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Research Article

IN VITRO CYTOTOXIC ACTIVITY OF THREE EGYPTIAN SOYBEAN CULTIVARS

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ABSTRACT

Functional foods play a key role in traditional indigenous medical system that have continued to cure many diseases in developing countries. In order to find new potent source of bioactive principles, the present study was designed to evaluate the cytotoxic effect of the defatted extract from soybean flour of three Egyptian soybean cultivars (Giza 22, Giza 35 and Giza 111). Subsequently, MTT (Methylthiazolyl diphenyl-tetrazolium bromide) assay was used to determine IC_{50} (half-maximal inhibitory concentration compared with control) of the different extracts against six cancer cell lines, namely HCT-116, EAC, HepG2, CCRF-CEM, PC-3 and MCF-7. The extracts of the three cultivars demonstrated IC_{50} at values less than 100 µg/mL against all the tested cell lines except CCRF-CEM. Among the studied cell lines, IC_{50} values below 20 µg/mL were recorded for all extracts against EAC cells. Giza 35 and Giza 111 extracts showed good cytotoxicity against HCT-116 and MCF-7 cells, respectively. Finally, these results provided corroborative information for the possible use of soybean flour as a natural source in the control of cancer disease.

Keywords: cytotoxicity, cancer cell lines, soybean flour, MTT assay, functional foods

INTRODUCTION

Since ancient times, people have derived immense pharmacological benefits from natural remedies. Some plants through the multiplicities of their chemical constituents have traditionally been used in folk medicine to treat various diseases[1]. When foods are consumed at adequate levels to provide health benefits as a part of a varied diet on a regular basis they can be considered as functional foods. Functional foods and molecular nutrition represent one of the most broadly endorsed and intensively inspected areas in the nutrition and food sciences today. However, scientific evidence for the medicinal properties of such foods should be demonstrated as not all food products today that are alleged to be functional foods are supported by sufficient strong data to justify such claims[2].

Cancer chemotherapy, a rapidly growing field of oncology, can be achieved by administration of various chemical drugs. Accordingly, new natural therapeutic strategies to treat cancer with less toxic effects on the host organs have become desirable[3]. Furthermore, certain plant secondary metabolites (e.g. flavonoids), especially those ingested in the human diet when used as adjunctive therapy, play a beneficial role in the prevention of certain types of cancer, and exhibit anticancer properties[4].

Soybean was recognized as an outstanding source of food all over the world, due to its high oil content and the high quality of its proteins. Soy flour applications include uses such as a source material for soymilk, cereal applications and ground meats[5]. Soybean is an annual crop rich in diverse functional constituents, consisting of some proteins, phytosterols, fibers, saponins as well as isoflavones like daidzein and genistein[6]. Recent expert reviews suggested a protective effect of isoflavones against breast, endometrial and prostate cancers. However, their efficacy remains insufficient to be fully proven, so investigations on benefits and risks are required[7, 8].

In this study, we have explored three types of defatted soy flour, i.e. Giza 22, Giza 35 and Giza 111 for their cytotoxic activity against HCT-116, EAC, HepG2, CCRF-CEM, PC-3 and MCF-7 cell lines.

MATERIALS AND METHODS

Soybean Material and Extraction

Flour of three soybean cultivars, Giza 22, Giza 35 and Giza 111, were obtained from Agricultural Research Center, Giza, Egypt. About 50 g flour of each soy cultivar was defatted using *n*-hexane, filtered and then the residue was extracted with 500 mL of 80% methanol using Soxhlet apparatus. The resulting extracts were concentrated under reduced pressure and stored at 5 °C until required. Different concentrations of the extracts (from 0.01 to 100 µg/mL) were prepared in 0.1% DMSO for determining cytotoxicity.

Chemicals

Doxorubicin (Sigma-Aldrich, Munich, Germany) was used as a positive control and all other chemicals used were of analytical grade.

Cell Lines and Culture Condition

EAC murine mammary adenocarcinoma cells were purchased from the Pharmacology and Experimental Oncology unit of the National Cancer Institute, Cairo University, Egypt. HCT 116 human colon carcinoma (ATCC No CCL-247), HepG2 human

hepatocellular carcinoma (ATCC No 59194), CCRF-CEM human T-acute lymphocytic leukemia (ATCC No CCL-119), PC-3 human prostate adenocarcinoma (ATCC No CRL-1435) and MCF-7 human breast adenocarcinoma (ATCC No HTB-22) cell lines were obtained from the ATCC Organization, Manassas, VA, USA.

All cell lines were maintained in a humidified incubator at 37 °C and 5% CO_2 in complete RPMI-1640 (Life Technologies, Rockville, MD, USA) with 10% FBS, 100 U/mL penicillin/streptomycin and 2 mM/L glutamine. To study the cytotoxic activity of soy flour extracts, cells were seeded at 1 × 10^5 cells/well in 200 µL of medium/well in 96 well microtiter plates. The cells were incubated overnight for attachment.

