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Therapeutic effects of *Nephthea pacifica* ethanolic extract in DMBA-induced breast cancer in adult female rats

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Abstract

Breast cancer is a complex disease that can rapidly progress due to abnormalities in several cellular signaling pathways. The purpose of the study was to evaluate the anti-cancer properties of *Nephthea pacifica* ethanolic extract (NEE) against DMBA induced breast cancer in adult female albino rats. 5 Groups of adult female albino rats (160-200 G) 5 Groups (10 animals each): group (1) healthy rats were used as the control group, group (2) normal rats were given a daily injection of 100 µg/kg of NEE, group (3) animals with DMBA-induced breast cancer, group (4) NEE extract (100 µg/kg/day, ip) was administered to rats with breast cancer models for a period of six weeks and (5) breast cancer-modeled animals were treated with 5UR reference drug (10 µg/kg/weekly, ip) for six weeks. The administration of NEE was found to significantly improve the induced breast cancer disorders. This was evidenced by a noteworthy decrease in the levels of CEA, CA15.3, CA19.9, TNF-α, IL1β, DNA fragmentation, ALAT, ASAT, urea, creatinine, cholesterol, and triglycerides, as well as mammary MDA and NO. Additionally, there was a notable increase in mammary SOD, CAT and GPx activity, and GSH level. Additionally, the histological results demonstrated the therapeutic potential of NEE, which was successful in preventing the development of mammary gland tumors. This study demonstrates how NEE can prevent DMBA-induced breast cancer in rats by enhancing the immune system, lowering inflammation, and restoring damaged oxidative stress.

Keywords: Breast cancer, DMBA, Antitumor, *Nephthea pacifica*, Anti-inflammatory, Oxidative stress.

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1. Introduction

The fifth most common cause of death for women worldwide, female breast cancer is the most common type of cancer diagnosed in this age group [1]. Approximately 2.3 million female breast cancer cases will be newly diagnosed globally in 2020, according to the International Agency for Research on Cancer [2]. Environmental variables, such as exposure to polycyclic aromatic hydrocarbons from burning coal, factories, and automotive gasoline, can raise the risk of breast cancer in addition to genetic factors and age. 7, 12-dimethylbenz (a) anthracene (DMBA) is one of the carcinogenic polycyclic aromatic hydrocarbons that is used to cause breast cancer in laboratory animals. Because rat cancer closely resembles human breast cancer, the rat model of breast cancer is the most often used model. This carcinogen is activated into DMBA-3, 4-diol-1, 2-epoxide (DMBA-DE) in the liver and breast, where it disrupts and interrupts the redox balance of the tissue and causes oxidative stress. Reactive oxygen species are produced as a result, which can damage DNA or a protein cell cycle and cause uncontrollable cell division and proliferation. The incidence of cancer that is estrogen receptor-positive is rising whereas the incidence of cancer that is estrogen receptor-negative is falling [2]. Estrogen influences cancer that is estrogen receptor-positive, which aids in the growth of cancer cells [3]. Medication for breast cancer includes those that shield DNA, interrupt the cell cycle and division, boost programmed cell death, and block some of the processes that lead to aberrant cell growth, like the expression of estrogen receptors [3]. When compared to synthetic pharmaceuticals, anticancer medications made from plants, such as the vinca alkaloids vinblastine and vincristine and paclitaxel from Taxus brevifolia, have higher therapeutic efficacies and lower toxicity [3]. Furthermore, it has been proposed that chemoprevention is a useful tactic for reducing the harmful effects of genotoxins and environmental carcinogens [4].

Through a variety of modes of action, such as anti-proliferation, anti-estrogenic activity, induction of apoptosis, antioxidation, and stimulation of detoxifying enzymes, these chemo-preventive chemicals can prevent cancer [3, 5]. The biological effects of marine natural products are diverse and have a vital role in the creation of molecules that are crucial for medication development [6]. A known genus of the Nephtheidae family (20 genera) that includes 12 species is marine *Nephthea sp*. These corals are often referred to as carnation corals, tree corals, or colt corals. The Indo-Pacific area is where they are dispersed [7]. Due to the existence of several chemical entities including steroids, sesquiterpenes, and diterpenes, 18 members of the genus Nephthea demonstrate a variety of bioactivities, including cytotoxic, anti-tumor, antiviral, antihypertriglyceridemia, and antiphlogistic properties [8-9]. Therefore, the purpose of this study is to examine if *Nephthea pacifica* extract has anti-tumor therapeutic potential against mammary tumors that are produced in female rats. This goal was accomplished through biochemical, immunological, and histological examinations.

