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Research Article

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Naringin Attenuates DMBA-Induced Mammary Carcinogenesis in Rats via Regulating the Oxidative Stress and Antioxidants Status

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ABSTRACT

The present study was aimed to investigate the antioxidant and anti-carcinogenic efficacy of naringin on DMBA-induced mammary carcinogenesis in Sprague Dawley (SD) rats. Female SD rats were induced orally with a single dose of DMBA (80 mg/kg b.wt). Naringin (30 mg/kg b.wt. twice a week) was orally administered before and after tumor induction for 16 weeks. The levels of serum tumor markers viz. CEA, AFP and CA 15-3 were quantified by ELISA method. Biochemical analysis showed that serum LPO, NO, AST, ALT, ALP, ACP, LDH, 5'ND and GGT were increased while enzymatic and non-enzymatic antioxidants were significantly ($P<0.05$) reduced on DMBA-induced rats. Interestingly, naringin treatment inhibited the incidence rate and tumor volume as well as regularized the levels of biochemical parameters and antioxidants. Additionally, altered ductal epithelial architectures and membrane ruffles were recovered upon naringin treatment as revealed in histological and scanning electron microscopy respectively. Together, our results suggest that naringin could attenuate the mammary carcinogenesis by balancing the oxidative stress and augmenting antioxidant status.

Keywords: Naringin; DMBA; Oxidative stress; Antioxidants; CEA; Chemoprevention

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Abbreviations: DMBA: 7,12-dimethylbenz(a)-anthracene; LPO: Lipid Peroxidation; NO: Nitric Oxide; CEA: Carcinoembryonic Antigen; AFP-α: Feto Protein; CA15-3: Cancer antigen 15-3; LDH: Latate dehydrogenase

INTRODUCTION

Breast cancer (BC) is the second most common malignancy among women in worldwide, with increasing incidence in recent years in India. The National Cancer Registry Programme (NCRP) of India reported that 0.15 million new cases and about 76,000 deaths were recorded to BC [1]. BC originates from the breast tissue, most commonly from inner lining of milk ducts (DCIS) or lobules (LCIS) that supply the ducts with milk which are susceptible regions for tumor microenvironment [2]. Majorly there are known risk factors such as diet, hormonal status, genetic factors, radiation exposure and environmental pollutants as associating influences in the etiology of the disease. DMBA (7,12-dimethylbenz(a)-anthracene) a potent environmental carcinogen, is known to induces several cancers viz. breast, lung, oral, and skin. The carcinogenic action is initiated by the enzymatic reduction of its nitro group, generating free radicals that result in oxidative stress and cellular dysfunctions which leads initiation of neoplasm [4,5]. Oxidative stress has been influenced by an imbalance between the antioxidants and production of free radicals such as superoxide anion ($O2^{-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^{-}) which causing oxidative damage and tissue injuries [6]. Recently, increasing evidence indicated that an excessive amount of ROS is positively associated with the impaired functions of cellular macromolecules such as lipids, proteins and DNA; further altering the intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, inhibition of protein synthesis and DNA adduct formation which drives mutagenesis and malignant transformation [7]. Further, ROS-mediated DNA damaged to futile DNA

repair mechanisms are studied both *invtro* and *invivo* experiments [8]. Therefore, the model of DMBA-induced breast cancer in rats was considered as one of the well established model to study about breast carcinogenesis in rats which mimics the morphology and histology of humans [9]. Although, wether the oxidadive stress influnced breast cancer meadiated by imbalanced antioxidants remains unclear.

In recent years, cancer chemotherapy is increasingly recognized as a significant factor in cancer treatment response. There are several neoplastic chemotherapy drugs available to the treat breast cancer patients (10), through above said modality is not satisfactory due to recurrence and unfavorable side effects. Recently, plant derived bioactive compounds have shown that flavonoids have the tendency to modulate the biological actions in several cancers. Flavonoids are a group of natural polyphenolic compounds which owns flavones, flavanones and isoflavones exists in fruits and vegetables regularly consumed by humans [11]. Naringin (4,5,7-trihydroxyflavanone 7-rhamnoglucoside), a flavonone glycoside abundantly present in citrus fruits that has broad spectrum of pharmacological activities including anti-oxidant, anti-tumor, neuroprotective and anti-inflammatory properties [12,13]. Pervious evidence showed that naringin and hesperidin combination has potential anti-inflammatory effects on the rat air pouch model [14]. However, there is limited knowledge regarding the chemoprotective effect of naringin alone on experimental mammary carcinogenesis in rats. Hence, the present study was embarked to investigate the antioxidants and anti-carcinogenic efficacy of naringin on DMBA induced mammary cancer in female Sprague Dawley rats.

EXPERIMENTAL SECTION

Chemicals

Naringin (4,5,7-trihydroxyflavanone 7-rhamnoglucoside) and 7,12-dimethylbenz[a]anthracene (DMBA) were procured from Sigma Chemicals Co. (St. Louis, MO, USA). Corn oil was purchased from Aroma essential oils, Haryana, India. All other chemicals used were of analytical grade (AR), purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Animals

Virgin female Sprague-Dawley rats 50-65 days old weighing around 110-130 g were obtained from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai, India. They were housed in clean polypropylene cages, maintained in air-conditioned animal house with standard temperature (26 ± 2°C) of 12 h light/dark cycle. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics committee (IAEC) Guidelines. During the experiments, all efforts were made to minimize animal suffering.

