

Anti-Tumor Effects of *Dendronephthya putteri* Ethanolic Extract in DMBA-Induced Breast Cancer in Adult Female Rats

Abeer Z.F. Maued¹, Amira El-Tohamy¹, Mahmoud Ashry^{2*}

¹Zoology Department, Faculty of Science, Al-Azhar University, Girl Branch, Cairo, Egypt

²Zoology Department, Faculty of Science, Al-Azhar University, Assuit, Egypt

*Corresponding author: Mahmoud Ashry, Mobile: (+20) 01009715105, E-Mail: mahmoud_ashry20@yahoo.com

ABSTRACT

Background: A number of physiological cell signaling pathways can develop aberrations that lead to the complex breast cancer sickness.

Objective: The effectiveness of *Dendronephthya putteri* ethanolic extract (DEE) against breast cancer caused by 7, 12-dimethylbenz[a]anthracene (DMBA) in adult female albino rats was examined in this study.

Materials and Methods: Adult female albino rats were divided into five groups of 10, each weighing between 160 and 200 g: group (1) normal rats serving as controls, group (2) normal rats receiving daily injections of DEE at a dose of 100 g/kg/day, group (3) animals with DMBA-induced breast cancer, group (4) animals modeled with breast cancer, and group (5) animals modeled for breast cancer receiving reference drug 5-Fluorouracil® (5UR) (10 g/kg/weekly, ip) treatment for six weeks. **Results:** DEE injection considerably improved illnesses brought on by breast cancer, as seen by the sharp decline in serum cancer antigen 15.3 (CA15.3), carcinoembryonic antigen (CEA) and cancer antigen 19.9 (CA19.9) levels. Mammary malondialdehyde (MDA) and nitric oxide (NO), along with mammary superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), the activity and appropriateness of reduced glutathione (GSH) level, were all considerably increased. Other significant elevations were seen in tumor necrosis factor alpha (TNF- α), interleukin-1beta (IL-1 β), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, cholesterol, and triglycerides. **Conclusion:** By enhancing immune function, lowering inflammation, and reversing oxidative stress, this study shows that DEE can prevent DMBA-induced breast cancer in rats.

Keywords: Breast cancer, DMBA, Antitumor, Oxidative stress, *Dendronephthya putteri*

INTRODUCTION

The most prevalent kind of cancer and the main reason for cancer-related morbidity and death in women throughout the world is breast cancer ⁽¹⁾. In developing nations, where the illness has historically been rare, the incidence and death of breast cancer have lately increased ⁽²⁾. Every year, it targets more than 500,000 women. Enormous cell proliferation, unbalanced cell differentiation, and insufficient apoptosis are characteristics of breast cancer ⁽³⁾. Rats with artificially induced breast tumors have been used for some time to simulate the growth of human breast cancer. Single doses of carcinogens like DMBA or nitrosomethyl urea can cause breast cancer in sensitive rat strains. Rat tumors are weakly invasive, highly hormone-dependent, have a brief latency, and seldom metastasize. This model's tumor's shape and histology are similar to estrogen-dependent actual breast cancer ⁽⁴⁾.

A high-ranking polycyclic aromatic hydrocarbon called dimethylbenz (a) anthracene (DMBA) is a genotoxic and tumor-causing invasive environmental pollutant. The DMBA-generated mammary tumor is a crucial preclinical animal model of breast cancer ⁽⁵⁾. The DNA is harmed by the metabolite of DMBA that results from the addition of adenine and guanine residues. p53, BRCA, Bcl2, and p63 are only a few of the biochemical and molecular markers that are expressed in DMBA-induced breast tumors in both rats and people ⁽⁶⁾. Apoptosis, which causes malignancy, as is well known, inhibits the development of cancer. Breast cancer and conditions associated with cancer have been treated with surgery, chemotherapy, radiation therapy, or a combination of these. But

regardless of these treatment choices, cancer still has a high mortality rate ⁽⁷⁾. The fact that the majority of those affected by breast cancer cannot afford the pricey sort of treatment is one of the largest issues with the disease's management ⁽⁸⁾. Additionally, low- and middle-income people have been shown to account for more than 70% of all cancer-related deaths ⁽⁹⁾.

