Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 142 - 149

Research Article Bio chemistry

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

EVALUATION OF THE ANTICYTOTOXIC ACTIVITY OF FICUS GLOMERATA ROXB.LEAF EXTRACT AGAINST DMBA INDUCED MAMMARY CARCINOMA IN RATS

V.DHANA RANGESH KUMAR*[1], G.JEYANTHI[2] AND K.GURUSAMY[1]

[1]*Department of Biochemistry, Dr. N. G. P Arts and Science College, Coimbatore.*
[2] *Department of Biochemistry, Govt. Women's Arts and Science College, Krishnagiri.*

ABSTRACT

The aim of the present study is to evaluate the *in vivo* anticancer activity of ethanolic extract of F*icus glomerata* on DMBA induced mammary carcinoma in rats. *Ficus glomerata* Roxb is an indigenous medicinal plant, belonging to the family Moraceae. It is largely used in herbal and herbo-mineral preparation to treat cancer, cardiovascular diseases, asthma, viral hepatitis and diabetes. DMBA at a dose of 20mg/kg body weight (gastric intubation) significantly elevated the xenobiotic markers such as cytochrome P_{450} reductase and cytochrome b_5 reductase in group (II) rats. On the treatments with the ethanolic extract of *Ficus glomerata* leaf extract (150 mg/kg bw p.o.) significantly decreased these activities in a group (III) rats when compared to DMBA induced rats, which confirmed its anticancer potential. The TCA cycle marker enzymes namely succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase and NADH dehydrogenase were significantly decreased by DMBA induction in group (II) rats, these enzyme levels were reverted to near normal by the supplementation of *F. glomerata* in group (III) rats. The results of our present study clearly showed that the *F. glomerata* possess significant anticancer activity.

KEYWORDS: Ficus glomerata, cytotoxic, 7, 12-dimethylbenz (a)anthracene (DMBA), cytochrome P_{450} reductase, cytochrome b_5 reductase

V.DHANA RANGESH KUMAR
Department of Biochemistry, Dr. N. G. P Arts and Science College, Coimbatore

Corresponding author

INTRODUCTION

Breast cancer represents the most common neoplastic disease in females, accounting for up to one third of new diagnoses of women's cancer in certain regions of the world. In developing countries traditionally known for low incidence of breast cancer, increase in both incidence and mortality have been recently detected[1]. Increased lifetime exposure to endogenous or exogenous estrogen is recognized as the single most important risk factor in the development of breast cancer[2].Estrogens are metabolized by the combined action of phase I and phase II enzymes in the mammary gland as well as in the liver. The phase I enzymes cytochrome P_{450} reductase and cytochrome b_5 reductase catalyses oxidative metabolism of estrogens, while the phase II enzymes are involved in detoxification[3]. The mitochondria is an important site for the production of cellular reactive oxygen species (ROS). The mitochondrial electron transport chain consumes oxygen through oxidative phosphorylation to form cellular energy in the form of ATP. During this process, 2% of the consumed oxygen is released in the mitochondria as superoxide free radicals[4, 5].The rat mammary gland is widely used as a system for a broad range of studies in basic sciences, including molecular biology and endocrinology. 7, 12-dimethylbenz (a) anthracene (DMBA) mammary carcinogenesis in rats has been widely used in various mammary cancer chemopreventive studies[6].Medicinal plants rich in radical scavenging antioxidants such as *Garcinia mangostana, litchi, Semecarpus anacardium Linn*. nut and *Operculina turpethum* have been demonstrated to exhibit antiproliferative and apoptotic effects against human breast cancer cells as well as DMBA-induced mammary carcinomas[7, 8, 9]*Ficus glomerata* Roxb. syn*. F. racemosa* L. (Family: Moraceae), commonly known as Gular in Hindi and cluster fig in english. It is medium sized to large evergreen or occasionally deciduous tree and found all over India and Southeast Asia. Its fruits are mixed with rice for making bread and used in several dishes. It has been reported to have many medicinal properties[10]. Fruits of *F. glomerata* contain glauanol, glauanol acetate, β-sitosterol, lupeol acetate[11, 12.] The aerial part of the plant contains β-sitosterol, lupeol and quercetin as major active constituents[13]. In the previous study, fruits of *F. glomerata* showed significant gastroprotective activity on physically and chemically induced gastric ulceration in rats[14]. In the present study we have investigated the effect of ethanolic extract of *F. glomerata* leaf on xenobiotic enzymes and TCA cycle marker enzymes in DMBA induced mammary carcinoma rats.