MTT Assay

The MTT assay of different cancer cell lines was performed according to Hayon, et. al.[9]. Cells were treated with different extracts and incubated for 48 hours in 5% CO_2 at 37 °C. Negative control wells containing vehicle DMSO (0.1%) were used, while Doxorubicin (added to reach final concentrations of 0.01 - 40 µg/mL) was used as a standard drug. At the end of incubation, 0.5 mg/mL of MTT (yellow colored) was added to each well. Four hours later, the formazan product (purple colored) of MTT reduction was dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader, with three replicates each. Percentage of inhibition was calculated by using the following formula,

$$\text{Inhibition \%} = 100\ \text{X}\left[1 - \left(\frac{\text{sample absorbance}}{\text{control absorbance}}\right)\right]$$

Statistical Analysis

The data were expressed as mean±standard deviation (SD) for at least three independent determinations in three replicate for each experimental point. The percentages of cell growth inhibition were used to construct the full dose response curves and to determine the IC_{50} values. The data was processed using Probit Analysis of SPSS package. The one way analysis of variance at 95% confidence level ($p < 0.05$) was considered as statistically significant.

Table 1: IC_{50} of Soybean cultivar extracts against cancer cell lines as determined by MTT assay

Studied samples	HCT-116	EAC	HepG2	CCRF-CEM	PC-3	MCF-7
Giza 22	50.7	14.1	62.8	*	49.6	24.5
Giza 35	15.0	17.9	76.1	*	27.3	45.9
Giza 111	58.6	18.4	41.3	*	66.5	16.4
Doxorubicin	12.3	5.2	11.5	8.7	2.1	3.9

(*): > 100 µg/mL

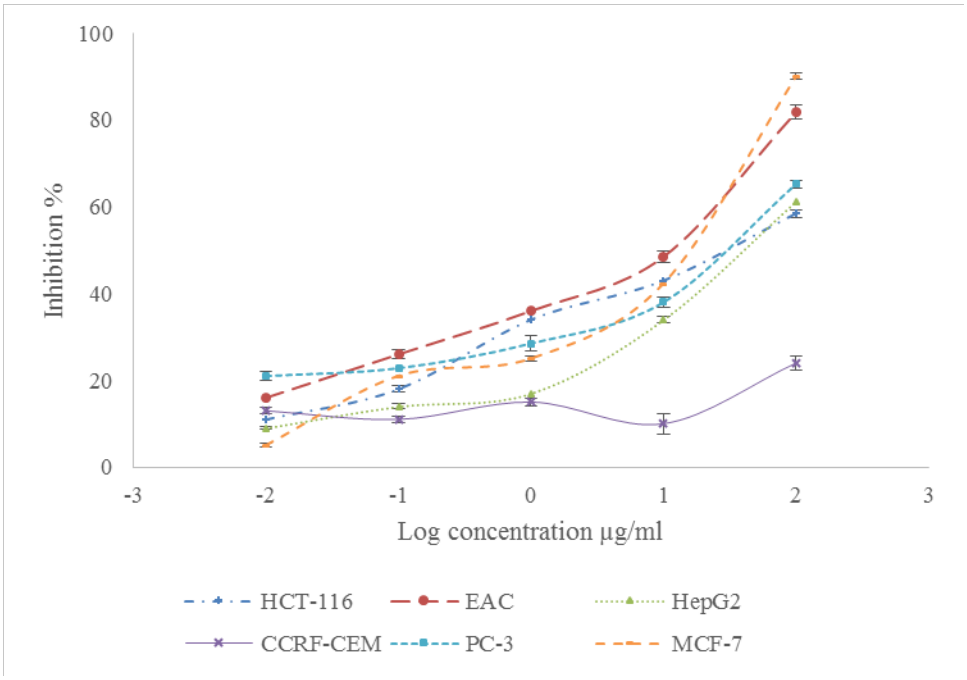

Fig. 1: Percentage of cell growth inhibition of various concentrations of Giza 22 extract against six cell lines

