International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.13, pp 250-256, 2017

Assessment of Protective Effect of a Bioflavonoid Quercetin in Dimethyl Benzanthracene-Induced Breast Cancer in Female Wistar Rats

Rajat Rana[1]*, Aneena Suresh [2]

[1]Unit of Pharmacology, Orrotta Medical College and Hospital, Asmara College of Health Sciences, Eritrea 560032
[2]Lecturer, Department of Pharmacy Practice, JSS College of Pharmacy (Jagadguru Sri Shivarathreeshwara University), Rocklands, Udhagamandalam, Tamil Nadu, India-643001

Abstract : The objective of this study wasto assess the role of Quercetin in 7, 12-Dimethyl benzanthracene (DMBA)induced breast cancer in female Wistar rats**.** A total of 30 female Wistar rats (total 6 groups, *n* = 5 per group) 6 - 8 weeks old, weighing 150 gm were used in the study. DMBA was given at the dose of 7.5 mg/kg subcutaneously in the mammary region once a week for 4 consecutive weeks in experimental group. Vincristine was given in the dose of 500 mcg/kg intraperitoneally every week for 4 consecutive weeks in group (E1). Quercetin was given orally in a dose of 200, 400, 800 mg/kg in group E200, E400, and E800 respectively. The statistical significance of the data was determined using one way analysis of variance. It was evident that Quercetin 200, 400, 800 mg/kg /oral for 120 days treated rats resulted comparable effects to that of standard vincristine and control groups. The outcomes indicated that Quercetin improved the antioxidant levels in plasma erythrocyte lysate and breast tissue and was effective in averting DMBA induced oxidative damage. Quercetin was found to be either equally effective or more effective than Vincristine in all the factors studied.
Keywords: Quercetin, Dimethyl benzanthracene, antioxidant, erythrocyte lysate.

Introduction

Breast cancer is the second commonest type of malignancy causing high morbidity and mortality among women worldwide[1, 2].The new versionof International Agency for Research on Cancer(IARC) online database, GLOBOCAN 2012 predicts a substantial increase to 19.3 million new cancer cases by 2025,due to growth and ageing of the global population[3]. Although there are manychemotherapeutic drugs for the treatment of breast cancer, they usually are associated with a number of side-effects. Therefore, the consideration and improvement of unique antitumor agents is critical to the improvement of breast cancer chemotherapy[4].

Quercetin is a major polyphenolic bioflavonoid compound known as antioxidant due to formation of less reactive phenoxy radicals from the reactivity of phenolic part with free radical species[5, 6].

In the recent years, many researches have indicated the anti-cancer properties of Quercetin. Also previous studies have indicated that Quercetin can induce apoptosis in various cancer cell lines and inhibit the growth of cancer cells notably in gastric, ovarian and oesophageal cancers[7, 8]. However, the studies investigating

the role of Quercetin in breast cancer are rare and have low evidence. Therefore the aim of this study was to investigate the effect of Quercetin on Super oxide Dismutase(SOD), lipid peroxidation in plasma in breast tissue, the antioxidant enzyme ThioBarbituric Acid Reactive Substance(TBARS) and to compare the efficacy with vincristine in breast cancer chemotherapy.

Experimental

Materials

Quercetin was purchased from sigma (St.louis, MO, USA) and DMBA from sigma (St.louis, MO, USA). Other chemicals and reagents that were used were of analytical grade.

Methods

Formulation of drug

In 0.1% of carboxy methyl cellulose, Quercetin powder was added to make a homogenous suspension. DMBA was uniformly dissolute in an emulsion of sunflower oil (0.75 ml) and physiological saline (0.25ml) just prior to use. Each rat received a daily dose of 200, 400 and 800mg/kg body weight of Quercetin in 2ml of suspension.

Experimental animals

A total of 30 female Wistar rats (Total 6 groups,n=5 per group) weighing around 150g, 6-8 weeks old were randomly divided into control(C) and experimental groups(E). Experimental group was divided further into four groups (E0, E1, E200, E400 and E800). Breast cancer can be induced outwardly through DMBA a procarcinogen, upon metabolic activation by mixed function oxidases located in Wistar rat liver microsomal enzymes resulting carcinogenesis[4].

