INNSPUB

Journal of Biodiversity and Environmental Sciences | JBES
ISSN: 2220-6663 (Print); 2222-3045 (Online)
Website: https://www.innspub.net
E-mail contact: info@innspub.net
Vol. 29, Issue: 2, p. 22-28, 2026

RESEARCH PAPER OPEN ACCESS

Modulation of estrogen and progesterone receptor expression by fish oil and tamoxifen in 7,12 dimethyl benz(a) anthracene induced mammary carcinogenesis in female sprague dawely rats

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Key words: Breast cancer, Tamoxifen, Fish oil, Omega-3 PUFAs, ER/PR expression, Mammary carcinogenesis, Endocrine modulation

Received: 19 July, 2026 **Accepted:** 29 July, 2026 **Published:** 05 August, 2026

DOI: https://dx.doi.org/10.12692/jbes/29.2.22-28

ABSTRACT

Cancer remains a primary global health challenge characterized by uncontrolled cellular proliferation and significant mortality. Within this landscape, breast cancer represents one of the most prevalent and hormone-dependent malignancies, contributing substantially to cancer-related deaths. While endocrine therapy with tamoxifen is a standard treatment for estrogen receptor-positive (ER+) breast cancer, its efficacy is often hampered by dose-associated toxicity and inconsistent outcomes. This study evaluates the potential of fish oil, rich in omega-3 polyunsaturated fatty acids, as an adjunct to tamoxifen in modulating hormonal imbalance and estrogen/progesterone receptor (ER/PR) expression. A 7,12-dimethylbenz(a)anthracene (DMBA)–induced mammary tumor model was established in female Sprague Dawley rats to assess the effects of fish oil alone and in combination with low and high-dose tamoxifen. Plasma estradiol and progesterone levels were quantified via ELISA, while ER/PR expression in mammary tissues was evaluated by immunohistochemistry. DMBA induction significantly increased ER and PR expression, indicating hormonal dysregulation. Fish oil treatment alone produced a moderate reduction in receptor expression. However, the combination of fish oil with tamoxifen markedly suppressed ER and PR levels. Notably, the fish oil low-dose tamoxifen combination produced effects comparable to the high-dose combination, suggesting that fish oil may enhance tamoxifen efficacy and allow effective endocrine regulation with a lower drug dose and potentially reduced toxicity. Fish oil maintained physiological hormone levels in control animals. In conclusion, fish oil enhances the endocrine-modulating effects of tamoxifen and may serve as a beneficial supplementary strategy to improve therapeutic efficacy while potentially minimizing adverse effects.

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INTRODUCTION

Cancer persists as a leading cause of mortality worldwide, continuing to limit gains in global life expectancy. According to GLOBOCAN 2022, approximately 20 million new cancer cases and 9.7 million cancer-related deaths were reported globally. Female breast cancer has emerged as the most commonly diagnosed malignancy, accounting for nearly 2.30 million new cases, followed by lung, colorectal, prostate, and stomach cancer (Bray *et al.* 2024 and National Cancer Institute, 2026). Despite significant advances in breast cancer management including surgery, radiotherapy, and chemotherapy treatment-related toxicity, high economic burden, and inconsistent therapeutic responses remain major challenges contributing to sustained mortality rates.

Breast cancer is a complex and diverse disease influenced by genetic and environmental factors, resulting in abnormal cell growth through multiple signalling pathways. Nearly two-thirds of breast cancers are hormone-dependent, regulated by estrogen and progesterone via Estrogen Receptors (ER) and Progesterone Receptors (PR) (Freihat *et al.*, 2025). These hormone signalling mechanisms contribute to cancer initiation, progression, metastasis, and maintenance of normal breast tissue.

Environmental exposures have been implicated in a substantial proportion of cancer cases, with estimates suggesting a contribution of up to 75%. Among these, polycyclic aromatic hydrocarbons (PAHs) are recognized as potent carcinogens. These compounds are generated through incomplete combustion processes such as fossil fuel burning, tobacco smoke, diesel exhaust, and consumption of charred foods. Due to their lipophilic nature, (Rengarajan *et al.*, 2015 and Rodgers *et al.*, 2018) PAHs tend to accumulate in adipose tissue, making breast tissue particularly susceptible to carcinogenic effects and thereby promoting tumor development.