Nigeria, one of the tropical countries, is home to a variety of plants that have been shown to have medicinal anti-cancer properties ⁽⁷⁾. The production of compounds important for drug development depends on the various biological effects of marine natural products ⁽¹⁰⁾. A special class of metabolites discovered in soft corals displays a wide-ranging bioactivities and structural diversity. Research on marine soft corals will produce a wide-ranging chemically and physiologically diverse substance that could be used in the pharmaceutical industry as a result ⁽¹¹⁾; the Nephtheidae family, which is made up of 20 species and is a substantial source of metabolites with therapeutic purposes ⁽¹²⁾. The most high-ranking metabolites are terpenes and steroids, which have bacterial, inflammatory, and cancer-preventative characteristics ⁽¹³⁾. The Red Sea and Indo-Pacific Oceans are home to the renowned Nephtheidae genus *Dendronephthya putteri*. *Dendronephthya*, a genus of marine soft corals, produces a variety of terpenoids. These metabolites are commonly considered as potential chemotherapeutic agents due to their anticancer properties. The genus *Dendronephthya putteri* is capable of producing up to 250 bioactive compounds, the majority of which are polyhydroxy steroids, sesquiterpenes, and diterpenes. These

secondary metabolites have been shown to have fascinating biological effects, particularly in the treatment of cancer, malaria, inflammation and a range of infectious disorders, where little structural variations fundamentally impact the potency and selectivity⁽¹⁴⁾.

The current work examined the anticancer therapeutic potential of DEE against an induced mammary tumor in female rats using biochemical, pro-inflammatory and oxidative stress.

MATERIALS AND METHODS

Chemicals: In the United States, Sigma Aldrich in St. Louis, Missouri, sold us DMBA (7, 12-Dimethylbenz (a) anthracene).

Preparation of *Dendronephthya putteri* ethanolic extract

A qualified professional identified the soft coral sample as (*Dendronephthya putteri*) after it was obtained in the winter of 2021 on the Red Sea coast of Egypt, immediately brought to the lab for the extraction procedure. After being divided into smaller bits, the 1.25 kg of material was allowed to macerate in ethanol at room temperature. To obtain 40 g of dry coral powder, Whatman filter paper (Merck, Darmstadt, Germany) was first used to filter the mixture. The solvent was subsequently evaporated at 50°C using a rotary evaporator. The powder was then kept at -80°C until use.

Animals: Adult female albino rats (weighing 160–200 g) were bought from the National Research Center's animal colony. They were given free access to food and water for a week prior to the experiment to allow for acclimatization.

Induction of breast cancer

Breast cancer induction: At the age of 7 weeks, the animals were given a single dosage of DMBA (20 mg/animal), diluted in 1 ml of soy oil, and given by gavage. This was accomplished using the methods described by **Chatterjee et al.**⁽¹⁵⁾. Each rat got a weekly physical examination, which included feeling, touching, and palpating each of the six pairs of mammary glands. Analyses were performed at the 8th and 13th weeks following DMBA ingestion to guarantee tumor formation.

Animals' grouping

Animals simulating breast cancer and healthy animals were sorted into five groups of ten rats each using the following formula: group (1) normal rats serving as controls, group (2) normal rats receiving daily injections of DEE at a dose of 100 mg/kg/day, group (3) animals with DMBA-induced breast cancer, group (4) animals modeled with breast cancer induced by DMBA then treated with DEE at a dose of 100 mg/kg/day for six weeks, and group (5) animals modeled for breast cancer induced by DMBA then receiving reference drug 5UR (10 g/kg/weekly, ip) treatment for six weeks. At the conclusion of the trial, blood samples from each animal were collected, separated, and kept at -80°C until biochemical and immunological analyses. Animals were immediately

beheaded to cause death after blood was collected, and mammary tissues were subsequently extracted and homogenized.

Assessment of biochemical markers

Serum triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, urea, creatinine, and the ASAT and ALAT activities were all measured spectrophotometrically using reagent kits obtained from Biodiagnostic, Giza, Egypt.