Experimental Protocol

Experimental rats were randomly divided into five groups (n=6 rats/group) as depicted in Figure 1

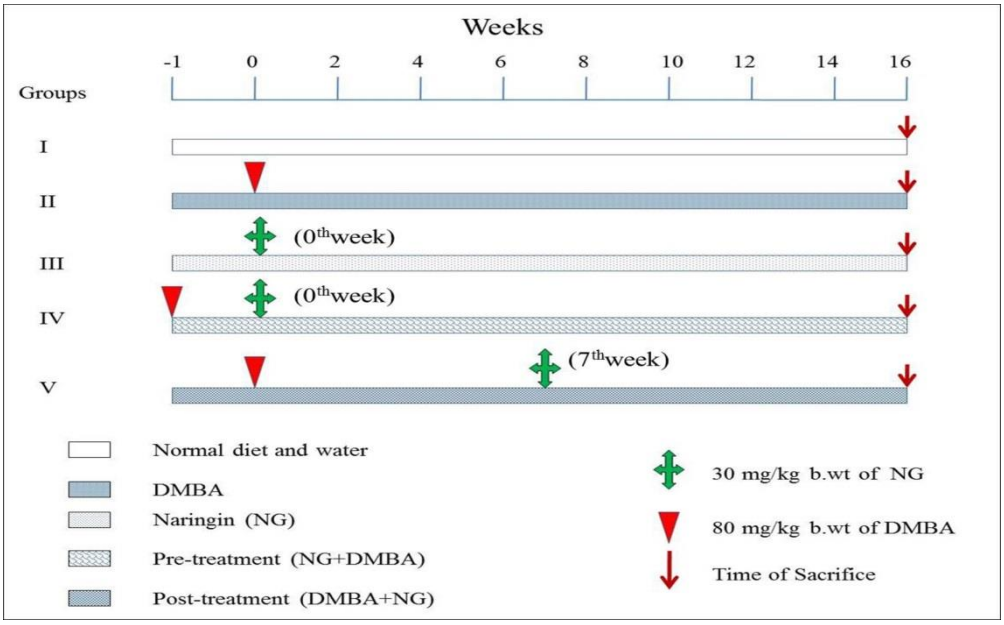

Figure 1: Schematic representation of experimental design

Group 1 normal control rats received 0.5 mL of corn oil for weekly twice and reset of the days receiving pure drinking water.
Group 2 rats were administered with DMBA (80mg/kg.b.wt dissolved in 0.5 ml of corn oil) at once.
Group 3 rats received naringin alone twice a week at a dose of 30 mg/kg.b.wt for 16 weeks.
Group 4 rats served as pre-treatment subjected by naringin (30 mg/kg.b.wt) 1 week prior to the administration of DMBA as shown in the group 3.
Group 5 rats served as post-treatment induced by DMBA after 7 weeks of mammary carcinoma, the rats were treated with naringin 30 mg/kg.b.wt for the entire experimental period.
Changes in the body weight of control and experimental groups of rats were monitored periodically during the experimental period. Incidence and tumors weight was estimated according to the method of Harris et al., [15]. At the end of 16th weeks, rats were fasted overnight and euthanized. Whole blood was collected. Serum was obtained after blood coagulation and centrifugation at 10,000 rpm for 10 min, and stored at -80ºC for further analyses. The breast tumors were excised and rinsed with ice-cold saline to remove the residual blood and stored at -80C for future analysis. A portion of the breast tissue was homogenized in 0.1 M Tris buffer, pH 7.4, and used for the further analysis.

Scoring
After the 16 weeks of experimental period, rats were anesthetized using intramuscular injection of ketamine (40–87 mg/kg) and xylazine (5–13 mg/kg) and sacrificed by cervical decapitation. For each animal, the entire intact lower abdominal mammary gland was removed and cut longitudinally, cleaned with saline to remove blood residues, blotted dry on a paper towel, weighed, and photographed.

Histopathological Examination
Hematoxylin-eosin staining was performed by the method of Elston et al. [16]. Scanning electron microscopy was carried out as described in earlier [17]. Briefly, after sacrifice, breast tissues were dissected out from rats and washed with phosphate-buffered saline (PBS). Tissues were fixed in Karnovsky's fixative (2.5 % glutaraldehyde and 2 % paraformaldehyde, in 0.1 M phosphate buffer, pH 7.4) for 6 hrs at 4 °C. Then, tissues were dehydrated using ascending series of graded alcohol concentrations (30-100%). Samples were cut into small pieces and placed on the stubs and put into E-1010-Hitachi Ion coater for gold coating and examined with a Zeiss EVO40 SEM instrument and photomicrographs were taken.

Biochemical Parameters
Total protein content was estimated by the method of Lowry et al. [18]. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) [19], alkaline phosphatase (ALP), acid phosphatase (ACP) [20], lactate dehydrogenase (LDH) [21], 5'-nucleotidase (5'ND) [22] and γ-glutamyl transpeptidase (GGT) [23] were determined in serum.