The control group(C) was given distilled water daily. In group E0 (cancer control) breast cancer was induced by giving 7.5mg/kg of DMBA subcutaneously in mammary region once a week for 4 consecutive weeks. Group E1 standard drug regimen (DMBA + Vincristine), vincristine was injected intraperitoneally in the dose of 500mcg/kg every week for 4consecutive weeks. Group E200 test drug medium dose (DMBA + Quercetin 200mg/kg/wt.) orally every 16 weeks. Group E400 was given test drug high dose (DMBA + Quercetin 400mg/kg/wt.) orally every 16 weeks. Group E 800 was given test drug very high dose (DMBA + Quercetin 800mg/kg/wt.) orally every 16 weeks.

The animals were provided with vitamin enriched pellet diet consisting of 60% roasted Bengal gram powder, 23% wheat flour, 5% skimmed milk powder, 4% casein, 4% refined oil, salt mixture with 4 % starch and choline. Water supplied to both groups was distilled with inverse osmotic pressure water purifier and was given spontaneously.

The rats were housed in animal house, Department of Pharmacology, Gautham College of Pharmacy in an air conditioned room with 12 hours light and dark cycle under optimum humidity. The standard procedures were followed in accordance with the Indian National Law on animal care and use for maintaining the experimental animals. During the total study period of 16 weeks the weight of the rats were recorded at beginning of experiment, at weekly intervals and at the end of the study period. At the end of 16 weeks after overnight fasting the rats were sacrificed [Fig 1].

Blood collection and Erythrocyte lysate preparation

The blood was collected in 0.5 ml heparinized tubes after sacrificing the rats. The plasma separation was carried out by centrifugation at 800rpm for 8 min at 4˚c. The buffy coats along with other supernatants were observed at the top after plasma separations which were discarded. Physiological phosphate- buffered saline (PBS) containing 5.5 mm maintained at 2˚c was used to wash packed cells three times.

Molecular damage in mammalian erythrocytes is very typical and highly sensitive due to high concentration of iron in erythrocytes which causes conversion of ferric ion to ferrous ion on interaction with reactive oxygen species and forms highly reactive hydroxyl ions[9, 10]. Erythrocyte lysate was separated by adding two volumes of distilled water to packed erythrocytes and centrifuging at 3000rpm for 10 min at 4˚c.

Tissue homogenate preparation

Breast tissues were excised immediately and washed with ice cold isotonic saline. 0.1M Tris- HCL buffer at pH 7.2 was used in preparing tissue homogenate. Further the homogenate was centrifuged at 800rpm for 5 min and the supernatant was used for assays.

Estimation of ThioBarbituric Acid Reactive Substance(TBARS)

Tissue and plasma TBARS were assessed by method of Ohkawa[11]. Superoxide dismutase was estimated using method of Kakkar *et al*[12].The results were compared with vincristine group.

Statistical analysis

The final end points analyzed were body weight, tumor incidence, tumor multiplicity and tumor volume and for all the groups. The data was presented as mean ± SD. Statistical analysis was performed with one way analysis of variance. *P < 0.05 was considered to indicate the statistically significant difference.

Results

General observation

No change was observed in the final body weight (g) body weight gain/loss (g) food intake (g/d), food efficiency (body weight gain (g/d)/food intake) and it remained same in all the experimental rats.Further no significant toxicity was observed apart from mild confusion in one subject and cachexia was not witnessed even in DMBA- treated rats. Body weight and growth rate was also same and no changes were observed during the experiment[Figure 1].

