Original Research Article

Preclinical evaluation of anti-cancer effect of naringenin in 7, 12-dimethyl benzanthracene induced breast cancer in female rats

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Abstract

Background: The present study is aimed to screen the anti-cancer effect of naringenin in 7,12-dimethyl benzanthracene induced breast cancer in female Wistar albino rats. **Methodology:** The study was conducted in the Division of Pharmacology, Annamalai University, Tamil Nadu. Female Wistar Albino rats weighing 140-150gm were selected in this study. All the animals were kept in Central Animal House under standard conditions. Total 36 rats were divided into six groups, each of 6 rats. G-1 (Control), G-2- (7,12-Dimethyl benzanthracene (25mg/kg/BW/sc), G-3 (7,12-Dimethyl benzanthracene(25mg/kg/sc)+Vincristine(500µg/kg/BW/ip),G-4(7,12-Dimethylbenzanthracene(25 g/kg/sc)+Naringenin (50 mg/kg/BW/PO), G-5 (7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO) and G-6 (7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (200 mg/kg/BW/PO). Inducing drug was administered only once. All the drugs were given upto 16 weeks. At the end of study period rats were sacrificed. The breast tissue was insolated and used for estimation of anti-oxidant enzymes like TBARS, Super oxide dismutase, Catalase, Glutathione and Glutathione peroxidase. **Results:** Group-2 showed significant changes compared to group-1. Group-3 and 5 showed significant difference compared to Group-2. Group-5 did not show significant difference compared to Group-3 in TBARS, super oxide disumutase, catalase, glutathione and glutathione peroxidase enzymes. **Conclusion:** Naringenin showed significant dose dependent anti-cancer effect in this study.

Key Word: Anti-cancer, breast tissue, Naringenin, 7,12-dimethyl benzanthracene, histology, Wistar Albino rats

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INTRODUCTION

Breast cancer remains the second leading cause of cancer death next to lung cancer. It is the leading cause of non preventable cancer death among women. The two most common types of breast cancer are ductal and lobular carcinoma named after their origin in breast tissue. Ductal carcinomas make 85% to 90%, and whereas, 8% are lobular breast cancers. Other types include invasive (infiltrating) and inflammatory breast cancer. The incidence of breast cancer in India is on the rise annually with roughly 53 deaths per 100,000. This rise is probably due to lifestyle changes in women and lack of awareness programmes. It is rapidly becoming the number one cancer in females (Radha Munagala *et al*; 2011). Each year more than 210,000 women are diagnosed with invasive breast cancer in the United States. In addition approximately 35,000 cases of in situ carcinoma are also found annually. Approximately 40,000 women die annually from this cancer (American cancer society. 1995). Different classes of anti-cancer drugs were currently available for the treatment of breast cancer. Natural products were most commonly used for the

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treatment of breast cancer. Treatment with these drugs leads to development of various adverse effects. There is a requirement of various new drugs with lesser adverse effects for the treatment of breast cancer. 7, 12-dimethyl benzanthracene is commonly used to induce breast cancer in experimental animals. Naringenin is a newer compound with anticancer and anti-oxidant property. The present study is aimed to screen the anti-cancer effect of naringenin in 7, 12-dimethyl benzant hracene induced breast cancer in Wistar Albino female rats.

MATERIALS AND METHODS

Study settings and period

The study was conducted in the Division of Pharmacology, Annamalai University, Tamil Nadu. The study was approved by Institutional Animal Ethics Committee (160/1999/CPCSEA).

Animals

Wistar Albino female rats weighing 140-150 gm were included in the study. Animals were housed in well ventilated room (temperature 23 $\pm$ 2^{o}C, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals". Female wistar rats, housed in polypropylene cages under hygienic conditions adapted to the laboratory conditions for a week were used for the study.

Study group

Total 36 rats were divided into six groups each of six rats.

Group-1- Normal saline
Group-2- 7,12-Dimethyl benzanthracene (25 mg/kg/BW/sc)
Group-3- 7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Vincristine (500 µg/kg/BW/ip)
Group-4-7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (50 mg/kg/BW/PO)
Group-5-7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO)
Group-6-7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (200 mg/kg/BW/PO)