Estimation Antioxidants Status
The activities enzymatic and non-enzymic antioxidant such as superoxide dismutase (SOD) [24], catalase (CAT) [25], glutathione peroxidase (GPx) [26], glutathione reductase (GR) [27], glutathione-S- transferase (GST) [28], vitamin C [29], vitamin E [30] and reduced glutathione (GSH) [31] were estimated in breast tissue homogenate. The levels of Nitric oxide (NO) [31] and lipid peroxidation (LPO) [32] products were estimated in both serum and breast tissue.

Quantification of Tumor Markers
The levels carcinoembryonic antigen (CEA), α-feto protein (AFP) and Cancer antigen 15-3 (CA15-3) were determined based on solid phase enzyme linked immunosorbent assay (ELISA) using the UBI MAGIWELL (USA) enzyme immunoassay kit [33].

Statistical Analysis
Data were expressed as mean ± Standard Deviation (S.D). Statistical analysis was carried out using one-way analysis of variance followed by Tukey's post hoc test using GraphPad Instat (version 5.0 for Windows 7; Graph Pad Software, San Diego, CA). Statistical significance was defined as p values less than 0.05. All the experiments were performed at least three times.

RESULTS

Naringin Inhibits Tumor Weight and Incidence Rate
The average body weight, tumor weight and incidence rate of control and experimental group of rats were summarized in Table 1 DMBA-induced Group II rats showed reduced average body weight, subsequent tumor weight and incidence was markedly increased (3-fold) and (90%) respectively as compared to normal control

Group I rats. Whereas, naringin (30 mg/kg b.wt) pre and post-treated rats exhibited significantly increased average body weight, simultaneous tumor weight and incidence was reduced (2-fold) and (69%) respectively than DMBA induced rats. There were no such changes (average body weight, tumor weight and incidence) observed in normal control Group I and naringin alone treated Group III rats. Interestingly, all these results were found to be statistically significant ($p< 0.05$).

Table 1: Effect of naringin on tumor weight and incidence of control and experimental rats. Values are expressed as mean ± SD (n=6 rats/each group). Statistical significance was set at p<0.05 compared with aControl, bDMBA, cDMBA, and #Control (non-significance) based on Tukey's post hoc multiple test

S.No	Experimental Groups	Initial Body weight(g)	Final Body weight (g)	Tumor incidence (%)	Average Tumor weight (g)
1	Control	123.16 ± 6.58	218.16 ± 10.49	--	--
2	DMBA	125.00 ± 6.38	178.00 ± 7.26a	90 a	7.9±1.81 a
3	Naringin	121.16 ± 6.40	219.83±10.77 #	--#	-- #
4	NG +DMBA	124.00 ± 6.09	201.16 ± 8.93 b	23 b	2.1±0.78 b
5	DMBA+NG	123.83 ± 6.94	183.50 ± 7.84 c	69 c	5.2±1.12 c

Effect of Naringin on Mammary Neoplasm

Figure 2 shows the gross examination of breast tumors in control and experimental group of rats. DMBA-induced group II rats showed malignant tumor mass present in mammary gland of lower abdominal right flank (Figure 2b). Whereas, naringin pre and post-treated rats showed palpable tumor at the armpit (Figure 2d) and pre-malignant tumor in lower abdomen left flank (Fig. 2e) respectively. While, there is no visible tumor was observed in the mammary gland of normal control and naringin alone treated rats (Figure 2a and 2c).

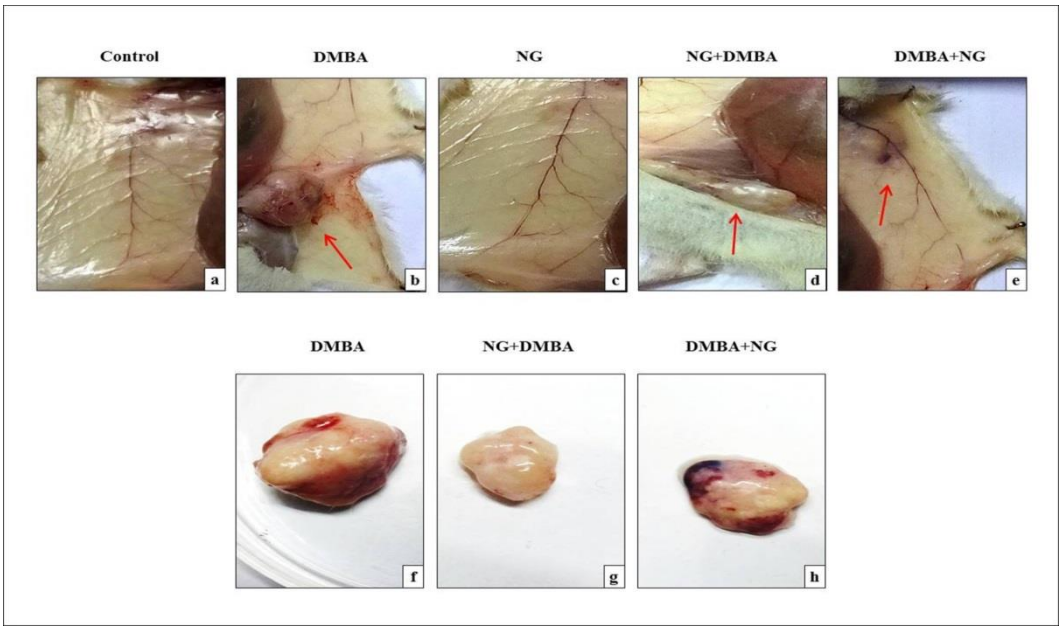

Figure 2: Effect of naringin on mammary neoplasm. a. control and c. naringin (↔ showed intact mammary gland), b. DMBA (↑ depicted solid tumor mass in lower abdomen), d and e. naringin pre and post-treatment (↑ indicates benign and premalignant tumor in right flank inguinal region respectively), f, g and h. showed tumor mass, benign and premalignant tumors respectively

