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Myrtenal downregulates hormonal receptors in DMBA induced mammary carcinoma in Sprague-Dawley rats

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ABSTRACT

Myrtenal, a natural phytocompound, has shown promising biological activities, including antioxidant and anti-inflammatory properties, which could be beneficial in modulating cancer-related pathways. This study evaluates the dose-dependent effects of myrtenal on the immunoexpression pattern of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2/neu), in the mammary tissues of mammary tumor bearing Sprague-Dawley rats. This study induced mammary carcinoma in Sprague-Dawley rats by injecting a single subcutaneous injection of 7,12-dimethylbenz(a)anthracene (DMBA; 25 mg/kg body weight) in the mammary gland. Immunohistochemical analysis revealed that all the three hormonal receptors were overexpressed in breast tissues of rats treated with DMBA alone. Oral administration of myrtenal at different doses (100, 200 and 400 mg/kg body weight) resulted in dose-dependent downregulation. These findings suggest that myrtenal has shown potent anticancer effect by modulating the expression of receptors in DMBA induced triple positive breast cancer.

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INTRODUCTION

Globally, the most prevalent cancer among women is the breast cancer and stands as a primary contributor to cancer-related fatalities. It is caused by abnormal proliferation of cells in the breast driven by hereditary, reproductive and lifestyle factors (Obeagu and Obeagu, 2024; Ye *et al.*, 2025). The main risk factors associated with human mammary carcinoma are long-term exposure to estrogen, early onset of menstruation and late menopause. Environmental factors, such as exposure to polycyclic aromatic hydrocarbons found in pollutants also plays a key role in the development of mammary carcinoma (Kay *et al.*, 2022; Al-Shami *et al.*, 2023). Worldwide, around 2.3 million new cases are diagnosed annually and around 670,000 mammary cancer related deaths were reported by the year 2022 (Kim *et al.*, 2025). In USA, around 316,950 new cases of invasive breast cancer will be diagnosed in women by the year 2025. The number of breast cancer cases in India will rise from 1.46 million (2022) to 1.57 million by 2025 (Siegel *et al.*, 2025; Vemula and Dhakshanamoorthy, 2025). Despite advances in treatment, breast cancer remains a significant public health problem with high mortality in both developed and developing countries, especially where early diagnosis and treatments are limited (Xiong *et al.*, 2025).

DMBA is widely used to induce mammary carcinomas in experimental rats. The Sprague-Dawley (SD) rat model is shown to be highly sensitive for DMBA-induced mammary tumors and seems to be an ideal experimental model for the study of human breast cancer (Sewoyo *et al.*, 2024). These tumors have morphological and molecular similarities with human breast cancer that helps to understand carcinogenic mechanisms (Shaban *et al.*, 2023).

Breast cancer prognosis can be most accurately predicted by the expression pattern of the ER and PR, located on normal and malignant breast tissues (Kunstič *et al.*, 2023). These receptor levels differ in various tissues and are affected by factors such as age, obesity and carcinogenesis. Prolonged exposure to endogenous or exogenous estrogen is considered as the most significant risk factor for the development of breast cancer (Kim and Munster, 2024). Estrogen and progesterone activate their corresponding receptors, triggering signaling pathways that promote carcinogenesis. The ER-α and ER-β receptors act as ligand-activated transcription factors. They bind to promoter regions of estrogen-regulated genes (Conzen, 2008). However, abnormal ER signalling is linked to breast cancer cell proliferation and growth (Miziak *et al.*, 2023). The significance of ER-β in breast tumor is unclear, however activation of ER-α is thought to drive tumour growth. ER-positive breast cancers are the most common molecular subtype of breast cancer, comprising about 70% of all cases worldwide (Acs *et al.*, 2024; Kim and Lukong, 2025).

