WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 6, 1499-1508. Research Article ISSN 2277– 7105

TUMOR GROWTH INHIBITORY EFFECTS OF B16F10 TUMOR MODEL BY SOY FLOUR AND ITS ISOFLAVONES

Pratima Sharma, P. Uma Devi, Indu Thakur* and Anil Prakash

Jawaharlal Nehru Cancer Hospital and Research Centre Idgah Hills Bhopal.

Article Received on
19 April 2017,

Revised on 10 May 2017,
Accepted on 30 May 2017

DOI:10.20959/wjpr20176-8678

***Corresponding Author**
Dr. Indu Thakur
Jawaharlal Nehru Cancer Hospital and Research Centre Idgah hills Bhopal.

ABSTRACT

Soy beans the very few that provide a complete protein sources. The present study was aimed to explore the tumor inhibiting activity of Soy flour and Isoflavones against B16F10 melanoma induced in C57BLmice.First group was treated orally with full fat soy flour and Isoflavones simultaneously with intradermal inoculation of tumor (Tumor growth inhibitory effect). 10 mice were taken in each group for the following study: Control and experimental i.e. Tumor growth inhibiting effect and Growth Delay with silent period has been analyzed. Both Soyflour and its Isoflavones have been proved to have antitumor activity against B16F10 melanoma tumor model.

KEYWORDS: Soy flour, Isoflavones, C57BL mice, B16F10 melanoma, Anti-tumor.

INTRODUCTION

Soybeans are the rich source of protein and used widely, for many foods products, soya has transcontinental its Asian origins and the richest and possibly the only known dietary source of isoflavones, epidemiologist have indicated generally those population have been used regularly soy foods have lower incidences of breast, colon and prostate cancers.[1] Their Isoflavones are genistein and daidzein, mostly found in soy in amounts of ~1–3 mg/g.[2] are may responsible for the anti-cancer activity. Biochemical components in soybean that is responsible for the cancer risk-lowering effects.[3] Some studies have been conducted on cancer tumor while in cancer patients tumor growth depends on the proliferating cell pool (growth fraction) in the tumor mass, a concept introduced by Mendelsohn (1960).[4] According to this concept, the rate of growth and the doubling time of small tumors are largely, but not entirely, dependent upon the percentage of cells in the mitotic cycle. Solid tumors have a high growth fraction initially, but as the tumor size increases the growth

fraction decreases. This is reflected in the progressive reduction in growth rate as the tumor grows in size. Tumor growth is generally expressed in terms of volume doubling time (VDT) and response to cytotoxic treatments is assessed from changes in VDT and growth delay (GD). This study has been shown that soy flour and its isoflavones have capabilities to enhance the VDT and significantly delay the growth of tumors.[5]

MATERIAL AND METHODS

Preparation of soy flour and Isoflavones

One g soy flour was solubilized in 22.5ml sterile distilled water and 0.8ml was used daily at particular time period orally. This is equivalent to 44.988mg for an adult of 60 kg. Pure Isoflavones 0.88 mg crystals were dissolved in 20 ml sterile distilled water and 0.4 ml daily fed to mice up to one month at the same time. This is also equivalent to 44.988mg that is a recommended dose for an adult. Doses standardized in our laboratory by Dr. P. Uma Devi.

Experiment design

The animals were treated according to the requirements of bioethics and according to the procedures of the CPCESA guidelines. In this experimental design, agouti mouse treated with Soy flour diet and isoflavones has been tested to examine their comparative efficacy. A mouse has been divided into three groups of ten each. First, Control group has been treated with double distilled water from the day of tumor injection. Second, oral doses of Soy flour diet has been given for one month from the day of tumor inoculation in agouti mice. Third group, Isoflavones orally has been given daily for one month at the time of tumor inoculation.

Animal Model

CPCSEA registration number CPCSEA/a/500/2001and IAEC latter Reference number 186/Research/01, dated 21/07/05.The agouti strain (C57BL strain X Swiss albino) were selected from a random breed colony and maintained in the animal house Department of Research, Jawaharlal Nehru Cancer Hospital, and Research Centre, Bhopal, Madhya Pradesh, India. The mice were housed in polypropylene cages containing sterile paddy husk as bedding material and maintained under controlled conditions of temperature (23 ± 2 0C), humidity (50 ± 5 %) and light (12h: 12 h of light dark respectively). The animals were fed standard mice feed and filtered acidified water ad labitum. Mice of either sex, 6 – 8 weeks old and weighing 23 ± 2 g were selected from the above colony for the experiments.