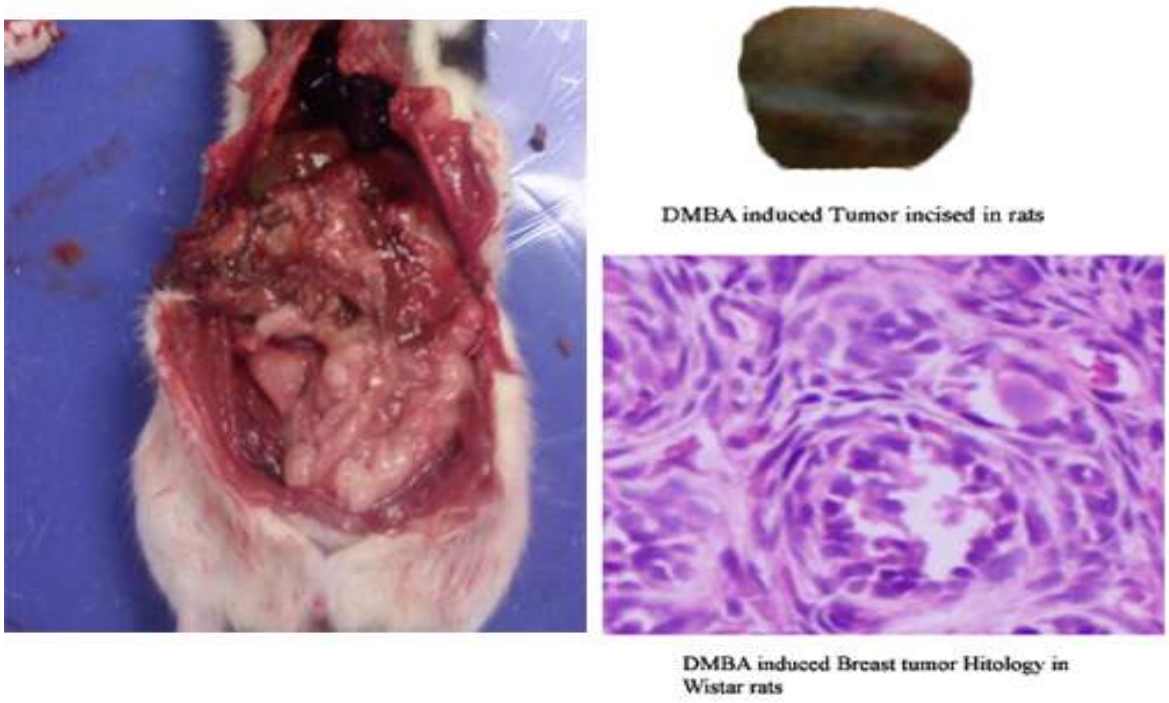

Fig. 1.Tumors produced in the Female Wistar treated with 7, 12-Dimethyl Benzanthracene

Plasma TBARS

In comparison to the DMBA group (E0) the plasma TBARS levels of all groups that is Vincristine group (E1) Quercetin treated groups (E200, E400, E800)are significantly lowered and statistically significant[Figure2a].There was a dose dependant reduction in plasma TBARS levels in Quercetin treated groups. The plasma TBARS reduction in E1 group and E800 group was almost equally efficacious[Table 1].

