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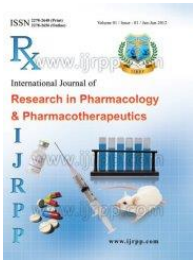

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Screening of anti-cancer effect of naringenin in 7, 12-dimethyl benzanthracene induced breast cancer in female Wistar Albino rats

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ABSTRACT

Aim and Objectives

This study is aimed to screen the anti-cancer action of naringenin in 7, 12-dimethyl benzanthracene induced breast cancer in female Wistar albino rats.

Materials and Methods

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-I (Control), G-II- **(**7,12-Dimethyl benzanthracene (25 mg/kg/BW/sc), G-III (7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Vincristine (500 µg/kg/BW/ip), G-IV (7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (50 mg/kg/BW/PO), G-V (7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO) and G-VI (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 and blood samples were collected to estimate the anti-oxidant enzymes like TBARS, Super oxide dismutase, Catalase, Glutathione and Glutathione peroxidase.

Results

Group-II showed significant changes compared to group-I. Group-III and V showed significant difference compared to Group-II. Group-V did not show significant difference compared to Group-III in TBARS, super oxide disumutase, catalse, glutathione and glutathione peroxidase enzymes.

Conclusion

Naringenin (100 mg/kg/BW/) showed significant anti-cancer effect compared to other doses.

Keywords: Anti-cancer, Anti-oxidants, Naringenin, vincristine, 7, 12-Dimethyl bensanthracene, Plasma

INTRODUCTION

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).

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. Breast cancer can be treated with various classes of anti-cancer drugs. Existing drugs produce the various adverse effects. 7, 12-dimethyl benzanthracene is commonly used to induce the 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 benzanthracene 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-I- Normal saline

Group-II- 7,12-Dimethyl benzanthracene (25 mg/kg/BW/sc)

Group-III- 7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Vincristine (500 µg/kg/BW/ip)

Group-IV-7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (50 mg/kg/BW/PO)

Group-V-7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO)

Group-VI-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 & 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. Blood samples were collected in centrifuge tubes using sodium citrate as anticoagulant and the plasma separated was used for the determination of TBARS, Superoxide dismutase, Catalase, Glutathione, and Glutathione peroxidase by standard procedures.

RESULTS

TBARS level of naringenin treated groups, naringenin 50 mg/kg/BW (Group-IV), naringenin 200 mg/kg/BW (Group-VI) and vincristine treated group (Group-III) showed statistically significant difference. No statistically significant difference was seen between control group (Group-I), group-III (vincristine treated group) and Group-V (Naringenin 100 mg/kg/BW).

When compared with the control group the plasma superoxide dismutase levels of all naringenin treated groups, naringenin 50 mg/kg/BW (Group-IV), naringenin 100 mg/kg/BW (Group-V), naringenin (200 mg/kg/BW) (Group-VI) and vincistine (Group-III) were statistically significant. No statistically significant difference was seen between Group-III (vincristine treated group) and Group-V naringenin (100 mg/kg/BW) (Table-1).

When compared with the control group the plasma catalase levels of all naringenin treated groups, naringenin 50 mg/kg/BW (Group-IV), naringenin 100 mg/kg/BW (Group-V), naringenin 200 mg/kg/BW (Group-VI) and vincistine group (Group-III) were statistically significant. No statistically significant difference was present between Group-III (vincristine group) and Group-V Naringenin (100 mg/kgBW). No statistically significant difference between Group-IV Naringenin 50 mg/kg/BW) and Group-VI naringenin (200 mg/kg/BW) was noted.

GSH levels of all naringenin treated groups, naringenin Group-IV, VI and III were statistically significant. There is no statistically significant difference noted between Group-I, III, V and VI groups (Table-2). The similar results were observed in glutathione peroxidase enzyme (Graph-1).

Table-1: Effect of naringenin on plasma levels of TBARS and super oxide dismutase

Groups	Treatment	TBARS (MEAN±SD)	Suoeroxide dismutase (MEAN±SD)
Group-I	Normal saline	2.68±0.03	4.09±.17
Group-II	7,12-Dimethyl benzanthracene (25 mg/kg/BW/sc)	3.71±0.05*	2.61±0.08*
Group-III	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Vincristine (500 µg/kg/BW/ip)	2.70±0.03$^{\#}$	3.95±0.17*,$^{\#}$
Group-IV	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+ Naringenin (50 mg/kg/BW/PO)	3.20±0.02*	3.06±0.11*,$^{\#}$
Group-V	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO)	2.84±0.10$^{\#}$	3.82±0.08*,$^{\#}$
Group-VI	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (200 mg/kg/BW/PO)	3.07±0.06*	3.2±0.01*,$^{\#}$