Effect of Naringin on Breast Histology

Figure 3 shows the histological examinations of breast tissues of control and experimental group of rats. DMBA-induced group II rats (Fig. 3b) (↔) showed the altered epithelial architectures, which was characterized by massive epithelial proliferation and invasive ductal carcinoma. While, naringin pre and post-treated rats (Fig. 3d, e) (→) shows improved ductal architecture with lesser proliferation and mild hyperplasia. The control and naringin alone treated rats (Fig. 3a, c) showed intact ductal architecture with uniform epithelial cells.

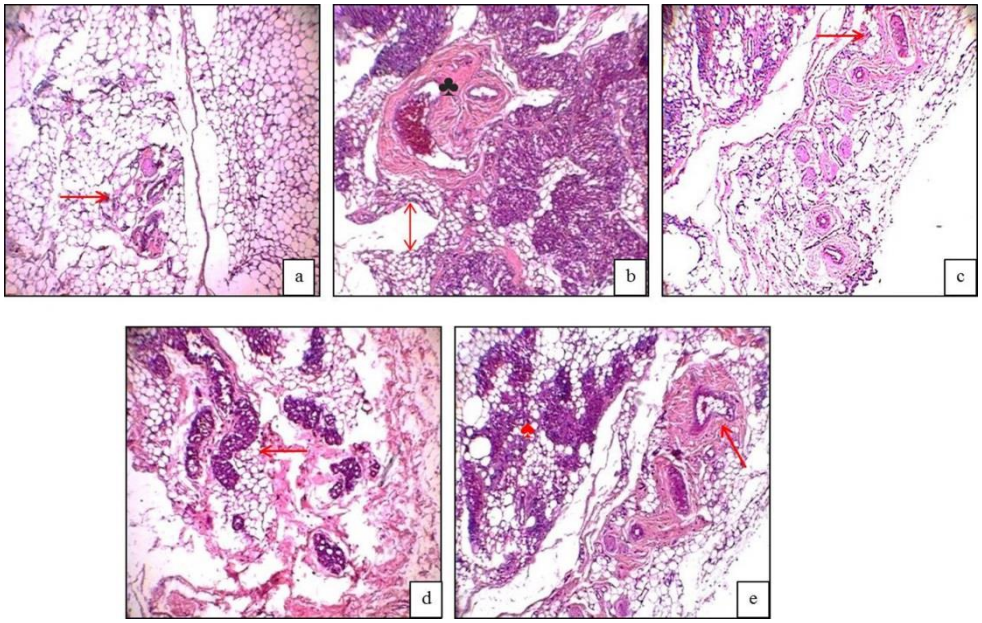

Figure 3: Histopathological observations of breast sections of control and experimental group of rats (hematoxylin and eosin staining-10x). a. control and c-naringin (↑ showed intact ductal architecture with clear lumen), b. DMBA (↔ indicates ductal hyperplasia with increased cluster of multiple groups of adenoma cells, ♣ stromal fibrosis and lymphocytic infiltration), d and e. naringin pre and post-treatment (↑ indicates improved cellular architecture with reduced mammary gland damage and ♠ moderate adenocarcinoma and lymphocytic infiltration respectively)

Effect of naringin on structural changes

The scanning electron microscopic images of breast tissues of control and experimental group of rats were represented in Figure 4. The control and naringin alone treated rats (Figure 4a and 4c) (*) showed organized breast lobules with smooth and supple texture. Whereas, DMBA-induced rats (Figure 4b) (↕) showed scattered cell surface with ruptured membrane ruffles and distorted surface territories. However, naringin pre and post-treated rats (Figure 4d and 4e) showed reverted membrane ruffles with regularized blebs.