2. Materials and Methods

2.1. Chemicals

7-Demethylbenz (a) anthracene, or DMBA, was supplied by Sigma Aldrich (St. Louis, MO, USA)

2.2. Nephthea pacifica collection, identification, and extraction

In January 2021, snorkeling was used to collect the soft coral under investigation from the Red Sea's shoreline in Hurghada, Egypt. The specimen was authenticated by Dr. Abdallah Alian (Zoology Department, Faculty of Science, Al-Azhar University, Assuit, Egypt). *Nephthea sp*. (500g) was divided into small slices, kept at 4°C, extracted multiple times with ethanol, and then vacuum-concentrated to obtain 6g. The resultant extract was separated using n-hexane, dried to produce 2g, and stored at 4°C for additional analysis.

2.3. Animals

The National Research Center's animal colony provided adult female albino rats weighing 160–200g. A week before to the experiment's commencement, the animals were allowed unrestricted access to food and water to allow for acclimation; the animals were given human care in accordance with the institution's standard procedures for the use and care of experimental animals, as outlined by the Faculty of Science's ethical committee at Al-Azhar University in Assuit, Egypt. This committee's approval number for this protocol is AZHAR 14/2022.

2.4. Induction of breast cancer

Following their acclimation, the animals were subjected to breast cancer induction using the techniques of Barros et al. [10]**.** At seven weeks of age, animals were given a single gavage dose (20 mg/animal) of DMBA dissolved in 1 ml of soy oil. Every week, rats underwent physical examinations. The six pairs of mammary glands on each rat were examined by palpation, touching, and inspection. To confirm that tumor induction had occurred, analyses were carried out eight and thirteen weeks after DMBA consumption.

2.5. Animals' grouping

After a breast tumor was induced, five groups of healthy and breast cancer-modeling rats were randomly assigned (10 rats each) as follows: group (1) included healthy animals and served as control group, group (2) comprised six weeks of daily oral soy oil treatment combined with intraperitoneal injections of NEE (100 µg/kg/day) into healthy rats, group (3) comprised animals models with breast cancer that were not given any treatment and acted as a positive control, group (4) Models of breast cancer rats treated a daily intraperitoneal injection of 100 µg/kg NEE for six weeks and group (5) Animals modeled with breast cancer and given reference medication 5UR® (10 µg/kg/weekly, ip) for a period of six weeks [11]. All animals had blood samples taken at the conclusion of the trial, which were then divided and kept at -80°C until biochemical and immunological assessments. Following blood collection, the animals were quickly beheaded as a form of sacrifice, and their mammary tissues were removed, homogenized, and subjected to histological study.

2.6. Determination of immune-cytokines and tumor markers

Tumor necrosis alpha (TNF-α), interlukin-1 beta (IL-1β), CEA, CA15.3 and CA19.9 levels were measured by ELISA technique (Dynatech Microplate Reader Model MR 5000) using rat reagent ELISA-kits from SinoGeneClon Biotech Co., Hang Zhou, China.

2.7. Assessment of biochemical markers

Spectrophotometric measurements were made of serum triglycerides, total cholesterol, HDL, LDL cholesterol, urea and creatinine levels as well as ASAT and ALAT activity using reagent kits purchased from Biodiagnostic, Giza, Egypt.

2.8. Determination of oxidative stress markers in mammary tissue

After homogenizing breast tissue in phosphate buffer, reagent kits from Biodiagnostic, Giza, Egypt were used to measure the levels of nitric oxide (NO), reduced glutathione (GSH), and lipid peroxidation end products (malondialdehyde, MDA), as well as the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT).

