti-inflammatory activity with the one of soy total extract.

Material and method: Soybean seeds were grounded and a solvent formed of DMSO-ethanol-water in rapport 5-70-25 v/v/v 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 50 °C. Daidzein was acquired from Extrasynthese (France). Animal studies were conducted on C57BL/6J female mice of 8 weeks. Edema was induced in both ears of each mouse by the topical application of 2 µg TPA dissolved in 20 µL of acetone to both the inner and outer ear surfaces. Thirty minutes after the application of TPA, the inner and outer surface of each ear was treated with 2 mg of soy total extract (group C) and 2 mg of daidzein (group D). Mice were killed after 24 h by cervical dislocation and ears were collected. Ears were weighed and histological analyze were made. For the histological analyze, tissue samples (skin) were fixed in 10% formalin solution and were embedded in paraffin and cut at 4 microns. Finally after deparaffinized the samples were stained with H&E (hematoxylin-eosin) and microscopically analyzed.

Results: The obtained results suggest that both of the samples used for the treatment are active agents against inflammation but with different empowering. Soy total extract is in charge of reducing the massive under epithelial inflammation with numerous granulocytes to a discrete under epithelial inflammatory infiltrate. Daidzein successfully reduced the massive under epithelial inflammation with numerous granulocytes to a discrete under epithelial edema.

Conclusion: Both daidzein and soy total extract present an anti-inflammatory action on animal model but daidzein is a better therapeutic choice since it has a stronger anti-inflammatory action.

Keywords: soy total extract, daidzein, inflammation, mouse

Introduction

Natural products have a long history of use in the service of mankind for the prophylaxis and treatment of several diseases. A remarkable amount of research regarding the health effects of soy consumption was carried out during the past 20 years, and the conclusions were that in most of the cases positive effects can be attributed to the presence of isoflavones, a class of organic compounds produced almost exclusively by the members of the family Fabaceae/Leguminosae (bean) [1].

Daidzein is the aglycone of daidzin, the second most plentiful isoflavone in soy-Glycine max Fam. Fabaceae after genistein. Soy isoflavones are known to exhibit certain biological activities including inhibition of protein tyrosine kinase, topoisomerase II, phytoestrogenic activity, inhibition of phosphatidylinositol turnover. According to these mechanisms they possess many biological functions such as prevention of coronary heart disease, as well as osteoporosis, antioxidant, anti-cancerous and anti-inflammatory activity [2–6]. But, soybeans and soy-based foods have hundreds of phytochemical components. In recent years, accumulating evidence has suggested that the isoflavones or soy proteins stripped of phytochemicals only reflect cer-

tain aspects of health effects associated with soy consumption. The benefices of soy may be attributed also to other phytochemicals, either alone or in combination with isoflavones or soy protein [7].

The aim of this study is to investigate the anti-inflammatory action of daidzein on animal model a less studied isoflavone than genistein on this topic and to compare it's anti-inflammatory activity with the one of soy total extract.

Material and method

Daidzein was acquired from Extrasynthese (France), 12-O-tetradecanoylphorbol-13-acetate (TPA) from Sigma Aldrich, Germany. Soy seeds were kindly provided from University of Agricultural Sciences and Veterinary Medicine, Timisoara, Romania, Department of Plant Culture. In a previous study seeds were considered for quantitative analysis of total lipids (Soxhlet), proteins (Kjeldahl), polyphenols (Folin Ciocâlțeu) and isoflavones – daidzin, genistin, daidzein and genistein (HPLC). Analysis showed 21.4% total lipids/100g sample, 39.40% proteins, 303 mg total polyphenols/100 g sample, heterosides: daidzin 819.0 mg/g, genistin 905.6 mg/g and aglicons: daidzein 91 mg/g, and genistein 119 mg/g. Values fit literature data [8].