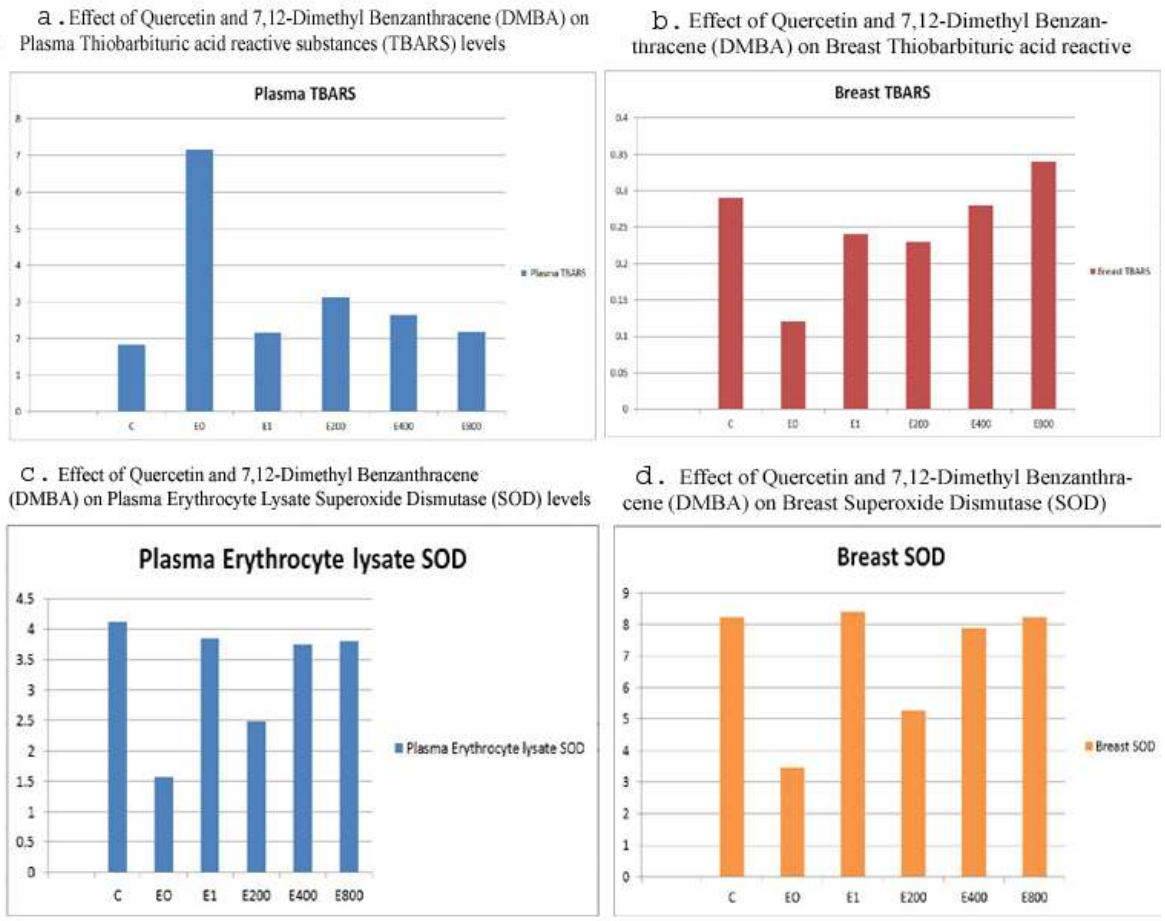

Fig. 2. a) Effect of Quercetin and 7,12-Dimethyl Benzanthracene (DMBA) on Plasma Thiobarbituric acid reactive substances (TBARS) levels; b) Effect of Quercetin and 7,12-Dimethyl Benzanthracene (DMBA) on Breast Thiobarbituric acid reactive substances (TBARS) levels; c) Effect of Quercetin and 7,12-Dimethyl Benzanthracene (DMBA) on Plasma Erythrocyte Lysate Superoxide Dismutase (SOD) levels; d) Effect of Quercetin and 7, 12-Dimethyl Benzanthracene (DMBA) on Breast Superoxide Dismutase (SOD) levels

Table 1.Effect of Quercetin and 7,12- Dimethyl Benzanthracene (DMBA) on Thiobarbituric acid reactive substances (TBARS) levels of control and experimental groups

Groups	**Group Codes**	**Plasma TBARS (nmol/ml plasma)**	**Breast TBARS (mmol/mg tissue)**
Control	C	1.84±0.14	0.29±0.04
DMBA	EO	7.16±0.62	0.12±0.06
DMBA+ Vincristine	E1	2.14±0.25	0.24±0.02
DMBA+QUERCETIN (200 mg/kg b.wt.)	E200	3.12±0.24	0.23±0.05
DMBA+QUERCETIN (400 mg/kg b.wt.)	E400	2.63±0.17	0.28±0.02
DMBA+QUERCETIN (800 mg/kg b.wt.)	E800	2.19±0.22	0.34±0.05

