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RESEARCH ARTICLES

Studies on the biochemical and molecular effects of some natural herbs on experimental-induced breast cancer in Wistar rats

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ABSTRACT

Background: Breast cancer is the most frequent malignant tumor in women, and its metastatic state represents the second cause of mortality. To treat cancer, we must prevent tumor cell proliferation and angiogenesis. The usage of herbal medication for treatment of some diseases as diabetes is increased nowadays for their wide safety margin. **Aims and Objectives:** Therefore, the current study was designed to examine the protective effect of ginger extract (GE) and cinnamon extract (CE) in controlling the mammary gland incidence in female Wistar rats. **Materials and Methods:** A total of 75 female virgin Wistar rats were allocated into 5 groups. Control group without any treatment; mammary gland group administered 7, 12-dimethyl-benz[a]anthracene at a dose of 20 mg/kg orally in corn oil. Rats in 3-5 served as tumor groups and received GE for group 3 (0.125% in water), CE for group 4 (100 mg/kg), and a mixture of GE plus CE for group 5. GE and CE were administered 2 weeks before cancer induction and continued for 4 months. Serum levels of breast cancer biomarker (CA125) and oxidative stress biomarkers were measured. Tissues of tumors were examined at molecular and histopathological levels. **Results:** There was a significant increase in the serum levels of CA125 in untreated mammary gland tumor group when compared with untreated healthy rats. There was a significant decrease in the serum levels of glutathione (GSH) peroxidase, catalase, superoxide dismutase, and GSH reductase in the untreated mammary gland tumor groups compared to control healthy rats. Administration of ginger and CE normalized the decrease in antioxidants levels. Induction of mammary gland tumor upregulated the genes associated with tumor incidences such as GST-P, CYP1A1, CYP1B1, and vascular endothelial growth factor-receptor 1 and administration of both herbal plants normalized these changes. Coadministration of GE and CE-induced additive inhibitory effect on genes upregulated due to tumor incidence. Bax expression was downregulated in tumor group and increased after GE and CE expression either alone or in combination. At histopathological levels, breast cancer group showed adenocarcinoma that is decreased with plants extracts administration when compared with breast cancer and control group. **Conclusion:** Results suggest that extracts of ginger and cinnamon have anticarcinogenic protective activities and support the hypothesis that these plants help in the prevention of mammary cancer by controlling the expression of carcinogenesis-associated genes.

KEY WORDS: Herbal Medication; Carcinogenic Activity; Rats

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INTRODUCTION

Breast cancer is the most frequent malignancy diagnosed in women, and only second to lung cancer as a cause of cancer-related death.[1] Its incidence is increasing in all industrialized nations. The etiology of breast cancer is multifactorial, and the risk factors include early menarche, late menopause,

nulliparity, and late age at first birth, postmenopausal obesity, extended use of oral contraceptives, hormone replacement therapy, family history, and previous benign breast disease.[2] In addition to this, the common risk factor in the development of breast cancer is the increased lifetime exposure to endogenous or exogenous estrogens. In addition, oxygen free radicals generated by a number of processes *in vivo* are highly reactive and toxic.[3]

However, biological systems have evolved an array of enzymatic and non-enzymatic antioxidant defense mechanisms to combat the deleterious effects of oxygen free radicals. It is a well-known fact that oxidative stress arises when there is an imbalance between oxygen free radicals formation and scavenging by antioxidants. Excessive generation of oxygen free radicals can cause oxidative damage to biomolecules resulting in lipid peroxidation (LPO), mutagenesis, and carcinogenesis.[4] Free radicals are often generated by various environmental contaminants when exposed to living systems such as polycyclic aromatic hydrocarbons (PAHs). Sources of PAHs include industrial and domestic oil furnaces, gasoline, and diesel engines. PAHs are widely distributed in our environment and are implicated in various types of cancer. Enzymatic activation of PAHs leads to the generation of active oxygen species such as peroxides and superoxide anion radicals, which induce oxidative stress in the form of LPO. 7, 12-dimethyl-benz[a]anthracene (DMBA) acts as a potent carcinogen by generating various reactive metabolic intermediates leading to oxidative stress.[5] Moreover, DMBA-induced changes in breast gland progression and survival.

Human body is equipped with various antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), glutathione (GSH), ascorbic acid (Vitamin C), and α-tocopherol (Vitamin E), which can counteract the deleterious action of ROS and protect from cellular and molecular damage induced by some substances such as DMBA.[6]

Bioactive compounds of some herbs, such as ginger and cinnamon, have the potential to subside the biochemical imbalances induced by various toxins associated with free radicals. They provide protection without causing any side effects, and therefore, development of drugs from plant products is desired. Many plant extracts and plant products have been identified as good protectors against the free radicals by triggering antioxidant gene expression and probably affect the gene expression of tumor-associated factors.[7] For that account, natural antioxidants from plant sources have been viewed as promising therapeutic drugs.[8,9]

Ginger is belonging to a tropical and sub-tropical family – Zingiberaceae, originating in South-East Asia and introduced to many parts of the globe, has been cultivated for thousands of years as a spice and for medicinal purposes.[10] Ginger has numerous functions among which are the anticarcinogenic activities. Cinnamon is a popular flavoring ingredient, widely used in food products. In addition to its flavoring application, cinnamon has exhibited health beneficial properties, such as antimicrobial activity, for controlling glucose intolerance and diabetes, inhibiting the proliferation of various cancer cell lines, and treating common cold.[11,12] Cinnamon extracts (CE) were able to reduce LPO and exhibited a protective capacity against irradiation-induced LPO in liposomes.[12]

Because of the wide safety margin of herbal plants medication, the current study was outlined. Therefore, this study was aimed to examine the protective effect of both ginger and CE to prevent the progression and changes in genes expression associated with induction of mammary gland cancer. The changes were examined at biochemical, molecular, and histopathological levels in female virgin Wistar rats.