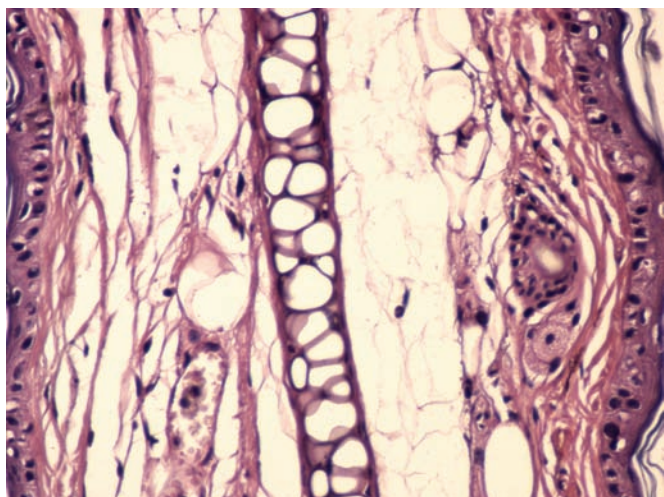


Fig. 1. Epithelial tissue and cartilage with discrete under epithelial edema Group A (HE x 400)

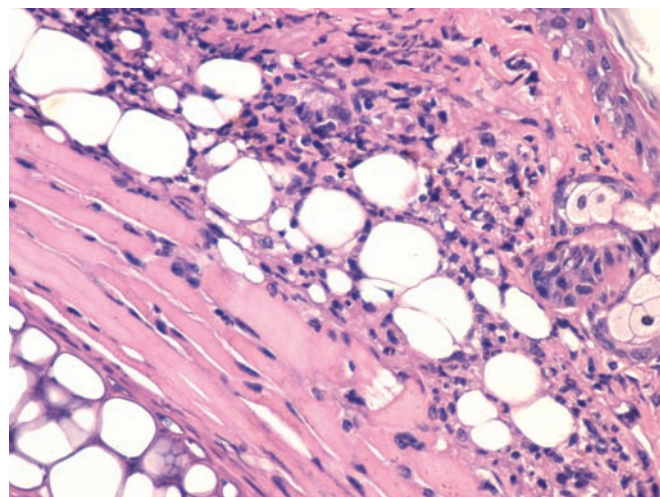


Fig. 2. Massive under epithelial inflammation with numerous granulocytes Group B (HE x 400)

Soybean seeds were grounded and a solvent formed of DM-SO-ethanol-water in rapport 5-70-25 v/v/v was prepared [9]. 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.

Animal studies were conducted on C57BL/6J female mice of 8 weeks. Mice were purchased from Charles River (Germany). The work protocol followed all NIAH-National Institute of Animal Health rules: animals were maintained during the experiment in standard conditions: 12 h light-dark cycle, food and water ad libitum, temperature 24 °C, humidity above 55%. The number of mice taken into study was twenty and they were divided in four groups of five mice per group as it follows:

- ▶ group A: blank group
- ▶ group B: mice on which it was administrated on the ear TPA in acetone
- ▶ group C: mice on which it was administrated on the ear TPA in acetone and after 30 minutes soy total extract
- ▶ group D: mice on which it was administrated on the ear TPA in acetone and after 30 minutes daidzein.

Edema was induced in both ears of each mouse by the topical application of 2 µg TPA dissolved in 20 µL of acetone to both the inner and outer ear surfaces. Thirty minutes after the application of TPA, the inner and outer surface of each ear was treated with 2 mg of soy total extract (group C) and 2 mg of daidzein (group D). Mice were killed after 24 h by cervical dislocation and ears were collected. Ears were weighed and histological analyze were made. For the histological analyze, tissue samples (skin) were fixed in 10% formalin solution and were embedded in paraffin and cut at 4 microns. Finally after deparaffinized the samples were stained with H&E (hematoxylin-eosin) and microscopically analyzed.

Results

The average weight values after years were the following: 0.147 g for group A, 0.183 g for group B, 0.164 g for group C and 0.155 g for group D.