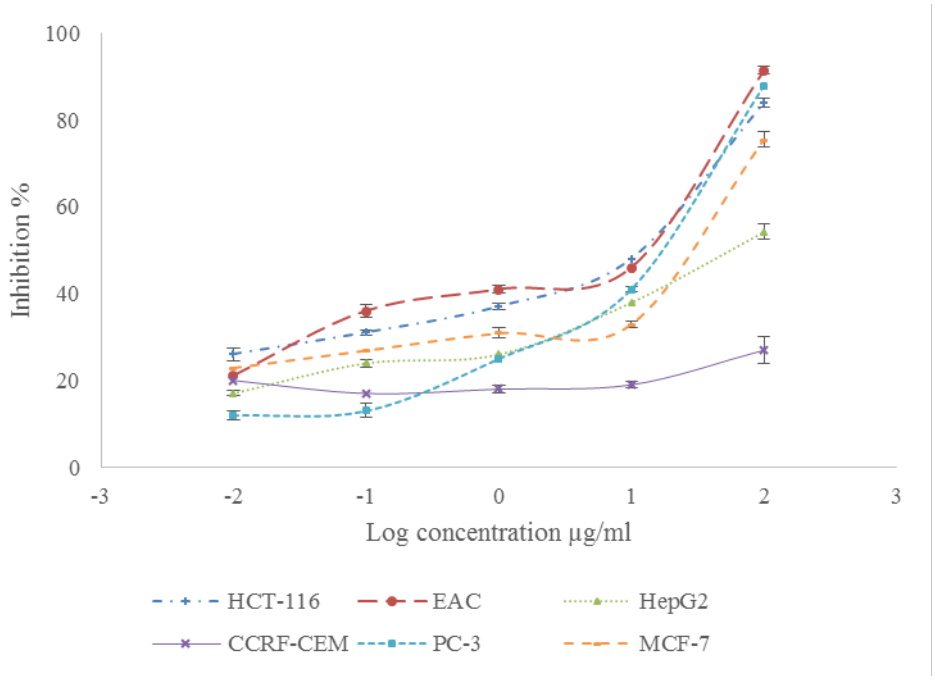

Fig.2: Percentage of cell growth inhibition of various concentrations of Giza 35 extract against six cell lines

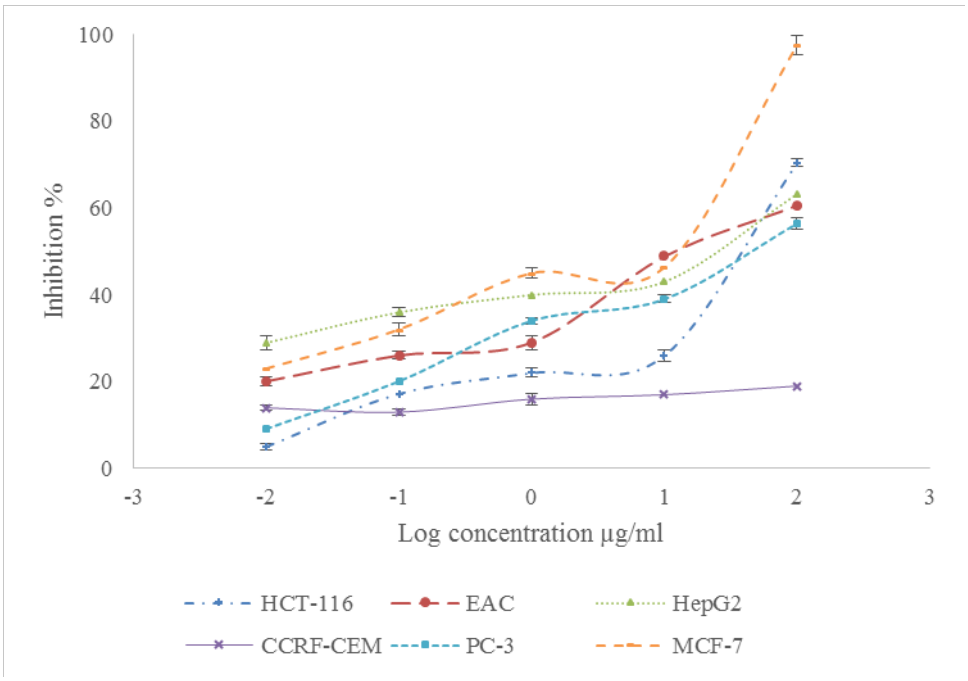

Fig.3: Percentage of cell growth inhibition of various concentrations of Giza 111 extract against six cell lines

