Assessment of Wheat Germ Oil Role in the Prevention of Induced Breast Cancer in Rats

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ABSTRACT: Breast cancer is the most predominant cause of death in women globally. The current study was performed to evaluate the possible protective role of wheat germ oil (WGO), wheat germ powder (WGP), and vitamin E (Vit E) against breast carcinoma induced by the environmental carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) in Sprague Dawely albino rats. Eighty female rats were divided into eight groups, each of ten rats. All protective agents were taken 21 days prior to DMBA treatment. Group I served as the normal control. Group II received Vit E (100 mg/kg BW/d) by gavage. Group III was fed a 20% WGP enriched basal diet. Group IV received WGO (270 mg/kg BW/d) by gavage. Group V received DMBA (50 mg/kg body weight/subcutaneous injection). Group VI received Vit E + DMBA. Group VII received WGP + DMBA. Group VIII received WGO + DMBA. The investigation focused on bodyweights, complete blood picture (CBC), cancer antigen 15.3 (CA15.3), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and histopathological and immunohistochemical analyses. Results showed that all cancer protective agents significantly improved CBC parameters, proliferating cell nuclear antigen (PCNA), and the histopathology picture, with the best improvement in the WGO group. In addition, WGO, WGP, and Vit E decreased the CA15.3 and MDA levels and elevated both the SOD and CAT levels compared to the DMBA group. Consequently, supplementation with WGO, WGP, and Vit E protects against lipid peroxidation and oxidative stress and reduces breast cancer.

INTRODUCTION

Breast cancer is a life-threatening malignant neoplasm among women (30% of all new cases of cancer) globally. It is considered the second largest cause of death in women.1 In Egypt, it represents 18.9% of total cancer cases (35.1% in women and 2.2% men).2

Early diagnosis of breast cancer became possible due to improved diagnostic techniques.3 A wide variety of recent clinical approaches, including hormonal therapy, radiotherapy, chemotherapy, and surgery, are applied for treatment of breast cancer, but there is still no effective therapy for most patients having advanced breast cancer.4 Therefore, the search for newer and more preventive approaches, biomarkers, and drugs that can minimize the incidence of breast cancer is necessary.4 So, the discovery of new drugs from natural sources is highly important to reduce the side effects of synthetic drugs, that should be highly selective, have low toxicity, and can directly kill the cancer cells.5

Medicinal plants are one of the promising chemopreventive options that have a long history in both breast cancer prevention and treatment since medicinal plants have anticarcinogen components. According to a literature survey, plant derived compounds constitute more than 50% of anticancer agents.5

Wheat germ (WG) is the part of the wheat kernel which is responsible for helping the plant reproduce and germinate to grow into new wheat. It is a nutrient-rich food source that contains a high amount of proteins (26–35%), sugars (17%), lipids (10–15%), fibers (1.5–4.5%), minerals (4%), and trace elements (i.e., zinc).6 It is an important derived byproduct from wheat milling and is separated from the endosperm during its manufacture. WG is a good source of wheat germ oil (WGO). It contains ~11% oil and a significant amount of bioactive compounds. For this reason, WG can be used in various applications, such as food, pharmaceutical, and other biological purposes. However, refined WG that removes the...
bran and oil significantly reduces the end product’s nutritional value.1–5

Several researchers have recently shown that the chemopreventive mechanisms of WG and WGO include anti-inflammatory, antioxidant, and anticarcinogenic activities in most cancer models.6,7 They induced potent protective effects as a result of their higher concentrations of vitamin E and essential fatty acids.8 Moreover, WGO can ameliorate lipid metabolism and minimize oxidative stress.9

Vitamin E is a fat-soluble antioxidant that comes in eight natural isomers: (α, β, γ, δ) tocopherol isomers and (α, β, γ, δ) tocotrienol isomers. Multiple studies have been carried out to assess the anticancer potential of α-tocopherol, a main isomer of vitamin E, based on the idea that many malignancies are characterized by high levels of oxidative stress.10 However, several clinical trials with α-tocopherol failed to produce meaningful results because α-tocopherol can interfere with the anticancer isomers among the tocotrienols when it was taken as antioxidant.10,11 Recent studies examined other isoforms of vitamin E, especially γ-tocopherol, δ-tocopherol, and γ-tocotrienols.11 Thus, vitamin E, as taken in the diet or in supplements that are rich in γ- and δ-tocopherols, has a vital role in delaying the pathogenesis of different diseases, e.g., cancer, inflammatory diseases, neurological disorders, and chronic vascular diseases, through its function of inhibiting free radical-mediated tissue damage.12 It can enhance the generation of humoral antibodies and consequently improve the cell-mediated immunity in humans and experimental animals.10,11,13

**MATERIALS AND METHODS**

**Wheat Germ Preparation.** Wheat germ was ground into powder (mixer), stored in a tightly sealed container, and refrigerated (at 4 °C) until used to prevent spoilage and maintain its nutritive value.

**Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Wheat Germ Powder.** GC-MS analysis of WGP was carried out at National Research Center, Dokki, Giza, Egypt. Its chemical composition was detected using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-SMS (30 m × 0.25 mm × 0.25 μm film thickness). The column oven temperature was initially held at 50 °C and then increased by 5 °C/min to 250 °C and held for 2 min. It then increased to the final temperature 300 °C by 30 °C/min and held for 2 min. The injector and MS transfer line temperatures were kept at 270 and 260 °C, respectively; helium was used as a carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 4 min, and diluted samples of 1 μL were injected automatically using Autosampler AS1300 coupled with a GC in the split mode. Electron impact mass spectra were collected at 70 eV ionization voltages over the range m/z 50–650 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with the mass spectral database of the WILEY 08 and NIST 014 libraries.

**Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Wheat Germ Oil.** GC-MS analysis of WGO was performed similarly to the previously discussed procedure for WGP.