Progesterone receptors (PR) also play an essential role in breast cancer. PR-positive tumors generally exhibiting a better response to hormone therapy. PR expression is regulated by estrogen and ER-positive tumors usually express PR, representing hormone sensitivity. The expression of ER and PR in breast cancer is usually associated with less aggressive tumors and a more favourable prognosis. The ER and PR positive tumors tend to be sensitive to hormone treatment (Thakkar and Mehta, 2011; Lu *et al.*, 2025). Around 65% of breast carcinoma are PR-positive, which is associated with high levels of circulating progesterone observed in postmenopausal women, increasing the breast cancer progression by 16% (Li *et al.*, 2022). PR positive tumors are frequently co-expressed with ER-positive tumors, often corresponding to the luminal A molecular subtype associated with good prognosis and survival (Geyer *et al.*, 2011).

HER2/neu is involved in the regulation of cell growth, survival and proliferation. In response to autophosphorylation, HER2 causes receptor dimerization and activates signaling pathways for increased cell growth and tumorigenesis (Rubin *et al.*, 2024). Approximately 15–20% of women with breast cancer have HER2 gene amplification, resulting in overexpression and tumours with worse prognosis (Hou *et al.*, 2023). For regional HER2-positive breast cancer, the 5-year relative survival outcome is between 97.7% and 99.5%. However, the five year relative survival outcome is

only about 40.8% to 46.7% for those diagnosed with metastatic HER2-positive tumor (Kesireddy *et al.*, 2025).

The current treatments for breast cancer like tamoxifen, doxorubicin and paclitaxel are expensive and have severe side effects such as cardiotoxicity and carcinogenic side effects (Haque *et al.*, 2025). Chemotherapy drugs typically lead to debilitating side effects such as nausea, fatigue and hair loss (Hashim *et al.*, 2024). These limitations indicate the need of natural plant-based products as a potent alternative to treat mammary carcinogenesis. Various plants and their bioactive compounds are employed as agents to mitigate neoplastic transformations (Iqbal *et al.*, 2017; Atuahene *et al.*, 2025). Numerous studies indicate that monoterpenes can inhibit tumor growth in experimental animals across different organs (Machado *et al.*, 2022). Therefore, treatment of cancer with therapeutic agents from natural origins has gained attention for their effectiveness.

Myrtenal, a bicyclic monoterpene exhibits a wide range of pharmacological activities, including anti-malarial, anti-plasmodial and anti-radicular effects. It acts as a cyclooxygenase-inhibitor (COX-2) and has gonadotrophic, hypocholesterolemic and immunostimulant properties. Additionally, it possesses antioxidant, anti-inflammatory, anti-apoptotic and anti-tumor effects. Myrtenal also demonstrates neuroprotective, antihyperglycemic, anticancer and acetylcholinesterase-inhibiting activities. Furthermore, it showed bronchodilatory, antihemolytic and antibacterial properties (Lin *et al.*, 2017; Dragomanova *et al.*, 2018). Myrtenal exhibited cytotoxicity in different human cancer cell lines such as A2780 (ovarian), Caco-2 (colon), MCF-7 (breast) and LNCaP (prostate) which further highlights its broad-spectrum anticancer potential (Korkmaz and Tekin, 2024). Evidence from various animal studies also demonstrated that myrtenal can modulate tumor-associated pathways and has been found to inhibit chemically induced tumor growth in various types of cancers (Dragomanova *et al.*, 2023). Recently, our laboratory reported that myrtenal suppresses tumors in DMBA-induced rat mammary cancer by reducing oxidative stress and modulating detoxifying enzymes activities (Pethanasamy *et al.*, 2025). The current study attempts to scientifically evaluate the modulating effect of myrtenal on the immunoexpression pattern of hormonal receptors in DMBA-induced breast carcinogenesis in Sprague-Dawley rats.

MATERIALS AND MEHODS

Animals

Female Sprague-Dawley (SD) rats, 7 - 8 weeks old, weighing between 130 and 140 grams, were acquired from Biogen, located in Bengaluru, India. These animals were housed at the Central Animal House at Annamalai University. The research was conducted in compliance with the CCSEA guidelines. The experimental protocol was approved by Institutional Animal Ethics Committee (GMCHC-IAEC/1379/3/24) at the Government Medical College and Hospital, Annamalainagar. All experiments were conducted under standard laboratory conditions (temperature 24 ± 2°C), relative humidity (50 ± 10%) and a 12-hour light/dark cycles. During the investigational period, animals were given a pellet diet and had free access to water.