MATERIALS AND METHODS

Chemicals and Kits

Ethidium bromide and agarose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Female Wistar rats were purchased from King Fahd Center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia. Kits for CAT, malondialdehyde (MDA), GSH-Px and SOD, creatinine, and urea were purchased from Bio-diagnostic Co., Dokki, Giza, Egypt. The deoxyribonucleic acid (DNA), 100 bp ladder was purchased from MBI, Fermentas, Thermo Fisher Scientific, USA. Qiazol for ribonucleic acid (RNA) extraction and oligo dT primer were purchased from QIAGEN (Valencia, CA, USA).

Herbal Plants Preparation

Dry matter of the fruits of Ginger (*Zingiber officinale* Rosc) was extracted repeatedly with hot water and dried in vacuo. The dry extract was dissolved with tap water at the concentration of 0.125%. For CE, dried bark of cinnamon was obtained from local markets in Taif, Saudi Arabia. 1 kg was ground, powdered, and macerated in 1000 ml of 80% methanol and water for 3 days. This procedure was repeated 3 times. The extract was filtered and concentrated using vacuum dry. The solid residue was weighted and kept in refrigerator till use. Cinnamon was used in a dose of 100 mg/kg BW orally (Figure 1).

Experimental Design, Induction of Mammary Gland Tumor and Sampling

This study was approved by the Ethical Committee Office of the dean of scientific affairs of Taif University (project number 4409/36/1), Saudi Arabia. 75 virgin female Wistar rats, 7 weeks old, weighing 100-120 g were used for this study. Rats were handled manually, daily, and kept under observation for

one week for complete acclimatization. Rats were kept at 12-h light-dark cycle and gained access to food and water ad libitum for the first week. Next, rats were allocated into 5 groups (15 per group), control group without any treatment; mammary gland group administered DMBA at a dose of 20 mg/kg orally in corn oil. Rats in 3-5 served as breast cancer groups and received ginger extract for group 3, CE for group 4, and mixture of both for group 5. The doses of ginger are 0.125% in water and were 100 mg/kg BW orally for cinnamon based on previous studies.[13,14] Animals were kept under observation for 3 months and inspected by palpation for the tumor occurrence. Herbal plants were administered 2 weeks before DMBA and continued for 4 months. At the end of experimental design, all rats were sacrificed after anesthetization by diethyl ether inhalation. Blood and mammary tissues were collected from slaughtered rats in sterilized vacutainer tubes. Serum was extracted after centrifugation of clotted blood for 10 min at 4000 ×*g* and kept at −20°C till assays. For gene expression, mammary tissues with tumor were kept in Qiazol reagent at −80°C for RNA extraction. For histopathological examination, sections from mammary tissues were inserted in 10% neutral buffered formalin (NBF) at room temperature for 24 h.

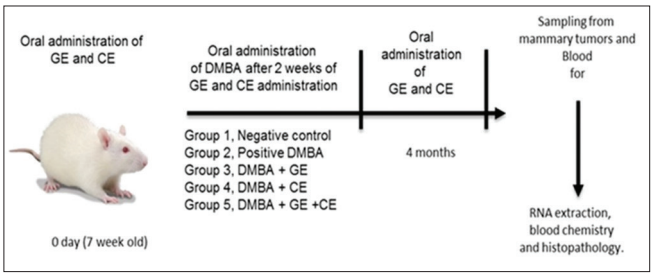

Figure 1: Methodology and experimental design

Serum Biochemical Assays

MDA, GSH-Px, Catalase, SOD, urea, creatinine, glutamate pyruvate transaminase (GPT), and glutamate oxaloacetate transaminase (GOT) were measured using commercial spectrophotometric analysis kits (Bio-Diagnostic Company, Giza, Egypt). Tumor marker of breast cancer (CA125) levels was measured using commercial ELISA kits bought from MyBioSource, Co, San Diego, CA 92195-3308, USA.

RNA Extraction, cDNA Synthesis, and Gene Expression Analysis

Total RNA was extracted from tissue samples as previously discussed.[15] The RNA integrity was confirmed after running in 1.5% denaturated agarose gel stained with ethidium bromide. A mixture of 2 µg of total RNA and 0.5 ng oligo dT primer (Qiagen Valencia, CA, USA) were used for cDNA synthesis.[19] For semi-quantitative gene expression and reverse transcription-polymerase chain reaction (RT-PCR) analysis, specific primers for examined genes (Table 1) were designed using Oligo-4 computer program, synthesized and ordered by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 pM of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI, USA); the volume was adjusted to 25 µl using sterilized, deionized water. PCR was carried out using Bio-Rad T100™ Thermal Cycle machine with the cycle sequence at 94°C for 5 min one cycle, followed by 33 cycles (Table 1), each of which consists of denaturation at 94°C for 1 min, annealing at the specific temperature corresponding to each primer (Table 1), and

Table 1: Primers sequence and PCR conditions of examined genes in mammary gland tumors of Wistar rats

Gene	Primer sequence and direction (5'-3')	Annealing temperature	Band size
GAPDH	AGATCCACAACGGATACATT (F)	52	309 bp
	TCCCTCAAGATTGTCAGCAA (R)		
GSH-Px	AAGGTGCTGCTCATTGAGAATG (F)	57	406 bp
	CGTCTGGACCTACCAGGAACTT (R)		
SOD	AGGATTAACTGAAGGCGAGCAT (F)	55	410 bp
	TCTACAGTTAGCAGGCCAGCAG (R)		
GST-P	TCATCTACACCAACTATGAG (F)	55	226 bp
	GCCACATAGGCAG AGAGCAG- (R)		
VEGF-R1	AGGAGAGGACCTGAAACTGTCTT (F)	59	230 bp
	ATTCCTGGGCTCTGCAGGCATAG (R)		
CY1B1	CACTGCCAACACCTCTGTCTT (F)	60	331 bp
	CAAGGAGCTCCATGGACTCT (R)		
CYP1A1	AAGTGCAGATGCGGTCTTCT (F)	58	419 bp
	CACCTCCGTGCCAGTATTTT (R)		
Bax	ACCAAG CTGAGCGA GTGTC (F)	55	374 bp
	ACAAAGATGGTCACGGTCTGCC (R)		

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, GSH-Px: Glutathione peroxidase, SOD: Superoxide dismutase, GST-P: Glutathione-S transferase peroxidase, VEGF-R1: Vascular endothelial growth factor-receptor 1

extension at 72°C for 1 min with an additional final extension at 72°C for 7 min. The expression of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was used as a reference. PCR products were run in 1.5% agarose (Bio Basic, Markham, ON, Canada) gel stained with ethidium bromide in Tris-Borate-EDTA buffer and visualized under UV light gel using gel documentation system.