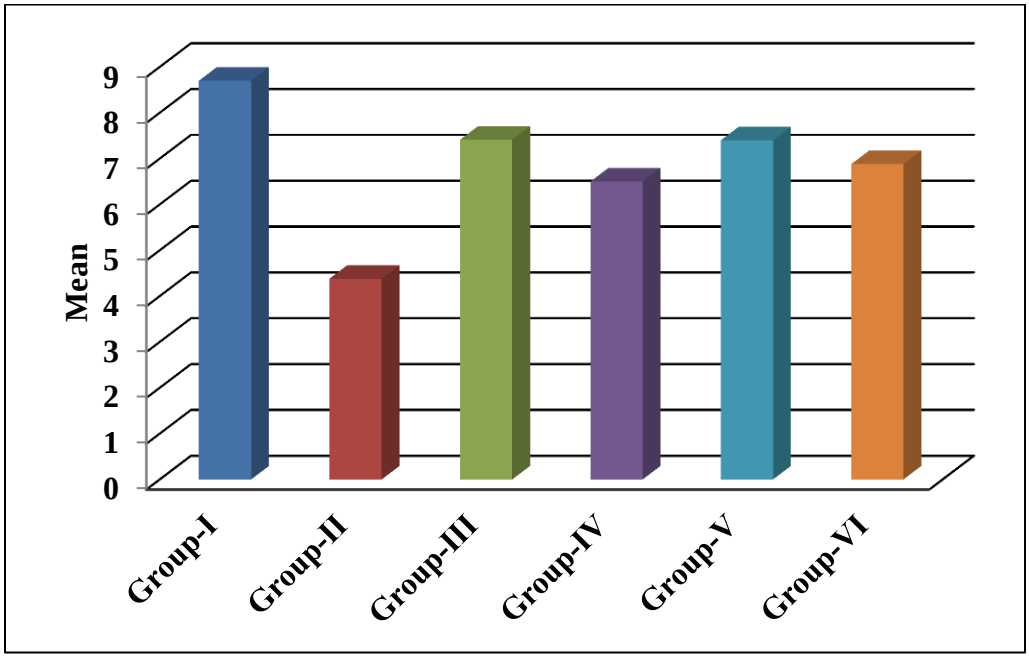
(*p<0.05 significant compared with Group-I with others,
$^{\#}$p<0.05 significant compared Group-II with other groups)

Table-2: Effect of naringenin on plasma levels of catalase and glutathione

Groups	Treatment	Catalase (MEAN±SD)	Glutathione (MEAN±SD)
Group-I	Normal saline	30.11±0.02	41.10±0.42
Group-II	7,12-Dimethyl benzanthracene (25 mg/kg/BW/sc)	20.09±0.31*	30.31±0.35*
Group-III	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Vincristine (500 µg/kg/BW/ip)	27.65±0.51$^{\#}$	42.25±0.49$^{\#}$
Group-IV	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (50 mg/kg/BW/PO)	26.51±0.07*,$^{\#}$	40.26±0.14$^{\#}$

Group-V	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (100 mg/kg/BW/PO)	27.52±0.33[#]	42.49±0.03[#]
Group-VI	7,12-Dimethyl benzanthracene (25 mg/kg/sc)+Naringenin (200 mg/kg/BW/PO)	26.17±0.24*,[#]	42.05±0.06[#]

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



Graph-1: Effect of naringenin on plasma levels of glutathione peroxidase

DISCUSSION

In India, breast cancer is the second most common cancer (after cervical cancer). Breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents 13.7% of all cancer deaths (Ferlay J BF et al ;2001). In metropolitan cities, breast cancer is the leading cancer in women, with rates nearly twice as common as cervical cancer. The risk factors for breast carcinoma include older age group (>60 yrs),family history of breast cancer, history of atypical hyperplasia, lobular carcinoma in situ or ductal carcinoma in situ , mutations in BRCA1(that controls cell growth) or BRCA2 (that suppresses cell growth), early menarche (<12 years), late menopause ,menopausal hormone therapy and nulliparity.

Naringenin belongs to the class of flavonoids called the flavanones. The flavanones are abundant in citrus fruits such as the grapefruit and oranges. Literature indicates the role of naringenin in the treatment of cancer (Wilcox et.al.1999). Naringenin is 2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one . A number a studies have detected naringenin in human urine and plasma following oral doses of pure naringin (Ameer B et.al.1996). These results showed that naringenin can be absorbed from diet. As naringenin is generally present in foods bound to sugars as b-glycosides (i.e., naringin), it was originally thought that absorption from the diet would be negligible.

Only few toxicity studies of naringenin *perse* have been done. However flavonoids are generally considered to have low toxicity. 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 analyze the anti-cancer activity of naringenin. Naringenin is shown to lower proliferation, increase apoptosis and so may contribute towards colon cancer prevention (Leonardi T, et.al. 2010, Travis et.al. 2005). It is also claimed to be effective in lung cancer of mice (Qin L, et. al; 2011). In the present study, we determined the efficacy of naringenin by evaluating its action on

plasma and breast lipid peroxidation, erythrocyte lysate superoxide dismutase, glutathione peroxidase and plasma catalase. Oxidative reaction plays a major role in the growth and development of cancer. Evidence points towards the association of oxidative stress in cancer cell, where there is decreased antioxidant defence (Cerutti 1994). Increased levels of reactive oxygen species and toxic degradation products of lipid peroxidation have been found in the plasma of DMBA induced carcinogenic animal models (Hu JW et. al. 2010). Both vincristine and naringenin 100 mg resulted in neutralization of toxic effect of DMBA on breast cancer cells.

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 the cell from carcinogen induced oxidative damage (Oberley, 1979). 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, and there is accumulation of hydrogen peroxide in cancer cells. DMBA decreases catalase level in plasma and breast tissue when compared to control. Naringenin at 100 mg dose increased breast tissue and plasma catalase level. Moreover its action on breast tissue was superior to Vincristine action and in plasma it increased catalase levels on par with vincristine group. Also in plasma, 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 plays a crucial role in the prevention of breast cancer by increasing the levels of Glutathione. Naringenin is found to be effective in the prevention of breast cancer. The Plasma values of Glutathione in Naringenin 100mg and 200mg groups was found to be on par with the Vincristine and control group, suggesting the anticancer activity of Naringenin at such dose levels. 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. Similarly naringenin at 100 mg dose increased the plasma glutathione peroxidase level on par with vincristine group. 200 mg of naringenin did not show any further benefit in the levels of both plasma and 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.

Conflict of interest: Nil

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