2.9. DNA fragmentation percentage

As previously mentioned the percentage of DNA fragmentation was identified [12]; cleaved DNA samples by centrifuging them, measuring the amount of DNA in the pellet and supernatant, and grading the degree of DNA damage using the diphenylamine test. The degree of DNA fragmentation was calculated using the ratio of the total DNA in the supernatant and pellet to the DNA present in the supernatant. Using the following formula, the percentage of fragmented DNA was determined from the absorbance measurement at 578 nm:

$$\text{DNA fragmentation } \% = \frac{\text{A supernatant}}{\text{A supernatant} + \text{A pellet}}$$

2.10. Histopathological examination

Rats of each group were scarified under anesthesia, their mammary glands tissues were collected, dissected immediately after sacrifice, and were used for the

histopathological analysis. For that, specimens were sectioned, fixed in 10% buffered formalin solution for 48 hours, dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin blocks. Serial sections of 5µm mounted on glass slides, then washed in water bath and left in oven for dewaxing. Finally, sections were stained with Hematoxylin and Eosin. Histological changes were assessed with electrical light microscope Olympus CX 41. Photomicrographs were taken using Adobphotoshop version 8.0 was used for image processing.

2.11. Statistical analysis

The Statistical Analysis System's General Linear Model Procedure was utilized to do an analysis of variance (ANOVA) on all the data (SAS 1982). The Waller-Duncan k-ratio was used to assess the importance of the variations between the various treatment groups [13]. Every significant statement relied on the likelihood of $p < 0.05$.

3. Results

The administration of DMBA treated resulted in a notable increase in tumor markers, pro-inflammatory cytokines, liver and kidney function markers as well as mammary DNA fragmentation. This was demonstrated by the significantly higher levels of serum CEA, CA15.3, CA19.9, TNF-α, IL-1β, ALAT, ASAT, urea and creatinine as well as mammary DNA fragmentation in comparison to normal rats. In contrast, the administration of healthy rats with the NEE never disrupted tumor markers, pro-inflammatory cytokines, liver and kidney function as well as mammary DNA fragmentation. The tumor markers, pro-inflammatory cytokines, liver and kidney function markers as well as mammary DNA fragmentation deteriorations caused by DMBA were dramatically improved in rats treated with NEE in a breast cancer model (Table 1). When healthy rats were administered NEE, their serum lipid profile did not deteriorate in comparison to the control group. However, when they were intoxicated with DMBA, the serum lipid profile significantly increased in terms of total cholesterol, triglycerides, and LDL cholesterol, while HDL cholesterol significantly decreased. This resulted in the onset of atherosclerosis. In contrast, when compared to the group of animals with breast cancer models, the post-treatment of rats with NEE significantly improved all lipid profile markers (Table 2). Additionally, compared to the normal control group, the treated of DMBA into the rats' mammary tissue resulted in abrupt disruptions in the tissue's oxidative status, as evidenced by a significant drop in the antioxidant battery (GSH, SOD, CAT and GPx) and an increase in the oxidative power (MDA and NO). Fortunately, NEE post-treatment of breast cancer-model rats decreased the mammary NO and MDA level compared to the corresponding values of the group of cancer-modeled animals that were not treated; additionally, NEE significantly increased SOD, GPx and CAT activities as well as GSH levels (Table 3).