Experimental design

A total of 48 female SD rats were acquired. Following a one-week acclimatisation period, the rats were randomly divided into six groups, with each group containing 8 animals. Group 1 (Control) received a single dose of 1 ml of sunflower oil and physiological saline by oral gavage throughout the study. Group 2 (DMBA-alone) was administered a single subcutaneous injection of 25 mg of DMBA dissolved in 1 ml of sunflower oil and physiological saline to induce mammary carcinoma. Groups 3, 4 and 5 received oral doses of myrtenal at 100 mg/kg, 200 mg/kg and 400 mg/kg body weight, respectively, beginning one week prior to DMBA exposure and continuing until the rats were sacrificed. Group 6 received myrtenal orally (400 mg/kg body weight) throughout the study. The experimental period lasted for 16 weeks and at the end of which all rats were anesthetized and sacrificed.

Immunohistochemical staining

Tissues from the mammary glands were removed and preserved for 24 hours in 10% buffered formalin. The

fixed tissues were treated and embedded in paraffin and serial sections of 2–3 µm thickness were obtained using a rotary microtome. After mounting the sections on glass slides, they were deparaffinized in xylene and rehydrated using a series of graded ethanols. Endogenous peroxidase activity was inhibited by incubating the sections in 3% hydrogen peroxide for 10 minutes at ambient temperature, followed by Power Block™ for 15 minutes at the room temperature. Antigen retrieval was conducted, followed by overnight incubation of sections with primary antibodies targeting ER, PR and HER2 at 4°C. Following primary antibody incubation, slides were exposed to horseradish peroxidase-conjugated secondary antibodies for 30 minutes at the room temperature. Immunoreactivity was detected by utilizing diaminobenzidine (DAB) as the chromogenic agent. Finally, the sections were counterstained with hematoxylin, dehydrated, cleared and mounted for microscopic examination using an EVOS XL Core Cell Imaging System (Invitrogen/AMEX 1200).

RESULTS

The immunoexpression pattern of ER, PR and HER2/neu markers in experimental animals are presented in Figs. 1–3 (A-F) respectively.

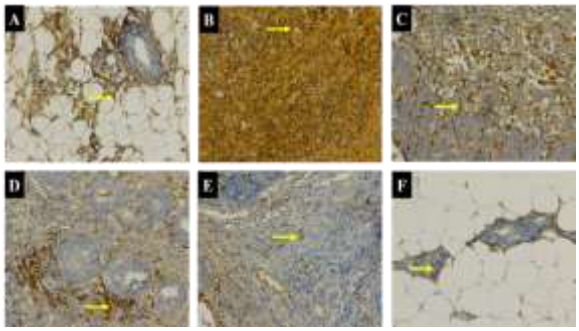

Fig. 1. ER immunohistochemical expression pattern in the breast tissues of control and experimental rats (40X)
Yellow arrows indicate areas with nuclear staining for ER.
A and F: Control and myrtenal-alone (400 mg/kg) groups show normal ductal epithelial cells with mild nuclear staining for ER.
B: DMBA-alone group shows tumor cells exhibiting strong nuclear overexpression of ER.
C: DMBA + myrtenal 100 mg/kg group shows severe dysplastic cells with moderate nuclear staining for ER.
D: DMBA + myrtenal 200 mg/kg group shows moderate dysplastic cells with mild to moderate nuclear staining for ER.
E: DMBA + myrtenal 400 mg/kg group shows mild dysplastic cells with mild nuclear staining for ER.