**Experimental Rats.** Eighty female albino rats (Sprague Dawely strains), weighing 80–100 g, were purchased from National Research Center, Dokki, Giza, Egypt. All animals were preserved in metallic cages (5/cage) on wood-shaving bedding at Laboratory Animal House of Faculty of Veterinary Medicine, Suez Canal University (SCU), Egypt. Rats were preserved under standard natural day-light rhythm with a temperature of 25 °C (±1 °C) and a relative humidity of 60 ± 5%. Rats were permitted to diet (casein 4.12%, corn 70.24%, soya 8.80%, bran 14.34%, salt 0.50%, vitamin 1%, mineral 1%) and water ad libitum. They were maintained and treated as required by the ethical guidelines of the Faculty of Veterinary Medicine, SCU, with committee approval number 2019023.

**Induction of Breast Cancer.** Mammary tumors were induced by a single dose of 50 mg/kg body weight of DMBA obtained from Sigma-Aldrich (St. Louis, MO, USA) dissolved in 2 mL of corn oil and then subcutaneously injected in the mammary gland of apparently healthy rats (~55 days of age, average weight 120–130 g) as previously described by Nguedia et al.14 Then the rats were allowed to develop tumors for 120 days.

**Experiment Design.** After 5 days of adaptation, the rats were randomly divided into eight equal groups (10 rats/group): Group I received corn oil (0.2 mL/rat) (Sigma-Aldrich, St. Louis, MO, USA) by oral gavage and was kept as a control. Group II was kept as a Vit E control and received Vit E (Pharco Pharmaceutical Company, Egypt) in a dose of 100 mg/kg BW/day dissolved in 4 mL of corn oil (0.2 mL/rat) by oral gavage.15 Group III was kept as a WGP (Abu Auf Company, Cairo, Egypt) control group fed on a WGP enriched basal diet containing 20% WGP and received 0.2 mL of corn oil according to Abdel-Rahim and Mahmoud.15 Group IV was kept as a WGO (El-Captain Company, sixth October City, Egypt) control and received WGO in a dose of 270 mg/kg BW/day dissolved in 4 mL of corn oil by oral gavage.16 Group V received DMBA (50 mg/kg body weight/subcutaneously injection) as previously described by Nguedia et al.14 Group VI received Vit E for 21 days prior to DMBA injection. Group VII received WGP for 21 days prior to DMBA injection. Group VIII received WGO for 21 days prior to DMBA injection. All treatments were taken 21 days prior DMBA injection and lasted for four months after DMBA injection. Rats were weighed weekly and palpated twice a week to check the presence of palpable mammary tumors. Animals that died throughout the experiment were autopsied, while rats that became moribund were humanely sacrificed for examination. After 141 days of treatment all survivors were sacrificed by decapitation after being anesthetized with diethyl ether.

**Determination of Body Weight (g).** The initial body weights of the rats were recognized at the beginning of the experiment. The body weights were monitored weekly until the completion of the experiment.

**Blood and Tissue Sampling.** After 141 days (end of the experiment period) the rats were anesthetized with diethyl ether. Blood samples were withdrawn from the retro-orbital plexus of 12 h fasted rats using capillary sterile glass tubes. Each blood sample was split into two parts: the first part was collected in a plain centrifuge tube to separate sera for biochemical examination; the remaining part was collected in EDTA tubes for complete blood picture analysis. Therefore, rats were sacrificed by decapitation, and mammary tissues were removed and then fixed in formalin for histopathological and immunohistochemical studies.

**Hematological and Biochemical Analysis.** Hematological Analysis. The influences of WGO, WGP, and Vit E on the
immunological system were evaluated through determination of the red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), hematocrit (Hct), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) values in the blood. This was done using an Auto Hematology Analyzer MINDRAY BC-2800 (Shenzhen, China).

**Serum Tumor Marker Cancer Antigen 15.3.** The biomarker CA15.3 is the most prognostic circulating factor for breast cancer. It was measured in sera using a commercial ELISA kit provided by Elabscience Biomedical, Houston, Texas, USA (Cat. No.: E-EL-R0615 96T) according to Ngueda et al. Lesions in ten rats in different groups were collected and instantly fixed in 10% neutral buffered formalin. After fixation, the tissue specimens were processed as described by Bancroft and Gamble. Scoring of mammary lesions was performed as reported by Gibson-Corley et al. Lesions in ten fields selected randomly from each slide for each animal were obtained and averaged. The lesions were scored in a blinded manner [score scale: 0 = normal; 1 ≤ 25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%].

**Histopathological Examination.** The breast tissues of the rats in different groups were collected and instantly fixed in 10% neutral buffered formalin. After fixation, the tissue specimens were processed as described by Bancroft and Gamble. Scoring of mammary lesions was performed as reported by Gibson-Corley et al. Lesions in ten fields selected randomly from each slide for each animal were obtained and averaged. The lesions were scored in a blinded manner [score scale: 0 = normal; 1 ≤ 25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%].

**Immunohistochemical Analysis.** For immunohistochemistry, cell proliferation in mammary tumors induced by DMBA was determined by the expression of PCNA according to Bishayee and Dhir. The ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used for the quantification of immunostaining intensities. The inverse mean density was estimated as reported by Vis et al. in 10 randomly selected fields from tissue sections of 10 experimental rats in each group to obtain the average of the PCNA labeling index (LI).

**Statistical Analysis.** The current results were analyzed using Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago) for windows. Results were expressed as mean ± SE (n = 10). One way ANOVA followed by a posthoc Duncan test was used for analysis. A P value less than 0.05 was considered significant.