3.1. Histopathological finding

Table 4 demonstrated the types and incidence of the histopathological changes in rat mammary gland tissue sections of the experimental groups. The mammary gland tissues of the control and NEE groups showed normal ductuloalveolar structure surrounded by a small amount of fibroblastic stroma and normally appearing adipose tissue that formed most of the gland without any abnormal proliferations (Figure 1). In rats treated with DMBA, the mammary glands developed various abnormal morphological patterns with varied incidence, including ductal apocrine metaplasia (50%), micropapillary ductal carcinoma in situ (50%), invasive ductal carcinoma (40%), and fibrosarcoma (20%). In ductal apocrine metaplasia, dilated duct lined by columnar epithelial cells with basally located round nuclei, abundant foamy eosinophilic cytoplasm, and apical snouts at the luminal surface were evident (Figure 2 A & B). Ductal carcinoma could be divided into micropapillary carcinoma in situ and invasive ductal carcinoma. The micropapillary carcinoma in situ had an intermediate nuclear grade and showed numerous micropapillae of different shapes. The micropapillae were formed by cells that exhibited pleomorphic nuclei with occasional nucleoli. Small foci of microcalcification were also seen within neoplastic ducts (Figure 2 C). Invasive ductal carcinoma displayed proliferation of intraductal neoplastic epithelial cells with remarkable variations in the cellular and nuclear sizes and shapes, which invaded the neighbor stroma (Figure 2 D). In fibrosarcoma, there were fibroblastic pleomorphic neoplastic cells organized in interlacing bundles. Nuclear atypia was evident and was represented by anisokaryosis, hyperchromacia, prominent nucleoli, and distinct irregular nuclear membrane. Also, intra-tumoral hemorrhages and angiogenesis were obviously seen (Figure 2 E & F). After treatment with NEE (DMBA ~ NEE group), the histomorphological profile of mammary ducts was apparently normal without any proliferative lesions. These ductular structures appeared to be surrounded by normal, but disorganized adipose tissue, in which the adipocytes were loosely connected (Figure 2 G). In the DMBA~5UR group, microscopic examination of mammary tissue sections exhibited a distinct reduction in the incidence of neoplastic development. Invasive ductal carcinoma was seen in 20% of the examined rats in this group (Figure 3 H). The adipose tissue surrounding the malignant ducts showed hemosiderin-laden macrophages (Figure 2 I).

4. Discussion

Group of animals modeled by untreated cancer: One of the most prevalent tumors in women and a major cause of cancer-related mortality in women is breast cancer [14]. Breast cancer, which accounts for around 29% of newly diagnosed cases and causes the highest cancer-related mortality rate among females worldwide, is still the most frequent malignant neoplasm, according to the American Cancer Society [15]. Thus, the current study's objective was to assess the potential therapeutic benefits of *Nephthea pacifica* of coral (NEE) ethanolic extract against DMBA-induced breast cancer in adult female albino rats.

The investigation shown that, in comparison to the control group, DMBA markedly increased the serum levels of pro-inflammatory cytokines (TNF-α and IL1β) and tumor markers (CEA, CA15.3 & CA19.9). These results are consistent with previous studies Kubata et al. [16]; Gul et al., [15] & El-Fakharany et al. [17] They claimed that the largest amount of mammary tumors produced by DMBA, either in single or multiple doses, closely resemble human breast cancer in terms of form and the expression of biochemical and molecular markers. Costa et al. [18] suggested that because DMBA raises the expression of CYP enzymes

involved in the metabolism of estrogen, it accumulates in the adipose tissue of the mammary gland and promotes the induction of cancer. CYP1B1 induction caused DNA damage from reactive oxygen species, which led to oncogenic transformation [19].

Tumor growth is significantly influenced by inflammation; transcription factors and pro-inflammatory cytokines NF-kB is a major molecular contributor to a number of diseases, including cancer and inflammation. TNF-α is an essential cytokine in inflammatory reactions; additionally, mounting evidence suggests that TNF-α may function as an endogenous tumor promoter; furthermore, DMBA increased the amount of IL-1β, a key mediator of inflammation in the pathogenesis of cancer [20]. Remarkably, serum levels of CEA, CA15.3, CA19.9, TNF-α and IL1β were much lower in rats treated with NEE for generated mammary tumors than in the group of rats with breast cancer. The current study found that breast cancer model rats given NEE had considerably lower levels of immune-inflammatory markers (TNF-α and IL-1β) as well as CA15.3, CA19.9, and CEA than the control group. This impact activity may be caused by a variety of pathways, including as the immunoregulatory, extracellular, and intracellular effects of its primary constituents [11].

The liver and kidney are vital organs in our body, and when their regular functions are disrupted, it can significantly impair the metabolism of different chemotherapy medications, increasing the toxicity of the drug to the body as a whole. The liver is the principal organ involved in the metabolism of xenobiotic; chemical agents have the potential to harm it as well. A significant increase in serum ALAT and ASAT activity was observed after DMBA intoxication relative to the control group; this increase is thought to be suggestive of hepatic damage caused by DMBA. DMBA metabolism produces carcinogenic metabolites and ROS, which are thought to be the cause of liver degeneration [21]. In this instance, NEE lowers hepato-nephrotoxicity through both altering the antioxidant defense system and lowering lipid peroxidation or by providing free radicals with an electron to lessen their reactivity [11].