In light of this, there is increasing interest in identifying alternative and supportive therapeutic approaches that target hormonal dysregulation with reduced toxicity. Natural bioactive compounds derived from dietary sources have gained attention for their potential to enhance treatment efficacy while minimizing adverse effects (Bidinotto *et al.*, 2012 and Bougnoux *et al.*, 2009). Fish oil, rich in omega-3 polyunsaturated fatty acids (PUFAs) such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), has demonstrated antioxidant, anti-inflammatory, and antitumor properties. These fatty acids modulate membrane fluidity, reduce oxidative stress, and suppress pro-inflammatory mediators, thereby influencing estrogen biosynthesis and hormone-dependent signalling pathways (Fabian *et al.*, 2015). Additionally, their ability to regulate aromatase activity and improve antioxidant defences supports their role in modulating endocrine function during mammary carcinogenesis.

Tamoxifen, a selective estrogen receptor modulator, remains a cornerstone in the treatment of hormone-responsive breast cancer due to its ability to inhibit estrogen binding and suppress tumor growth. However, its long-term use is often limited by adverse effects and the development of resistance (Manni *et al.*, 2015). Emerging evidence indicates that the combination of fish oil and tamoxifen may enhance therapeutic efficacy through multiple mechanisms, including improved drug bioavailability, modulation of apoptosis, and reduction of inflammation. (Subbaramaiah *et al.* 2019), This combination has been shown to influence circulating hormone levels and downregulate ER and PR expression in tumor tissues. Collectively, these findings highlight the potential of fish oil as an effective adjuvant strategy to improve endocrine regulation and therapeutic outcomes in hormone-dependent breast cancer.

MATERIALS AND METHODS

Chemicals

7,12-dimethylbenz(a)anthracene (DMBA), Tamoxifen were purchased from Sigma-Aldrich Co.Ltd. The primary antibodies for ER and PR were procured from Santa Cruz Biotech, USA. All other chemicals used were of analytical grade purchased from local commercial sources.

Animals and diet

Female Sprague Dawley rats (130–150 g) were procured from the Biogen Laboratory Animal Facility, Bangalore, India. The animals were housed in well-ventilated polypropylene cages under standard laboratory conditions (temperature: 27 ± 2 °C; relative humidity: 55 ± 5%; 12 h light/dark cycle). The rats were provided with a standard pellet diet and water *ad libitum* at the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. All animals were acclimatized for one week prior to the commencement of the experiment. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Annamalai University (Registration No. AU-IAEC/1309/12/21; in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All procedures were conducted in strict compliance with Indian National regulations and the ethical guidelines prescribed by Annamalai University.

Tumour induction

7,12-Dimethylbenz[a]anthracene (DMBA) was employed as the mammary carcinogen for this study. Mammary tumors were induced via a single subcutaneous injection of DMBA at a dose of 25 mg/kg body weight. The carcinogen was prepared as a 1 ml emulsion consisting of sunflower oil (0.75 ml) and physiological saline (0.25 ml) per animal.

Experimental design

The experimental period is 16 weeks. The rats were divided into six groups with six rats in each group and given the dose regimen as follows (n=6) (Fig. 1). Group I and VI rats served as control and Fish oil (FO) (0.5ml) Groups II–V rats were received 25 mg/kg b.wt. of DMBA as a single subcutaneous injection during the first week of the experiment. After 8 weeks of tumor formation, Group III - V were administered with an optimum dosage of, Fish oil (FO) (0.5ml) Group IV and V were treated with Tamoxifen Low doses (50µg/kg b.wt) and High doses100µg/kg b.wt) along with Fish oil (FO) (0.5ml). The dosage applied in the current study is based on earlier analyses. The experiment was terminated at the end of 16 w, and all the rats were sacrificed. Levels of Estradiol and Progesterone in plasma were measured by using Enzyme-linked immunosorbent assay (ELISA) kits (Life Span Bio Sciences Inc, USA) as per the manufacturer's instructions. The tissues were promptly dissected, washed thoroughly with ice-cold saline, and were preserved in 10% formalin, paraffin-embedded, partitioned, and fixed on polyline coated glass slides for immunohistochemical staining.