## RESULTS

**GC-MS Analysis of Wheat Germ Powder.** GC-MS analysis of WGP showed 26 peaks in the GC-MS chromatogram, indicating the presence of 26 different phytochemical compounds. These compounds were identified by comparison with mass spectra via the database of the WILEY 08 and NIST 014 libraries as shown in Table 1 and Figure 1.

| PK  | Compound Name                        | RT  | Area % | CAS #  |
|-----|--------------------------------------|-----|--------|--------|
| 1   | 1,2-Dithiacyclopentene                | 3.38| 2.20   | 288-26-6 |
| 2   | Benzy1 chloride                      | 3.95| 6.56   | 100-44-7 |
| 3   | Allyl disulfide                       | 4.26| 8.44   | 2179-57-9 |
| 4   | Dialyl disulfide                     | 4.60| 0.56   | 2179-57-9 |
| 5   | Allyl trisulfide (Trisulfide, di-2-propenyl) | 7.97| 16.88 | 2050-87-5 |
| 6   | 5-Isopropyl-2-methylphenol           | 8.13| 2.48   | 499-75-2 |
| 7   | 5-Methyl-1,2,3,4-tetrahydronaphthalene | 9.23| 1.71   | 116664-30-3 |
| 8   | Dodecanal (Lauraldehyde)             | 10.07| 0.53   | 112-54-9 |
| 9   | 1-Chlorododecane                     | 11.26| 0.24   | 112-54-9 |
| 10  | Dodecan-1-ol                         | 11.36| 0.61   | 112-53-8 |
| 11  | Nizatidine                           | 11.93| 27.78  | 76963-41-2 |
| 12  | Allylterrasulfide                    | 12.62| 1.06   | 2444-49-7 |
| 13  | Dimethyl myristinatine               | 15.70| 11.54  | 112-75-4 |
| 14  | 1,6-Heptadien-4-ol, acetate          | 16.57| 0.40   | NA      |
| 15  | 1- Allyl-3-(2-(allylthio)propyl)trisulfane | 17.65| 0.65  | 193625-59 |
| 16  | Hexadecanoic acid, methyl ester      | 19.55| 1.80   | 112-39-0 |
| 17  | Hexadecanoic acid                    | 20.24| 0.90   | 57-10-3 |
| 18  | Octathiozone                          | 21.06| 0.40   | 7704-34-9 |
| 19  | Methyl (9E,12E)-9,12-octadecadienoate | 22.22| 1.66   | 2566-9-7-4 |
| 20  | Oleic acid, methyl ester             | 22.32| 2.21   | 112-62-9 |
| 21  | 1-(Dimethylamino)-2-(benzylamino)propane | 22.49| 6.21   | 125641-44-3 |
| 22  | Methyl stearate                      | 22.49| 0.41   | 112-61-8 |
| 23  | Octadec-9-enolic acid                | 22.99| 1.29   | 112-80-1 |
| 24  | Oleic acid                           | 25.53| 1.71   | 114250-58-7 |
| 25  | Linoleoyl chloride                   | 27.03| 1.07   | 7459-33-8 |
| 26  | Glycidyl oleate                      | 27.64| 0.70   | 5431-33-4 |

**Table 1. GC-Mass Analysis of WGP**

with mass spectra via the database of the WILEY 08 and NIST 014 libraries as shown in Table 2 and Figure 2.

After comparing the results of the GC-MS analyses of WGP and WGO with each other, we detected that both contained six similar compounds with different concentrations. These compounds are oleic acid, nizatidine, palmitic acid, glycylidoleate, linoleoyl chloride, and hexadecanoic acid methyl ester, as summarized in Table 3.

**DMBA-Induced Mammary Tumorigenesis.** Table 4 demonstrates the tumor incidence and mortality cases of DMBA groups with or without (Vit E, WGP, and WGO) treatment. In DMBA-treated rats the majority of tumors were extremely large. In the Vit E + DMBA group, Vit E decreased tumor incidence when compared to DMBA-treated rats. A further reduction in tumor incidence was recognized in animals given WGP. Interestingly, the WGO + DMBA group was the best to decrease tumor incidence as compared to the tumor incidence of any other DMBA-treated rats. Oral administration of Vit E, WGP, and WGO minimized the total tumor burden (50, 60, and 80%) in DMBA-treated groups, respectively.

**Body Weight.** The initial body weight of rats revealed that there was no significant difference between the control groups and DMBA-treated rats, while the rats’ final body weight and the weight gain in DMBA-treated groups significantly (P < 0.05) diminished compared to the animals in the control...
Lipid peroxidation indicated by the MDA serum level was activities of SOD and CAT were significantly reduced compared to the control group (P < 0.001). In parallel, there was no significant difference between treated groups in body weight when compared to each other (Table 5).

Hematological Parameters. Table 6 shows the effect of Vit E, WGP, and WGO on the hematological parameters. There was a significant (P < 0.001) elevation in WBC number and a reduction in RBCs, Hb, and Hct in the DMBA-treated group when compared to control groups. Treatment with Vit E + DMBA showed partial decrement in WBC number and partial elevation in RBCs, Hct, and Hb level compared to the DMBA group, while treatment with WGP + DMBA and WGO + DMBA showed significant improvement in RBCs, Hct, WBCs, and Hb compared to the DMBA group (P < 0.001). Control groups did not show any change in hematological parameters.

Serum Tumor Marker Cancer Antigen 15.3. The level of CA15.3 biomarker was significantly (P < 0.001) elevated in the DMBA-treated group compared to the control group, while in cancer-protected groups, the Vit E + DMBA, WGP + DMBA, and WGO + DMBA groups, the CA15.3 level significantly (P < 0.001) reduced compared to the DMBA-treated group. The WGO cancer-protected group was observed to have the lowest CA15.3 level in comparison with the DMBA-treated group followed by the WGP + DMBA and Vit E + DMBA groups. There was no significant difference between control groups (control, Vit E, WGP, and WGO) (Table 7).