Furthermore, increased lipid metabolizing activity is linked to cancer and the regulation of cholesterol metabolism changes as tumors grow [22]. Tumor cell membranes were shown to be enriched in cholesterol due to deregulated lipogenesis, which was linked to excessive production. Abnormal lipid profile levels and alterations in lipid metabolizing enzyme levels are associated with the disease stage. In the present study, rats with breast cancer generated by DMBA exhibited a significant increase in serum levels of triglycerides, LDL cholesterol, and total cholesterol, but no noticeable decrease in HDL cholesterol. Increased triglyceride and cholesterol levels change the lipid fluidity of tumor cell membranes, which encourages tumor cell growth and multiplication and increases the likelihood that a tumor will become malignant [22]. Positively, administering NEE to rats with breast tumor models allowed the lipid profile to be regulated to nearly normal levels. The improvement that is advantageous can be ascribed to its anti-tumor, anti-microbial, and antioxidant properties [23]

Antioxidants, such as SOD, CAT, and GPx enzymes as well as GSH levels, are essential in combating free radicals and/or reactive oxygen species that are produced [24]. The current study's findings showed that, in comparison to control rats, the mammary tissue of rats given DMBA-induced breast cancer had significantly lower levels of GSH, CAT, and SOD activity. This decrease in the mammary tissue's antioxidant battery (SOD, CAT and GPx activities, and GSH content) may be the result of their increased use to counteract the excessive production of ROS and free radicals following DMBA intoxication, weakening the body's defense mechanisms against oxidative stress [25]. It was in line with earlier research showing that decreased GSH levels in rats with breast cancer caused greater lipid peroxidation (higher MDA levels) and excessive antioxidant consumption for the growth of tumor cells [26, 15]. Nitric oxide synthase in immune cells may have been overstimulated during the adenoma of preneoplastic transition, as seen by the striking increase in NO levels seen in mammary tissue during the active breast cancer inflammation [27]. The results of the present investigation demonstrated that NEE therapy successfully decreased the malignant rats' mammary MDA and NO levels and raised the antioxidant battery (SOD, GPx, CAT, and GSH) in relation to the control group. The antioxidant potential of NEE-coral components may be responsible for the excessive blocking and/or stability of free radical formation, leading to this advantageous effect.

Table 1: Mean values of serum CEA, CA15.3, CA19.9, TNF-α, IL-1β, ALAT, ASAT, urea and creatinine as well as mammary DNA fragmentation of normal and breast cancer-treated animals' groups as compared to control group.

	Control	**NEE**	**DMBA**	**DMBA ~ NEE**	**DMBA ~ 5UR®**
CEA (U/mL)	30.17±1.2	28.9±2.9	96.4±5.4*	44.2±2.3#	48.2±3.6#
CA15.3 (U/mL)	16.42±0.95	16.02±1.29	62.7±6.6*	41.3±3.1#	48.0±4.4#
CA19.9 (U/mL)	36.06±4.27	34.4±3.4	206±4.3*	62.0±6.5#	75±5.9#
TNF-α (ng/L)	187.7±8.8	182.9±5.3	446±19.4*	220±15.9#	260±16.8#
IL-1β (ng/L)	43.7±2.5	41.3±4.3	171.7±4.3*	66.1±5#	75.0±5.9#
ALAT (U/L)	40.8±1.38	39.75±2.03	83.5±3.9*	49.75±2.01#	65.75±5.29#

ASAT (U/L)	50.6±2.02	47.4±1.57	99.8±3.1*	60.2±2.2#	73.2±4.7#
Creatinine (mg/dl)	0.735±0.04	0.72±0.06	2±0.319*	1.1425±0.15#	1.85±0.16#
Urea (mg/dl)	42.75±2.09	40±2.027	71.5±4.6*	51.5±2.91#	60.25±3.37#
DNA fragmentation (%)	4.2±0.8	4.3±0.96	24.8±2.1*	8.2±3.2#	12.5±2.1#

Data are presented as mean ± standard error; * is significantly different from control group, while # is significantly different from DMBA group ($p \leq 0.05$) using one way ANOVA. DMBA (Dimethyl Benz (α) Anthracene), NEE (*Nephthea pacifica* ethanolic extract), 5UR® (5 Fluorouracil®).

