VIVO SCIENTIA
Panacea Research Library
http://prlpublisher.com/journal/index.php/pjpps/index

Vivo Scientia
A National Journal of Science

Original Research Article **Volume 4 Issue 4**

ROLE OF THE PHYTOESTROGEN DAIDZEIN IN EXPERIMENTALLY INDUCED BREAST CANCER IN WISTAR RATS

[1] Dr. Vanitha Samuel* and [2] Dr. Parthasarathy Nirmala

[1] Professor in Pharmacology, [2] Professor & Head,
Division of Pharmacology, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, India

Article history:

Received: 24th Oct 2015

Received in revised form: Nov 2015

Accepted: Dec. 2015

Available online: 30th Dec 2015

Corresponding author:

Dr. Vanitha Samuel

Email: drvanisam@hotmail.com

***Present address*:**

Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, India

These authors have no conflict of interest to declare.

Abstract:

Background: Phytoestrogens have recently emerged as potential sources of chemo prevention especially in mammary carcinogenesis. Several controversies exist about their role in preventing cancer progression, has propelled a more profound research activity involving these flavonoids .

Aims & Objectives: This study was carried out to explore the anti - cancer potential of the isoflavone Daidzein using appropriate animal models, and to evaluate its effect on the oxidative stress induced by 7,12-dimethylbenz (a) anthracene (DMBA)

Materials & Methods: DMBA - a chemical carcinogen was administered (25mg/rat/ s.c) in 2 divided doses to induce mammary cancers in female Wistar rats. Daidzein at doses of 50mg/kg and 100mg/kg and Tamoxifen as reference standard (40mg/kg) was administered, starting from the 1st day. The growth of tumors was monitored after the treatment. The mammary tumors collected from rats were analyzed by estimation of anti-oxidant enzymes, 17β estradiol levels to determine their chemo preventive potential.

Results: Daidzein at a human equivalent dose suppressed the growth of DMBA-induced mammary tumors especially at higher doses of 100mg/kg, which were comparable to the effects of Tamoxifen ($P < 0.05$). Daidzein showed the potential of inhibiting the growth of mammary tumors in rodents and attenuating oxidative stress by modifying the effect of anti-oxidant enzymes.

Conclusion: Daidzein could be used as a core structure to design new drugs for breast cancer therapy. The outcome of results indicates that consumption of Daidzein may protect against breast cancer.

Key words: Chemoprevention, Daidzein, DMBA, isoflavone, mammary carcinogenesis, Phytoestrogens, Tamoxifen.

INTRODUCTION

Phytoestrogens are compounds of plant origin which possess estrogen like activity. They may act as weak agonists / antagonists or modulators of estrogen receptors in humans. The three major classes of Phytoestrogens include Lignans, Coumestans and Isoflavones [(1)]. The isoflavones Genistein & Daidzein are present in soybeans and soy proteins. The leguminous plant food Soya bean, is a cholesterol -free high protein source of the Isoflavone - Daidzein. Another source is Radix puerariae from the root of legume *Puerperia labata* used as Chinese folklore medicine [(2, 3)].

Mammary carcinogenesis is the second most common cause of cancer worldwide and the fifth common cause of cancer death. It has the tendency to metastasize if not treated promptly with surgery /radiotherapy/chemotherapy. Most cases remain undetected as cancer *in situ* and though genetic factors and heredity account for 10-15% of cases, lifestyle, environmental factors predispose women to this form of cancer. Women especially in the western world report 200,000million new cases and 50,000 deaths annually [(4)]. The multistage process of carcinogenesis involves generation of reactive oxygen species or free radicals which alter the redox balance, causing oxidative stress which consequently results in damage to normal cells. 7,12 ((DMBA Dimethylbenz [a] anthracene has been widely used to produce chemically induced carcinogenesis, with a cascade of events culminating in Oxidative stress [(5)]. The main target sites for the potent carcinogenicity of this chemical in rodents are the skin and the mammary gland.