Oxidative Stress and Lipid Peroxidation Parameters. Lipid peroxidation indicated by the MDA serum level was significantly elevated in the DMBA-treated group, while the activities of SOD and CAT were significantly reduced in comparison with the control group (P < 0.001), whereas the WGO cancer-protected group (WGO + DMBA) had the lowest MDA level and the highest SOD and CAT activities compared to the DMBA group followed by the WGP + DMBA and Vit E + DMBA groups. There was no significant difference between the control groups (control, Vit E, WGP, and WGO) (Table 7).

Histopathology of the Breast. The breasts of control groups (control, Vit E, WGP, and WGO) showed normal histological pictures of the mammary tissue. The glandular epithelium did not reveal any signs of hyperplasia or abnormal proliferations. In group V (DMBA-treated group), mammary tissues revealed moderate to severe anaplastic transformation of the glandular epithelium. There was high cancer cells’ proliferation of the lining of the epithelium of acini and ductules with hyperchromatic nuclei that showed multiple mitotic figures. Solid tumor masses invaded the mammary tissue with extensive areas of necrosis along with acute inflammations. Massive to severe angiogenesis was also observed. The breast tissues of group VI (Vit E + DMBA) showed reduction of neoplastic tissue alterations and reduction in inflammation and necrosis of the mammary tissue. Group VII (WGP + DMBA) showed a moderate improvement of the cellular architecture in tumor tissue and a reduction in inflammation and necrosis areas. Group VIII (WGO + DMBA) showed significant improvement of the cellular architecture in tumor tissue with minimum proliferation of the glandular epithelium and reduction in mitotic figures and nuclear changes (Figure 3).

Proliferating Cell Nuclear Antigen (PCNA) Immunohistochemistry. The expression of PCNA was analyzed by the immunohistochemical technique in tumor sections originating from several experimental groups to determine whether WGO, WGP, and vit E affect the cell proliferation induced by DMBA in mammary tumors or not. The DMBA-treated group tumor samples revealed significant (P < 0.001) elevation in PCNA-positive cells, indicating active cell proliferation compared to the normal control group. Although a marginal reduction in the proliferation of tumor cells was observed in group VI (Vit E + DMBA), a pronounced inhibition of cell proliferation in group VIII (WGO + DMBA) followed by group VII (WGP + DMBA) indicated the antiproliferative potential of this compound (Figure 4). As presented in Figure 5, the mean PCNA labeling index (LI) was found to be smaller in all cancer-protected groups. Interestingly, a statistically significant (P < 0.001) decrease in the PCNA LI was observed in the Vit E, WGP, and WGO groups exposed to DMBA as compared to the DMBA control.

**DISCUSSION**

Breast cancer is a common widespread type of cancer in women globally and in Egypt as well.2 In this article, several scientific researchers studied several natural products as chemopreventive agents that can ameliorate the growth of breast cancer cells in animals and/or prevent mammary tumors’ proliferation using a DMBA-induced breast cancer rat model.2,14
The current study was performed to estimate the preventive effects of vitamin E, wheat germ powder, and wheat germ oil against breast carcinoma induced by the environmental carcinogen DMBA in Sprague Dawely albino rats. In this study, we found that WGO followed by WGP and then Vit E pretreatments have an inhibiting effect against breast carcinoma induced by the environmental carcinogen DMBA in Sprague Dawely albino rats. In this study, we found that WGO followed by WGP and then Vit E pretreatments have an inhibiting effect against breast carcinoma induced by the environmental carcinogen DMBA in Sprague Dawely albino rats.

The results of GC-MS analysis of WGO and WGP revealed the preventive impact. Thus, oleic acid has the ability to induce apoptosis and inhibit cell proliferation in cancer cell lines. Several research reports have been published on its effect on breast cancer. Oleic acid, which has received a lot of attention in recent years, has long been thought to have a cancer-preventive effect. Several research reports have been published on its effect on breast cancer. Oleic acid has been shown to have a potential preventive impact. Thus, oleic acid has the ability to induce apoptosis and inhibit cell proliferation in cancer cell lines.