Induction of tumor and drug administration

Mammary cancer was induced in female Wistar Albino rats in groups II, III, IV, V and VI through a single subcutaneous injection of 25 mg/kg of 7, 12-Dimethyl benzanthracene dissolved in an emulsion of sunflower oil and 0.9 ml of normal saline. 7, 12-Dimethyl benzanthracene was injected in the mammary region of female rats (Ganesan Dhamodharan *et al*; 2012) on day one. Mammary tumors appeared by 7th - 9th week of the experimental period, while Group IV, V and VI received the Naringenin orally in three dose levels of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively for 16 weeks. Vincristine 500 µg/kg once a week for 4 weeks was administered to the rats in group III. (Natla Sashidhar Reddy *et al*; 2012). Group I was given the basal diet and water *ad libitum* throughout the experimental study period. At the end of 16 weeks the animals were sacrificed by cervical decapitation under ketamine anesthesia. The breast tissue was excised immediately and washed with chilled isotonic saline. The tissue homogenates were prepared in ice cold 0.1 M Tris-HCl buffer, pH 7.2 separately. The homogenate was centrifuged and the supernatant was used for the assay of clinical marker enzymes in the tissue and determination of biochemical parameters TBARS, Superoxide dismutase, Catalase, Glutathione, and Glutathione peroxidase by standard procedures.

RESULTS

Compared with DMBA group (group 2) the breast tissue TBARS of naringenin treated groups, naringenin 50mg/kg body weight (group 4) and naringenin 100 mg/kg body weight (group 5) and vincristine group (group 3) shows statistically significant difference. There is no statistically significant difference between group 3 (vincristine group) and group 5 (naringenin 100 mg/kg body weight). When compared with DMBA group (group 2) breast superoxide dismutase levels of all naringenin treated groups, naringenin 50mg/kg body weight (group 4), naringenin 100mg/kg body weight (group 5), naringenin 200 mg /kg body weight (group 6) and vincistine group (group 3) were statistically significant. No statistically significant difference was noted between group 3 (vincristine group) and group 5 (naringenin 100mg/kg body weight). When compared with DMBA group(group 2) the breast tissue catalase levels of all naringenin treated groups, naringenin 50mg/kgbodyweight (group 4), naringenin 100mg/kg body weight (group 5), naringenin 200 mg /kg body weight (Group 6) and vincistine group (group 3) were statistically significant. No statistically significant difference between group 3(vincristine group), group 5 (naringenin 100mg/kg body weight) and control group (group 1) was noted. No statistically signifincant difference between group 4(naringenin 50 mg/kg body weight) and group 6 (naringenin 200 mg/kg body weight) was seen. DMBA group (group 2) the breast tissue GSH levels of all naringenin treated groups, naringenin 50mg/kg body weight (group 4), naringenin 100mg/kg body weight (group 5), naringenin 200 mg /kg body weight(group 6) and vincistine group (group 3) were

statistically significant. Except DMBA group (group 2), no statistically significant difference was seen among all other groups like group 1 (control group), group 3(vincristine group), group 5 (naringenin 100mg/kg body weight), group 4 (naringenin 50 mg/kg body weight) and group 6 (naringenin 200 mg/kg body weight). When compared with DMBA group (group 2) the breast tissue GPx levels of all naringenin treated groups, naringenin 50mg/kg bodyweight (group 4), naringenin 100mg/kg body weight (group 5), naringenin 200 mg /kg body weight (group 6) and vincistine group (group 3) were statistically significant. No statistically significant difference was seen between group 3 (vincristine group), group 4 (naringenin 50mg/kg body weight) and group 5 (naringenin 100 mg/kg body weight). Also no statistically significant difference between group 4 (naringenin 50mg/kg body weight) and group 6 (naringenin 200 mg/kg body weight) was evident (Table-1,2 and 3).

Table 1: Effect of Naringenin on Bars and Super Oxide Dismutase of Breast Tissue

Groups	Treatment	TBARS (MEAN±SD)	Suoeroxide dismutase (MEAN±SD)
Group-I	Normal saline	1.60±0.03	2.94±0.16
Group-II	7,12-Dimethyl benzanthracene (25 mg/kg/BW/sc)	1.22±0.09*	1.55±0.04*
Group-III	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Vincristine (500 µg/kg/BW/ip)	1.76±0.02#	2.78±0.08#
Group-IV	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+ Naringenin (50 mg/kg/BW/PO)	1.55±0.03#	2.27±0.16#
Group-V	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO)	1.75±0.02#	2.73±0.04#
Group-VI	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (200 mg/kg/BW/PO)	1.27±0.09*	2.42±0.03#

(*p<0.05 significant compared with Group-I with others; #p<0.05 significant compared Group-II with other groups)

Table 2: Effect of Naringenin On Catalase and Glutathione Of Breast Tissue

Groups	Treatment	Catalase (MEAN±SD)	Glutathione (MEAN±SD)
Group-I	Normal saline	19.94±0.51	4.97±0.18
Group-II	7,12-Dimethyl benzanthracene (25 mg/kg/BW/sc)	14.02±0.13*	3.06±0.08*
Group-III	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Vincristine (500 µg/kg/BW/ip)	19.96±0.56#	5.07±0.25#
Group-IV	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (50 mg/kg/BW/PO)	13.28±0.29*	4.89±0.14
Group-V	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO)	20.02±0.34#	5.09±0.24*,#
Group-VI	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (200 mg/kg/BW/PO)	13.30±0.27*	5.00±0.17*,#