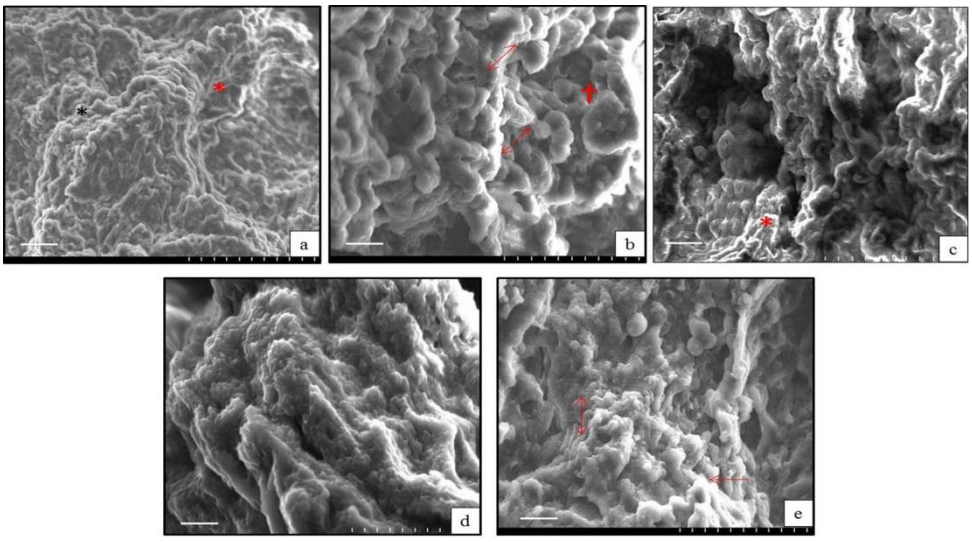

Figure 4: Scanning electron microscopic architecture of breast sections of control and experimental group of rats. a. control (* indicates high density of surface microvilli with apical cytoplasmic membrane, c. naringin was found to be similar to that of control, b. DMBA (↔ represents distorts villous pattern with irregular pitted epithelial surface and (†) depicts clefted surface territories. d and e. naringin pre and post-treated (♠ shows uniform microvillus over the membrane surface and (↔) regularized membrane blebs respectively. Scale bar-20 µm.

Effect of naringin in biochemical markers

The effect of naringin on the levels of biochemical markers in serum of control and experimental group of rats were portrayed in Table 2 The levels of AST, ALT, ALP, ACP, 5' NTD, GGT and LDH were significantly ($p<0.05$) increased in DMBA-induced group II rats as compared to normal control Group I rats. Contrary, naringin pre and post-treated rats exhibited significantly reduced levels of those markers as compared with

DMBA-induced rats. There were no such significant changes were observed in control and naringin alone treated group of rats.

Table 2: Effect of naringin on the levels of biochemical parameters. Values are expressed as mean ± SD (n=6 rats/each group). Statistical significance was set at p<0.05 compared with aControl, bDMBA, cDMBA, and #Control (non-significance) based on Tukey's post hoc multiple test. Units: GGT is expressed in µM of p-nitroaniline liberated/mg protein/min; 5'ND is expressed in µM of inorganic phosphate liberated/mg protein/min

Parameters	Control	DMBA	Naringin	NG+DMBA	DMBA+NG
AST	4.70 ± 0.41	6.37 ± 0.50 a	4. 71 ± 0.43 #	5.21 ± 0.38 b	5.57 ± 0.46 c
ALT	26.05±2.22	42.61±3.89 a	26.29±2.80 #	32.49±2.37 b	39.17±3.11 c
ACP	5.0±0.52	7.18±0.57 a	5.26±0.47 #	6.23±0.57 b	6.58±0.60 c
ALP	176.25±12.58	254.19±23.97 a	174.44±12.30 #	201.15±14.09 b	218.67±16.05 c
GGT	1.93±0.02	3.01±0.54 a	1.87±0.06 #	2.11±0.91 b	2.72±0.79 c
5'ND	2.72±0.29	4.70±0.45a	2.73±0.30#	3.25±0.38b	3.67±0.51c

Effect of naringin on the activities of antioxidants status

Figure 5a-5d shows the antioxidants activities in breast tissues of control and experimental group of rats. DMBA-induced Group II rats showed a significant regression in the activities of enzymic (SOD, CAT, GPx, GR, and GST) and non-enzymic (vitamin-C, vitamin-E and GSH) antioxidants than the normal control Group I rats. Naringin pre and post-treated rats showed a significant elevation in the levels of those antioxidants versus DMBA-induced rats. Whereas, control and naringin alone treated rats doesn't showed any significant changes in their antioxidant activities.