Table 2. GC-Mass Analysis of WGO

| PK | Compound Name | RT  | Area % | CAS  |
|----|---------------|-----|--------|------|
| 1  | 5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-Z) | 3.97 | 1.39 | 77509-05-8 |
| 2  | Nizatidine     | 11.92| 6.30  | 79695-41-2 |
| 3  | 9-Heptadecene-4,6-diyn-8-ol, (Z)- | 14.41| 0.82 | 32768-90-4 |
| 4  | Retinol       | 14.96| 0.97  | 11631-4 |
| 5  | (CAS)$S$ Vitamin A aldehyde | 15.07| 1.73 | 556-68-3 |
| 6  | Hexadecamethyl-cyclooctasiloxane | 15.73| 2.31 | 10049-60-2 |
| 7  | Benzene, (1-pentylheptyl)- | 16.14| 0.96 | 2719-62-2 |
| 8  | Benzene, (1-butyloctyl)- | 16.22| 0.89 | 2719-63-3 |
| 9  | (3S,6S)-2,2,6-Trimethyl-6-((S)-4-methylcyclohex-3-en-1-yl) tetrahydro-2H-pyran-3-ol | 16.49| 2.78 | 22567-36-8 |
| 10 | 1,3,5-Triazine-2,4-diamine,6-chloro-N-ethyl- | 17.39| 0.57 | 1007-28-9 |
| 11 | 1H-Purin-6-amine, [(2-fluorophenyl) methyl]- | 17.92| 2.99 | 74421-44-6 |
| 12 | Cyclopentane tridecanoic acid, methyl ester | 19.56| 1.29 | 24828-61-3 |
| 13 | Hexadecanoic acid | 20.26| 6.81 | 57-10-3 |
| 14 | (CAS)$S$ Palmitic acid | 20.73| 1.03 | 542-44-9 |
| 15 | 2,3-Dihydroxypropylpalmitate | 22.31| 1.46 | 56555-07-8 |
| 16 | 2-Methylenebexane | 22.50| 2.27 | NA |
| 17 | Octasiloxane,1,1,3,3,5,5,7,7, 9, 9,11,11,13,13,15,15Hexadecamethyl- | 22.84| 1.00 | 19095-24-0 |
| 18 | Oleic acid | 23.00| 5.59 | 112-80-1 |
| 19 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 24.99| 1.32 | 6068-80-0 |
| 20 | 2-Methylenebrexane | 25.22| 3.60 | NA |
| 21 | 1,2-Dihydroxyvitamin D3, TMS derivative | 25.54| 0.79 | 57559-94-9 |
| 22 | Linoleoyl chloride | 27.03| 13.39 | 7459-33-8 |
| 23 | Benzyl oleate | 27.22| 2.93 | 1007-28-9 |
| 24 | Benzyl oleate | 27.38| 0.91 | 2462-35-2 |
| 25 | Benzyl oleate | 27.64| 8.90 | 5431-33-4 |
| 26 | Dotriacontane | 27.95| 1.59 | 544-85-4 |
| 27 | Methyl (6E,9E,12E,15E)-6,9,12,15-docosatetraenoate | 28.61| 1.00 | 17364-34-8 |
| 28 | 6,8-Di-C-α-Glucosylluteolin | 28.84| 0.90 | 29428-58-8 |
| 29 | 9,12-Octadecadienoyl chloride, (Z,Z)- | 30.09| 2.28 | 7459-33-8 |
| 30 | 4H-1-Benzopyran-4-one,2(3,4-dimethoxy phenyl)-3,5-dihydroxy -7-methoxy- | 30.46| 1.00 | 6068-80-0 |
| 31 | Hexadecanoic acid, methyl ester | 31.19| 1.89 | 112-39-0 |
| 32 | 3',4',7'-Trimethylquercetin | 32.80| 2.74 | 6068-80-0 |
| 33 | Olein, 2-mono-9-octadecenoic acid,1,2,3-propanetriyl ester, (E,E,E)- | 33.69| 0.55 | 537-39-3 |
| 34 | 2-Hydroxy-3-[(9E)-9-octadecenoxyloxy] propyl(9Z,12Z)-9,12-octadecadienooate | 34.57| 2.00 | 2465-32-9 |
| 35 | Isochiapin B | 35.03| 2.67 | NA |
| 36 | 9-Octadecenoic acid-1,2,3-propanetriyl ester, (E,E,E)- | 35.54| 1.32 | 537-39-3 |
| 37 | 9,12,15-Octadeca trienoic acid, 2,3-bis [(trimethylsilyl)oxy] propyl ester, (Z,Z,Z)- | 35.97| 5.15 | 55521-22-7 |

$^a$PK = peak, RT = retention time, NA = not available, CAS = Chemical Abstracts Service, (CAS)$S$ = common name.

Hexadecanoic acid methyl ester has been found to have anti-inflammatory, antiandrogenic, antioxidant, hypocholesterolemic, S alpha reductase inhibitory, nematicide, and pesticide properties. The hexadecanoic acid methyl ester can also suppress vascular endothelial growth factor receptor 1 protein, showing anticolon cancer activity.

Oleic acid, which has received a lot of attention in recent years, has long been thought to have a cancer-preventive effect. Several research reports have been published on its effect on breast cancer. Oleic acid has been shown to have a potential preventive impact. Thus, oleic acid has the ability to induce apoptosis and inhibit cell proliferation in cancer cell lines.

Linoleoyl chloride (9,12-octadecadienoyl chloride, (Z,Z,Z)-) has been found to have several bioactive impacts in several studies. It has anticorony, hypocholesterolemic, hepatoprotective, nematicide, and antiarthritic properties. Further,
linoleoyl chloride also has been found to have antiandrogenic, 5-alpha reductase inhibitor, antihistaminic, insectifuge, anti-eczemic, nematicide, and anticancer properties, as reported by Vijayabaskar and Elango.28

Glycidyl oleate is a carboxylic ester and an epoxide that was found to has many beneficial health effects, such as antithrombotic and anti-inflammatory properties. In addition, it decreased the triglyceride and cholesterol levels in blood. Moreover, it had antioxidant activities through scavenging of free radicals or by enhancing the activity of antioxidant enzymes.29

It has been demonstrated that four compounds, oleic acid, palmitic acid, linoleoyl chloride, and hexadecanoic acid, methyl ester, have potential anticancer activity. Two of these four compounds, oleic acid and palmitic acid, have the most anticancer activity, as reported by Liu et al.27 and Sianipar et al.,30 respectively. Table 3 summarizes that oleic acid and palmitic acid have higher concentrations in WGO than in WGP. This result confirmed that WGO is more effective as an anticancer agent than WGP.