Preparations of reagents and solutions

A chemical has been purchased from Sigma U.S.A. Eagles, Minimum Essential Media, Methanol (Laboratory Grade), Trypan blue was dissolved in 100 ml of physiological saline (0.9%NaCl) and stored at the 4OC.

Tumor Transplantation

Melanoma B16F10 tumor model originally procured from Cancer Research Institute, Mumbai, India, was used in the study. These has been propagated and maintained in adult agouti mice. Tumor-bearing mice were sacrificed by cervical dislocation and the whole animal was dipped in 70% alcohol. The tumor was dissected out and single cell suspension was prepared in phosphate buffered saline by mechanical dispersion. The cell suspension was filtered through a 45µ nylon mesh and centrifuged at 800 rpm for 5 min. The supernatant was discarded and the pellet suitably diluted. Prior to transplantation, a small portion of the tumor cell suspension was treated for microbial contamination (Department of Microbiology, JNCH&RC) and the only contamination free tumors were used for propagation and experiment.[5]

Tumor volume Measurement

Tumors were grown on the dorsal skin of healthy adult mice by intradermal inoculation of 5X 105 viable cells. Once a palpable tumor has developed (after 5-6 days), the diameter was measured in three perpendicular planes (D1, D2, D3) using a Vernier calipers. The tumor volume (V) was calculated using the formula $V = \pi/6$ D1 D2 D3.Tumors measuring 100 ±10 mm3 were used for the experiments.[6] Volume doubling time (VDT) is calculated as the time in days required for the tumor to attain double time treatment volume. VDT for each tumor was calculated from the growth curve. Growth delay (GD) is measured as the difference in time between the treated (T) and untreated (C) tumors to reach five times the treatment volume. GD = T- C.

Body weight

The animal body weight was measured on alternate day.

Animal survival

The animals were observed for 120 days or till death. The mean survival time for each group were calculated. Increase in life span and %T/C value was calculated by the Formula:

Increase Life Span (%ILS) = (Mean Survival Time of treated group – Mean Survival Time of control group) × 100/ Mean Survival Time of control group.

Statistical analysis

All the values were expressed as Mean ± SE. The data of the volume doubling time and mean survival time were statistically analyzed by Student 't' test and data of growth delay were analyzed by one-way ANOVA using microcal origin version 6.0, Graph Pad In-Stat (GPIS) statistical software, U.S.A, and Chi plot. $P < 0.05$ was considered to be significant.[8]

RESULT

Experiment 1: Tumor Inhibitory effect at Soy flour and Isoflavones on Melanoma

(a) Silent period: The silent period (i.e. time taken for palpable growth) for the control group was found to be 7.4 days. In case of soy flour and isoflavone it was found to be 10 days and 11.62 days respectively and the delay in appearance of tumor was highly significant ($p<0.01$) compared to control. The isoflavones produced significant increase in silent period (Table 1).

(b)Time taken to reach 100 mm: In control group the time taken the 100mm volume was found to be 3.71 days. The time taken in case of soy flour and isoflavone treated groups was found to be 3.98 days and 3.85 respectively. Comparison between control group and those treated with soy flour and isoflavones showed that the effect was significant at ($p<0.05$) (Table 1).

(c) Volume Doubling Time (VDT): The VDT for the control groups was 1.31days. When it was compared with the treatment groups, an extremely significant increase in VDT was observed in Soy flour treated group 1.68 day (<0.05).Comparison between soy flour and isoflavones showed that the difference was very significant ($p<0.01$) (Table 1).

(d) Growth Delay (GD): Both the treatments resulted in significant delay in tumor growth compared to control. The GD was 2.84days in Soy flour treated group, was significant ($p<0.05$). Delay in growth was found to be 2.46 days in isoflavones treated group, which was very significant ($p<0.01$).Comparison between Soy flour and isoflavones showed that the difference was significant $p<0.05$ (Table1).

(e) Body weight: The animal body weight was measured on alternate day.

Experiment 2: Survival Analysis

(a) Percent Survival: None of treatments resulted in tumor see survival of animals up to 120 days. In animals treated with Soy flour survival only up to 41 days. The mean survival and median survival time were calculated for the different treatment groups. The percentage survival was plotted with Mean Survival time.