Mammary Gland (Breast Cancer) Histopathology

Mammary gland tissues were dissected after diethyl ether inhalation and sacrificing of rats and fixed overnight in a 10% NBF solution. Fixed tissues were processed routinely and washed and preserved in 70% ethanol, dehydrated in ascending grades of ethanol solution, cleared in xylene, paraffin wax embedded, casted and cut into 5 μm sections. Sliced sections were placed on top of glass slides. The slides were stained with Mayer's hematoxylin and eosin (H and E) and special stains based on previous stated protocols.[16] Tissue slides were visualized using a Wolfe S9-0982 microscope, and photos were captured using Canon Power-Shot SX500 IS digital camera.

Statistical Analysis

Data are represented as means ± standard error of means. Data analyzed using analysis of variance (ANOVA) and *post-hoc* descriptive tests by SPSS software version 11.5 for Windows (SPSS, IBM, Chicago, IL, USA) with $P < 0.05$ regarded as statistically significant. Regression analysis was performed using the same software.

RESULTS

Protective Effects of Ginger and CEs on CA125 Levels in Mammary Gland Tumors in Wistar Rats

Administration of GE and CE for consecutive 4 months decreased the increase in CA125 levels reported in rats with mammary gland tumors. The levels were with highly significant in the carcinogenic group (1.4 ± 0.01 μ/ml) compared to 0.14 ± 0.05 μ/ml for control rats. Administration of both ginger and cinnamon alone decreased significantly CA125 levels compared to breast cancer group (0.63 ± 0.01 μ/ml for ginger and 0.69 ± 0.12 μ/ml, respectively). Coadministration of GE and CE together induced additive inhibitor effect for the CA125 level (0.39 ± 0.08) compared to the levels of either ginger and cinnamon groups alone.

Protective Effects of Ginger and CEs on Kidney and Liver Biomarkers in Mammary Gland Tumors in Wistar Rats

Induction of breast cancer by DMBA increased significantly ($P < 0.05$) the serum levels of urea, creatinine, GPT, and GOT. Prior administration of GE and CE for 4 months normalized such increase reported in breast cancer group. Coadministration of GE and CE together induced additive action on examined parameters in DMBA administered rats as shown in Table 2.

Protective Effects of Ginger and CEs on MDA and Antioxidants Biomarkers in Mammary Gland Tumors in Wistar Rats

Induction of breast cancer in rats by DMBA-induced oxidative stress as MDA levels was increased significantly ($P < 0.05$) in mammary gland group compared to control one. Prior administration of either GE or CE for consecutive 4 months induced normalization in MDA compared to breast cancer group. Coadministration of GE plus CE induced additive normalization in MDA levels. Breast cancer group showed a significant reduction ($P < 0.05$) in CAT, GSH reductase, and SOD levels (Table 3). Administration of GE or CE normalized such reduction in antioxidants levels. Coadministration of GE plus CE together showed a range of

Table 2: Serum changes in kidney and liver function parameters in mammary gland tumors and protection by ginger and cinnamon extracts in Wistar rats

Group	Urea (mg/dL)	Creatinine (mg/dL)	GPT (U/L)	GOT (U/L)
C	71.6±6.5	0.4±0.06	38.4±5.7	71.6±7.6
MT	219.4±14.8*	0.89±0.1*	69.6±7.1*	155.4±15.1*
GE	169.0±11.4$^{\#}$	0.63±0.07$^{\#}$	52.3±2.3$^{\#}$	131.7±9.9$^{\#}$
CE	171.7±8.5$^{\#}$	0.67±0.09$^{\#}$	50.6±1.9$^{\#}$	120.5±8.3$^{\#}$
GE+CE	134.2±10.5$^{\$}$	0.57±0.02$^{\$}$	48.9±2.2$^{\$}$	98.7±5.9$^{\$}$

Values are means±SEM for 3 independent experiments per each treatment. Values are statistically significant at *P<0.05 versus control; $^{\#}P$<0.05 versus tumor group; $^{\$}P$<0.05 versus either GE or CE group. SEM: Standard error of mean, MT: Mammary tumor, GE: Ginger extract, CE: Cinnamon extract, GPT: Glutamate pyruvate transaminase, GOT: Glutamate oxalacetate transaminase, C: Control

Table 3: Serum changes in oxidative stress and antioxidants biomarkers in mammary gland tumors and protection by ginger and cinnamon extracts in Wistar rats

Group	MDA (nmol/g tissue)	CAT (U/g tissue)	GR (U/g tissue)	SOD (U/g tissue)
C	5.3±1.1	21.4±3.0	9.3±0.6	12.5±0.8
MT	35.3±2.1*	9.7±0.9*	3.3±0.3*	3.7±0.2*
GE	21.1±1.8$^{\#}$	18.1±1.3$^{\#}$	7.5±1.0$^{\#}$	8.9±1.1$^{\#}$
CE	19.1±1.2$^{\#}$	17.1±1.1$^{\#}$	6.6±0.09$^{\#}$	9.3±0.9
GE+CE	18.1±2.1$^{\#}$	19.1±1.2$^{\#}$	8.1±0.1$^{\#}$	10.9±0.5$^{\#}$

Values are means±SEM for 3 independent experiments per each treatment. Values are statistically significant at *P<0.05 versus control; $^{\#}P$<0.05 versus tumor group; $^{\$}P$<0.05 versus either GE or CE group. MDA: Malondialdehyde, CAT: Catalase, GR: Glutathione reductase, SOD: Superoxide dismutase, SEM: Standard error of mean, MT: Mammary tumor, GE: Ginger extract, CE: Cinnamon extract, C: Control

15-20 additive normalization in antioxidants that reduced in breast cancer group (Table 3).