In the current study, rats’ final body weight was decreased in group V (DMBA-treated group) during the whole experimental period compared to the control group. The reduction

Table 3. GC-MS Concentrations of Different Compounds in WGP and WGO

| Compound Name                  | Area (%) in WGP | Area (%) in WGO |
|--------------------------------|-----------------|-----------------|
| Oleic acid                     | 1.29            | 5.59            |
| Nizatidine                     | 11.54           | 6.30            |
| Palmitic acid                  | 0.9             | 6.81            |
| Glycidyl oleate                | 0.7             | 8.90            |
| Linoleoyl chloride             | 1.07            | 13.39           |
| Hexadecanoic acid methyl ester| 1.80            | 1.89            |

linoleoyl chloride also has been found to have antiandrogenic, 5-alpha reductase inhibitor, antihistaminic, insectifuge, anti-eczemic, nematicide, and anticancer properties, as reported by Vijayabaskar and Elango.28

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Table 4. Effects of Vitamin E, Wheat Germ Powder, and Wheat Germ Oil on DMBA-Induced Breast Cancer Female Albino Rats

| Parameter                  | Experimental Group | Control | Vit E Control | WGP Control | WGO Control | DMBA | Vit E + DMBA | WGP + DMBA | WGO + DMBA |
|----------------------------|--------------------|---------|---------------|-------------|-------------|------|--------------|------------|------------|
| No. of rats with tumors/total rats | 0/10               | 0/10    | 0/10          | 0/10        | 10/10       | 5/10 | 4/10         | 2/10       |
| Tumor incidence (%)       | 0                  | 0       | 0             | 0           | 100         | 50   | 60           | 20         |
| Inhibition (%)            | 100                | 100     | 100           | 100         | 100         | 50   | 60           | 80         |
| Mortality cases           | 0                  | 0       | 0             | 0           | 2           | 0    | 0            | 0          |

Table 5. Effects of Vitamin E, Wheat Germ Powder, and Wheat Germ Oil on Body Weight (g) in Normal and Induced Breast Cancer Female Albino Rats

| Parameter | Experimental Group | Control | Vit E Control | WGP Control | WGO Control | DMBA | Vit E + DMBA | WGP + DMBA | WGO + DMBA |
|-----------|--------------------|---------|---------------|-------------|-------------|------|--------------|------------|------------|
| Initial BW (g) | 85.33 ± 3.05a    | 86.75 ± 2.98a | 87.25 ± 2.05a | 88.40 ± 2.99a | 87.26 ± 3.13a | 89.20 ± 3.00a | 86.50 ± 2.68a | 89.70 ± 2.59a |
| Final BW (g)  | 213.00 ± 6.42a   | 212.50 ± 6.50a | 215.25 ± 6.59a | 217.50 ± 6.48a | 174.26 ± 6.66a | 202.40 ± 6.20ab | 205.00 ± 6.48ab | 209.80 ± 6.58ab |
| Weight gain   | 127.67 ± 3.37a   | 127.75 ± 3.52a | 128.00 ± 4.54a | 129.10 ± 3.49a | 87.00 ± 3.53a | 113.20 ± 3.20ab | 118.50 ± 3.80ab | 120.20 ± 3.69ab |

**Each value represents the mean ± SE (n = 10). Abbreviations: vitamin E (Vit E), wheat germ powder (WGP), wheat germ oil (WGO), 7,12-dimethylbenz[a]anthracene (DMBA), bodyweight (BW). Means in the same row with different superscript letters are significantly different at (P < 0.05).**
### Table 6. Effects of Vitamin E, Wheat Germ Powder, and Wheat Germ Oil on Hematological Parameters in Different Groups

| Parameter | Control | Vit E | WGP | WGO | Vit E + DMBA | WGP + DMBA | WGO + DMBA |
|-----------|---------|-------|-----|-----|-------------|------------|------------|
| RBCs (×10⁶/μL) | 5.34 ± 0.76 | 8.53 ± 0.42 | 6.39 ± 0.78 | 11.68 ± 0.91 | 3.81 ± 0.09 | 8.17 ± 0.08 | 16.17 ± 0.91 |
| Hct (%) | 49.93 ± 1.16 | 49.97 ± 0.40 | 49.97 ± 0.40 | 50.00 ± 0.40 | 49.87 ± 0.40 | 50.00 ± 0.40 | 50.00 ± 0.40 |
| MCV (fL) | 59.57 ± 0.54 | 57.03 ± 0.54 | 62.77 ± 0.54 | 57.03 ± 0.54 | 57.77 ± 0.54 | 60.70 ± 0.54 | 57.77 ± 0.54 |
| MCH (pg) | 19.03 ± 0.23 | 18.77 ± 0.23 | 18.53 ± 0.23 | 18.77 ± 0.23 | 18.53 ± 0.23 | 18.53 ± 0.23 | 18.53 ± 0.23 |
| MCHC (g/dL) | 31.97 ± 0.87 | 31.57 ± 0.87 | 31.40 ± 0.87 | 31.57 ± 0.87 | 31.40 ± 0.87 | 31.40 ± 0.87 | 31.40 ± 0.87 |
| WBCs (10³/μL) | 9.57 ± 0.90 | 10.23 ± 0.90 | 9.50 ± 0.90 | 10.23 ± 0.90 | 9.50 ± 0.90 | 10.23 ± 0.90 | 9.50 ± 0.90 |
| N (%) | 51.90 ± 2.34 | 49.60 ± 2.34 | 46.67 ± 2.34 | 49.60 ± 2.34 | 46.67 ± 2.34 | 46.67 ± 2.34 | 46.67 ± 2.34 |
| L (%) | 52.33 ± 1.67 | 52.33 ± 1.67 | 52.33 ± 1.67 | 52.33 ± 1.67 | 52.33 ± 1.67 | 52.33 ± 1.67 | 52.33 ± 1.67 |
| M (%) | 9.00 ± 0.00 | 9.00 ± 0.00 | 9.00 ± 0.00 | 9.00 ± 0.00 | 9.00 ± 0.00 | 9.00 ± 0.00 | 9.00 ± 0.00 |
| B (%) | 0.00 ± 0.00 | 0.67 ± 0.00 | 0.33 ± 0.00 | 0.67 ± 0.00 | 0.33 ± 0.00 | 0.33 ± 0.00 | 0.33 ± 0.00 |
| PLT (x10⁴/μL) | 599 ± 27.32 | 618 ± 20.00 | 599 ± 27.32 | 618 ± 20.00 | 599 ± 27.32 | 618 ± 20.00 | 599 ± 27.32 |

Each value represents the mean ± SE (n = 10). Means in the same row with different superscript letters are significantly different (P < 0.05). P < 0.01, different compared to the control group. P < 0.001, different compared to the control group. P < 0.0001, different compared to the control group.

