International Journal of Zoology and Applied Biosciences
Volume 11, Issue 1, pp: 283-292, 2026
https://doi.org/10.55126/ijzab.2026.v11.i01.038
ISSN: 2455-9571
http://www.ijzab.com
Research Article

COMBINATORIAL EFFECT OF FISH OIL AND TAMOXIFEN IN 7,12 DIMETHYL BENZ(A) ANTHRACENE DMBA INDUCED RAT MAMMARY CARCINOGENESIS

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Article History: Received 13th November 2025; Accepted 12th January 2026; Published 31st January 2026

ABSTRACT

Breast cancer is the most frequently diagnosed malignancy worldwide and a leading cause of cancer-related mortality among women. Tamoxifen (TAM), a selective Estrogen receptor modulator (SERM), is extensively used in breast cancer therapy; however, its prolonged use is often associated with adverse metabolic and hepatic effects. Marine fish oil (FO), rich in omega-3 fatty acids, exhibits potent antioxidant and therapeutic properties. The present study examined the therapeutic efficacy of fish oil (FO) alone and in combination with tamoxifen (TAM) in attenuating 7,12 Dimethyl Benz(a) anthracene DMBA-induced mammary carcinogenesis in female Sprague–Dawley rats. Mammary tumors were induced by a single oral dose of DMBA (25 mg/kg body weight). After an eight-week tumor development period, tumor-bearing rats were treated with Fish oil FO (0.5 mL) alone or in combination with Tamoxifen TAM at doses of 50 µg/kg and 100 µg/kg body weight. Plasma and tissue samples were analyzed for carcinogenic, biochemical, antioxidant parameters, and histopathological evaluations of the mammary, liver, kidney, and uterus tissues were performed using Hematoxylin and Eosin staining(H&E). DMBA-induced rats showed a significant reduction in final body weight and oxidative stress. Treatment with FO and TAM significantly increased body weight and restored antioxidant defense systems compared with the DMBA control group. Histopathological analysis revealed severe structural abnormalities in the DMBA group, whereas considerable improvement in tissue architecture was observed in the treated groups, particularly in the FO and TAM-treated group. Overall, fish oil administered with tamoxifen confers enhanced therapeutic and antioxidant benefits against DMBA-induced rat mammary carcinoma and improves systemic tissue integrity, highlighting its potential role in breast cancer treatment.

Keywords: Breast cancer, Tamoxifen, Fish oil, DMBA-induced carcinogenesis, Antioxidants.

INTRODUCTION

Breast cancer remains the most prevalent cancer in women worldwide, necessitating the urgent need for novel approaches to diagnosis and treatment. It has a heterogeneous disease with a broad spectrum of pathological, clinical, and molecular characteristics. According to 2022 statistics, breast cancer was the most widely diagnosed cancer in women globally, with an estimated 2.3 million new cases and approximately 670,000 deaths worldwide (Bray *et al*., 2023). Breast cancer (BC) pathology is influenced by a combination of genetic, environmental, and lifestyle factors, making it multifactorial in nature. Exposure to environmental pollutants is a known risk factor for breast cancer, in addition to other common hazards. These pollutants include polycyclic aromatic hydrocarbons (PAHs), which are found in grilled foods, cigarette smoke, vehicle exhaust, and emissions from sources like industrial processes, domestic kerosene stoves, and gasoline and diesel engines (Liang *et al*., 2025). DMBA, a polycyclic aromatic hydrocarbon, is used to induce mammary tumors in animal models, mimicking human breast cancer characteristics, making it crucial for carcinogenesis research and treatment. High levels of free radicals and a weak antioxidant defence system can cause oxidative stress, which harms the cells and tissues involved in chemical carcinogenesis (Russo and

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Russo 1996). To counter this damage, antioxidants work by neutralizing free radicals and other reactive oxygen species (ROS). These antioxidant defence pathways evolved alongside aerobic metabolism to protect against the harmful effects of ROS and other cellular damage.