Determination of tumor markers and immune-cytokines

Using rat reagent ELISA-kits acquired from SinoGeneClon Biotech Co, Hang Zhou, China, the levels of CEA, CA19.9, CA15.3 level, tumor necrosis alpha (TNF- α), interleukin-1 beta, and (IL-1 β) were assessed using the ELISA technique (Dynatech Microplate Reader Model MR 5000).

Determination of oxidative stress in mammary gland tissue:

Breast tissue levels of nitric oxide (NO), lipid peroxidation end product (malondialdehyde, MDA), reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were spectrophotometrically measured using reagent kits obtained from Biodiagnostic, Giza, Egypt.

Regarding temperature, ventilation, humidity, and a regular dark/light cycle, animals were handled in the same degree of the recommendations for the use and care of laboratory animals. Over the course of the project, the animals had a diet of rat chow and were permitted free use of water.

Ethical approval

The Al-Azhar University Ethics Board gave the study its approval. The Animal Care and Use Committee at Cairo, Egypt's Al-Azhar University's Faculty of Science approved the ethical norms and guidelines used in this work. All the experimental procedures conformed to "Guide for the care and use of Laboratory Animals" for the use and welfare of experimental animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Statistical analysis

Using the SAS General Linear Model method (SAS 1982), the analysis of variance (ANOVA) method was used to statistically examine all data. The Waller-Duncan k-ratio was used to assess the significance of changes between the treatment groups⁽¹⁶⁾. All claims of significance were supported by the probability of $p \leq 0.05$.

RESULTS

It was discovered that the levels of CEA, CA19.9, CA15.3, TNF- α , and IL-1 β were considerably greater in the DMBA-induced breast cancer group than in the control group. DEE therapy reduced serum levels of CEA, CA19.9, CA15.3, TNF- α , and IL-1 β to levels that were comparable to those of the control group, as opposed to the group that had breast tumor models (Table 1).

Table (1): Mean values of serum IL-1 β , TNF- α , CEA, CA15.3 and CA19.9 of normal and breast cancer-treated animals' groups as compared to control group.

	Control	DEE	DMBA	DBMA~ DEE	DMBA ~ 5UR
CEA (U/mL)	30.1 \pm 1.2	28.2 \pm 2.7	62.4 \pm 5.436*	44.2 \pm 2.3 [#]	58.2 \pm 3.6 [#]
CA15.3 (U/mL)	16.0 \pm 0.95	16 \pm 1.01	206.4 \pm 8.5*	34 \pm 3.6 [#]	48.07 \pm 4.4 [#]
CA19.9 (U/mL)	36.06 \pm 4.2	33.8 \pm 2.9	171.7 \pm 4.3*	66.1 \pm 5.04 [#]	42.8 \pm 4.3 [#]
IL-1 β (ng/L)	43.7 \pm 2.5	42.9 \pm 3.6	456.4 \pm 19.717*	85 \pm 6.8 [#]	75.07 \pm 5.9 [#]
TNF- α (ng/L)	187.7 \pm 8.8	184.7 \pm 8.4	233.4 \pm 5.302*	194.4 \pm 14.6 [#]	190.7 \pm 5.1 [#]

Data are presented as mean standard deviation, and one way ANOVA demonstrates that * is substantially different from the control group and # is fundamentally different from the DMBA group (p \leq 0.05). DMBA (Dimethyl Benz (α) Anthracene), DEE (*Dendronephthya putteri* ethanolic extract); cancer antigen 15.3 (CA15.3), carcinoembryonic antigen (CEA) and cancer antigen 19.9 (CA19.9); tumor necrosis factor alpha (TNF- α), interleukin-1beta (IL-1 β)

One orally administration of DMBA had a significant impact on markers of liver and kidney function, as seen by notably elevated blood liver enzymes (ALT and AST) and increased serum renal markers (urea and creatinine) when compared to normal rats. The liver and renal functions of healthy rats receiving DEE extract were never hampered. In rats modeled breast cancer, DEE dramatically reduced DMBA-induced decreases in liver and kidney function (Table 2).

Table (2): Mean values of serum ALAT, ASAT, creatinine and blood urea of normal and breast cancer-treated animals' groups as compared to control group.