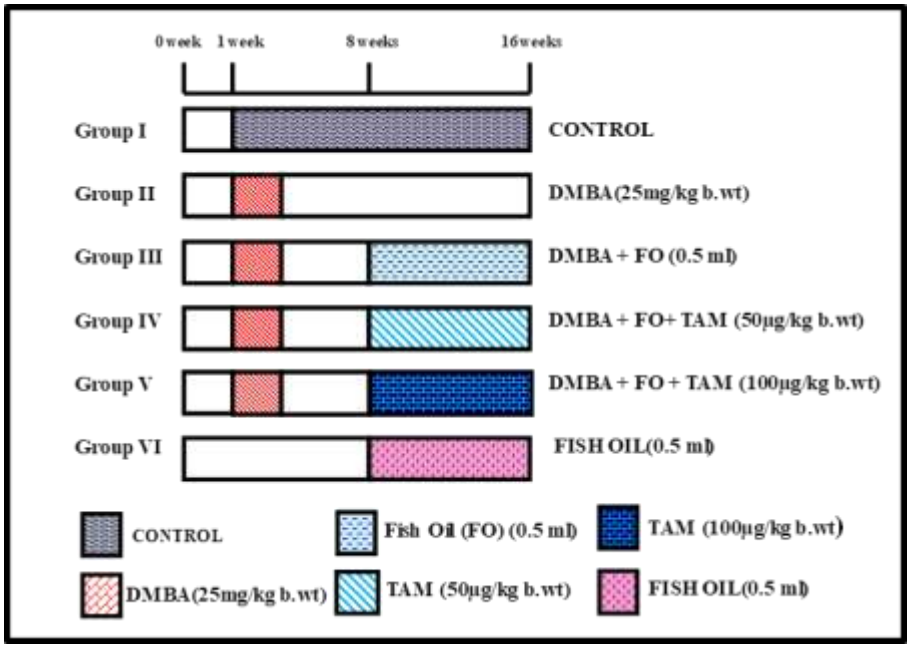

Fig. 1. Schematic representation of an experimental design

Immunohistochemistry

The paraffin-embedded tissue sections were first deparaffinized by heating at 60 °C for 10 min, followed by three rinses in xylene. The sections were then rehydrated through a series of graded alcohols. Antigen retrieval was performed by heating the slides in citrate buffer (pH 6.0) using a microwave oven for two cycles of 5 min each. After allowing the sections to cool for 20 min, they were rinsed with Tris-buffered saline (TBS). Endogenous peroxidase activity was quenched by treating the sections with 3 % hydrogen peroxide prepared in distilled water for 15 min.

To reduce non-specific antibody binding, the sections were incubated with normal goat serum for 20 min. This was followed by incubation with mouse monoclonal primary antibodies against ER, and PR for 1 hr at room temperature. After washing with TBS, the slides were incubated with biotin-conjugated anti-mouse/anti-rabbit secondary antibodies for 30 min at room temperature, followed by streptavidin–biotin–peroxidase complex treatment.

Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) as the chromogen, and the sections were counterstained with hematoxylin. Negative control sections were processed by replacing the primary antibody with TBS, while positive controls were run concurrently for each antibody.

RESULTS

Effect of fish oil and tamoxifen on hormonal status in plasma of control and experimental rats

Hormonal analysis revealed that DMBA-induced rats (Group II) exhibited significantly elevated plasma estradiol and progesterone levels compared with the control group (Group I), confirming hormone-dependent tumor progression. Administration of fish oil alone (Group III) moderately normalized the altered hormone levels, whereas combined treatment with fish oil and tamoxifen at both low (Group IV) and high (Group V) doses resulted in a pronounced restoration of estradiol and progesterone levels toward near-normal values. In contrast, no significant hormonal alterations were observed in the fish oil–treated control group (Group VI) when compared with normal control rats (Group I), indicating the safety and non-hormonal disruptive nature of fish oil under physiological conditions (Fig. 2).

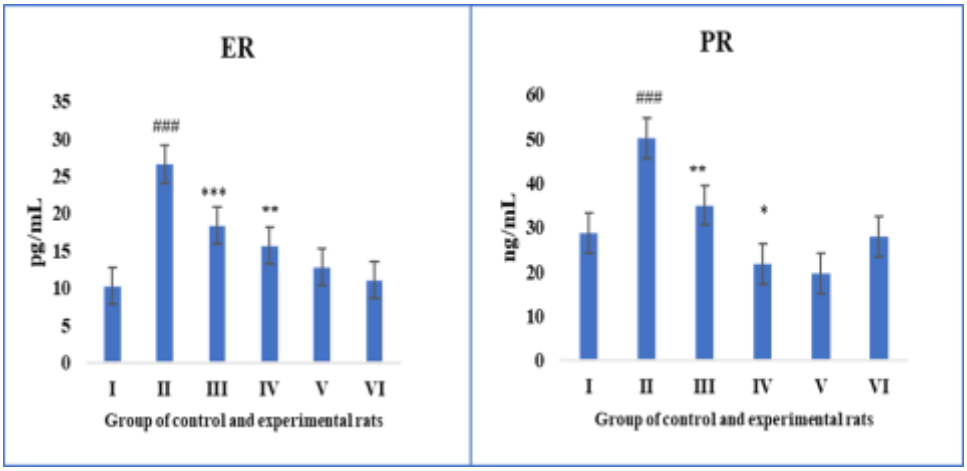

Fig. 2. Effect of fish oil and tamoxifen on hormonal status in plasma of control and experimental rats