Protective Effects of Ginger and CEs on GSH-Px and SOD Expression in Mammary Gland Tumors in Wistar Rats

We examined the effect of breast cancer induction on the mRNA expression of oxidative stress biomarkers. As seen in Figure 2a, breast cancer decreased the expression of GSH-Px and SOD significantly compared to control rats. Administration of GE normalized GSH-Px expression to control levels. CE administration upregulated and induced 1-fold increases in GSH-Px expression (Figure 2b). Coadministration of GE plus CE induced 0.8-fold increase in GSH-Px expression. In parallel, mammary gland tumor induced downregulation in SOD expression (Figure 3). GE induced highly significant upregulation in mRNA expression, whereas CE normalized SOD expression to normal control levels and co-administration of GE and CE showed potency in SOD expression (Figure 3a and b). The normalization of reduction in antioxidants expression confirmed the antioxidative stress properties of GE and CE.

Protective Effects of Ginger and CEs on Cell Survival and Proliferation Biomarkers in Mammary Gland Tumors in Wistar Rats

As seen in Figure 4, induction of breast cancer upregulated the expressions of GST-P that is associated with sell survival and proliferation in cancer. GE showed a slight reduction in GST-P expression, whereas CE showed more significant reduction in mRNA expression. Coadministration of GE and CE extract together and induced more significant and synergistic reduction in mRNA expression of GST-P as seen in Figure 4a and b.

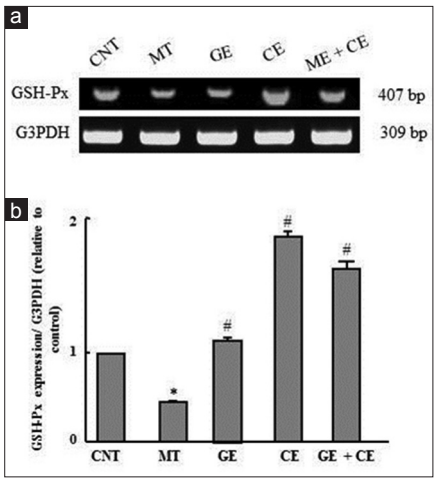

Figure 2: (a and b) Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of glutathione peroxidase micro ribonucleic acid (RNA) expressions and their corresponding glyceraldehyde-3-phosphate dehydrogenase in mammary gland tumor. RNA was extracted and reverse-transcribed (3 μg), and RT-PCR analysis was carried out for examined genes as described in the materials and methods. Densitometry analysis was carried for 5 different rats per each group. Values are means ± standard error of mean obtained from 5 rats per group. *$P < 0.05$ versus control group, and $^{\#}P < 0.05$ versus tumor group

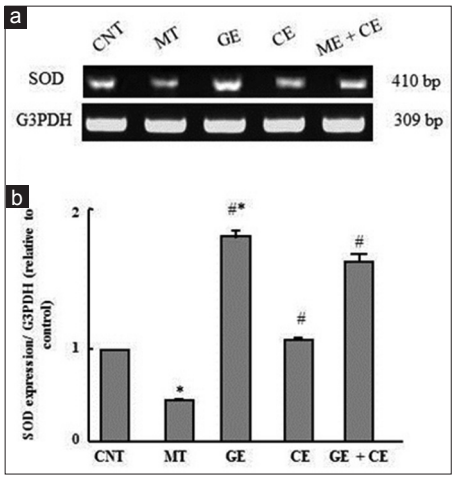

Figure 3: (a and b) Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of superoxide dismutase micro ribonucleic acid (RNA) expressions and corresponding glyceraldehyde-3-phosphate dehydrogenase in mammary gland tumor. RNA was extracted and reverse-transcribed (3 μg), and RT-PCR analysis was carried out for examined genes as described in the materials and methods. Densitometry analysis was carried for 5 different rats per each group. Values are means ± standard error of mean obtained from 5 rats per group. *$P < 0.05$ versus control group, and $^{\#}P < 0.05$ versus tumor group

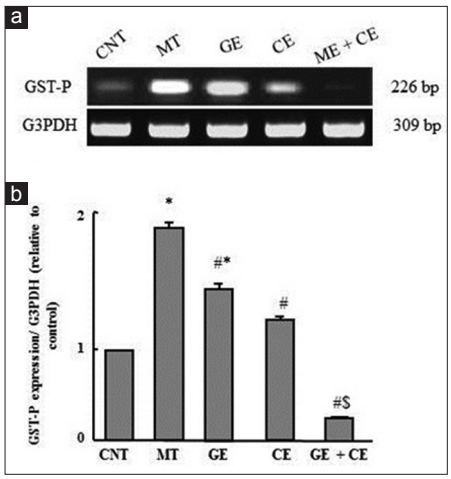

Figure 4: (a and b) Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of glutathione-S transferase peroxidase micro ribonucleic acid (RNA) expressions and corresponding glyceraldehyde-3-phosphate dehydrogenase in mammary gland tumor. RNA was extracted and reverse-transcribed (3 μg), and RT-PCR analysis was carried out for examined genes as described in the materials and methods. Densitometry analysis was carried for 5 different rats per each group. Values are means ± standard error of mean obtained from 5 rats per group. *$P < 0.05$ versus control group, and $^{\#}P < 0.05$ versus tumor group

Protective Effects of Ginger and CE on Carcinogenesis Metabolism Biomarkers in Mammary Gland Tumors in Wistar Rats

The expression of CYP1A1 showed an increase in mRNA expression in breast cancer group compared to normal rats. Administration of GE, CE, or both together showed reduction in CYP1A1 expression relative to breast cancer group (Figure 5a and b). Moreover, the expression of CYP1B1 mRNA was increased in breast cancer group and decreased after administration of GE or CE alone (Figure 6a and b) similar to that reported in CYP1A1. Coadministration of GE and CE induced additive inhibitor effect on mRNA expression of CYP1B1 (Figure 6).