(b) Mean survival Time (MST): The MST was 30.76 days in control group and 39.25 days in soy flour treated group, which was 8.49 days increase than an the control group. MST for isoflavones group was 41.5 days which was 10.74 days more than in control. Inter group comparison revealed that response to isoflavones in terms MST was significantly higher (<0.05) than that to Soy flour (Table 2).

(c) Median Survival time (MdST): MdST has been calculated for the soy flour and isoflavones treatments, which were significant ($p<0.05$) compared to the control soy flour (40days) and isoflavones (41.5days) treated groups at the dose of 1g/kg and 0.88mg/kg respectively. When isoflavones treated group was compared with soy flour treated group, significant ($p<0.05$) increased in the Survival has been observed. (Table 2).

(d) Percent Increase in life span (ILS%): ILS was calculated from MST for different treatment group. It was 33.33% for Soy flour and 38.33% for isoflavones treatments. (Table2).

In all treatments it has been shown that delayed the growth of tumors was observed by an increase in the silent period, Volume Doubling Time, and the Growth Delay. The VDT for the control group was 1.31days. When it was compared with the treatment groups, an extremely significant increase in VDT was observed in Soy flour treated group 1.68 days (<0.05) were significant.

Comparison between soy flour and isoflavones showed that the difference was very significant ($p<0.01$). Both the treatments resulted in significant delay in tumor growth compared to control. The GD was 2.84days in Soy flour treated group, was significant ($p<0.05$). Delay in growth was found to be 2.46 days in case of isoflavones treated group, which was very significant ($p<0.01$).

Experiment 1: Tumor growth inhibitory effects

Table 1: Effect of Soyflour and Isoflavones treatment (1gm/kg, 2mg/kg, oral daily for 1 month) on tumor take in Hybrid mice. B16F10 melanoma cells.

S. No.	Treatment groups	No. of animals	Silent Period (Mean ±SE)	Time taken to reach 100 mm^3 (Mean ±SE)	VDT (Days) (Mean ±SE)	GD (Days) (Mean ± SE)
1.	Control (DDW)	10	7.43±0.12	3.71 ± 0.20	1.31± 0.12	0
2.	Group I Soyflour 1g/kg	10	10 ± 0.15[a]	3.98± 0.24	1.68± 0.15[c]	2.48 ± 0.26[a]
3.	Group II Isoflavones 0.88 mg/kg	10	11.62± 0.15 [a]	3.45 ± 0.15[c]	1.77 ± 0.18[c]	2.46 ± 0.24 [a]

a: p <0.05, b : p <0.01, c: p <0.001 compared to control; compared to Isoflavones

Experiment 2: Tumor growth inhibitory effects

Table 2: Long- term survivals of mice bearing Melanoma tumor treatment with Soyflour and Isoflavones of Soybeans.

S. No.	Treatment group	Mean Survival Time (Days) (Mean ±SE)]	Median Survival Time (days) (Mean ±SE)	ILS (%)
1.	Control	30.76 ± 0.23	30 ± 0.23	0
2.	Group I Soyflour	39.25 ± 0.76[c]	40 ± 0.0.76 [a]	33.33
3.	Group II Isoflavones	41.5 ± 0.94 [a]	41.5 ± 0.94[c]	38.33

a – Significance between Control and soyflour (FFSF)

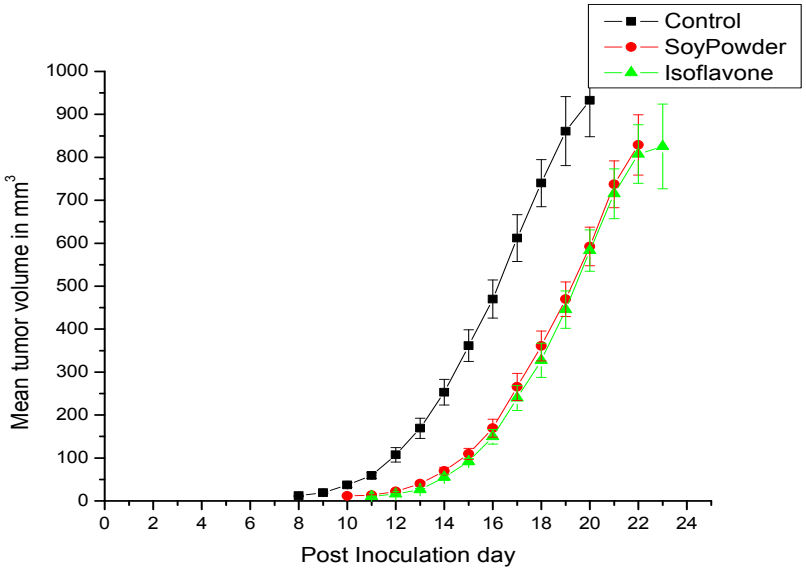
c - Significance between Control and Isoflavones