Table 2: Mean values of serum total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol of normal and breast cancer-treated animals' groups as compared to control group.

	Control	**NEE**	**DMBA**	**DMBA ~ NEE**	**DMBA ~ 5UR®**
Total cholesterol (mg/dl)	150±5.1	146.5±7.3	226.75±12.9*	166±6.0#	168.5±13.5#
Triglycerides (mg/dl)	152.7±2.7	153±4.0	207±9.4*	161.75±15.4#	187.75±6.7#
HDL- cholesterol (mg/dl)	43.7±1.6	44.25±1.3	28.25±3.0*	35.5±0.8#	26.75±1.9#
LDL- cholesterol (mg/dl)	1.9±3.5	108.25±5.5	177±5.3*	133.25±8.3#	143.75±10.4#

Data are presented as mean ± standard error; * is significantly different from control group, while # is significantly different from DMBA group ($p \leq 0.05$) using one way ANOVA. DMBA (Dimethyl Benz (α) Anthracene), NEE (*Nephthea pacifica* ethanolic extract), 5UR® (5 Fluorouracil®).

Table 3: Mean values of mammary gland oxidant-antioxidant markers of normal and breast cancer-treated animals' groups as compared to control group.

	Control	**NEE**	**DMBA**	**DMBA ~ NEE**	**DMBA ~ 5UR®**
MDA (nmol/g)	749.3±64.1	747.2±64.0	1407.9±35.9*	890.7±35.2#	972.4±30.5#
NO (µmol/g)	124.74±7.2	125.6±4.5	262.4±18.7*	160.3±5.8#	177.1±4.3#
GSH (mmol/g)	54.26±3.9	57.8±2.7	25.04±2.6*	41.5±2.5#	35.16±3.2#
SOD (U/g)	2501.7±90.1	2560.3±73.1	1163.7±61.8*	2027.2±58.3#	1778.8±51.5#
GPx (U/g)	365.54±13.2	372.08±10.8	166.4±6.8*	293.4±5.8#	252.5±35.2#
CAT (U/g)	31.68±2.0	32.2±2.2	14.4±1.6*	26.9±1.4#	21.9±2.4#

Data are presented as mean ± standard error; * is significantly different from control group, while # is significantly different from DMBA group ($p \leq 0.05$) using one way ANOVA. DMBA (Dimethyl Benz (α) Anthracene), NEE (*Nephthea pacifica* ethanolic extract), 5UR® (5 Fluorouracil®).