RESULTS AND DISCUSSION

In the present study, *in vitro* cytotoxicity assay of flour extracts of three soybean cultivars, Giza 22, Giza 35 and Giza 111, was carried out at different concentrations level on a variety of cancer cell lines (Fig.1, 2 and 3). IC_{50} values were determined and the results were recorded in Table 1. It was found that the growth inhibition rate of all cells, except CCRF-CEM leukemia cells, was enhanced with increasing concentrations of soy flour extracts. At concentration of 100 µg/mL, Giza 111 extract showed a highly potent activity against MCF-7 cells followed by Giza 35 extract on EAC cells. The IC_{50} values were in a range from 14.1 µg/mL (EAC cells) to 62.8 µg/mL (HepG2 cells) for Giza 22, from 15.0 µg/mL (HCT-116 cells) to 76.1 µg/mL (HepG2 cells) for Giza 35 and from 16.4 µg/mL (MCF-7 cells) to 66.5 µg/mL (PC-3 cells) for Giza 111. Remarkably, none of the tested extracts could inhibit the growth of CCRF-CEM cells at a concentration of 100 µg/mL by more than 50%. For the control drug doxorubicin, the IC_{50} values were in a range from 2.1 µg/mL (PC-3 cells) to 12.3 µg/mL (HCT-116 cells). Moderate cytotoxicity was detected by Giza 111 and Giza 35 extracts on EAC cell lines. It is also worthy to mention that Giza 22 had the least significant activity against MCF-7 cell lines. On the other hand, Giza 111 showed its most pronounced activity against MCF-7 cells, while Giza 22 and Giza 35 had strong activity against EAC and HCT-116 cell lines, respectively.

The results obtained in the current study are coming in agreement with those previously recorded. Kim, et. al.[10] determined IC_{50} values of 100–250 µg/mL for individual fractions of wild soybean extract against MCF-7 cells. MTT assay of wild soybean extract on HCT-116 cells exhibited IC_{50} value of 97.56 µg/mL[11]. In an *in vitro* study, Wang, et. al.[12] obtained IC_{50} values of 10–160 µg/mL from soybean cake fractions against PC-3 cells. Another study on anticancer effects of soybean cake fractions against HepG2 cells showed IC_{50} values of 9–197 µg/mL[13].

Soybean contains several components with anticancer activity, such as isoflavones, protease inhibitors, saponins and phytates[14].

CONCLUSION

The study provides an evidence for a variable *in vitro* cytotoxic activity of the three studied soybean cultivars against all the tested cell lines except for CCRF-CEM, suggesting their potential application as an integral part of functional foods. Further research is necessary on the active constituents for proper assessment of their antiproliferative properties.

REFERENCES

1. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 2012; 75: 311-35.
2. Ozen AE, Pons A, Tur JA. Worldwide consumption of functional foods: a systematic review. Nutr Rev 2012; 70: 472-81.
3. Xiao Z, Morris-Natschke SL, Lee KH. Strategies for the Optimization of Natural Leads to Anticancer Drugs or Drug Candidates. Med Res Rev 2016; 36: 32-91.
4. Aghajanpour M, Nazer MR, Obeidavi Z, Akbari M, Ezati P, Kor NM. Functional foods and their role in cancer prevention and health promotion: a comprehensive review. American Journal of Cancer Research 2017; 7: 740-69.
5. Singh P, Kumar R, Sabapathy SN, Bawa AS. Functional and Edible Uses of Soy Protein Products. Comprehensive Reviews in Food Science and Food Safety 2008; 7: 14-28.
6. Ahmad A, Hayat I, Arif S, Masud T, Khalid N, Ahmed A. Mechanisms Involved in the Therapeutic Effects of Soybean (*Glycine Max*). International Journal of Food Properties 2014; 17: 1332-54.
7. Andres S, Abraham K, Appel KE, Lampen A. Risks and benefits of dietary isoflavones for cancer. Crit Rev Toxicol 2011; 41: 463-506.
8. Messina M, Messina VL. Exploring the Soyfood Controversy. Nutrition Today 2013; 48: 68-75.
9. Hayon T, Dvilansky A, Shpilberg O, Nathan I. Appraisal of the MTT-based assay as a useful tool for predicting drug chemosensitivity in leukemia. Leuk Lymphoma 2003; 44: 1957-62.
10. Kim Y, Kim D, Woo S, Kim H, Lee Y, Kim H, et al. Antioxidant activity and cytotoxicity on human cancer cells of anthocyanin extracted from black soybean. Korean Journal of Crop Science 2008; 53: 407-12.
11. Hidayat DA, Dwira S. Phytochemical analysis and *in vitro* cytotoxicity test of black soybean (*Glycine soja* L.) ethanolic extract as a growth inhibitor of the HCT-116 colon carcinoma cell line. Journal of Physics: Conference Series 2018; 1073: 032041.
12. Wang BF, Wang JS, Lu JF, Kao TH, Chen BH. Antiproliferation effect and mechanism of prostate cancer cell lines as affected by isoflavones from soybean cake. J Agric Food Chem 2009; 57: 2221-32.
13. Kao T-H, Huang R-F, Chen B-H. Antiproliferation of Hepatoma Cell and Progression of Cell Cycle as Affected by Isoflavone Extracts from Soybean Cake. International Journal of Molecular Sciences 2007; 8: 1095-110.
14. Isanga J, Zhang G-N. Soybean Bioactive Components and their Implications to Health—A Review. Food Reviews International 2008; 24: 252-76.

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