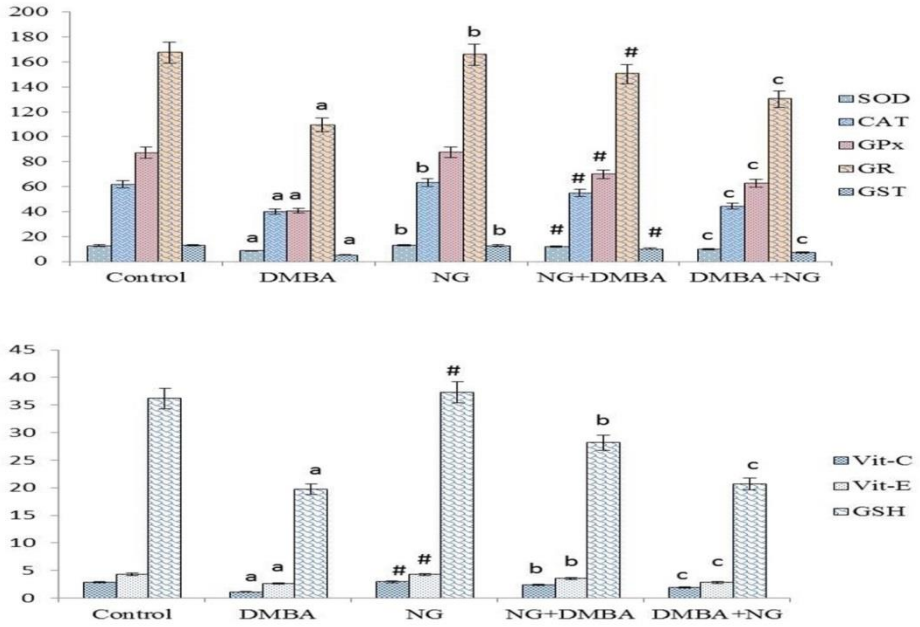

Figure 5: Effect of naringin on the activities of antioxidants of control and experimental rats. Values are expressed as mean ± SD (n=6 rats/each group). Statistical significance was set at p<0.05 compared with aControl, bDMBA, cDMBA, and #Control (non-significance) based on Tukey's post hoc multiple test. Units: SOD: 50% inhibition of adrenaline auto oxidation/min; CAT: lM H2O2 consumed/mg protein/min; GPx: lg GSH utilized/mg protein/min; GR: nM NADPH oxidized/mg protein/min; GST: µmol CDNB-GSH conjugate formed/min/Hb

Assessment of oxidative stress markers

The effect of naringin in the levels of LPO and NO in both serum and tissue of control and experimental groups of rats were depicted in Figure 6a and b respectively. In DMBA induced group II rats shows that there was a significantly ($p<0.05$) increased activities of LPO and NO when compared with normal control group I rats. Whereas, naringin pre and post-treated rats showed significant reduction in their levels when compared to DMBA-induced rats. However, no adverse changes were observed in normal control Group I and naringin alone treated Group III rats.

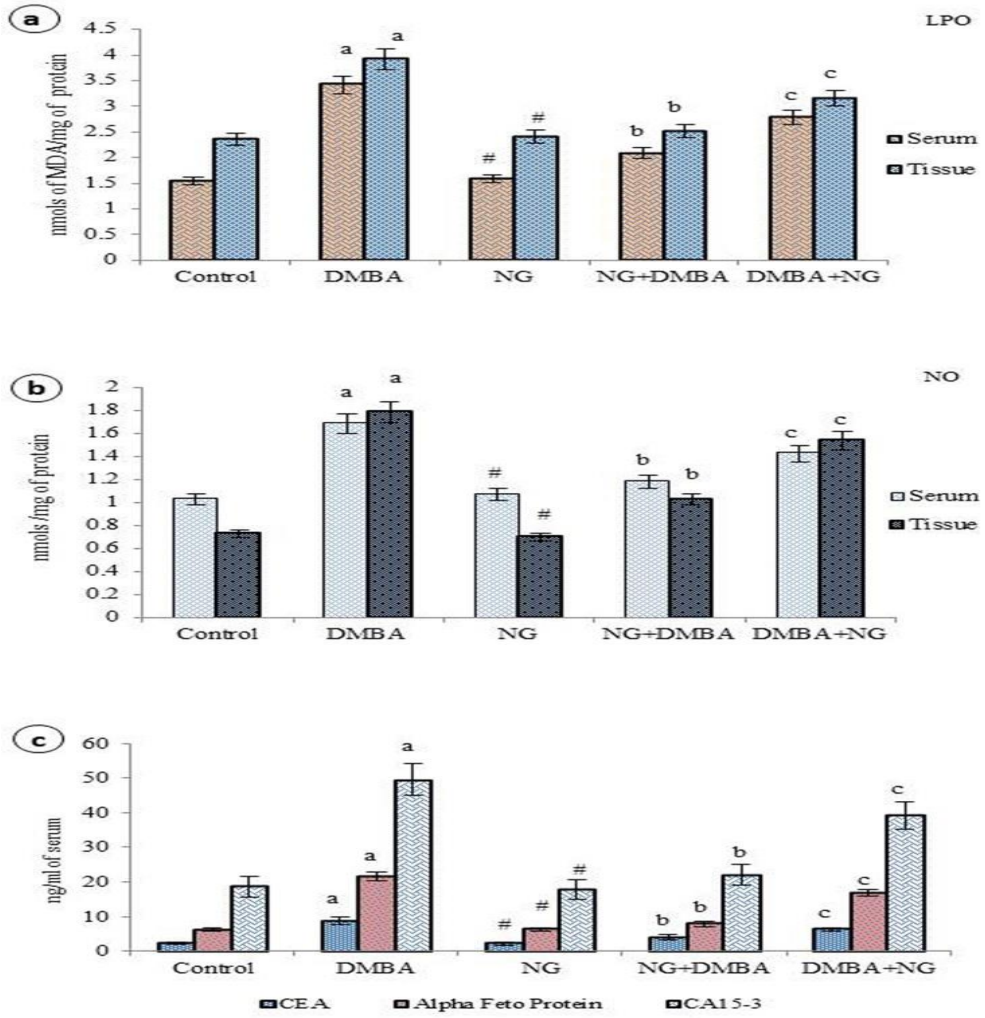

Figure 6: Effect of naringin on the levels of oxidative stress markes and tumor markers. (a) LPO, (b) NO and (c) CEA in serum and tissue of control and experimental group of rats. Values are expressed as mean ± SD (n=6 rats/each group). Statistical significance was set at p<0.05 compared with aControl, bDMBA, cDMBA, and #Control (non-significance) based on Tukey's post hoc multiple test

Effect of naringin on the levels of tumor marker

Figure 6c depicts the levels of CEA in serum of control and experimental group of rats. DMBA-induced group II rats showed a significant ($p<0.05$) elevated levels of CEA than the normal control Group I and naringin alone treated Group III rats. Whereas, naringin pre and post-treated rats exhibits significantly reduced CEA levels as compared to DMBA induced rats.