Several Chemo preventive agents / Chemo sensitizers are in vogue, which modify the course of therapy. Although the mechanism of anticarcinogenic activity is still unclear, Daidzein and its glycosides, with weak estrogenic effects have proven effective in prostate and breast cancers [(6)]. *In vitro* studies have demonstrated anti-proliferative, induction of cell cycle arrest and apoptosis, modification of antioxidant enzymes, regulation host immune system and free radical scavenging properties of some compounds, but several controversies exist in preclinical *in vivo* studies in animals and in epidemiological studies in humans [(7)].

Since the result of several epidemiologic studies of this association are highly variable, experimental evidence is suggestive of estrogenic activity of Soy isoflavones which could also be potentially risk enhancing. However, the dose of the compounds the duration and route of administration and the time of exposure have posed numerous unanswered questions. Thus the present study was designed to evaluate the effect of Daidzein in DMBA induced mammary carcinogenesis in comparison with Tamoxifen [(8)], a SERM (Selective Estrogen Receptor Modulator) used as an adjuvant in most types of breast cancer .

Materials & methods

Animals: - Female Wistar albino rats (*Rattus norvegicus*) 7 - 8 weeks old weighing 150 – 180 Gms were used for the study. The animals were obtained from Central Animal House, RMIHS, Annamalai University, Chidambaram, India. The rats were housed in polypropylene cages at room temp, (27 ± 2° C) with relative humidity 55 ± 5%, in an experimental room. The LD cycle of about 12: 12 hr was maintained. The local IAEC (Reg no. 160/ 1999 /CPCSEA), Annamalai University, Annamalai nagar approved the experimental design. Animals were maintained as per the principles and guidelines of the IAEC in

accordance with the laws of animal care and use. Animals were also provided standard pellet feed and water *ad libitum*.

Chemicals: The carcinogen 7, 12 DMBA and biochemicals such as reduced glutathione were obtained from Sigma – Aldrich Chemicals, Pvt, Ltd, Bangalore, India. DMSO from Sun Pharma, India. Heparin, Thiobarbituric acid (TBA) and related chemicals were from Hi – Media Laboratories. Daidzein from Ascent Scientific Ltd UK, Tamoxifen from Mankind Pharma. All other chemicals used were of Analar grade.

Induction of Mammary Cancer: Mammary carcinogenesis was induced in female Wistar rats by *Intra peritoneal* injection of 25mg / rat of 7, 12 DMBA (dimethyl benzanthracene, in two divided doses in the 4th week & 5th week. The drug being lipid soluble was administered in 1ml emulsion of sunflower oil (0.75ml) and physiological saline (0.25ml) to each rat in groups 2 to 5. **(9)**

Experimental design:- Thirty female Wistar rats were divided into 5 groups of six rats each .

Group I – Normal controls with 2% DMSO as vehicle, *po*

Group II – DMBA (25mg/rat/S.C in 2 divided doses) at 4th & 5th week

Group III– Tamoxifen (40mg/kg, po) + DMBA

Group IV–Daidzein – (50mg/Kg/, po) + DMBA

Group V – Daidzein – (100mg/kg, p.o) + DMBA

The drugs were dissolved in 2% DMSO and administered from the 1st day till the 4th week before the exposure to the carcinogen and continued throughout the experimental period (100days) with a wash out period of a fortnight. The experiment was terminated at the 16th week and evaluated for chemo preventive effect. The rats were sacrificed by cervical dislocation after ketamine anaesthesia at the completion of study.

Biochemical Analysis: - Blood samples were collected in heparin mixed tubes. Plasma was separated by centrifugation at 4500rpm for 15min.Chemiluminiscent immunoassay (CLIA) was used to estimate serum 17-B estradiol (E2). **(10)**

Tissue samples were washed with ice cold saline, dried between folds of filter paper, weighed, homogenized using appropriate buffer in an all glass homogenizer and used for biochemical estimations.