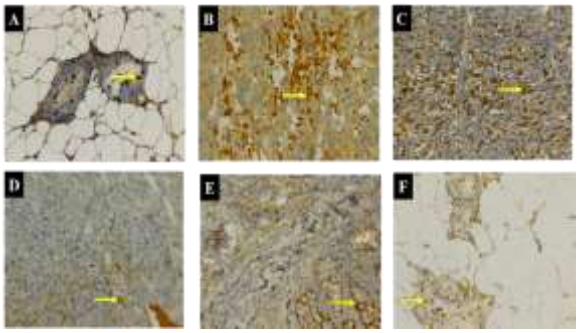

Fig. 2. PR immunohistochemical expression pattern in the breast tissues of control and experimental rats (40X)
Yellow arrows indicate areas of nuclear positivity for PR.
A and F: Control and Myrtenal alone (400mg/kg) group shows normal ductal epithelial cells with mild nuclear positivity for PR.
B: DMBA-alone group shows tumor cells with strong nuclear overexpression for PR.
C: DMBA + Myrtenal 100 mg/kg group shows necrotic cells with moderate nuclear positivity for PR.
D: DMBA + Myrtenal 200 mg/kg group shows moderate dysplastic cells with mild nuclear positivity for PR.
E: DMBA + Myrtenal 400 mg/kg group shows mild dysplastic cells with mild nuclear positivity for PR.

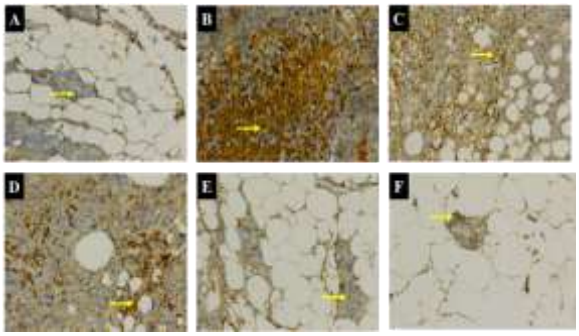

Fig. 3. HER2/neu immunohistochemical expression pattern in the breast tissues of control and experimental rats (40X)
Yellow arrows indicate areas of membranous positivity for HER2.
A and F: Control group and Myrtenal alone (400mg/kg) group shows mild membranous positivity for HER2 in ductal epithelial cells.

B: DMBA-alone group shows membranous overexpression for HER2 in tumor cells.
C: DMBA + Myrtenal 100 mg/kg group shows moderate membranous positivity for HER2 in adenoma cells.
D: DMBA + Myrtenal 200 mg/kg group shows moderate membranous positivity for HER2 in tumor cells.
E: DMBA + Myrtenal 400 mg/kg group shows mild membranous positivity for HER2 in mild dysplastic cells.

Overexpression of all three receptors was observed in DMBA alone treated rats, showing strong nuclear positivity for ER and PR and intense membrane staining for HER2/neu. Oral administration of myrtenal at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg to DMBA-treated rats resulted in a dose-dependent decrease in the expression of ER, PR and HER2/neu. The most significant reduction was observed in Group 5 animals (DMBA + myrtenal 400 mg/kg). The receptor expression pattern in the rats receiving myrtenal alone was similar to that of control rats.

DISCUSSION

The ER is critically involved in mammary carcinogenesis and studies in both animal models and humans have demonstrated that ER plays an essential role in controlling cancer cell proliferation and the progression of breast cancer (Platet *et al.*, 2004). Long-term exposure to endogenous or exogenous estrogen has been pointed out as a major risk factor for the development of breast cancer and 70% of mammary tumors are reported to express ERs (Malik and Mukherjee, 2025). Estrogen interacts with ER-α and ER-β to stimulate the transcription of estrogen-responsive genes that drive tumor cell proliferation and growth of estrogen-dependent mammary tumors (Mangani *et al.*, 2024). Earlier reports had demonstrated increased ER-α: ER-β ratio in breast tumorigenesis, which is significantly associated with the higher level of cell proliferation in human breast tumors (Mashat *et al.*, 2021).