Breast TBARS

In comparison to DMBA group (E0) the breast TBARS levels of all groups that are Vincristine group (E1), Quercetin treated groups (E200, E400, E800) are statistically significant[Figure 2b].When compared to

group E1, E400 and E800 were superior in efficacy. Group E800 was statistically significant when compared to control group(C)

Erythrocyte Lysate Superoxide Dismutase (SOD)

In comparison to DMBA group (E0) the Erythrocyte Lysate Superoxide Dismutase (SOD) levels of all groups that are Vincristine group (E1), Quercetin treated groups (E200, E400, E800) are statistically significant. E200 was not statistically significant when compared to group E1[Figure 2c]. There was a dose dependant increase in erythrocyte lysate levels in Quercetin treated groups. Group E800 and E1 were almost equally effective. None of the experimental groups were significant in comparison to the control group [Table 2].

Table 2.Effect of Quercetin and 7,12‑Dimethyl Benzanthracene (DMBA) on Superoxide Dismutase (SOD) levels of control and experimental groups

Groups	Group Codes	Plasma Erythrocyte lysate SOD (U/mg Hb)	Breast SOD (U/mg protein)
Control	C	4.12±0.16	8.23±0.42
DMBA	EO	1.57±0.17	3.46±0.29
DMBA+ Vincristine	E1	3.85±0.29	8.41±0.37
DMBA+QUERCETIN (200 mg/kg b.wt.)	E200	2.48±24	5.28±0.23
DMBA+QUERCETIN (400 mg/kg b.wt.)	E400	3.75±0.27	7.88±0.63
DMBA+QUERCETIN (800 mg/kg b.wt.)	E800	3.81±0.16	8.24±0.58

Breast Superoxide Dismutase (SOD)

In comparison to DMBA group (E0) the breast Superoxide Dismutase (SOD) levels of all groups that are Vincristine group (E1), Quercetin treated groups (E200, E400, E800) are statistically significant. There was a dose dependant increase in breast Superoxide Dismutase (SOD) levels in Quercetin treated groups. Group E1 was statistically significant and found superior over group E800[Figure 2d].Group E800 was statistically significant in comparison to control group (C) and almost equally effective. The breast superoxide dismutase (SOD) levels were higher in E1 group in comparison to control group (C) [Table 2].

Discussion

Breast cancer is most common cancer and a leading cause of high fatality rates among women. The treatment options available are very limited. Thus, there is urgent need of newer noble agents which are highly efficacious and have reliable safety profile. Quercetin is the major bioflavonoid in human diet and also extracted from various fruits and vegetables. Previous researches have demonstrated that Quercetin inhibits proliferation of colon, pancreas, bladder, stomach and ovarian cancers and also induces tumour cell apoptosis[13].

Present study was envisioned to assess the effectsof Quercetin in management of breast cancer in Wistar rats. The parameters tested were lipid peroxidation (TBARS) and Superoxide Dismutase (SOD). The anticancer effects of Quercetin were studied by measuring the TBARS and SOD levels in plasma and breast tissues. The Quercetin treated groups were compared with Vincristine, a standard drug in chemotherapeutic regimen.

In DMBA induced breast cancer there is a significant rise in TBARS levels in plasma which causes reduction in lipid peroxidation. The reduced lipid peroxidation indicates increased cellular proliferation and differentiation in animal treated with a carcinogen[14]. In this study it was observed that the groups treated with Quercetin illustrated a dose dependant reduction in TBARS levels. The inhibition of DMBA induced elevated TBARS levels highest in E800 group which indicates that Quercetin is more effective in higher doses. The efficacy was highest in E800 group even when compared to Vincristine treated group in reducing plasma TBARS.