MATERIALS AND METHODS

(i) Chemicals

DMBA (7, 12 Dimethylbenz (a) anthracene) was purchased from sigma chemical company St.Louis, MO, USA. All other chemicals used in this study were of analytical grade which were brought from Himedia laboratories private ltd., Mumbai, India.

(ii) Plant

Ficus glomerata leaves were collected from the trees located in and around Coimbatore. The leaf samples were authenticated by Dr.V.S.Ramachandran, Professor, Department of Botany, Bharathiar University, Coimbatore-46.

(iii) Preparation of extract

F. glomerata leaves were shade dried and powdered. 10gram of this powder was extracted with 250ml of ethanol in a soxhlet apparatus. The obtained residues were vaccum dried and used for the study.

(iv) Experimental animals

Female Sprague dawley rats (180-200g) obtained from the small animal breeding station, Mannuthy, Thrissur, Kerala, India. The animals

were housed and maintained in clean polypropylene cages. The animals were fed with standard pellet diet (M/s.Hindustan lever ltd, Mumbai, India) and water *ad libitum*. The experimental protocol was carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), India and approved by the animal ethical committee.

(v) Experimental design

A total of 30 rats were used. The animals were divided into five groups of six animals each as follows. Group I served as control rats receiving olive oil, a vehicle alone. Group II rats were induced with a single dose of DMBA (20mg/rat, dissolved in olive oil) by oral gavage at the first day of the experimental period. Groups III rats received *F. glomerata* extract (150 mg/kg b.wt.) orally for 14 days after 90 days of DMBA induction. Group IV received Tamoxifen (20 mg/kg b.wt.) orally for 14 days after 90 days of DMBA induction. Group V rats received *F. glomerata* extract alone (150 mg/kg b.wt.) Orally for 14 days. At the end of the experimental period (i.e. on the 15thday) all animals were euthanized and the liver, kidney, mammary tissues were excised immediately and rinsed with ice cold saline, dried with blotting paper, weighed, cut into pieces and 1 gram of each sample (liver, kidney, mammary tissue) was homogenized with 0.25M sucrose, 1mM Tris HCL and 1mM EDTA, (pH 7.2-7.4) using a motorized glass-Teflon homogenizer to get a 10% homogenate. The 10% homogenate was centrifuged at 600x g for 5 minutes to remove the nuclear fraction and broken cell debris and then supernatant was collected. That supernatant was centrifuged at 1200x g for 10 minutes to sediment the mitochondrial fraction. Mitochondrial pellet was finally suspended in 0.25M sucrose solution. All operations were performed at 4°C. The mitochondrial fraction was used for the following biochemical studies.

(vi) Phase I mitochondrial xenobiotic enzymes

The mitochondrial fraction of liver, kidney, mammary tissue homogenates were used for assaying cytochrome P_{450} reductase[15] and cytochrome b_5 reductase[16].

(vii) Mitochondrial TCA cycle marker enzyme

The Mitochondrial fraction of liver, kidney and mammary tissue homogenates were used for assaying TCA cycle marker enzymes namely succcinate dehydrogenase (SDH) [17], malate dehydrogenase (MDH) [18], NADH dehydrogenase (NADH) [19] and isocitrate dehydrogenase (ICDH)[20].