Oxidative stress, caused by an imbalance of reactive oxygen species (ROS), can disrupt the levels of key antioxidant enzymes and non-enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH), and vitamins E and C. This disruption impacts critical cellular processes like cell division and growth (Bel'skaya and Dyachenko 2024). Furthermore, the accumulation of highly reactive free radicals can induce oxidative stress in the body's lipid rafts, leading to cellular damage and compromising the integrity of cell barriers. Existing breast cancer treatments often have undesirable side effects, highlighting a critical need for novel, less toxic therapeutic agents. Consequently, there is a compelling rationale for exploring alternative prophylactic and therapeutic approaches. The use of potent, yet low-toxicity, natural compounds derived from dietary sources is considered a highly promising strategy to mitigate the morbidity and mortality associated with breast cancer. In recent years, significant attention has focused on natural bioactive compounds as complementary approaches in breast cancer therapy (Hardman 2004). Fish oil, which is high in omega-3 polyunsaturated fatty acids (PUFAs) such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), was shown to have strong antioxidant, anti-inflammatory, and anti-tumor properties. Omega-3 fatty acids regulate membrane fluidity, decrease the emission of pro-inflammatory eicosanoids, and inhibit key tumor-promoting processes including oxidative stress, angiogenesis, and uncontrolled cell proliferation. Evidence from various studies indicates that fish oil enhances the body's antioxidant defence mechanisms, thereby reducing ROS-induced cellular damage and potentially lowering the risk or progression of breast cancer (Calder, 2017). Tamoxifen (TAM), a widely prescribed selective Estrogen receptor modulator (SERM), serves as a key therapeutic agent for the management of hormone-responsive breast cancer. It exerts its antineoplastic effect primarily by competitively antagonizing Estrogen binding to Estrogen receptors, thereby suppressing tumor progression and cellular proliferation. However, the clinical effectiveness of tamoxifen is often compromised by dose-related adverse reactions and the development of drug resistance (Jordan 2006).

Accumulating evidence suggests that co-administration of fish oil with tamoxifen can potentiate antitumor efficacy. Omega-3 polyunsaturated fatty acids have been demonstrated to enhance the pharmacological activity of tamoxifen by improving its bioavailability, attenuating inflammatory responses, regulating apoptosis-related signalling pathways, and reinforcing endogenous antioxidant defense systems. Through these complementary mechanisms, the synergistic interaction between fish oil and tamoxifen may lead to superior therapeutic outcomes and represents a promising strategy for optimizing breast cancer management.

MATERIALS AND METHODS

7,12-Dimethylbenz(a)anthracene (DMBA), were purchased from Sigma-Aldrich Co.Ltd. Thiobarbituricacid (TBA), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), reduced glutathione (GSH) and dimethyl sulphoxide (DMSO) were purchased from Himedia. All other chemicals used were of analytical grade procured from local commercial sources.

Experimental Rats and Diet

The study was conducted on 8 to 10 weeks old healthy female Sprague Dawley rats, weighing approximately 130–150g were obtained from Biogen Laboratory Animal Facility, Bangalore, India and maintained in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India. The rats were housed in well-ventilated large spacious polypropylene cages (six rats /cage) lined with husk under temperature (24 ± 2°C) with relative humidity 50 ± 10% and photoperiod of 12 h light/dark. The rats were given standard pellet diet and water throughout the experimental period.

Induction of Mammary Carcinogenesis

In current research, DMBA was used as a chemical carcinogenic agent. Mammary tumor was induced in female Sprague Dawley rats by using single subcutaneous injection (near the mammary gland) of DMBA 25mg/kg b.wt diluted in 1mL emulsion (0.75mL of sunflower oil and 0.25mL of physiological saline).

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) (Figure 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). Blood has collected in heparinized tubes and the plasma was utilized for further analysis. Mammary, uterus, and liver tissues were separated without interruption and washed well with freezing saline and homogenized in Tris–HCL buffer (0.1 M, pH 7.4) before existence centrifuged at 3000g for 10 minutes at 40°C. Supernatants were collected and processed to determine special biochemical parameters. The remaining tissue was conserved using 10% formalin for histochemical analysis.