	Control	DEE	DMBA	DBMA~ DEE	DMBA ~ 5UR
ALAT (U/L)	40.8 \pm 1.389	40 \pm 2.2	83.5 \pm 3.9*	45 \pm 3.8 [#]	65.7 \pm 5.2 [#]
ASAT (U/L)	50.6 \pm 2.027	48.6 \pm 0.7	99.8 \pm 3.1*	55 \pm 4.3 [#]	73.2 \pm 4.7 [#]
Creatinine (mg/dl)	0.73 \pm 0.0463	0.7 \pm 1.7	2.0 \pm 0.3*	1.5 \pm 0.2 [#]	1.8 \pm 0.16 [#]
Urea (mg/dl)	42.7 \pm 2.092	40.5 \pm 1.6	71.5 \pm 4.6*	49 \pm 3.5 [#]	60.2 \pm 3.3 [#]

Data are presented as mean standard deviation, and one way ANOVA demonstrates that * is substantially different from the control group and # is fundamentally different from the DMBA group (p \leq 0.05). DMBA (Dimethyl Benz (α) Anthracene), DEE (*Dendronephthya putteri* ethanolic extract); 5-Fluorouracil® (5UR); alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT)

DEE delivery to healthy rats did not affect the blood lipid profile compared to the healthy control group, but DMBA intoxication triggered atherosclerosis start. This was made possible by noticeably higher serum levels of LDL cholesterol, triglycerides, and total cholesterol, along with noticeably lower serum levels of HDL cholesterol. As opposed to the group of breast cancer model animals, DEE enzyme post-treatment of breast cancer model rats fundamentally improved all lipid profile parameters (Table 3).

Table (3): Mean blood concentrations of HDL cholesterol, LDL cholesterol, triglycerides, and total cholesterol in normal and breast cancer-treated animal groups compared to the control group.

	Control	DEE	DMBA	DBMA~ DEE	DMBA ~ 5UR
Total cholesterol (mg/dl)	150 \pm 5.1	147.5 \pm 5.2	226.7 \pm 12.9*	176 \pm 9.6 [#]	168.5 \pm 13.5 [#]
Triglycerides (mg/dl)	152.7 \pm 2.7	151 \pm 5.8	207 \pm 9.4*	164.5 \pm 13.4 [#]	187.7 \pm 6.7 [#]
HDL-C (mg/dl)	43.7 \pm 1.6	44.5 \pm 1.4	28.2 \pm 3.0*	34 \pm 2.3 [#]	26.7 \pm 1.9 [#]
LDL-C (mg/dl)	76.7 \pm 3.5	72.5 \pm 3.2	156.5 \pm 5.3*	100.5 \pm 3.3 [#]	104.75 \pm 10.4 [#]

Data are presented as mean standard deviation, and one way ANOVA demonstrates that * is substantially different from the control group and # is fundamentally different from the DMBA group (p \leq 0.05). DMBA (Dimethyl Benz (α) Anthracene), DEE (*Dendronephthya putteri* ethanolic extract); HDL-C (high density lipoprotein-cholesterol); LDL-C (low density lipoprotein-cholesterol)

In addition to having significant changes in the oxidative status of their mammary tissue, rats receiving DMBA injections also showed significant differences from the healthy control group. This was demonstrated by a sudden drop in the antioxidant batteries (GSH, SOD, and GPx) and a simultaneous increase in the oxidative potency (MDA and NO). Fortunately, post-treatment with DEE of animal model breast cancer rats fundamentally increased SOD activity and fundamentally decreased low mammary GSH. Additionally, DEE was able to lower the breast level of NO and MDA in comparison to the similar values of the group of cancer model rats who had not received DEE (Table 4).

Table (4): Mean values of mammary gland oxidant-antioxidant indicators in the control group and normal and breast cancer-treated animal groups

	Control	DEE	DMBA	DBMA~ DEE	DMBA ~ 5UR
MDA (nmol/g)	749.3±64.1	743.8±92.2	1407.9±35.9*	870.6±52.5#	972.4±30.5#
NO (µmol/g)	124.7±7.2	123.1±6.6	262.4±18.7*	154.9±10.06#	177.1±4.3#
GSH (mmol/g)	54.2±3.9	55.7±2.6	25.04±2.6*	39.2±3.2#	35.16±3.2#
SOD (U/g)	2501.7±90.1	2581.1±129.2	1163.7±61.8*	1990.4±94.6#	1778.8±51.5#
GPx (U/g)	365.5±13.2	371.1±14.2	166.4±6.8*	286.3±11.4#	252.5±35.2#
CAT (U/g)	31.6±2.04	33.5±1.7	14.4±1.6*	25.7±3.0#	21.9±2.4#