Immunohistochemical findings

Immunohistochemical (IHC) examination of mammary tissues from DMBA-induced tumor-bearing rats (Group II) showed increased expression of estrogen receptor (ER) and progesterone receptor (PR) when compared with control rats (Group I), confirming the hormone receptor–positive nature of mammary carcinoma (Fig. 3&4).

In contrast, administration of fish oil (FO) at 0.5 mL (Group III) and combined treatment with fish oil (FO, 0.5 mL) along with low-dose tamoxifen (50 µg/kg

body weight) (Group IV) and high-dose tamoxifen (100 µg/kg body weight) (Group V) markedly reduced the expression of ER and PR when compared with DMBA-induced rats (Group II). No significant alterations in ER and PR expression were observed in fish oil–treated control rats (Group VI) when compared with control rats (Group I). Notably, combined treatment with fish oil and high-dose tamoxifen exhibited greater efficacy in suppressing abnormal ER and PR expression when compared with fish oil alone and low-dose tamoxifen treatment. Notably, combined treatment with fish oil and both low- and high-dose tamoxifen demonstrated comparable efficacy in suppressing abnormal ER and PR expression. This suggests that the lower dose of tamoxifen may be sufficient to achieve therapeutic effects, potentially minimizing toxicity while maintaining efficacy.

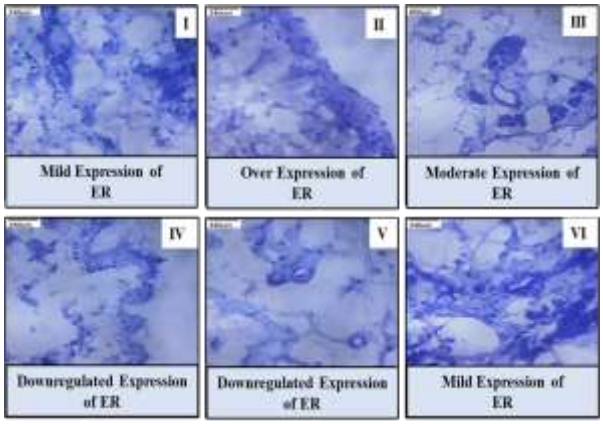

Fig. 3. Immunohistochemical expressions of ER

Note: Immunohistochemical analysis of ER expression in the mammary tissues of control and experimental rats. Immunohistochemical on mammary tissues of control (a) and Fish oil (F) alone treated rats showed normal mammary tissue staining; Mammary tissues of DMBA induced (B) and Fish oil (0.5ml)(C) treated rats showed increased expression of ER; Mammary tissues of Fish oil and tamoxifen 50µg/kg b.wt(D) and Fish oil and tamoxifen 100µg/kg b.wt (E) treated rats showed diminished expression of ER as compared to DMBA induced rats (B).

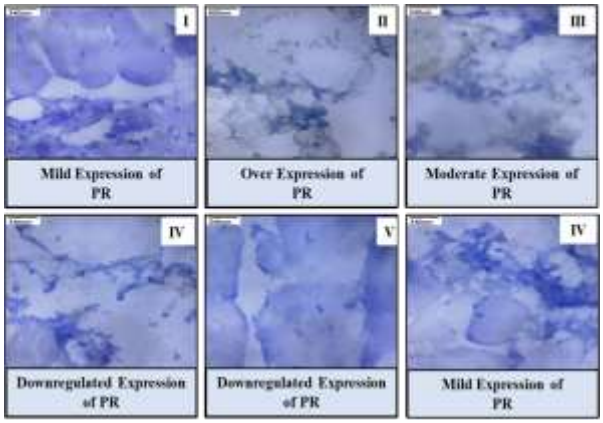

Fig. 4. Immunohistochemical expressions of PR

Note: Immunohistochemical analysis of PR expression in the mammary tissues of control and experimental rats. Immunohistochemical on mammary tissues of control (a) and Fish oil (F) alone treated rats showed normal mammary tissue staining; Mammary tissues of DMBA induced (B) and Fish oil (0.5ml)(C) treated rats showed increased expression of ER; Mammary tissues of Fish oil and tamoxifen 50µg/kg b.wt(D) and Fish oil and tamoxifen 100µg/kg b.wt (E) treated rats showed diminished expression of ER as compared to DMBA induced rats (B).