Abbreviations: Hb, hemoglobin; RBCs, red blood cells; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBCs, white blood cells; M, monocytes; L, lymphocytes; E, eosinophils; N, neutrophils; B, basophils; PCT, platelet count.

in body weight could be due to the alterations induced by DMBA injection, as DMBA alters the normal biochemical processes, changed the activities of several antioxidant enzymes, induced apoptosis of epithelial cells, and elevated the lipid peroxidation and free radical levels that damage the membrane of the organelles and the structure of the cell, including nucleic acids, proteins, and lipids, leading to decreased body weight, as recorded by Wang and Zhang.31 This result was in accordance with those of Rojas-Armas et al.4

Meanwhile, the body weights of DMBA-treated rats in the vitamin E, wheat germ powder, and wheat germ oil pretreated groups were increased and found to be near the normal control groups. These results were in accordance with those of Abo-Elmaaty et al.,15 who assessed the protective effect of Vit E at a dose of 100 mg/kg BW for 15 consecutive days against the acute kidney injury induced by cisplatin in rats, as it returned the body weight to the control ranges. Moreover, Nagib30 studied the protective effect of WGP at a dose of 20 g/100 g diet for 6 weeks on metabolic disturbances induced by a high fat and high fructose diet in rats. WG showed a significant elevation in body weight gain as compared to the negative control group. Also Khalifa et al.33 showed that rats fed on a diet supplemented with treatment doses of wheat germ oil at a level of 200 mg/kg diet for 30 days significantly increased the body weight when compared to the chlorpyrifos-treated group.

The data of the hematological parameters in this study revealed that DMBA injection in group V caused a significant reduction in RBCs, Hb, and Hct as compared with group I (control) and all other treated groups, indicating a tendency to anemia.34 The occurrence of anemia is a logical consequence of malignancy and is estimated to occur in up to 60% of cancer cases. The most common anemia in cancer is anemia of inflammation/chronic disease, occurring due to iron deficiency, since cancer can slow down the production of red blood cells or interfere with iron stored in cells and decrease iron absorption.35 The MCHC, MCV, and MCHC levels demonstrated that the anemia is of the normocytic normochromic type. These results were in agreement with those of Zinge et al.36 Furthermore, the number of WBCs was significantly elevated compared to control groups. The elevation in WBCs is due to the inflammatory response in rats with large tumors that increases the risk of developing invasive breast cancer.37 The results of Nguedia et al.15 were consistent with the present study.

As compared to group V (DMBA group), pretreatment of rats with Vit E, WGP, or WGO greatly ameliorated the hematological parameters to each respective control values. The improvement of the hematological parameters in the Vit E-, WGP-, and WGO-treated groups might be due to the stabilize-antioxidant function. Vitamin E treatment avoids polyunsaturated fatty acids’ oxidation in the RBCs’ membrane, consequently enhancing erythropoiesis and improving blood hemoglobin levels.38 Moreover, WGP and WGO contain vitamins (A and D) which regulate the cellular growth, act as anti-inflammatory agents, differentiate erythropoiesis in bone marrow cells, and are essential for the formation of RBCs.39

These results were in accordance with those of Abdou et al.,40 who showed that intraperitoneal injection of vitamin E at the dose 100 mg/kg BW/day and oral administration of WGO at the dose 54 mg/kg BW/day for 13 days ameliorated all hematological parameters against cadmium chloride-induced toxicity. These results also were consistent with those of Saleh et al.,41 who assessed that administration of WG as 20% of the...
diet for 45 days improved the hematological parameters to normal control values against the toxic effects of chlorpyrifos given at the dose 50 mg/kg/day for the last 3 days of experiment by gavage.

Serum tumor biomarker CA15.3, which is the most widely used biomarker for breast cancer diagnosis, is frequently applied for screening and monitoring of metastatic breast cancer and is a possible factor that may regulate it. CA15.3 is a mucinous glycoprotein that is produced by the Mucin1 gene found in epithelial cells.14

In our study, the level of CA15.3 was significantly increased in group V (DMBA-induced breast cancer rats) in comparison with control rats. This elevation is attributed to the increase in the Mucin1 gene found in epithelial cells, which confirmed the induction of breast cancer. This result was consistent with that of Karimi et al.,31 who revealed that treatment of rats with 50 mg/kg of DMBA dissolved in sesame oil by oral gavage showed increased levels of CA15.3 compared to control rats.

Administration of WGO, WGP, and Vit E along with DMBA significantly ameliorated the CA15.3 level. Such a decline is associated with WGO-, WGP-, and Vit E-treated groups, denoting the beneficial effect of these substances on breast cancer prognosis and outcomes. This amelioration was due to the anti-inflammatory, antioxidant, and anticarcinogenic activities of these materials. These results were in agreement with those of Helal et al.,32 who assessed the improved effect of Vit E (100 g BW/day) on the oxidative stress induced by chronic administration of bisphenol A.

Oxidative stress plays a vital role in the pathogenesis of different diseases. MDA, SOD, and CAT were evaluated on serum to investigate the possible mechanism by which WGO,
WGP, and Vit E abolished breast carcinoma induced by the environmental carcinogen DMBA in albino rats. ROS are directly eliminated by SOD enzymatically. Moreover, catalase catalyzes the reduction of hydrogen peroxides and protects the tissues from reactive hydroxyl radicals, since DMBA initiates tumorigenesis through production of ROS that leads to the initiation and promotion of cancer.