Morphopathological analysis of collected years is presented in Figures 1–4. Figure 1 presents results from the Blank group: epithelial tissue and cartilage with discrete

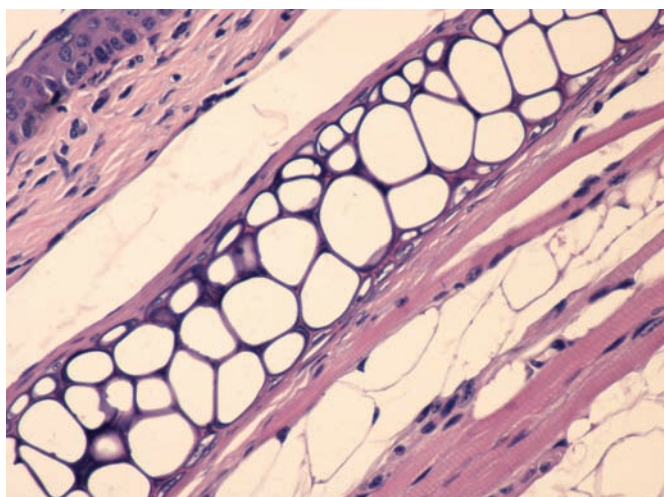


Fig. 4. Group A after treatment (HE x 400)

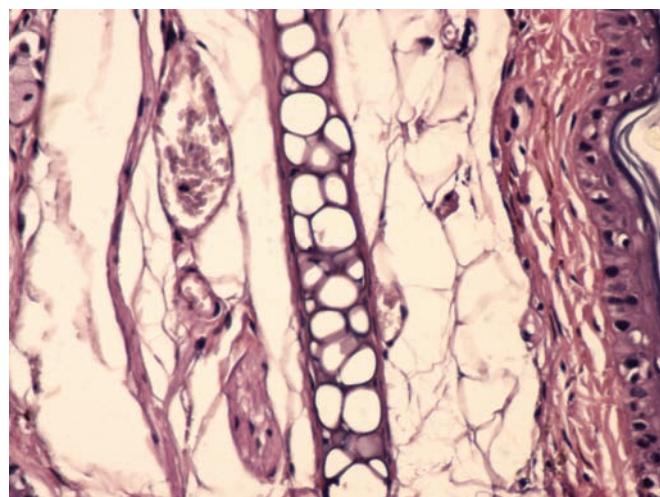


Fig. 5. Group B after treatment (HE x 400)

under epithelial edema. The under epithelial edema is present due to the physical injury caused when samples were collected. Figure 2 presents results from group B: mice on which it was administered on the ear TPA in acetone and one can observe massive under epithelial inflammation with numerous granulocytes; acute inflammation is present. Figures 3 and 4 present results obtained after treatment was applied. One can observe the epithelial tissue, cartilage and muscle with discrete under epithelial inflammatory infiltrate in case of Group C on which it was administered on the ear TPA in acetone and after 30 minutes soy total extract and epithelial and cartilaginous tissue with discrete under epithelial edema and rare vessels with hyperemia in case of Group D on which it was administered on the ear TPA in acetone and after 30 minutes daidzein,

Discussions

One can observe the difference between the average weight of years in group A – blank group and group B-mice on which it was administered on the ear TPA in acetone. These results suggest that inflammation occurred due to the increase values obtained for years in group B, because of the presence of edema. Treated mice, group C and group D, showed lower values, so from the macroscopically analyze we can conclude that the tested samples, soy total extract and daidzein present anti inflammatory action, with a stronger action in case of daidzein compared to the one of soy total extract.

Regarding morpho- pathological analyze one can observe different stages of acute inflammation depending on the group of mice. Acute inflammation is the earlier, almost immediately reaction of tissue to harmful stimuli such as pathogens, damage cells and irritants and it is the first phase of wound healing. Acute inflammation occurs before the immune system becomes established and aims primarily of removing the injury causing agent and eliminating the tissue damage. Acute inflammation occurs in two overlapping stages: vascular and cellular [10–12]. Obtain results in group B show that that topical application of 2 µg TPA dissolved in 20 µL of acetone to both the inner and outer ear surfaces is a good, reproducible model for initiating acute inflammation in animal model.