Protective Effects of Ginger and CE on Angiogenesis Biomarkers in Mammary Gland Tumors in Wistar Rats

Vascular endothelial growth factor (VEGF) is a marker for vascular growth characterizes cancer progression. Therefore, we examined VEGF receptor expression, breast cancer, and in general, tumors are characterized by increase in vasculatures and angiogenesis. Therefore, as seen in Figure 7, mammary gland tumor upregulated significantly the expression of VEGF-R1 compared to normal control rats. Administration of GE, CE alone or in combination downregulated VEGF-R1 expression compared to breast cancer group.

Protective Effects of Ginger and CE on Apoptosis Gene Biomarkers in Mammary Gland Tumors in Wistar Rats

Induction of breast cancer decreased Bax expression compared to control rats (Figure 8a and b). The current study shows that GE administration normalized Bax expression around 90%, whereas CE showed around 50% normalization and co-administration of GE and CE showed normalization with the percentage of 70% compared to breast cancer (Figure 8a and b).

Protective Effects of Ginger and CE on Histology of Mammary Gland Tumors in Wistar Rats

Finally, we tested the effect of DMBA on breast cells and the possible protection by GE and CE in rats. The mammary gland of control rats consisted of lobules, separated by CT septa. Each lobule consisted of alveoli, which lined by low cuboidal or squamous cells with centrally located nuclei and eosinophilic cytoplasm (Figure 9a). In mammary gland tumor group, the mammary alveoli lost its architecture with hyperplasia (adenocarcinoma) of the epithelium and hemorrhage in the blood vessels (Figure 9b). In tumor group administered ginger, the alveoli appeared as solid cell masses with faint Periodic Acid Schiff reaction (Figure 9c). In cinnamon administered group, it showed same as in the ginger group beside that the collagen fibers extend between

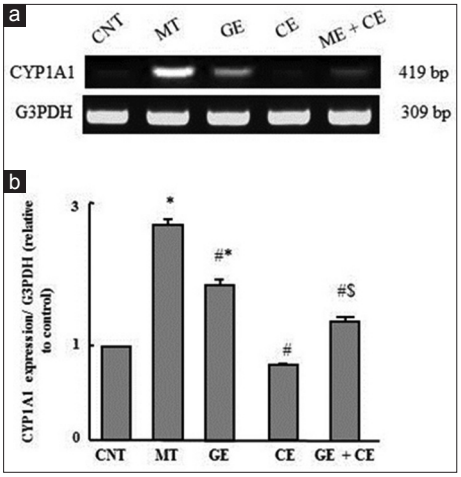

Figure 5: (a and b) Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of CYP1A1 micro ribonucleic acid (RNA) expressions and corresponding glyceraldehyde-3-phosphate dehydrogenase in mammary gland tumor. RNA was extracted and reverse-transcribed (3 μg), and RT-PCR analysis was carried out for examined genes as described in the materials and methods. Densitometry analysis was carried for 5 different rats per each group. Values are means ± standard error of mean obtained from 5 rats per group. *$P < 0.05$ versus control group, and #$P < 0.05$ versus tumor group

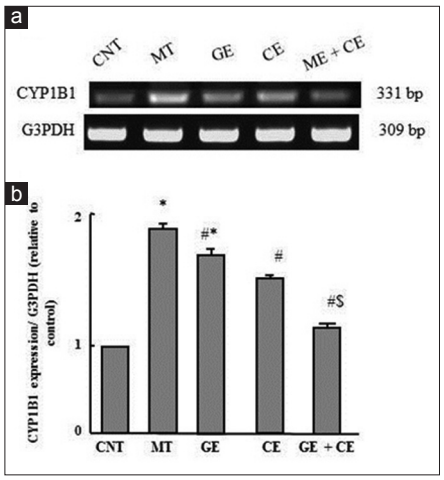

Figure 6: Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCRI) analysis of CYP1B1 micro ribonucleic acid (RNA) expressions and corresponding glyceraldehyde-3-phosphate dehydrogenase in mammary gland tumor. RNA was extracted and reverse-transcribed (3 μg), and RT-PCR analysis was carried out for examined genes as described in the materials and methods. Densitometry analysis was carried for 5 different rats per each group. Values are means ± standard error of mean obtained from 5 rats per group. *$P < 0.05$ versus control group, and #$P < 0.05$ versus tumor group

the alveolar masses (Figure 9d). Coadministration of ginger and cinnamon to tumor group (group 5), there are a decrease in the areas of alveolar masses and increase in connective tissue fibers between the alveolar tissues and appearance of new alveoli and new ducts between the affected alveoli (Figure 9e and f).

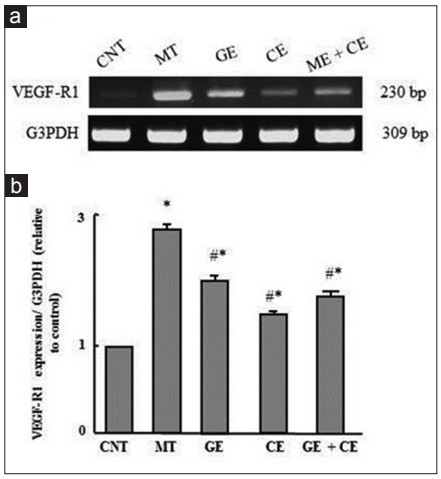

Figure 7: (a and b) Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of vascular endothelial growth factor-receptor 1 micro ribonucleic acid (RNA) expressions and corresponding glyceraldehyde-3-phosphate dehydrogenase in mammary gland tumor. RNA was extracted and reverse-transcribed (3 μg), and RT-PCR analysis was carried out for examined genes as described in the materials and methods. Densitometry analysis was carried for 5 different rats per each group. Values are means ± standard error of mean obtained from 5 rats per group. *$P < 0.05$ versus control group, and $^{\#}P < 0.05$ versus tumor group