Anti-oxidant enzymes:-

Estimation of Tissue T Bars: Lipid peroxidation in tissue (TBARS) was estimated by the method of Ohhawa *et al.* **(11)**; to 0.2 ml of Tissue homogenate, 0.2 ml of 81 % sodium dodecyl sulphate and 1.5 ml of glacial acetic acid 20 % were added. pH was adjusted to 3.5 with NaOH, then 1.5 ml of 0.8 % TBA was added to the mixture and volume made up to 4 ml. Reaction mixture was heated in oil bath at 95° C for 60 min. 1 ml distilled water added after cooling with5 ml n – butanol pyridine and shaken vigorously. After centrifugation at 4000 rpm for 10 min the supernatant was removed and absorbance was read at 620 nm.

Estimation of Tissue Anti-oxidant Enzymes: Estimation of SOD (Kakkar *et al*) **(12)**. Catalase was assayed by the method of (Sinha *et al*) **(13)** and glutathione peroxide by the method of Rotruck *et al* **(14)**.

Statistical Analysis: The values are expressed as Mean ± SD statistical comparisons were performed by analysis of Variance (ANOVA), followed by Dunett's multiple range test DMRT (using SPSS version12.0 for Window) P-Values less than 0.05 was considered to be statistically significant.

Results:

The results of the present study evaluated the effect of Daidzein, with the anti-estrogenic drug Tamoxifen as the standard positive control. Estimation of TBARS showed a marginal decrease in all drug treated groups in tissue & erythrocyte lysate (table: 1). An overall decrease in Anti-oxidant enzymes SOD, CAT & GPx was observed in the DMBA treated groups when compared to control group. In comparison levels of these antioxidants enzymes was marginally increased in the Daidzein and the Tamoxifen group. This increase in Anti-oxidant enzymes in group 5 corroborated well with other findings in this group.

17-β Levels were found to be elevated in DMBA treated group in comparison with controls suggestive of chemical alterations, resulting in tumour development. The levels in the drug treated groups were comparatively lower especially in Tamoxifen treated rats implying anti-estrogenic activity (table: 2).

TABLE: 1 Results of anti-oxidant Enzymes.

Groups	SOD	GPX	CAT	TBARS
Group-I	10.17±0.24	74.88±0.41	46.54±1.29	13.33±0.68
Group-II	1.21±0.34	14.52±0.98	9.464±0.89	53.55±0.50
Group-III	6.24±0.25	56.44± 0.76	39.34±4.84	18.172±0.73
Group-IV	3.68±0.49	45.89±0.17	22.345±1.52	27.586±0.15
Group-V	5.94 ±0.37	52.71±0.15	29.704±1.43	20.514±0.23
ANOVA "f" "P" Value	644.672 <0.05	10235.7 <0.05	1105.462 <0.05	137.175 <0.05

Values are expressed as Mean ± SD for 6 animals in each group (n=6), Mean, P<0.05 as compared to control and DMBA.

TABLE 2: Results of plasma 17-β estradiol (E2) in control & drug treated groups

S.No.	Groups	17-β Estradiol
1.	Control	45.1±2.2
2.	DMBA	55.6±3.1
3.	Daidzein 50mg/kg + DMBA	38.1±0.3
4.	Daidzein 100mg/kg + DMBA	35.4±2.6
5.	Tamoxifen + DMBA	30.3±5.6

Values are expressed as mean ±SD (n=6); values that are not sharing a common superscript letter in the same column differ significantly at $p < 0.05$ (DMRT)

Discussion : Cancer is a clonal disease of a single transformed cell with a tendency to under mutational changes to eventually express its full malignant potential. 7,12 DMBA induced mammary carcinogenesis is a suitable model to study the *in vivo* tumour genesis in rats. The oxidation of DMBA by Cyt P_{450} enzyme produces metabolites, that form covalent adducts with DNA and result in formation of depurinated sites within DNA. Excess production of reactive oxygen species which results in metabolic activation, may causes oxidative damage to the structure and function of DNA, protein and lipids, contributing to neoplastic transformation **(16, 17).** Using rat models of chemically induced tumourogenesis, the effect of the tumour incidence and increase in tumour latency in young adult females was compared with Tamoxifen as the conventional anti-tumour agent. Phytoestrogens are thought possibly to inhibit binding of the more potent endogenous estrogens and decrease their potential effects on breast cancer risk by competing for estrogen receptor. Isoflavones, which exhibit multiple biological effects and, in some systems, operate through estrogen receptors, upon which they may act as agonists, antagonists or mixed agonist-antagonists and can thus, be used for modulation of ERs functioning **(15)**. Having the capability to modulate ER activity, isoflavone Daidzein and its derivatives thereof may be useful to treat or prevent a variety of diseases and conditions related to estrogen receptor functioning in mammals, preferably humans.