(viii) Statistical analysis

The values were expressed as mean ± SD. Data were analyzed for the statistical significance by one way analysis of variance (ANOVA) followed by the group means were compared with Dunnet`s multiple comparison test using a statistical software SPSS version 10 and value of $P<0.05$ was considered to indicate a significant difference between the groups.

RESULTS

(i) Body weight and organ weight

The effect of *Ficus glomerata* leaf extract on body weight and organ weight of control and experimental rats were given in table 1.

Table 1
Effect of Ficus glomerata leaf extract on body weight and organ weight of control and experimental rats

Groups	Body weight (g)	Liver weight (g)	Kidney weight (g)
Group I	189.17 ± 7.86	5.54 ± 0.29	1.11 ± 0.06
Group II	166.00 ± 7.85a	3.84 ± 0.20a	0.90 ± 0.05a
Group III	180.9 ± 4.08b	5.12 ± 0.17b	1.05 ± 0.04b
Group IV	182.67 ± 7.56abc	5.26 ± 0.07abc	1.07 ± 0.04abc
Group V	190.33 ± 3.54a	5.50 ± 0.13a	1.12 ± 0.07ns

Values are expressed as mean ± SD of six observations
Comparisons are made between: a –Group I vs Group II, IV & V; b – Group II vs Group III &IV; c – Group III vs Group IV

Statistical significance: a,b,c significant at $p<0.05$, ns-not significant.

The body weight and organ weight of mammary carcinoma bearing (Group II) rats were significantly ($P<0.05$) reduced when compared with control rats. Body and organ weight were steadily increased significantly and recovered back to near normal conditions after treatment with *F.glomerata* leaf extract in Group III rats. Group V plant extracts administered control rats did not show any significant variation in body and organ weight when compared with control rats.

(ii) Phase I mitochondrial xenobiotic enzymes

The activities of mitochonrial xenobiotic enzymes such as cytochrome P_{450} reductase and cytochrome b_5 reductase in liver, kidney and mammary tissue of control and experimental rats were presented in tables 2, 3, 4.

Table 2
Effect of Ficus glomerata on Phase I xenobiotic enzymes in liver mitochondrial fraction of control and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V
CytochromeP_{450} reductase	0.55 ± 0.09	1.86 ± 0.16a	0.85 ± 0.35b	0.72 ± 0.03bc	0.57 ± 0.04ns
Cytochromeb_5 reductase	0.26 ± 0.06	0.88 ± 0.12a	0.50 ± 0.02b	0.38 ± 0.04bc	0.27 ± 0.06ns

Table 3
Effect of Ficus glomerata on Phase I xenobiotic enzymes in kidney mitochondrial fraction of control and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V
CytochromeP_{450} reductase	0.66 ± 0.17	1.97 ± 0.18a	0.89 ± 0.46b	0.78 ± 0.00bc	0.65 ± 0.21ns
Cytochrome b_5 reductase	0.09 ± 0.01	0.32 ± 0.02a	0.19 ± 0.02b	0.15 ± 0.03bc	0.11 ± 0.02ns

Table 4
Effect of *Ficus glomerata* on Phase I xenobiotic enzymes in mammary tissue mitochondrial fraction of control and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V
Cytochrome P_{450} reductase	0.74 ± 0.14	2.40 ± 0.17a	1.01 ± 0.07b	0.97 ± 0.06bc	0.69 ± 0.11a
Cytochrome b_5 reductase	0.12 ± 0.02	0.29 ± 0.03a	0.18 ± 0.03b	0.19 ± .01bc	0.12 ± 0.02ns

Units: Cytochrome P_{450} reductase – μmol of NADPH oxidised/min/mg protein Cytochrome b_5 reductase- μmol of NADH oxidised FeCN/min/mg proteinValues are mean ± SD of six observations Comparisons are made between: a –Group I vs Group II & V; b – Group II vs Group III & IV; c – Group III vs Group IV Statistical significance: a,b,c significant at p<0.05, ns-not significant.