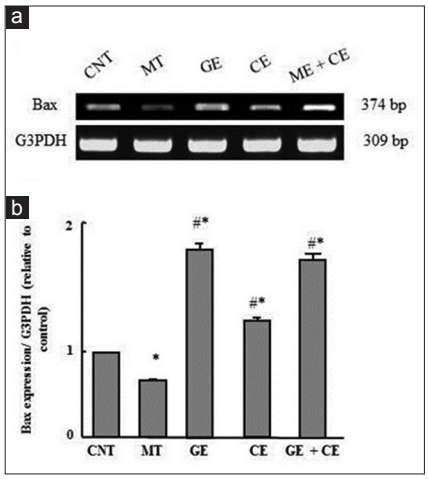

Figure 8: (a and b) Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of Bax micro ribonucleic acid (RNA) expressions and corresponding glyceraldehyde-3-phosphate dehydrogenase in mammary gland tumor. RNA was extracted and reverse-transcribed (3 μg), and RT-PCR analysis was carried out for examined genes as described in the materials and methods. Densitometry analysis was carried for 5 different rats per each group. Values are means ± standard error of mean obtained from 5 rats per group. *$P < 0.05$ versus control group, and $^{\#}P < 0.05$ versus tumor group

DISCUSSION

Cancers are the most life-threatening health problems in the world.[17] Although many different types of antitumor agents are available, severe side effects and toxicity limit their applications.[18] Recently, herbal medicine is becoming a popular treatment for various cancers, especially breast

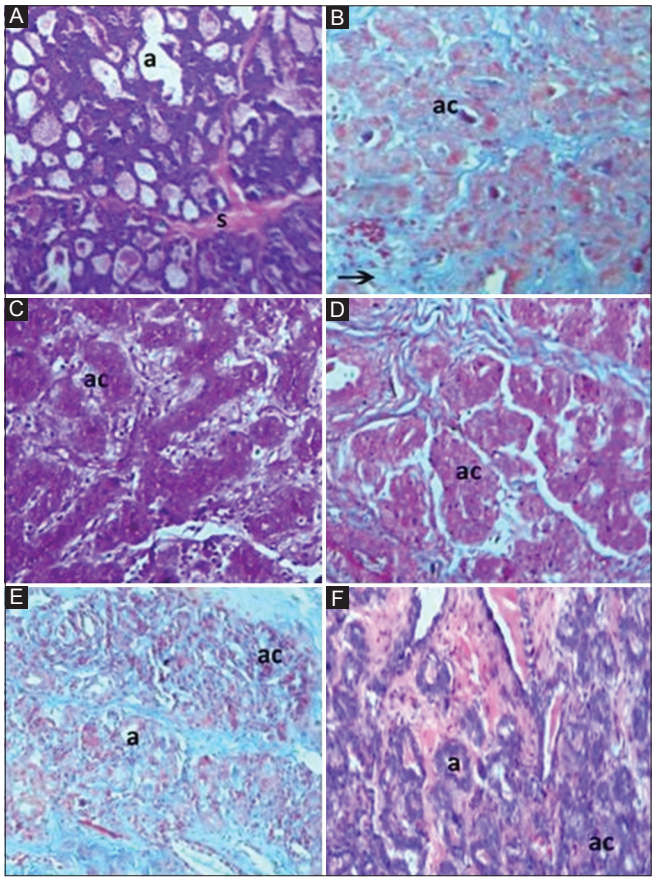

Figure 9: Protective effect of ginger and cinnamon on the histological changes in mammary gland tissues after tumor induction. (A) The mammary gland of the rats showed mammary lobules separated by CT septa (s) and alveoli (a) (H and E, ×20). (B) The mammary gland of the tumor rats showed adenocarcinoma of the mammary alveoli (ac) and hemorrhage of the blood vessels (arrow) (Masson trichrome, ×40). (C) The mammary gland of the ginger administered rats showed adenocarcinoma of the mammary alveoli (ac) with faint Periodic Acid-Schiff (PAS) reaction (PAS, ×40). (D) Mammary gland tissues of cinnamon administered rats showed adenocarcinoma of the mammary alveoli (ac) and increased CT between the alveoli (arrow) (Masson trichrome, ×20). (E) The mammary gland of ginger and cinnamon administered rats showed decreased adenocarcinoma of the mammary alveoli (ac) and appearance of new alveoli between the affected alveoli (Masson trichrome, ×10). (F) The mammary gland of ginger and cinnamon administered rats showed a decrease in adenocarcinoma of the mammary alveoli (ac) and appearance of new ducts between the affected alveoli (H and E, ×10)

cancer.[19] Oriental herbal medicine including traditional and folk-healing methods have been used for the treatment of malignancies for several years. Currently, numerous scientific researches support herbal medicine as a potent anticancer drug.[20] However, the development of herbal medicine as an anticancer agent needs substantial research for it to meet strict criteria such as those on standardization, quality control, safety, toxicity, and clinical trials.[21]

In this study, we have demonstrated the potent antitumor efficacy of both ginger and CE. Both herbal medications

downregulated the tumor marker CA125 and both together showed synergistic action. Moreover, GE and CE decreased the increase in expression of GST-P, CYP1A1, CYP1B1, and VEGF-R1 reported in tumor groups. On the same time, both GE and CE upregulated the decrease in GSH-Px, SOD, and Bax expression reported in positive tumor groups.