DISCUSSION

Steroid hormones, particularly estradiol and progesterone, play a crucial role in the initiation and progression of hormone-dependent breast cancer. In the present study, DMBA-induced tumor-bearing rats (Group II) exhibited significantly elevated plasma estradiol and progesterone levels compared with control rats (Group I), confirming the estrogen-dependent nature of DMBA-induced mammary carcinogenesis. Increased hormone levels are known to stimulate mammary epithelial proliferation and support tumor progression. Similar findings have been reported by (Yager and Davidson 2006), who demonstrated that dysregulated steroid hormone production contributes to breast tumor development.

Treatment with fish oil alone (Group III) moderately reduced circulating estradiol and progesterone levels, indicating its hormone-modulatory potential. This effect may be attributed to the presence of omega-3 polyunsaturated fatty acids, particularly EPA and DHA, which are reported to regulate estrogen metabolism, suppress aromatase activity, and interfere with steroid hormone synthesis. Studies by

(Manni *et al.*, 2020) have shown that fish oil supplementation can attenuate hormone-driven breast tumor progression through modulation of endocrine signalling pathways.

More pronounced normalization of plasma estradiol and progesterone levels was observed in rats treated with the combination of fish oil and tamoxifen (Groups IV and V). Tamoxifen, a selective estrogen receptor modulator, inhibits estrogen-mediated signalling by competitively binding to estrogen receptors, thereby suppressing tumor cell proliferation. The enhanced hormonal restoration observed with combination therapy suggests a synergistic interaction between fish oil and tamoxifen, where fish oil may enhance tamoxifen efficacy by modulating hormone synthesis, membrane lipid composition, and downstream estrogen-responsive pathways. Similar synergistic effects between omega-3 fatty acids and tamoxifen have been reported by (Manni *et al.*, 2010) who demonstrated improved endocrine control and enhanced anti-tumor activity with combined treatment.

Immunohistochemical analysis further revealed marked overexpression of estrogen receptor (ER) and progesterone receptor (PR) in DMBA-induced mammary tissues (Group II), confirming the hormone receptor–positive nature of the tumors. Fish oil treatment alone reduced ER and PR expression, supporting its ability to interfere with receptor-mediated estrogen signalling. However, the greatest suppression of ER and PR expression was observed in animals receiving combined fish oil and tamoxifen therapy, particularly in the high-dose tamoxifen group (Group V). This enhanced receptor downregulation indicates that fish oil may potentiate the anti-estrogenic action of tamoxifen and improve therapeutic efficacy against hormone-dependent breast cancer (Cao *et al.*, 2012).

Importantly, fish oil-treated control rats (Group VI) showed no significant alterations in plasma hormone levels or ER and PR expression compared with normal control rats, indicating that fish oil does not disrupt physiological hormonal homeostasis under normal conditions. These findings collectively suggest that fish oil supplementation may serve as a safe and effective adjuvant strategy to enhance tamoxifen therapy and improve endocrine regulation in hormone receptor–positive breast cancer.

CONCLUSION

Fish oil exhibits significant chemotherapeutic potential against DMBA-induced mammary carcinogenesis in female rats. The present investigation evaluated its endocrine-modulatory role both independently and as an adjunct to tamoxifen therapy. Fish oil effectively reduced hormonal imbalance and downregulated estrogen and progesterone receptor expression associated with tumor progression. Adjunct administration of fish oil with both low and high doses of tamoxifen resulted in comparable therapeutic efficacy, demonstrating significant restoration of hormonal homeostasis and suppression of hormone-dependent tumor growth. Importantly, the low-dose tamoxifen regimen achieved similar antitumor effects without the moderate adverse effects observed at the higher dose. Furthermore, fish oil supplementation did not alter normal hormonal parameters in control animals, indicating its safety. These findings suggest that fish oil as a promising adjunct to tamoxifen therapy, enabling effective tumor suppression at lower tamoxifen doses while minimizing dose-related adverse effects in hormone receptor–positive breast cancer.

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