The activities of xenobiotic enzymes cytochrome P_{450} reductase and cytochrome b_5 reductase in liver, kidney and mammary tissue were significantly ($p<0.05$) increased in DMBA induced (GroupII) rats when compared to control (Group I) rats. In *F.glomerata* leaf extract treated (GroupIII) rats showed significant ($p<0.05$) decrease in the levels of cytochrome P_{450} reductase and cytochrome b_5 reductase when compared with DMBA induced rats. Tamoxifen administered (Group IV) rats also showed the above mentioned enzyme activities were near normal to control rats. Plant extract alone administered rats (Group V) did not show any significant changes when compared to control rats.

(iii) Mitochondrial TCA cycle marker enzymes

The activities of ICDH, MDH, SDH and NADH in liver, kidney and mammary tissue of control and experimental rats were represented in the tables 5, 6, 7.

Table 5
Effect of TCA cycle marker enzymes in liver of control and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V
SDH	38.38 ± 0.04	22.52 ± 0.63a	32.51 ± 0.06b	34.57 ± 0.41bc	39.17 ± 0.13a
MDH	339.17 ± 13.73	269.93 ± 11.68a	290.60± 17.00b	297.97 ± 8.69b	338.65±20.32ns
ICDH	0.69 ± 0.11	0.41 ± 0.04a	0.57 ± 0.02b	0.60 ± 0.01bc	0.70± 0.11 ns
NADH	20.23 ± 0.96	9.32 ± 0.36a	15.37 ± 0.46b	16.60 ±1.33bc	20.31 ± 0.98 ns

Table 6
Effect of TCA cycle marker enzymes in kidney of control and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V
SDH	28.50 ± 2.80	16.42 ± 0.44a	24.36 ± 1.81b	25.73 ± 0.55bc	28.62 ± 0.54 ns
MDH	274.53 ± 15.24	189.33 ± 8.20a	230.38±8.11b	239.92±10.40b	275.3±13.75 ns
ICDH	0.62 ± 0.08	0.46 ± 0.03a	0.54 ± 0.04b	0.54 ± 0.08bc	0.63± 0.04 ns
NADH	8.23 ± 0.35	4.78 ± 0.27a	6.47 ± 0.26b	7.01 ± 0.13bc	8.22 ± 0.36 ns

Table 7
Effect of TCA cycle marker enzymes in mammary tissue of control and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V
SDH	25.95 ± 0.58	12.51 ± 0.37a	19.30 ± 0.51b	19.07 ± 0.44b	26.15 ± 0.32a
MDH	285.52 ± 20.12	177.85 ± 26.81a	242.43±19.14b	253.21 ± 4.57b	286.48±22.37ns
ICDH	0.42 ± 0.08	0.19 ± 0.06a	0.33 ± 0.04	0.35 ± 0.02bc	0.42 ± 0.09 ns
NADH	12.34 ± 0.50	7.31 ± 0.13a	9.99 ± 0.33b	10.20 ± 0.37b	12.15± 0.66 ns

Units: SDH – µmoles of succinate oxidised /min/mg protein MDH - nmol of NADH oxidised/min/mg protein ICDH - nmol of pi liberated/min/mg protein NADH dehydrogenase- - µmol of NADH oxidised/min/mg protein
Values are expressed as mean ± SD of six observations Comparisons are made between: a –Group I vs Group II & V; b – Group II vs Group III & IV; c – Group III vs Group IV Statistical significance: a,b,c significant at p<0.05, ns-not significant.

The activities of ICDH, MDH, SDH and NADH were significantly (p<0.05) decreased in carcinoma bearing (Group II) animals when compared to control (Group I) rats. In *F. glomerata* leaf extract treated (Group III) rats, the activities of ICDH, MDH, SDH and NADH were significantly increased (p<0.05) in liver, kidney and mammary tissues when compared to mammary carcinoma induced (Group II) rats. Tamoxifen administered (Group IV) rats also showed the above mentioned enzymes activities were near normal to control rats. No significant variations were observed in plant extract control (Group V) rats when compared to control rats.