Data are presented as mean standard deviation, and one way ANOVA demonstrates that * is substantially different from the control group and # is fundamentally different from the DMBA group (p< 0.05). DMBA (Dimethyl Benz (α) Anthracene), DEE (*Dendronephthya putteri* ethanolic extract); malondialdehyde (MDA); nitric oxide (NO); superoxide dismutase (SOD); catalase (CAT); glutathione peroxidase (GPx); reduced glutathione (GSH).

DISCUSSION

One of the principal causes of death for women and one of the most common malignancies in women is breast cancer (17). Conforming to the American Cancer Society, breast cancer continues to kill the most people worldwide and affects the most women of all cancers (18). The current investigation sought to ascertain whether *Dendronephthya putteri* (DEE) might be used to treat adult female albino rats that had developed breast cancer as a result of DMBA. They may be good candidates for the treatment of breast cancer because the therapeutic actions of DEE did not result in any harm in adjacent healthy tissues or mammary gland cells.

The results, which are consistent with earlier research, showed that the blood levels of the tumor markers CA15-3 and CA19.9 along with the pro-inflammatory cytokines TNF-α, IL-1β, and CD4 were fundamentally higher in the DMBA-treated group than in the control group(19,20). They found that whether it was given once or multiple times, DMBA produced the greatest breast tumors with biochemical and molecular markers that closely mimicked the symptoms of human breast cancer. Conforming to research, DMBA builds up in the fatty tissue of the mammary glands and encourages the development of cytochrome (CYP) enzymes that are involved in the metabolism of estrogen Costa *et al.* (21). Cancer has been linked to cytochrome P450 (CYP1B1) overexpression and reactive oxygen species harm to DNA (22). The transcription factor NF-kB and pro-inflammatory cytokines are significant molecular contributors to a range of processes, including cancer and inflammation. A crucial element in the development of tumors is inflammation. A growing body of data also points to TNF-α potential role as an endogenous tumor promoter. IL-1β, a crucial component in the connection between inflammation and cancer etiology, was likewise elevated by DMBA (23). Intriguingly, as compared to rats with breast cancer, DEE treatment dramatically decreased blood levels of CEA, CA15-3, CA19.9, TNF-α, and IL1β in rats with produced mammary tumors.

Conforming to the current study, model prostate cancer-bearing rats received considerably less DEE

than the control group in terms of immunoinflammatory markers (TNF-α and IL-1 β), CA15-3, CA19.9, and CEA. Numerous elements, including the immunoregulatory and anti-inflammatory abilities of its main ingredients, intracellular and extracellular effects, may affect this impact activity (20). The therapeutic advantages of DEE shield the host from ROS-induced cell and tissue harm by binding free iron and regulating crucial antioxidant enzymes.

As essential organs, the kidneys and liver's ability to break down chemotherapy drugs is substantially reduced when their regular function is interfered with, increasing the body's overall toxicity (24). The liver, which plays a major role in the metabolism of xenobiotic chemicals, is also vulnerable to chemical agents' harmful effects. While administration of the prepared therapeutic effects of the DEE had no impact on the hepatic and renal functions of the treated rats, single DMBA intraperitoneal injection fundamentally elevated indicators of kidney and liver function. This is shown by noticeably higher blood levels of the renal indicators; urea and creatinine along with the liver enzymes AST and ALT when compared to the rats' normal levels.

In comparison to the control group, serum ALT and AST activity fundamentally increased after DMBA intoxication; this increase is thought to be a symptom of liver harm brought on by DMBA. Conforming to Dakrory *et al.* (25), liver deterioration is assumed to be a result of DMBA metabolism's production of ROS and carcinogenic compounds. Similar to this earlier discovery, the results of the current study demonstrated that the DEE had hepatoprotective effects against DMBA in a rat model of breast cancer via reducing hepatic inflammation. The poisonous metabolic byproducts of DMBA have a detrimental effect on this significant organ, much as the kidney, which excretes a range of dangerous metabolic wastes.