Results obtained suggest that both of the samples used for the treatment are active agents against inflammation but with different empowering. One can observe the epithelial tissue, cartilage and muscle with discrete under epithelial inflammatory infiltrate in group C where the treatment was soy total extract. Soy total extract is in charge of reducing the massive under epithelial inflammation with numerous granulocytes to a discrete under epithelial inflammatory infiltrate. Similar results were obtained by Huang et al who studied the effect of soy isoflavone extract (aglycone and acetylglucoside form) from soybean cake for the protective effects on UVB-induced damage, including inflammation and concluded that isoflavones from soybean cake are a desirable anti-photoaging agent for skin care [13]. A better result against inflammation was observed in case of soy

isoflavonoid daidzein; mice in group D showed epithelial and cartilaginous tissue with discrete under epithelial edema. Edema is translated by an increased permeability of blood vessels resulting in an exudation of plasma proteins and fluid into the tissue, so in case of mice treated with daidzein it is present only a phase of acute inflammation: the discrete under epithelial edema and rare vessels with hyperemia. Daidzein successfully reduced the massive under epithelial inflammation with numerous granulocytes to a discrete under epithelial edema. Lee et al also underlined the anti-inflammatory action of daidzein on animal model. Genistein, another isoflavonoid from soybean has been studied for the anti inflammatory action [14,15]. This study has chosen daidzein because the literature does not present as many data as in case of genistein. Obtained results suggest that from the variety of phyto compounds from soy total extract, isoflavonoids are responsible for the anti inflammatory action.

Conclusions

Both daidzein and soy total extract present an anti-inflammatory action on animal model but daidzein is a better therapeutic choice since it has a stronger anti-inflammatory action.

References

- Messina M. – A Brief Historical Overview of the Past Two Decades of Soy and Isoflavone Research. *J. Nutr.* 2010, 140(7), 1350S-1354S
- Lee JS, Son KH, Sung MK et al. – Anticarcinogenic properties of a daidzein-rich fraction isolated from soybean. *J. Med. Food* 2003 Fall; 6(3):175-81
- Perabo FGE, Von Löw EC, Ellinger J et al. - Soy isoflavone genistein in prevention and treatment of prostate cancer. *Prostate Cancer Prostatic Disc.* 2008; 11(1):6-12
- Singh-Gupta V, Zhang H, Yunker CK, et al – Daidzein effect on hormone refractory prostate cancer in vitro and in vivo compared to genistein and soy extract: potentiation of radiotherapy. *Pharm. Res* 2010; 27(6):1115-27.
- Dalais F.S., Ebeling P.R., Kotsopoulos D., McGrath B.P., Teede H.J. – The effects of soy protein containing isoflavones on lipids and indices of bone resorption in postmenopausal women. *Clin. Endocrinol. (Oxf.)* 2003, 58, 704-70
- Wei H., Saladi R., Lu Y., Wang Y, Palep S.R., Moore J., Phelps R., Shyong E., Leibold M.G. – Isoflavone Genistein: Photoprotection and Clinical Implications in Dermatology. *J. Nutr.*, 2003, 133:3811S-3819S
- Kang J., Badger T.M., Ronis M.J. and Wu X. – Non-isoflavone Phytochemicals in Soy and Their Health Effects. *J. Agric. Food Chem.*, 2010, 58 (14), 8119–8133
- Bruneton J – Pharmacognosie. *Phytochimie. Plantes medicinales, in Composés du métabolisme primaire.* Paris, France, 1999, 123-183
- 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
- Calixto J.B., Campos M.M., Otuki M.F., Santos A.R. – Anti-inflammatory compounds of plant origin. Part II. modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Med.*, 2004, 70(2):93-103
- Vane J.R., Botting R.M. – New insight into the mode of action of antiinflammatory drugs. *Inflamm. Res.* 1995, 44, 1-10
- Serhan, C.N. – Controlling the resolution of acute inflammation: a new genus of dual anti-inflammatory and proresolving mediators. *J Periodontol*, 2008, 79(8): 1520-1526
- Huang C.C, Hsu B.Y., Wu N.L., Tsui W.H., Lin T.J., Su C.C., Hung F.C. – Anti-Photoaging Effects of Soy Isoflavone Extract (Aglycone and Acetylglucoside Form) from Soybean Cake. *Int. J. Mol. Sci.* 2010, 12, 4782-4795
- Lee S.J., Baek H.J., Lee C.H., Kim H.P. – Antiinflammatory activity of isoflavonoids from *Pueraria radix* and biochanin A derivative. *Archives of Pharmacal Research*, 1994, 17(1):31-35
- Bandara M., Arun S.J., Allanson M., Widyarini S., Chai Z., Reeve V.E. – Topical isoflavonoids reduce experimental cutaneous inflammation in mice. *Immunology and Cell Biology*, 2010, 88, 727–733