Expression of ER was reported in 15.1–57.5% cases of DMBA-induced mammary tumors and strong ER expression was also detected in DMBA-triggered lesions like intraductal papilloma, fibroadenoma and adenocarcinomas (Maaruf *et al.*, 2023). The strong ER immunoreactivity in DMBA-induced mammary tumors emphasizes the critical role of ER signaling in the pathogenesis of chemically induced mammary carcinogenesis (Abba *et al.*, 2016). In the present investigation, ER expression was markedly elevated in the mammary tissue of rats treated with DMBA alone. This finding consistent with previous reports demonstrating the hormone-dependent nature of DMBA-induced mammary carcinogenesis (Lakshmi and Subramanian, 2013; Srinivasan *et al.*, 2017; Hamza *et al.*, 2022). Administration of myrtenal orally to DMBA-treated rats downregulated the ER expression in a dose-dependent manner.

PR is considered a critical predictive biomarker in mammary carcinogenesis and often co-expressed with estrogen receptor emphasizing its contribution in hormone-dependent tumors (Purdie *et al.*, 2013). In previous reports using the DMBA induced rat model, PR immunopositivity was observed in 11.1–61.1% of carcinomas, confirming the hormonally dependent nature of this model (Russo, 2014). Recent clinical data suggest that low PR expression in hormone receptor-positive breast cancers is significantly associated with worse prognosis and reduced overall survival, suggesting that PR status may serve as an independent prognostic marker (Hou *et al.*, 2025). In the present investigation, PR expression was overexpressed in the mammary tissue of rats treated with DMBA alone. This finding is consistent with previous studies that reported strong PR immunoreactivity in DMBA-induced mammary tumors and confirms the involvement of PR in chemically induced rat mammary carcinogenesis (Lakshmi and Subramanian, 2013; Srinivasan *et al.*, 2017; Thangarasu *et al.*, 2019). Administration of myrtenal orally to DMBA-treated rats down regulated the PR expression in a dose-dependent manner.

In breast cancer, HER2 signaling interacts with hormone receptor pathways, such as ER and PR and this interaction plays an important role in tumor development and progression (Pegram *et al.*, 2023). HER2 overexpression has been reported as a poor prognostic indicator and is associated

with inadequate differentiation, increased cell proliferation, lymph node metastasis and a higher risk of recurrence in mammary tumors (Cong *et al.*, 2020). In clinical practice, patients with HER2-positive breast cancer are routinely treated with various HER2-targeted drugs that include trastuzumab, pertuzumab, lapatinib and antibody–drug conjugates that have significantly increased survival as well as overall clinical outcomes (Wakasa *et al.*, 2025). In chemically induced carcinogenesis, earlier studies have documented overexpression of the HER2/neu protein in rat mammary tumors, indicating that HER2 deregulation contributes to tumor initiation and progression in DMBA-induced mammary carcinogenesis (Swain *et al.*, 2022). The rat neu gene, a counterpart of the human HER2 gene, was first identified as a potent oncogenic mutant and overexpression of its wild-type form has been demonstrated in several malignant neoplasms, including breast cancer (Fry *et al.*, 2016). In the present investigation, HER2 expression was markedly elevated in the mammary tissue of rats treated with DMBA alone, a finding that agrees with earlier reports describing strong HER2 immunoreactivity in chemically induced mammary tumors (Lakshmi and Subramanian, 2013; Srinivasan *et al.*, 2017; Adefisan-Adeoye *et al.*, 2025). Oral administration of myrtenal to DMBA-treated rats downregulated the HER2/neu expression in a dose-dependent manner.

The present study thus explores the ability of myrtenal in downregulating the expression of all the three hormonal receptors during DMBA induced mammary carcinogenesis in SD rats.

CONCLUSION

The present study thus concludes that myrtenal prevented the overexpression of ER, PR and HER2/neu in the DMBA induced mammary tumor tissues of Sprague–Dawley rats. The treatment groups exhibiting dose-dependent reductions in receptor expression. Future studies are needed to explore the molecular pathways underlying the modulatory effect of myrtenal in mammary carcinogenesis.

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