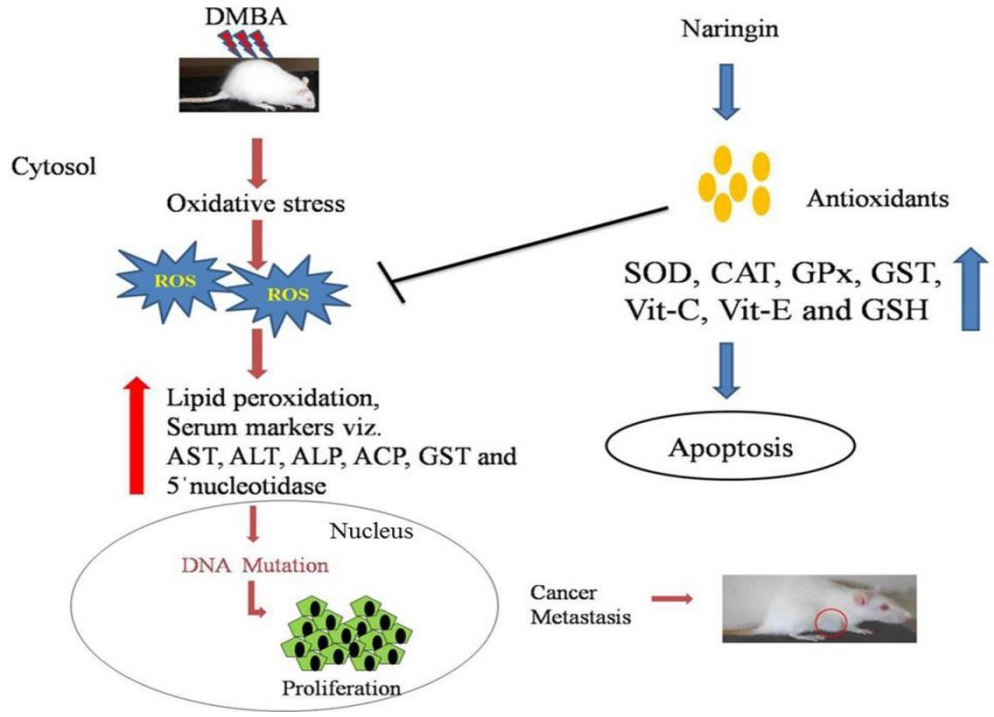

Figure 7: A simplified illustration showing the biochemical actions of naringin on DMBA-induced mammary carcinogenesis in rats. Naringin attenuate oxidative stress by augmenting antioxidants mechanism there by delays tumorgenesis formation

DISCUSSION

An increased free radical generation and/or reduced antioxidants may leads to several biochemical and pathological complications has been suggested to play key role in initiation of carcinogenesis [34]. In this study, we investigated the chemopreventive effect of naringin on DMBA-induced carcinogenicity in rats. DMBA-induced rats Table 1 showed a significant reduction in body weight due to their cancer cachexia which is directly correlates to cancer progression in patients [35] perhaps this may be due to the damage of skeletal muscle and adipose tissue. On naringin treatment opposes the loss of body weight by its counteractive and antioxidants property this can be evident from the gross evaluation of rats breast Figure 2 showed significant suppression of tumor formation. Besides, the constant intestinal absorption of naringin facilitates increased bioavailability, body weight gain and radical scavenging effects this could be positively coincided with Choudhury et al. 1999 [36]. Thus, it suggested that decreased tumor multiplicity, tumor incidence, and cancer neoplasm could be attributed in the setback of cancer initiation.

Oxidative metabolism of DMBA implicated the ROS production that capable of generating free radicals and depletion of antioxidants leads to peroxidation of membrane lipids results degeneration and/or tissue injury has been a sign of carcinogenesis [37]. Data also indicated that an increased lipid peroxidation and DNA adducts have been detected in mammary tissues of breast cancer patients Wang et al. [38]. Our results were in agreement with above findings in that we observed significant reduction in enzymic (SOD, CAT, GPx, GR, and GST) and non-enzymic (vitamin E, vitamin C and GSH) antioxidants as given in Figure 5a and 5b and subsequent increased levels of LPO and NO in Figure 6a and b respectively on cancer-bearing rats this may be due to the mutagenic potency of carcinogen it might be the primary events of mutagenesis. Conversely, naringin treatment significantly elevated antioxidants and reduces LPO and NO levels perhaps this might have their direct scavenging mechanism of ROS by donating hydrogen atom from secondary metabolites of flavonoids and also their total number of hydroxyl groups could be attributed to appreciably raise the influence of antioxidant activity. Concurrently, in our results narinign pre-treatment promoted the better results than the post-treatmet because of intoxication of carcinogen to rats. In early stages could actively combats whereas in post-treatment condition cancer can be in aggressive proliferation and malignant transformation which allows minimal therapeutic effects.