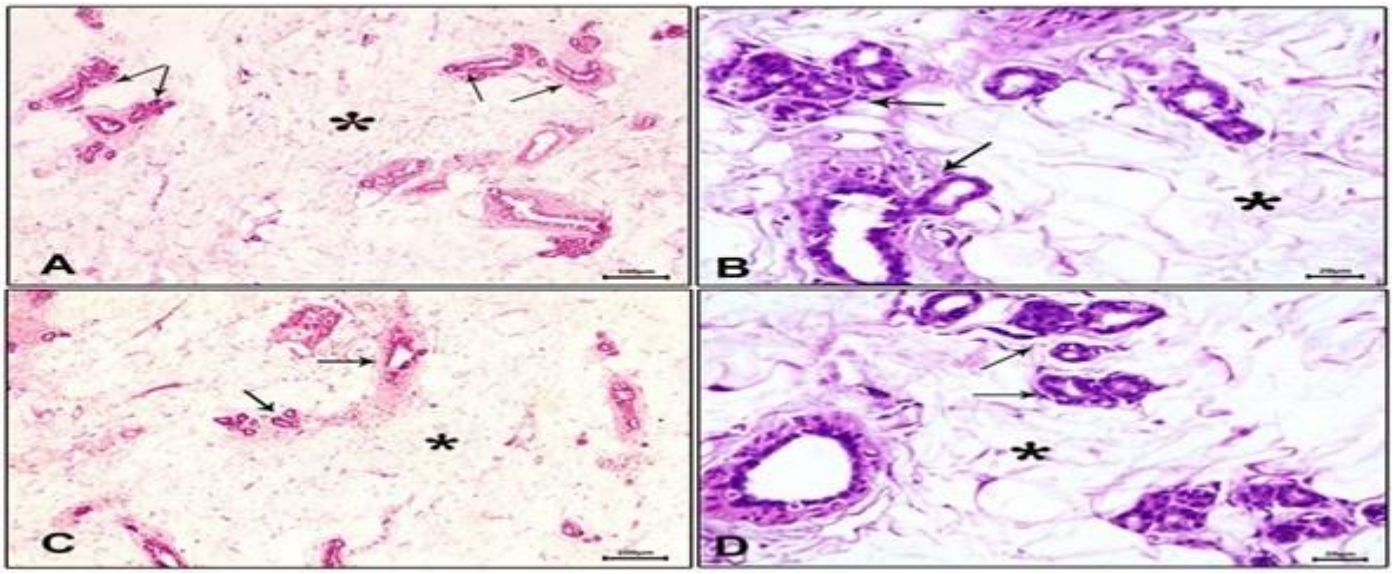

Figure 1: Representative micrographs of the mammary gland tissues stained by H&E. (A, B) control rats and (C, D) NEE showing normal small ductal structures surrounded by a small amount of fibroblastic stroma (arrow) and normally appeared adipose tissue (asterisk).

Table 4: Histopathological alterations of mammary gland of various studied groups.

Lesion	Control	NEE	DMBA	DMBA ~ NEE	DMBA ~ 5UR
Ductal apocrine metaplasia	0	0	5 (50%)	0	0
Micropapillary ductal carcinoma in-situ	0	0	5 (50%)	0	0
Invasive ductal carcinoma	0	0	4 (40%)	0	2 (20%)
Fibrosarcoma	0	0	2 (20%)	0	0

Data are given as number percentage. DMBA (Dimethyl Benz (α) Anthracene, NEE (*Nephthea pacifica* ethanolic extract).