DISCUSSION

Numerous studies have explained that DMBA induces substantial oxidative effects in liver and an oxidant closely associated with tumor promotion, is correlated with our study[21]. Mammary carcinoma bearing animals, there was a sharp drop in their body weight. This may be due to the cancer *cachexia.* Cancer *cachexia* result in progressive loss of body weight, which is mainly accounted by wasting of host body compartments such as skeletal muscles and adipose tissue[22].The inhibiting property of the plant extract may be due to the presence of flavonoids, as they have been reported to impart antiproliferative action on several cancer cells[23]. A counteractive property was observed in *F.glomerata* leaf extract treated rats, where the gain was observed.Cytochrome P_{450} reductase and Cytochrome P_{450} reductase levels were significantly elevated in group II rats, whereas their levels restored back to near normal in group III animals treated with *F.glomerata.* Although hepatic enzymes are likely to play a role in the metabolism of potential breast carcinogens. It is probable that enzymes locally expressed in the target site also have an important influence in modulating levels of DNA reactive species[24].*Ficus glomerata* extract with a similar potential could be notified pharmacological and antitoxicological agent. The obtained mitochondrial enzymes restorative in mammary tissues in addition to liver and kidney refers that extract may be potent with flavanoid that could suppress carcinogenic effects of DMBA induced carcinogenisis by reverting that xenebiotic phase I enzymes[9]. The reduction in the activities of MDH, SDH, NADH and ICDH in the mitochondrial fractions of mammary carcinoma rats might prove the defect in aerobic oxidation of pyruvate, which might cause the low production of ATP molecules[25]. Decreased activity of these enzymes might also be due to the alteration in the morphology and ultra structure of cancer cells[26]. Therefore, our results suggested that the DMBA, due to its ability to generate ROS, inactivate mitochondrial enzymes. *F.glomerata* treatment protects the mitochondria from peroxidation and subsequent inactivation of enzymes caused by mammary

carcinoma. This observation of *F.glomerata* treatment is supported by a previous report on mammary carcinoma where the reduced activities of TCA cycle enzymes were replenished by *Semecarpus anacardium* treatment[27].From the results of above mentioned biochemical parameters it was concluded that, *Ficus glomerata* leaf extract possess potent antioxidant and anticancer activities.