y - Significance between FFSF and Isoflavones

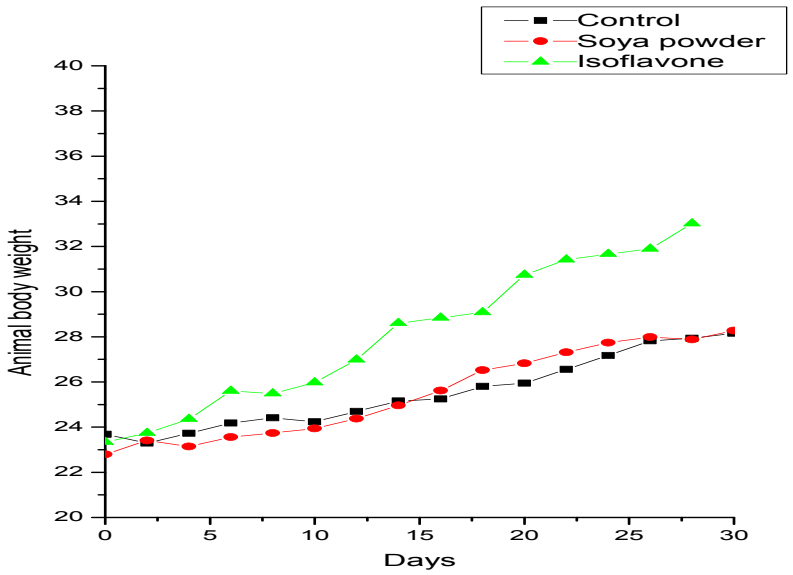
* 1g/kg daily 30 days orally,** 0.88 mg /kg daily for 30 days orally

The Data represents Mean ± SE of 10 animal

[a]P < 0.05, [C] P < 0.01 Compared to Control. [y]P < 0.01 compared to isoflavones.



Experiment 1: Fig 1: Effect of full fat Soy flour and pure Isoflavones on B16F10 mouse melanoma.



Experiment 2: Growth Curve for B16F10 melanoma after treatment 1g/kg and 0.88 mg/kg body weight with full fat soyflour and isoflavones.

DISCUSSION

The study indicates that oral administration of soy flour and isoflavones upon 1month feeding were having some tumor regression response by delaying the tumor growth. The Soy flour was having most significant effect in slowing the growth by increasing the volume doubling time and by increasing the growth delay. While the survival studies concluded that

isoflavones were the most effective in increasing the survival time during 120 days of the life span. The isoflavones have shown better mean survival time and significant percentage increase in life span. Isoflavones (Genistein and daidzein) could inhibit the CYP1A1 enzyme activity induced by 2,3,7,8 tetracholorodibenzo-p- dioxin (TCDD; dioxin).[19] CYP1A1enzyme 10 catalyzes the formation of benzo [a] pyrene (Bap) metabolites that will ultimately cause DNA mutation. Soybean and its isoflavones do not work at the transcription level against the activity of CYP1A1, as daidzein does against catalase. Instead, soy bean isoflavones compete non- competitively with the CYP1A1 substrate BaP for microsomal hydroxylation to protect against carcinogenesis caused by BaP. Genistein was also found to significantly inhibit the expression of TPA-induced proto-oncogene (c-fos), which may help prolong tumor latency and decrease tumor multiplicity[20] As there are to reports concerned so far on its antitumor property the present investigations on its antitumor effect of this plant shows that this could be effective in the treatment of cancer. The data obtained in this study are quite promising and open way for further investigation.

AKNOWLEDGEMENT

The authors Pratima Sharma are grateful to Central Institute of Agricultural Engineering (ICAR), Bhopal, Madhya Pradesh, for providing lab facility in the department of Microbiology for preparing Soy flour and Isoflavones.