DMBA is the PAHs and is widely used for induction of mammary gland tumors in rodents.[22] Administration of DMBA, in a single oral dose or multiple doses, yields maximum mammary tumors. Furthermore, these tumors closely mimic human breast cancer in morphology and the expression of biochemical and molecular markers.[23,24] DMBA is accumulated in adipose tissue of mammary gland and increase induction of cancer due to increase in the expression of CYP enzymes involved in the metabolism of estrogen.[24] Induction of CYP1B1 resulted in oncogenic transformation through reactive oxygen species (ROS)-induced DNA damage.[25] Here, both GE and CE downregulated the expression of CYP1A1 and CYP1B1 and control ROS-induced DNA damage.

p53 plays a major role in prevention of malignancy transformation. p53 exerts its tumor suppressor action by integrating multiple signaling pathways that regulate cell survival and cell proliferation.[26] Thus, downregulation in p53 in DMBA-induced mammary tumors may facilitate cell proliferation and survival by induction the expression of GST-P that is responsible for cell survival and proliferation in breast cancer.[27]

VEGF-R1, which binds with VEGF significantly, correlates with high metastasis risk and is considered as a marker for breast tumor aggressiveness.[28] The results of this study demonstrate that multiple signaling pathways including carcinogen metabolism, cell proliferation, apoptosis, invasion, metastasis, and angiogenesis are intricately interlinked in malignant transformation of the rat mammary gland by DMBA. CE was previously shown to inhibit the growth of hematologic tumor cell growth; however, the role of CE in *in vivo* tumor progression remained to be determined.[29] Tumor cells recruit new blood vessels by excessive production of pro-angiogenic factors that play a pivotal role in tumor progression and tumor survival. These include VEGF, basic fibroblast growth factor, interleukin 8, placenta-like growth factor, transforming growth factor beta, platelet-derived endothelial growth factor, pleiotrophin, and other factors.[30] Indeed, inhibition of tumor angiogenesis is thought to be a good target for cancer treatments as indicated by results obtained from administration of ginger and CEs.

It has been shown that ginger root (*Zingiber officinale*) and its main polyphenolic constituents (gingerols and zerumbone) have antioxidant,[31,32] anti-inflammatory,[33] and anticarcinogenic activity.[34] In particular, ginger root and its constituents can inhibit nuclear factor-Kb (NF-κB) activation induced by a variety of agents[35] and has been shown to downregulate NF-κB regulated gene products involved in cellular proliferation and angiogenesis including VEGF.[36] These factors have also been shown to promote tumor cell proliferation, angiogenesis, and affect apoptotic response in ovarian cancers.

Accumulating evidence suggests that many dietary factors may be used alone or in combination with traditional chemotherapeutic agents to prevent or treat cancer. Previous reports indicate that the ginger component 6-shogaol induces cell death in chemoresistant hepatoma cells and induce activity against breast cancer.[37,38] Cinnamomum cassia bark contains large amounts of bioactive molecules including essential oils (cinnamic aldehyde and cinnamyl aldehyde), tannin, mucus, and carbohydrates. Many studies have shown the diverse biological functions of cinnamon including anti-inflammatory,[39] antioxidant,[40] antimicrobial,[41] and anti-diabetic effects.[42] An antitumor effect of cinnamon was previously suggested *in vitro* without *in vivo* evidence or a working mechanism.[43] The active substances of cinnamyl aldehyde are responsible for cancer prevention.[38]

Taken together, the results from the current study confirmed that ginger and CEs had antioxidants activities and regulated the expression of genes associated with cell survival, proliferation, apoptosis, angiogenesis, and oxidative stress biomarkers. The co-administration of GE and CE together induced the additive effect in prevention the incidence of mammary gland tumors.

CONCLUSION

The present findings confirmed the anticarcinogenic activity of ginger and CEs in prevention of breast cancer induction in Wistar rats. The protective effects of herbal plants are regulated at molecular and cellular levels.

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REFERENCES

1. Kelsey JL, Horn-Ross PL. Breast cancer: Magnitude of the problem and descriptive epidemiology. Epidemiol Rev. 1993;15(1):7-16.
2. Mc Pherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. BMJ. 2000;321(7261):624-8.
3. Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis. 2000;21(3):361-70.
4. Hristozov D, Gadjeva V, Vlaykova T, Dimitrov G. Evaluation