The current investigation found that following DMBA poisoning, blood urea and creatinine levels fundamentally increased in comparison to the control group. These increased levels could be a sign of renal dysfunction and DMBA poisoning (24). At least a portion of DEE's therapeutic actions against DMBA-induced renal oxidative stress and tissue harm may be

ascribed to its antioxidant capacity ⁽²⁶⁾.

Additionally, increased lipid metabolizing activity has been linked to cancer, and cholesterol metabolism is changed during tumor formation ⁽²⁷⁾. The two suggested mechanisms by which DEE lowers hepato-nephrotoxicity in this instance are reducing lipid peroxidation and changing the antioxidant defense system or giving free radicals an electron to minimize their reactivity ⁽²⁸⁾. Dysregulated lipogenesis, which leads in enormous cholesterol synthesis and enrichment of tumor cell membranes, has been linked to malignant tumors. Atherosclerosis was brought on by DMBA poisoning, but it did so by dramatically lowering HDL cholesterol and fundamentally raising blood total cholesterol, triglycerides, and LDL cholesterol. Conforming to **Weng *et al.*** ⁽²⁹⁾, changes in lipid metabolizing enzymes and abnormal lipid profile levels are related to illness stage. Rats with DMBA-induced breast cancer had considerably greater levels of total blood cholesterol, triglycerides, and LDL cholesterol, but their levels of HDL cholesterol were also fundamentally lower. High triglyceride and cholesterol levels modify the lipid fluidity of tumor cell membranes, affecting tumor cell growth and reproduction and raising the risk of malignancy ⁽³⁰⁾.

Using DEE, lipid profiles were effectively restored to almost normal levels in breast tumor model rats. It has been discovered that boosting a number of antioxidant enzymes and maintaining the equilibrium between pro- and antioxidants can dramatically lower carcinogenicity. Additionally, rats given DMBA injections had their mammary tissue's oxidative state drastically altered. GSH, SOD, and GPx levels fundamentally decreased when compared to rats in the healthy control group, and MDA and NO levels correspondingly increased. Antioxidants protect cells from the harm that free radicals and reactive oxygen species (ROS) cause (SOD and GPx enzymes, along with GSH levels) ⁽³¹⁾.

Comparing rats with DMBA-induced breast cancer to control rats revealed fundamentally lower levels of GSH, SOD, and GPx enzyme activity. After DMBA poisoning, SOD and GPx activities, along with GSH levels, may have been used more frequently to combat the enormous production of free radicals and ROS, leaving them with weakened defense against free radicals, which may have contributed to the decline in the antioxidant capacity of breast tissue antioxidant stress defenses ⁽³²⁾. Reduced GSH levels were shown to increase lipid peroxidation (increased levels of MDA) and enormous antioxidant consumption for tumor cell growth in rats with breast cancer ⁽¹⁹⁾. This beneficial shift might be the result of enormous blocking and/or stability of free radical production.

Nitric oxide synthase in immune cells may have been overstimulated during the preneoplastic transition stage, conforming to the significant increase in NO levels observed in breast tissue during active breast cancer inflammation ⁽³³⁾. Soft corals' sesquiterpenoid

and diterpenoid metabolites mostly contribute to defensive reactions. Biologically active terpenoids are used in the treatment of cancer, malaria, inflammation, and a range of infectious diseases ⁽³⁴⁾. Conforming to the results of the current investigation, DEE treatment successfully decreased the breast MDA and NO levels of malignant rats while replenishing their antioxidant reserves (SOD, GPx, CAT, and GSH) to levels comparable to the healthy controls. The enormous suppression and/or stability of free radical production caused by the antioxidant capacity of DEE components may be the cause of this beneficial enhancement.

CONCLUSION

Conforming to the study's findings, the DEE has the potential to treat breast cancer. The much-improved data on biomarkers, immuno-inflammatory state, and oxidative condition allowed for this outcome. It might be claimed that DEE is a prime contender for breast cancer prevention along with treatment. The usage of DEE exhibitions is also secure.

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