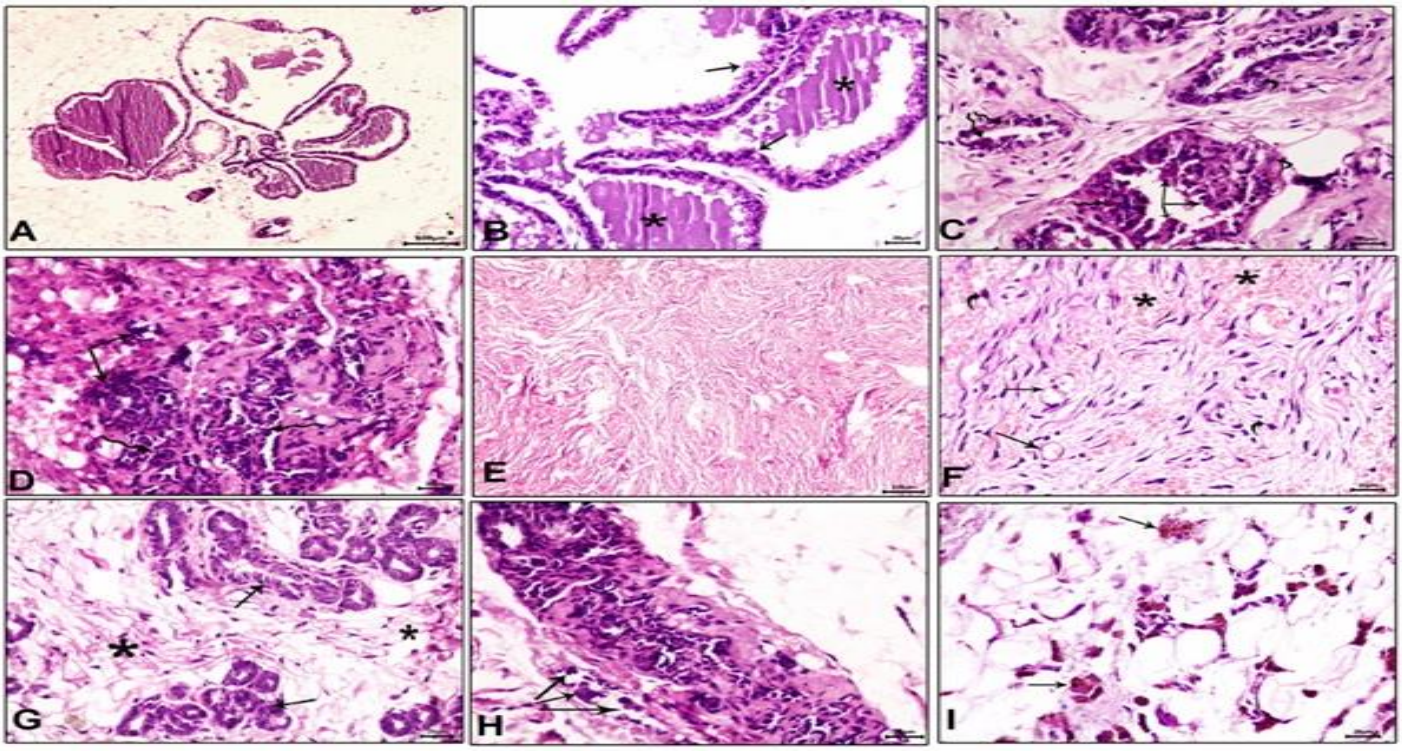

Figure 2: Representative micrographs of the mammary tissues stained by H&E. (A-F) DMBA-treated rats. A and B showing ductal apocrine metaplasia, characterized by dilated ducts lined by columnar cells with basally located nuclei, abundant foamy

eosinophilic cytoplasm, apical snouts (arrow), and luminal secretions (asterisk); C micropapillary carcinoma in situ showing micropapillae of neoplastic cells (arrow) with atypical nuclei (curved arrow) and small foci of microcalcification (wavy arrow); D invasive ductal carcinoma showing intraductal neoplastic cells (wavy arrow), which invaded the neighbor stroma (arrow); E and F fibrosarcoma showing fibroblastic pleomorphic neoplastic cells organized in interlacing bundles, nuclear atypia (curved arrow), intra-tumoral hemorrhages (asterisk) and angiogenesis (arrow). G DMBA ~ NEE-treated rats showing normal appearance of mammary ductules (arrow) and mildly disorganized adipose tissue, in which the adipocytes were loosely connected (asterisk). (H and I) DMBA ~ 5UR-treated rats; H invasive ductal carcinoma showing intraductal neoplastic cells invaded the neighbor stroma (arrow). I adipose tissue with hemosiderin-laden macrophages (arrow)

This decrease in the mammary tissue's antioxidant battery (SOD, CAT and GPx activities, and GSH content) may be the result of their increased use to counteract the excessive production of ROS and free radicals following DMBA intoxication, weakening the body's defense mechanisms against oxidative stress [19]. It was in line with earlier research showing that decreased GSH levels in rats with breast cancer caused greater lipid peroxidation (higher MDA levels) and excessive antioxidant consumption for the growth of tumor cells [20, 15]. Nitric oxide synthase in immune cells may have been overstimulated during the adenoma of preneoplastic transition, as seen by the striking increase in NO levels seen in mammary tissue during the active breast cancer inflammation [21]. The results of the present investigation demonstrated that NEE therapy successfully decreased the malignant rats' mammary MDA and NO levels and raised the antioxidant battery (SOD, GPx, CAT, and GSH) in relation to the control group. The antioxidant potential of NEE-phytochemical components may be responsible for the excessive blocking and/or stability of free radical formation, leading to this advantageous effect.

5. Conclusions

The current study's findings suggest that the ethanolic extract of coral *Nephthea pacifica* (NEE) has therapeutic potential for treating breast cancer. The significant improvement in the biomarkers, immunoinflammatory, oxidative state, and histological data led to this outcome. It is possible that NEE is a potent future medication for the treatment and most likely the prevention of breast cancer. Additionally, NEE exhibits are safe to use.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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