The present study showed that DMBA increased oxidative stress in cancer-induced rats. It significantly elevated the MDA levels compared to the normal control groups, whereas it significantly reduced the SOD and CAT levels. These results were in agreement with those of Nguedia et al., who found that DMBA caused an increase in the MDA level and a decrease of the antioxidant enzymes’ levels due to the higher production of ROS that damaged numerous biomolecules and exerted many molecular and cellular effects, including cytotoxicity and mutagenicity that can lead to the initiation and promotion of cancer.

In parallel, Vit E treatment in group VI (Vit E + DMBA) significantly reversed the DMBA effects and restored the functions of the antioxidant. As the Vit E antioxidant properties increased, the activities of both the SOD and CAT enzymes also increased. These results were in agreement with those of some earlier preclinical studies. Meanwhile the WGO-protected group (WGO + DMBA) had the lowest MDA level and the highest level in SOD and CAT activities compared to the DMBA group followed by the WGP-protected group (WGP + DMBA). These results revealed that WGO administration is the best at ameliorating the effect of DMBA and restoring the values of the parameters to the normal range compared to WGP and Vit E; this was in agreement with the results of Abdou et al. and Saleh et al. The amelioration is due to the anti-inflammatory, antioxidative, and antitumor activities that regulate the main function of cellular enzyme. The conditions produced by WGO, WGP, and Vit E in the herein breast cancer model demonstrated their cancer development inhibiting effect, whereas they lower the oxidative damage risk during carcinogenesis, therefore inhibiting cancer development.

Our results showed that induction of mammary carcinogenesis caused histopathological alterations in the breast tissues of cancer-induced rats. In group V (DMBA-treated group), mammary tissues revealed moderate to severe anaplastic transformation of the glandular epithelium. There was high cancer cells’ proliferation of the lining of the epithelium of acini and ductules with hyperchromatic nuclei that showed multiple mitotic figures. Solid tumor masses invaded the mammary tissue with extensive areas of necrosis along with acute inflammations. Massive to severe angiogenesis was also observed compared to normal breast tissues. The mechanism of DMBA-induced carcinogenesis is through upregulation of cytochrome P450 leading to transformation of DMBA into a mutagenic intermediate that causes DNA adducts proceeding to carcinogenesis. The present findings are in agreement with recent studies that declared the interruption effect of DMBA on the normal differentiation process of the mammary gland.

Cell proliferation plays a critical role in the investigation of breast cancer progression and the examination of substances that used are in the prevention of breast cancer and have an influence on cancer cell proliferation. PCNA is used to examine progression in mammary carcinoma and tumor cell proliferation. This study examined PCNA expression in breast tissues using the immunohistochemical technique for all experimental groups to investigate whether WGO, WGP, and Vit E affect cell proliferation induced by DMBA in mammary tumors or not.

Expression of PCNA is found to be elevated in DMBA-treated mammary tumor sections in group V compared to normal control groups, indicating active cell proliferation. This elevation occurred because DMBA increased cancer cell proliferation and invasion through the promotion of epithelial–mesenchymal transition inducing factors and β-catenin by upregulating specificity protein 1 activity. This result agreed with that of Mehraban et al., who studied the anticancer effect of Astragalus ovinus against DMBA (40 mg/kg BW)-induced breast cancer in rats. The result revealed that treatment with DMBA significantly increased the PCNA expression (80%) compared to the control group.

Moreover, reduction in the proliferation of tumor cells was observed in group VI (Vit E + DMBA), and a substantial inhibition of cell proliferation was observed in group VIII (WGO + DMBA) followed by group VII (WGP + DMBA). This result indicated the antiproliferative potential and protective effect of these compound. The reduction in PCNA expression along with lowering of the PCNA LI in the tumor tissues of rats treated with WGO and WGP might be attributed to the presence of four compounds, oleic acid, palmitic acid, linoleoyl chloride, and hexadecanoic acid, methyl ester, as detected in GC-MS analysis of WGO and WGP. These compounds are found in higher concentration in WGO than in WGP. They have been found to have potential anticancer activity, as they can suppress cell proliferation and induce apoptosis cancer cells. Moreover, they had antioxidant activities through scavenging of free radicals or by enhancing the activity of antioxidant enzymes. WGO is the best protective agent to ameliorate the DMBA effect and reduce PCNA expression.

CONCLUSION

The current study demonstrated that WGP, WGO, and Vit E administration provided an effective protection against DMBA-induced breast carcinogenesis in rats, since they can ameliorate serum biochemical parameters, reduce tumor size as well as MDA and CA15.3 levels, and increase antioxidant properties compared to the DMBA rats. Furthermore, they reduced the proliferation of mammary ducts with mild to moderate inflammatory responses.

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Notes

The authors declare no competing financial interest. The experimental design was performed according to the ethical guidelines set by the research ethical committee of Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

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■ ABBREVIATIONS

ANOVA, analysis of variance; BC, breast cancer; BW, body weight; CAT, catalase; CA15.3, breast antigen; CBC, complete blood picture; °C, degree Celsius; DMBA, 7,12-dimethylbenz[a]anthracene; E, eosinophils; EDTA, ethylenediaminetetraacetic acid; fL, femtoliter; gm, gram; H&E, hematoxylin and eosin; Hb, hemoglobin; Hct, hematocrit; kg, kilogram; L, lymphocyte; LI, labeling index; M, monocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MDA, malondialdehyde; mg, milligram; mL, milliliter; N, neutrophils; n, number; ng, nanogram; P, probability; PCNA, proliferating cell nuclear antigen; pg, picogram; RBCs, red blood cells; ROS, reactive oxygen species; SCU, Suez Canal University; SD, Sprague–Dawley; SE, standard error of the mean; SOD, superoxide dismutase; SPSS, Statistical Package for Social Sciences; Vit E, vitamin E; WBCs, white blood cells; WGO, wheat germ oil; WGP, wheat germ powder; µL, microliter; %, percent

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