ROS are generated in and around mitochondria and are regarded as the byproducts of normal cellular oxidative processes. It has been indicated that they can regulate initiation of apoptotic signaling **(18)**. These results confirmed that ROS were crucial in the induction of apoptosis and acted as upstream signaling molecules to initiate cell death. One of the prominent and medically most useful properties of flavonoids is their ability to scavenge the free radicals **(19)**.

The research demonstrated that Daidzein at dose levels of 50mg/kg did not show any significant regression in tumour growth, even though the levels of anti-oxidant enzymes were marginally elevated. At doses of 100mg/kg the percentage inhibition of tumour size and volume was significant with TBARS exhibiting an alleviation of oxidative stress induced by DMBA. Lipid peroxidation and disturbed anti-oxidant status was observed in tumour tissues compared to normal tissues. The increase in values of TBARS with a reduction in anti –oxidant enzymes was observed in the tumour tissues when compared to normal and drug treated groups. The decrease SOD and CAT levels, which are regarded as markers of malignant transformation further lend credibility to our study. Though estrogen has been implicated in mammary carcinogenesis, the exact mechanism of its action has been elusive.

Reports of several studies indicate lesser protective association of Daidzein when administered in lower doses or after the induction of carcinogenesis. Results of our study indicates that Daidzein 4weeks prior to tumour induction and continued till the end of the study period, reveal there was a mediocre response at 50mg/kg dose but a statistically significant response at doses of 100mg/kg. Numerous studies have shown that 7,12-dimethylbenz(a)anthracene (DMBA) can be used to induce experimental breast carcinomas in rats and that this process

involves disruption of tissue redox balance; in turn, this suggests that biochemical and pathophysiological disturbances may result from oxidative damage[20]. Under normal physiological conditions, any free radicals generated in sub cellular compartments would subsequently be scavenged by antioxidant defense systems of the corresponding cells [21]. However, such protective mechanisms can be broken easily by chemicals, such as DMBA, which disrupt the pro-oxidant–antioxidant balance, leading to cellular anomalies. Furthermore, owing to their high content of polyunsaturated fatty acids, cellular membranes are highly susceptible to lipid peroxidation, and adverse alterations of the cell membrane can result in a pathological outcome [22, 23].

Conclusion: To summarise, the research on Daidzein in rodents are suggestive of mild to moderate antiproliferative and anti-oxidant activity especially at higher doses and when administered prior to chemical carcinogenesis by DMBA.

References:

1. Rowland I, Faughnan M, Hoey L,, "Bioavailability of phyto-oestrogens," *Br J Nutr*, 2003; 89 Suppl 1:S 45-58.
2. Zhou, H. Y.; Wang, J. H.; Yan, F. Y. "[Separation and Determination of Puerarin, Daidzin and Daidzein in Stems and Leaves of *Pueraria thomsonii* by RP-HPLC]". 2007. 32 (10): 937-939.
3. Xu, H.-N.; He, C.-H. "Extraction of Isoflavones from Stem of *Pueraria lobata* (Willd.) Ohwi Using n-Butanol / Water Two-Phase Solvent System and Separation of Daidzein". *Separation and Purification Technology; 2007;* 56 (1): 255–262.
4. Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, CA. *Cancer J Clin* 2002; 52(1): 23–47.
5. Moselhy SS, Al Mslmani MAB. Chemopreventive effect of lycopene alone or with melatonin against the genesis of oxidative stress and mammary tumors induced by 7, 12 dimethyl(a)benzanthracene in *Sprague Dawley* female rats. Mol Cell Biochem;2008; 319:175–80.
6. Abdul Lateef, Abdul Quaiyoom Khan, Mir Tahir, Rehan Khan, Muneeb U Rehman. Androgen deprivation by flutamide modulates uPAR, MMP-9 expressions, lipid profile, and oxidative stress: amelioration by daidzein. Molecular and Cellular Biochemistry; 2013; 02: 01.
7. Constantinou AI, Lantvit D, Hawthorne M. Chemopreventive effects of soy protein and purified soy isoflavones on DMBA-induced mammary tumors in female Sprague–Dawley rats. *Nutr Cancer;* 2001; 41: 75–81.
8. Fisher B, Constantino JP, Wickerham DL, Redmond CK, Kavanah M, CroninWM, Vogel V, Robidoux A, Dimitrov N, Atkins J, *et al*.: Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst;* 1998, 90 (18):1371-1388.
9. Kadir Batcioglu, A. Burcin Uyumlu, Basri Satilmis, Battal Yildirim, NeslihanYucel Hakan Demirtas. Oxidative Stress in the *in vivo* DMBA Rat Model of Breast Cancer: Suppression by a Voltage - gated Sodium Channel Inhibitor. *Basic & Clinical Pharmacology & Toxicology*; 2012, 111, 137–141.
10. Buscarlet.L, H.Volland,J.Dupret-Carruel,M. Jolivet and J.Grassi. Use of free radical chemistry in an immunocentric assay for 17-B estradiol. *J.Clin.Chem*. 2001; 47:102-109.
11. Ohkawa.H,Ohishi.N,Yagi.K,Assay for lipid peroxides in animal tissues by

thiobarbituric acid reaction. *Anal .Biochem* ;1979;95:351-8.

12. Kakkar .D, Das. B, Viswanathan.PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophy;* 1984;21:130-2.
13. Sinha AK. Colorimetric assay of catalase. *Anal Biochem* ; 1972; 47: 389-394.
14. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG,Hoekstra WG. Selenium. Biochemical role as a component of glutathione peroxidase. Science; 1973; 179: 588-590.
15. Limer JL, Speirs V. Phyto-oestrogens and breast cancer chemoprevention. *Breast Cancer Res.* 2004; 6: 119–127.
16. Nandakumar N, Jayaprakash R, Rengarajan T, Ramesh V, Balasubramanian MP. Hesperidin, a natural citrus flavonoglycoside, normalizes lipid peroxidation and membrane bound marker enzymes in 7, 12-Dimethylbenz (a) anthracene induced experimental breast cancer rats. *Biomed Prevent Nutri* . 2011; 1: 255-262.
17. Selamoglu TZ, Yilmaz I, Ozdemir I, Ates B, Gok Y, Cetinkaya B. Role of synthesized organoselenium compounds on protection of rat erythrocytes from DMBA-induced oxidative stress. *Biol Trace Elem Res.* 2009;128:167–75.
18. Choi EJ. Antioxidative effects of hesperetin against 7, 12 dimethylbenz(a)anthracene-induced oxidative stress in mice. *Life Sci.* 2008; 82:1059–64.
19. Cavalieri E, Roth R, Rogan E. Mechanisms of tumor initiation by polycyclic aromatic hydrocarbons. *Carcinogenesis.* 1978; 3:273–87.
20. Agarwal R. (2000), "Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents" .*Biochem. Pharmaco.* (2000) ; 60: 1051-1059.
21. Ju YH, Fultz J, Allred KF, et al., "Effects of dietary daidzein and its metabolite, equol, at physiological concentrations on the growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in ovariectomized athymic mice," *Carcinogenesis*, 27(4):856-63, 2006.
22. Atkinson .C,Frankenfeld .CL, Lampe. JW :" Gut bacterial metabolism of Soy Isoflavone Daidzein;exploring the relevance to human health ", *Exp.Biol.Med*, 2005 ;230(3):155-170.
23. Mandlekar S, Kong AN: Mechanisms of tamoxifen induced apoptosis. *Apoptosis.* 2001, 6(6):469-477

No funding source.

Acknowledgement:

The presenting author wish to express sincere thanks to Dr. N. Chidambaram, Dean, RMMC and Dr. Nagini, HOD Biochemistry & Biotechnology.