In breast cells the TBARS levels in Quercetin E800 group was most effective which strongly indicates that Quercetin can be effectively used in breast cancer therapy and prophylaxis.DMBA induces oxidative damage due to highly reactive free radical formation and induces increased cellular differentiation. This oxidative damage is controlled by primary anti-oxidant Superoxide Dismutase (SOD) and protects the cells from such damage.The plasma erythrocyte lysate SOD levels and breast SOD levels markedly reduces in DMBA induced breast carcinoma[15, 16].

The plasma erythrocyte lysate levels were highest in Vincristine treated group (E1) and were equally effective to E800 group which indicates the high efficiency of Quercetin in increasing plasma SOD levels in higher doses. Quercetin elevated the breast SOD levels almost equally to the Vincristine treated group.Thus, Quercetin was found to be either equieffectiveor superior in all the studied parameters and epitomises a potential in breast chemotherapy.

Conclusion

This study confirms the anticancer property of Quercetin, a ubiquitous flavonoid molecule. It was observed that Quercetin inhibits tumour growth and may have a possible role in cell cycle regulation, inhibition of tyrosine kinase and oestrogen binding. More research is needed to elucidate the magnitude of anticancer effect of Quercetin in breast cancer management.

Authors Contribution

All the authors have contributed in various degrees to conception and design, acquisition of data, analysis and interpretation of data and writing present article.

Conflicts of Interests

All the author(s): Rajat Rana and Aneena Suresh declare that there is no conflict of interest regarding the publication of this paper.

References

1. Reddy NS, Nirmala P, Chidambaram N, Kumar PA. Quercetin in dimethyl benzanthracene induced breast cancer in rats. Am J PharmacolToxicol.,2012, 7, 68‑72.
2. Notani PN. Global variation in cancer incidence and mortality. CurrSci., 2001, 81, 465‑474.
3. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C *et al.* (2013). GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer.
4. Xiao-huideng, hai-yan song, ying-fengzhou, guo-yanyuan and feng-jinzheng. Effects of quercetin on the proliferation of breast cancer cells and expression of survivin in vitro.ExpTher Med., 2013 Nov,6(5),1155-1158
5. Cody V, ed. Plant Flavonoids in Biology and Medicine. ProgClinBiol Res.,1986,213.
6. Cody V, ed. Plant Flavonoids in Biology and Medicine, part II. ProgClinBiolRes., 1988,280.
7. Heinritz W, Paasch U, Sticherling M, *et al*: Evidence for a founder effect of the germlinefumaratehydratase gene mutation R58P causing hereditary leiomyomatosis and renal cell cancer (HLRCC). Ann Hum Genet 72 (Pt 1)., 2008, 35-40.
8. Comalada M, Camuesco D, Sierra S, *et al*: In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. Eur J Immunol35., 2005, 584-592.
9. Glass GA, Gershon H, Gershon D. The effect of donor and cell age on several characteristics of rat erythrocytes. ExpHematol.,1983, 11, 987‑995.
10. Masotti L, Casali E, Galeotti T. Lipid peroxidation in tumour cells.Free Radic Biol Med., 1988, 4, 377‑386.
11. Ohkawa H, Onishi N, Yagi K. Assay for lipid peroxides in animal tissueby thiobarbituric acid reaction. Anal Biochem., 1979,95,351‑358.

12. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J BiochemBiophys.,1984,21,130‑132
13. NTP Technical Report (no.409) on the toxicology and carcinogenesis studies of quercetin in F344/N rats. NIH Publication No. 91-3140 (1991). U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, ResearchTriangle Park, NC.
14. Navarro J, Obsrador E, Carretero J, Petschen I, Avino J, Perez P, *et al*. Changes in glutathione status and the antioxidant system in blood and in cancer cells associated with tumour growth *in vivo*.Free RadicBiolMed .,1999,26,410‑18.
15. Elucheri S, Overley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H, et al. CuZn SOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later inlife. Oncogene., 2005,24,367‑380.
16. Oberley LW, Oberley TD. Role of antioxidant enzymes in the cancer phenotype. In: Clerch LB, Massaro D, editors. Oxygen, Gene Expression and Cellular Function. Washington: Marcel Dekker., 1997, 279‑308.

*****