(*p<0.05 significant compared with Group-I with others; #p<0.05 significant compared Group-II with other groups)

Table-3: Effect of Naringenin on Glutathione Peroxidase Levels Of Breast Tissue

Groups	Treatment	Glutathione peroxidase (MEAN±SD)
Group-I	Normal saline	25.05±0.14
Group-II	7,12-Dimethyl benzanthracene (25 mg/kg/BW/sc)	18.10±0.20*
Group-III	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Vincristine (500 µg/kg/BW/ip)	27.06±0.50#
Group-IV	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (50 mg/kg/BW/PO)	26.76±0.44#
Group-V	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO)	26.90±0.39#
Group-VI	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (200 mg/kg/BW/PO)	26.40±0.01#

(*p<0.05 significant compared with Group-I with others; #p<0.05 significant compared Group-II with other groups)

DISCUSSION

A breast cancer rate tend to be higher in women of higher education and in specific communities that have adopted a more westernized lifestyle, such as Christians and Parsis, and is lowest in Muslim communities (Paymaster *et al*; 1970). The medical treatment of cancer has made substantial improvements since the early years of modern anti-tumour drug research. The identification and development of natural compounds and their derivatives have greatly contributed to this progress. Many of these compounds are now being used in clinical practice. Naringenin is structurally related to estradiol and other steroid hormones, thyroid hormone, retinoic acid, nucleosides, and folic acid. Many studies have been carried out to analyse the anti-cancer activity of naringenin. Naringenin is shown to lower proliferation and to increase apoptosis and so may contribute towards colon cancer prevention (Leonardi T et.al. 2011) and is also claimed to be effective in lung cancer of mice (Qin L, et. al; 2011). Oral administration is the most convenient route for patients, and improves their compliance and hence in our study, we opted for oral route of administration. In the present study, we determined the efficacy of naringenin by evaluating its action on breast lipid peroxidation, superoxide dismutase, glutathione peroxidase and catalase and the results were compared with vincristine, a standard drug used in the treatment of breast cancer. DMBA when administered in rats, decreases lipid peroxidation in breast tissue. This inverse relationship between lipid peroxidation and cellular proliferation in DMBA treated rats is reported by Das, 2002. Naringenin at 50 and 100 mg / kg body weight dose range increased the breast thiobarbituric acid reactive substances (TBARS) when compared to the DMBA group indicating their preventive effect on lipid peroxidation mediated breast cancer cellular proliferation. Naringenin at 50 mg / kg body weight dose was more effective compared to other two doses and was superior to the effect produced by vincristine. Both vincristine and naringenin 100 mg resulted in neutralization of toxic effect of DMBA on breast cancer cells. Naringenin at 50 mg dose was comparable to the control value indicating that a near 100% recovery can be achieved by naringenin even at a lowest dose of 50 mg/kg. Superoxide dismutase protects the cells from oxidative damage and its loss or inhibition results in greater metabolic consequence. Mutations in SOD can result in pathological changes in cells. Increasing the levels of SOD can protect a cell from carcinogen induced oxidative damage (Oberley, 1997). SOD is a cytoprotective antioxidant. Naringenin at 100 mg dose increased the SOD levels of plasma and breast tissue on par with vincristine group, indicating that it is very effective at that dose level. Catalase catalyses the decomposition of hydrogen peroxide to oxygen and water. It has a rapid turnover rate. Catalase activity has been reported to be low in cancer cells (Sun, 1990), and there is accumulation of hydrogen peroxide in cancer cells. DMBA decreases catalase level in breast tissue when compared to control. Naringenin at 100 mg dose increased breast tissue catalase level. Moreover its action on breast tissue was superior to Vincristine increased catalase levels on par with vincristine group. 50 mg and 200 mg dose levels of Naringenin increased the catalase levels comparable to vincristine group, indicating the anticancer potential of Naringenin in breast cancer. Glutathione is present in both reduced and oxidized forms. Glutathione acts as a co-factor for Glutathione Peroxidase and is also involved in many other metabolic processes, like ascorbic acid metabolism. It is a major non-enzymatic component of intracellular antioxidant defences. In our study all three naringenin treated groups' replenished glutathione to near normal values, similar to vincristine treated group. High levels of glutathione is claimed to reduce the rate of tumor growth, recurrence or secondary cancer. Glutathione is one of the main cellular scavengers of free radicals. Glutathione depletion in DMBA treated group (group 2) is due to either inhibition of its synthesis or acceleration of its efflux from mitochondria. Glutathione plays a crucial role in the prevention of breast cancer by increasing the levels of Glutathione. Naringenin is found to be more effective in the prevention of breast cancer. Glutathione peroxidase results in reduction of peroxidase to water and oxidation of Glutathione. Catalase and Glutathione peroxidase act synergistically in the removal of hydrogen peroxidase. Being a noted antioxidant, its level is crucial in the prevention of cancer. In our study the data indicates that naringenin at 50 mg and 100 mg dose increased the level of glutathione peroxidase in breast tissue comparable to vincristine group. 200 mg of naringenin did not show any further benefit in the levels of breast glutathione peroxidase. Since the inactivation of hydrogen peroxidase catalyzed by glutathione peroxidase needs glutathione as cofactor, the capacity of naringenin to increase both glutathione peroxidase and glutathione levels in DMBA treated groups (group 4, 5 6) is significant to prove its role in prevention of breast cancer. Naringenin is known to be a potent antioxidant (Cavia-Saiz, Busto MD *et al*; 2010).