Antioxidants are substances that detain, prevent or remove oxidative damage by chelating trace elements or by inhibiting the enzymes involved in free radical production. This goes in accordance with the findings of Hanasaki et al. 1994 [39] which implies that endogenous antioxidants certainly combating the reactive free radicals. On the other hand, vitamin C and E is the chief constituent of aqueous and lipid soluble environment; and they play eminent role in antioxidant defense system possibly by the mechanisms of catalysis, regulation,

electron transportation and in preserving the correct structure of proteins. Our results are very well coincide with earlier findings suggesting that reduced oxidative stress might be the reasoning of excessive utilization of antioxidants. Therefore, we could suggested that naringin has potent antioxidants and pro-oxidant properties which can positively the attenuated oxidative stress. Similarly, Prochazkova et al. and Miller et al. 2008 [12,40,41] also reported that strong correlation between antioxidants and pro-oxidant properties of flavonoids definitely influences the cancer chemoprevention.

Furthermore, reduction of NO levels could be maintained by naringin treatment perhaps due to the inhibition of NO synthase enzyme which catalysis the oxidation of L-arginine has mainly enabled by peroxynitrite that organized in the reaction of NO with O2-. Nitric oxide can act as a chain-breaking oxidant to LPO, which involves in the process of oxidative degradation of polyunsaturated fatty acids (PUFA) that occurs in biological membranes causing the impaired structural integrity and reduced membrane fluidity were presumably associated with increased free radicals [42]. In this study, naringin treatment actively counterbalances the toxic effects of ROS through inhibiting oxidative damage of cell membranes. Hence, the exact antiperoxidative and lipid lowering mechanism could have resulted in inhibition of mammary carcinogenesis. Similarly, Jung et al. 2003 [43] also suggested that naringin supplementation reduces the serum LPO and increases antioxidants activities in hypercholesterolemic subjects.

Biochemical markers enzymes viz. transaminases, phosphatase, LDH, 5'nucleotidase and GGT served as a useful indicator for prognosis prediction and understanding the metastasis of breast cancer patients [44]. The abnormalities in their activities are considered to be a good correlation with the number of transformed cells in cancer conditions of rodents which presumed by the action of tissue damaged thereby primarily leakage of pathophysiological enzymes from cytosol into blood stream [45]. In concurrent with the above findings an elevated levels of AST, ALT, ACP, ALP, γGT, 5'ND and LDH were observed in cancer induced rats were given in Table 2 this could be the effect of tissue injury and impaired cell membrane integrity resulting cytoplasmic leakage of enzymes into the blood stream, this could be reasoning for persistent liberation of those enzyme into serum; naringin treatment significantly attenuated this alterations thereby showing the anticarcinogenic activity. Further, histopathological (Figure 3) and surface architectural (Figure 4) observations were conforms the mammary tissue protection by recovering altered epithelial architectures and reduced clefted surface territories. Accordingly, we can suggest that naringin could protect tissue damage through maintaining the membrane integrity, permeability and minimizing enzyme leakage. Similarly, Dong et al., 2015 and Alam et al., 2013 [46,47] stated that naringin prevent liver damage and improves cardiovascular dysfunction by maintaining pathophysiological enzymes respectively these are very well coincides with our reports.

Circulating tumor markers are proteins liberated into the blood streem by cancer cells that has been generally accepted tool for metastatic patients; instead it could be minimally achieved in benign tumors because of their low sensitivity and specificity. Carcinoembryonic antigen (CEA), an oncofetal glycoprotein normally found in epithelial mucosal cells and overexpressed in adenocarcinoma of the breast [48]. Alpha-fetoprotein is the vital protein of fetal serum synthesized by the yolk sac and the liver during fetal advancement. Cancer antigen 15-3 (mucinous antigen) is a surface glycoproteins for cell adhesion with O-linked oligosaccharide chains encoded by MUC-1 gene, which accompany examine the response to chemotherapy in metastatic breast cancer [49]. All these tumor markers were elevated in cancer induced rats (Figure 6c), whereas naringin treatment bring back those markers to near normal. Thus, we can suggested that naringin could possibily reduces the serum tumor markers.

CONCLUSION

Together, results of the present study clearly demonstrates that naringin has potent chemopreventive efficacy on DMBA-induced mammary carcinogenesis in rats (Figure 7) and this can be credited to its propensity to enhance antioxidant profiles; attenuated oxidative stress; LPO, NO, AST, ALT, ALP, ACP, LDH, 5'ND, CEA, AFP and CA 15-3 and also inhibits tissue damage by soothing membrane ruptures. Thus, we postulated that naringin may develop as promising chemotherapeutic agent. Further studies are required to explore the molecular mechanisms involved to prove naringin as an anticancer agent.

HIGHLIGHTS

DMBA-induced rats showed an elevated serum tumor markers such as CEA, AFP and CA15-3 accompained with increased incidence and tumor volume.

Naringin treatment regulated the oxidative stress by augmenting antioxidants status.

Naringin modulated the biochemical parameters and recovered the altered ductal epithelial architectures and membrane ruffles.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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