of oxidative stress in patients with cancer. Arch Physiol Biochem. 2001;109(4):331-6.
5. Bishayee A, Oinam S, Basu M, Chatterjee M. Vanadium chemoprevention of 7,12-dimethylbenz(a)anthracene-induced rat mammary carcinogenesis: Probable involvement of representative hepatic phase I and II xenobiotic metabolizing enzymes. Breast Cancer Res Treat. 2000;63(2):133-45.
6. Cotgreave IA, Moldéus P, Orrenius S. Host biochemical defense mechanisms against prooxidants. Annu Rev Pharmacol Toxicol. 1988;28:189-212.
7. Mittal A, Pathania V, Agrawala PK, Prasad J, Singh S, Goel HC. Influence of *Sinopodophyllum hexandrum* on endogenous antioxidant defence system in mice: Possible role in radioprotection. J Ethnopharmacol. 2001;76(3):253-62.
8. Prakash J, Gupta SK. Chemopreventive activity of *Ocimum sanctum* seed oil. J Ethnopharmacol. 2000;72(1-2):29-34.
9. Vijayavel K, Anbuselvam C, Balasubramanian MP. Free radical scavenging activity of the marine mangrove *Rhizophora apiculata* bark extract with reference to naphthalene induced mitochondrial dysfunction. Chem Biol Interact. 2006;163(1-2):170-5.
10. Park EJ, Pezzuto JM. Botanicals in cancer chemoprevention. Cancer Metastasis Rev. 2002;21(3-4):231-55.
11. Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, et al. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem. 2004;52(1):65-70.
12. Murcia MA, Egea I, Romojaro F, Parras P, Jiménez AM, Martínez-Tomé M. Antioxidant evaluation in dessert spices compared with common food additives. Influence of irradiation procedure. J Agric Food Chem. 2004;52(7):1872-81.
13. Nagasawa H, Watanabe K, Inatomi H. Effects of bitter melon (*Momordica charantia* l.) or ginger rhizome *(Zingiber* offiifinale rosc) on spontaneous mammary tumorigenesis in SHN mice. Am J Chin Med. 2002;30(2-3):195-205.
14. Madkor HR, Mansour SW, Khalil MA. Antiangiogenic activities of cinnamon, black and green tea extracts on experimentally induced breast cancer in rats. Asian J Biochem. 2012;7(4):206-17.
15. Soliman MM, Baiomy AA, Yassin MH. Molecular and histopathological study on the ameliorative effects of curcumin against lead acetate-induced hepatotoxicity and nephrototoxicity in Wistar rats. Biol Trace Elem Res. 2015;167(1):91-102.
16. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 6th ed. Philadelphia, PA: Churchill Livingstone, Elsevier; 2008. p. 126-7.
17. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA. Cancer J Clin. 2008;57(1):43-66.
18. Cassileth BR, Lusk EJ, Strouse TB, Bodenheimer BJ. Contemporary unorthodox treatments in cancer medicine. A study of patients, treatments, and practitioners. Ann Intern Med. 1984;101(1):105-12.
19. Cassileth BR. Complementary and alternative cancer medicine. J Clin Oncol. 1999;17 11 Suppl:44-52.
20. Di Paola RS, Zhang H, Lambert GH, Meeker R, Licitra E, Rafi MM, et al. Clinical and biologic activity of an estrogenic herbal combination (PC-SPES) in prostate cancer. N Engl J Med. 1998;339(12):785-91.
21. Buchanan DR, White JD, O'Mara AM, Kelaghan JW, Smith WB, Minasian LM. Research-design issues in cancer-symptom-management trials using complementary and alternative medicine: Lessons from the National Cancer Institute Community Clinical Oncology Program experience. J Clin Oncol. 2005;23(27):6682-9.
22. Russo J, Russo IH. Experimentally induced mammary tumors in rats. Breast Cancer Res Treat. 1996;39(1):7-20.
23. Russo J, Russo IH. Atlas and histologic classification of tumors of the rat mammary gland. J Mammary Gland Biol Neoplasia. 2000;5(2):187-200.
24. Costa I, Solanas M, Escrich E. Histopathologic characterization of mammary neoplastic lesions induced with 7,12-dimethyl benz(alpha)anthracene in the rat: A com parative analysis with human breast tumours. Arch Pathol Lab Med. 2002;126(8):915-27.
25. Vinothini G, Murugan RS, Nagini S. Evaluation of molecular markers in a rat model of mammary carcinogenesis. Oncol Res. 2009;17(25):483-93.
26. Clerkin JS, Naughton R, Quiney C, Cotter TG. Mechanisms of ROS modulated cell survival during carcinogenesis. Cancer Lett. 2008;266(1):30-6.
27. Kumaraguruparan R, Seshagiri PB, Hara Y, Nagini S. Chemoprevention of rat mammary carcinogenesis by black tea polyphenols: Modulation of xenobiotic-metabolizing enzymes, oxidative stress, cell proliferation, apoptosis, and angiogenesis. Mol Carcinog. 2007;46(9):797-806.
28. Wu Y, Hooper AT, Zhong Z, Witte L, Bohlen P, Rafii S, et al. The vascular endothelial growth factor receptor (VEGFR-1) supports growth and survival of human breast carcinoma. Int J Cancer. 2006;119(7):1519-29.
29. Schoene NW, Kelly MA, Polansky MM, Anderson RA. Water-soluble polymeric polyphenols from cinnamon inhibit proliferation and alter cell cycle distribution patterns of hematologic tumor cell lines. Cancer Lett. 2005;230(1):134-40.
30. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer. 2003;3(6):401-10.
31. Ahmed RS, Seth V, Banerjee BD. Influence of dietary ginger (*Zingiber* officinales Rosc) on antioxidant defense system in rat: Comparison with ascorbic acid. Indian J Exp Biol. 2000;38(6):604-6.
32. Banerjee S, Bueso-Ramos C, Aggarwal BB Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: Role of nuclear factor-kappaB, cyclooxygenase 2, and matrix metalloprotease 9. Cancer Res. 2002;62(17):4945-54.
33. Grzanna R, Lindmark L, Frondoza CG. Ginger – An herbal medicinal product with broad anti-inflammatory actions. J Med Food. 2005;8(2):125-32.
34. Shukla Y, Singh M. Cancer preventive properties of ginger: A brief review. Food Chem Toxicol. 2007;45(5):683-90.
35. Aktan F, Henness S, Tran VH, Duke CC, Roufogalis BD, Ammit AJ. Gingerol metabolite and a synthetic analogue Capsarol inhibit macrophage NF-kappaB-mediated iNOS gene expression and enzyme activity. Planta Med. 2006;72(8):727-34.
36. Kim EC, Min JK, Kim TY, Lee SJ, Yang HO, Han S, et al. [6]-Gingerol, a pungent ingredient of ginger, inhibits angiogenesis *in vitro* and *in vivo*. Biochem Biophys Res Commun. 2005;335(2):300-8.
37. Elkady AI, Abuzinadah OA, Baeshen NA, Rahmy TR. Differential control of growth, apoptotic activity, and gene expression in human breast cancer cells by extracts derived

from medicinal herbs Zingiber officinale. J Biomed Biotechnol. 2012;2012:614356.
38. Chen CY, Liu TZ, Liu YW, Tseng WC, Liu RH, Lu FJ, et al.6-shogaol (alkanone from ginger) induces apoptotic cell death of human hepatoma p53 mutant Mahlavu subline via an oxidative stress-mediated caspase-dependent mechanism. J Agric Food Chem. 2007;55(3):948-54.
39. Lee SH, Lee SY, Son DJ, Lee H, Yoo HS, Song S, et al. Inhibitory effect of 2'-hydroxycinnamaldehyde on nitric oxide production through inhibition of NF-kappa B activation in RAW 264.7 cells. Biochem Pharmacol. 2005;69(5):791-9.
40. Lee JS, Jeon SM, Park EM, Huh TL, Kwon OS, Lee MK, et al. Cinnamate supplementation enhances hepatic lipid metabolism and antioxidant defense systems in high cholesterol-fed rats. J Med Food. 2003;6(3):183-91.
41. Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M. Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. Int J Food Microbiol. 2006;107(2):180-5.
42. Khan A, Safdar M, Ali Khan MM, Khattak KN, Anderson RA. Cinnamon improves glucose and lipids of people with type 2 diabetes. Diabetes Care. 2003;26(12):3215-8.
43. Kamei T, Kumano H, Iwata K, Nariai Y, Matsumoto T. The effect of a traditional Chinese prescription for a case of lung carcinoma. J Altern Complement Med. 2000;6(6):557-9.

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