REFERENCES

1. Lee YP, Gurley L, Duffy SW. Estevez J, Lee J, Day NE. Dietary effects on breast cancer risk in Singapore. Lancet, 1991; 337: 1197–1200.
2. Wang H. and P. A. Murphy. Effects of variety, crop year, and location. J. Agric. Food Chem., 1994; 42: 1674-1677.
3. Cline J.M. and Hughes. C.L. Jr. Phytochemicals for prevention of breast: and endometrial cancer. In Biological and Hormonal Therapies of cancer. Eds. K.A. Foon and H.B. Muss. Boston. MA: kluwer Academic publishers, 1998.
4. Mendelsohn, M. L. The growth friction: a new concept applied to tumors. Sciences, 1960; 132: 1496.
5. Chapman .J.D., Urtasun, R.C., Franko, A.J., Raleigh, J.A., Meeker, B. E. and McKinnon, S. A. The measurement of oxygenation status of individual tumors. In: Prediction of response in Radiation Therapy: The Physical and Biological Basis. American Institute of Physics Inc., New York, 1989; 49-60.

6. Steel, G. G. Cell Population kinetics of tumors in experimental animals. In: Growth Kinetics of Tumor. Clarenden Press, Oxford, 1977; 146-181.
7. Vaupel, P., Thews, O., Kelleher, D.K. and Hoeckel, M. Oxygenation of human tumors: the Mainz Experience. Strahlenther. Onkol, 1998; 174(IV): 6-12.
8. Rothman, K. J. Synergy and antagonism in cause-effect relationships. *Amer. J. Epidermiol.* 1974; 99: 601-607.
9. Stephens. F.O. The rising incidence of breast cancer in woman and prostate cancer in men. Dietary influences: a possible preventive role for nature's sex hormone modifiers – the phytoestrogens (review). Oncol. Reports, 1999; 6: s65–370.
10. Messina MJ, Persky V, Setchell KDR, Barnes S. Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data**.** *Nutr Cancer*, 1994; 21**:** 113-131.
11. Messina, M. Legumes and soybeans: Overview of their nutritional profiles and health effects. Am. J Clin. Nutr, 1999; 70: 439S-450S.
12. Anothony, M.S., Clarkson, T.B. and William, J.K. Effects of soy isoflavones on atherosclorosis: Potiential mechanism. *Am. J. Clinical Nutrition*, 1998; *68:* 139S–1393S.
13. Bourke G J, Daly L C & Gilvary J, *Interpretation and uses of medical statistics (Blackwell Scientific publishers, Oxford), 1985.*
14. Dietary isoflavones: biological effects and relevance to human health. *J. Nutr*, 1999; *129: 758S-767S.*
15. Griffiths, K., Denis L. Turkes. A. and Morron. M.S. Phytoestrogens and diseases of the prostate gland. *Bailliere Clin. Endocrinol. Metab*, 1998; 12: 625 –647.
16. Wang HZ, Zhang Y, Xie LP, Yu XY, Zhang RQ Effects of genistein and daidzein on the cell growth, cell cycle, and differentiation of human and murine melanoma cells. J Nutr. Biochem, 2002; 13: 421–426.
17. Kurbanov BM, Fecker LF, Geilen CC, Sterry W, Eberle J Resistance of melanoma cells to TRAIL does not result from upregulation of antiapoptotic proteins by NF-???B but is related to down regulation of initiator caspases and DR4. Oncogene, 2007; 26: 3364–3377.
18. Fernanda Maria Pinto Vilela, Deeba N. Syed, Jean Christopher Chamcheu, Laura A. Calvo-Castro, Vanessa Silveira, ortes, Maria José Vieira Fonseca, Hasan Mukhtar "Biotransformed Soybean Extract (BSE) Inhibits Melanoma Cell Growth and Viability *In Vitro*: Involvement of Nuclear Factor-Kappa B Signaling PLOS Published, July 29, 2014 http://dx.doi.org/10.1371/journal.pone.0103248.

19. Shertzer, H. G; Puga, A; Chang C; Smith, P; Nebert, D.W; Setchell, K. D; Dalton T.P. Inhibition of CYP1A1 enzyme activity in mouse hepatoma cell culture by soybean isoflavones. Chem. Biol. Interact, 1999; 123: 31-49.
20. Wei, H; Bowen, R; Cai; Barens, S; Wang, Y. Antioxidant and antipromotional effects of the soybean isoflavones genistein. Proc. Soc. Exp. Biol. Med., 1995; 208: 124.