CONCLUSION

The capacity of flavonols to act as antioxidant in cells definitely represents a fascinating potential in the field of oncology. Although basic research in cancer biology has

provided new targets into a sharp focus, new and novel approaches to cancer prevention and treatment are needed. Naringenin is given orally while vincristine can be given only through parenteral route. Also Naringenin can be given prophylactically at low doses in high risk individuals. The effect was either comparable or superior to the action of vincristine. However, further clinical trials are needed to prove its efficacy in breast cancer.

REFERENCES

1. Radha Munagala, Farrukh Aqil, Ramesh C.Gupta . Promising molecular targeted therapies in breast cancer.Indian j Phamacol.2011;43(3):236-45.
2. American cancer society. Breast cancer: cancer facts and figures. Atlanta. American cancer society; 1995.
3. Mohamed IN, Fatema E, Nada A, Kamilia A, Satyanarayana G. Breast cancer: Conventional diagnosis and treatment modalities and recent patents and technologies. BC 2015;9(2):17-34.
4. Dumitrescu RG, Cotarla I. Understanding breast cancer risk-where do we stand in 2005?. JCMM 2005;9(1):208-21.
5. Alfredo CS< Barros D, Elisa NK, Lincon JM. Induction of experimental mammary carcinogenesis in rats with7,12-dimethylbenz (a)anthracene. RHC 2004;59(5):257-61.
6. Ganesan Dhamodharan, Sankaran Mirunalini. Dose response study of Agaricus Bisporus (white button mushroom) and its Encapsulated chitosan Nanoparticles against 7, 12 – Dimethylbenz (a) anthracene induced mammary carcinogenesis in Female Sprague-Dawley rats .International journal of Pharmacy and Pharmaceutical sciences 2012;4(4): 20-25.
7. Natla Sashidhar Reddy, Parthasarathy Nirmala, Natesan Chidambaram, Pammi Ashok. Quercetin in Dimethyl Benzanthracene Induced Breast Cancer in Rats. American journal of Pharmacology and Toxicology 2012;7(2): 68-72.
8. Paymaster TC, Gangadharan P . Cancer in parsicommunity of Bombay. *Int J Cancer*, 5, 1970, 426-31.
9. Leonardi T, Vanamala J, Taddeo SS, et.al . Apigenin and Naringenin suppress colon Carcinogenesis through the aberrant crypt stage in azoxymethane –treated rats. Exp Biol Med (Maywood) .2010 Jun ;235(6): 710-7.
10. Qin L, Jin L, Lu L, Lu X, Zhang C, Zhang F, Liang W.Naringenin reduces lung metastasis in a breast cancer resection model .protein cell .2011 Jun ;2 (6): 507-16 .
11. Das U: A radical approach to cancer. Med.Sci.monit, 2002;8: 79-92.
12. Oberley LW, Bize IB, Sahu Sk, Leuthauses SWHC, Gruber HE: Superoxide dismutase activity of normal murine liver, regenerating liver and H6 hepatoma. J. Natl. Cancer Inst, 1997; 61: 375-379.
13. Sun Y: Free radicals, antioxidant enzymes and carcinogenesis. Free radic Biol Med, 1990; 8: 583-599.
14. Cavia-Saiz M, Busto MD, Pilar-Izquierdo MC, Ortega N, Perez-Mateos M, Muniz P. Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: A comparative study. Journal of the Science of Food and Agriculture, 2010;90:1238–44.

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