REFERENCES

1. Jemal A, Thomas A, Murray T, Thun M, Cancer statistics. CA.Cancer J.Clin, 52: 23-47, (2002).
2. Yager JD, Davidson NE, Estrogen carcinogenesis in breast cancer. N. Engl. J. Med, 354: 270–282, (2006).
3. Haas S, Pierl C, Harth V, Pesch B, Rabstein S, Bruning T, Ko Y, Hamann U, Justen Hoven C, Brauch H, Fischer HP, Expression of xenobiotic and steroid hormone metabolizing enzymes in human breast carcinomas. Int. J. Cancer, 119: 1785–1791, (2006).
4. Cadenas E, Davies KJA, Mitochondrial free radical generation, oxidative stress and aging. Free Radic. Biol. Med, 26: 222–230, (2000).
5. Gutierrez J, Ballinger SW, Darley-Usmar VM, Landar A, Free radicals, mitochondria, and oxidized lipids: the emerging role in signal transduction in vascular cells. Circ. Res., 99: 924–932, (2006).
6. Russo J, Russo IH, Experimentally induced mammary tumors in rats. Breast Cancer Res Treat, 39: 7-20, (1996).
7. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N, Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. J. Ethnopharmacol, 92: 161–166, (2004).
8. Anbuselvam C, Vijayavel K, Balasubramanian MP, Protective effect of *Operculina turpethum* against 7, 12-dimethylbenz(a)anthracene inducedoxidative stress with reference to breast cancer in experimental rats. Chem. Biol. Int, 168: 229–236, (2007).
9. Mathivadhan P, Shanthi P, Sachdanandam P, Effects of *Semecarpus anacardium* Linn. nut extract on mammary and hepatic expression of xenobiotic enzymes in DMBA-induced mammary carcinoma. Environ. Toxicol. Pharmacol, 23: 328–334, (2007).
10. Trivedi CP, Shinde S, Sharma RC, Preliminary phytochemical and pharmacological studies on *Ficus racemosa* extract (Gular). Indian J. Med. Res, 57 (6): 1070–1074, (1969).
11. Merchant JR, Bakshi VM, Engineer AB, Chemical investigation of fruits of *Ficus glomerata* Roxb. Indian J. Chem, 17B: 87–88, (1979).
12. Baruah KK, Gohain AK, Chemical composition and nutritive value of Dimaru (*Ficus glomerata* Roxb.) leaves. Indian J. Anim. Nutr, 9 (2): 107–108, (1992).
13. Khan N, Sultana S, Chemomodulatory effect of *Ficus racemosa* extract against chemically induced renal carcinogenesis and oxidative damage response in wistar rats. Life Sci, 77: 1194–1210, (2005).
14. Rao Ch.V, Verma AR, Vijayakumar M, Rastogi S, Gastroprotective effect of Standardized extract of *Ficus glomerata* fruit on experimental gastric ulcers in rats. J. Ethnopharmacol, 115: 323–326, (2008).
15. Omura T, Takasue S, A new method for simultaneous purification of cytochrome b_5 and NADPH-cytochrome c reductase from rat liver microsomes. J. Biochem, 67: 249-257, (1970).
16. Mihara K, Sato R, Partial purification of cytochrome b_5 reductase from rabbit liver microsomes with detergent and its properties. J. Biochem, 71: 725-735, (1972).

17. Slater EC, Bonner WD, Succinate dehydrogenase. Biochem. J, 2: 182-196, (1952).
18. Mehler AH, Kornberg A, Grisolia S, Ochoa S, The enzymatic mechanism of oxidation-reductions between malate or isocitrate and pyruvate. J. Biol. Chem, 174: 961-977, (1948).
19. Minakami S, Ringer RL, Singer TP, Studies on respiratory chain linked dihydrodiphosphopyridine nucleotide dehydrogenase. J. Biol. Chem, 237: 569-576, (1962).
20. Bernt E, Bergmeyer HV, Methods of enzymatic analysis. Academic Press, New York, (1974).
21. Izzotti A, Camoirano A, Cartiglia C, Grubbs CJ, Lubet RA, Kelloff GJ, DeFlora S, Patterns of DNA adduct formation in liver and mammary epithelial cells of rats treated with 7,12dimethylbenz(*a*)anthracene, and selective effects of chemopreventive agents. Cancer Res, 59: 4285–4290, (1999).
22. Argiles JM, Bieto JA, The metabolic environment of cancer. Mol. Cell. Biochem, 81: 3-17, (1988).
23. Williams JA, Philips DP, Mammary expression of xenobiotic metabolizing enzymes and their potential role in breast cancer. Cancer Res, 60: 4667-4677, (2000).
24. Brit DF, Hendrich S, Wang W, Dietary agents in cancer prevention; flavanoids and isoflavonoids. Pharmacol.Ther, 90: 157-177, (2001).
25. Senior AE, McGowan SE, Hilf R, A comparative study of inner membrane enzymes and transport systems in mitochondria from R3230AC mammary tumor and normal rat mammary gland, Cancer Res, 35: 2061–2067, (1975).
26. Pedersen PL, Tumor mitochondria and the bioenergetics of cancer cells, Prog. Exp. Tumor Res, 22: 190–274, (1978).
27. Arathi G, Sachidanandam P, Therapeutic effect of *Semecarpus anacardium* Linn. nut milk extract on carbohydrate metabolizing and mitochondrial TCA cycle and respiratory chain enzymes in mammary carcinoma rats, J. Pharm. Pharmacol, 55: 1283–1290, (2003).

This article can be downloaded from www.ijpbs.net