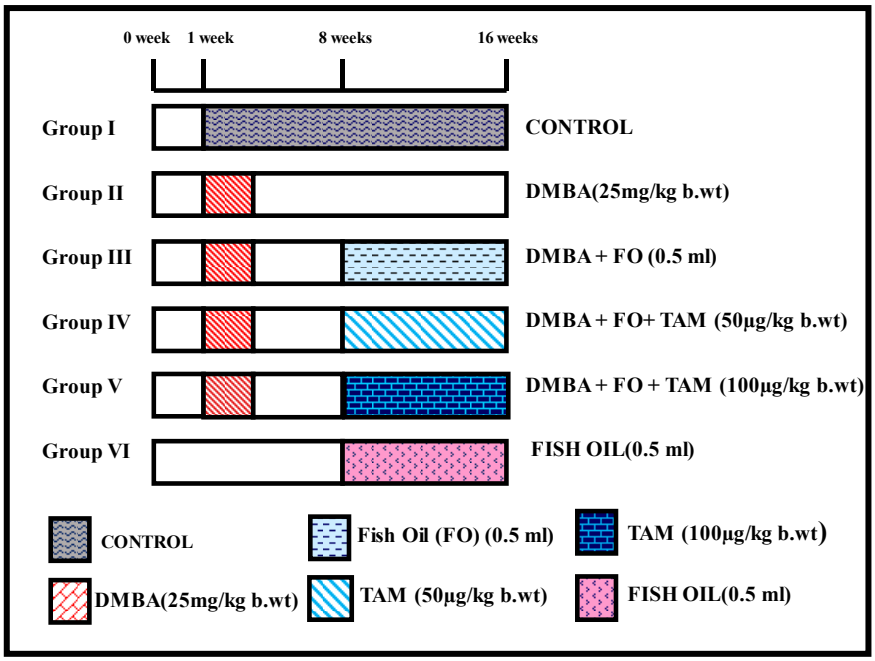

Figure 1. Schematic representation of an experimental design.

Estimation of Lipid Peroxidation

The lipid peroxidation was estimated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in plasma, liver and mammary tissues. The plasma TBARS was assayed by the method of Yagi and tissues TBARS (liver, Uterus and Mammary) were estimated according to the method of Yagi (1987).

Estimation of Enzymatic and Non enzymatic Antioxidants

The activities of enzymatic antioxidant such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in plasma and tissues (liver, Uterus and Mammary) were assayed by the methods of Kakkar *et al*. (1984), and Rotruck *et al*. and non-enzymatic antioxidants such as reduced glutathione (GSH), vitamin C (Vit C) and vitamin E (Vit E) levels in plasma and tissues (liver, Uterus and Mammary) were determined by the methods of Desai (1984), Ellman *et al*. (1961), and Omaye *et al*. (1979) respectively.

Histopathological Examination

The histopathology studies of liver, Uterus, and mammary tissues in experimental rats were sliced, immersed in neutral buffered formalin (10% formaldehyde) for fixation and dehydrated with ethanol solutions and then embedded in paraffin wax. Tissues (3-5 µm in thickness) were cut and stained with haematoxylin and eosin (H&E). Then, slides were observed under microscope (40×). All histopathological changes were examined by the pathologist.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD). A statistical analysis was carried out using SPSS V23.0 (IBM SPSS, USA) software package. The comparisons between groups were done using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 describes the average body weights of the control and experimental rats. Initially, there were no substantial differences in body weight between control and experimental rats. Finally, we observed a significantly diminished in the body weight of DMBA-induced tumour bearing rats (Group II) when compared to the control rats (Group I). However, no significant changes were found in Fish oil 0.5ml (Group III) treated rats when compared with DMBA induced rats (Group II). On the contrary, in tumor bearing rats treated with Fish oil and Tamoxifen 50µg/kg b.wt (Group IV) and Fish oil and Tamoxifen 100 µg/kg b.wt (Group V) treated rats showed significantly increased

the body weight when compared to DMBA induced rats (Group II). However, no significant differences were observed in Fish oil (Group VI) alone treated rats when compared to control rats (Group I). Predominantly, Fish oil and tamoxifen 50µg/kg b.wt (Group IV) was shown to be similar when compared to the Fish oil and tamoxifen 100 µg/kg b.wt (Group V).

Table 1. Effect of Fish oil and Tamoxifen on Body Weight.

Groups	Body Weight(g)	
	Initial (1st week)	Final (16th week)
I	154.37± 12.1	168.03± 13.1
II	159.62± 18.17	101.28± 6.24###
III	141.51± 18.17	128.01± 11.3*
IV	152.42± 19.8	143.18± 12.6**
V	156.97± 16.2	147.79± 12.8**
VI	137.91± 9.9	154.78± 18.9

Values are expressed as mean ± SD for six rats in each group. Significant levels are ###P < 0.001 when compared with control group and **P < 0.01, ***P < 0.001 when compared with DMBA group.

Table:2 portrays the carcinogenic parameters like tumor incidence, cumulative numbers of tumors, and tumor volume of both the control and experimental rats. This assessment revealed 100 % tumor incidence in DMBA administered rats (Group II). Whereas tumor bearing rats were treated Fish oil and tamoxifen 50µg/kg b.wt and Fish oil and tamoxifen 100 µg/kg b.wts was remarkably reduced the tumor incidence as 16% and 14% respectively when compared to DMBA induced rats (Group II) However (Group III) showed a significant reduction as 50% when compared to DMBA induced rats (Group II). When contrasted with control cohort rats (Group I), no discrepancies were recorded in solely addressed Fish oil alone (Group VI) rats. Therefore, Fish oil and tamoxifen 100 µg/kg b.wts suppressed tumor volume more productively than Fish oil and tamoxifen 50µg/kg b.wt.

Table 2. Effect of Fish oil and Tamoxifen on Carcinogenic Parameters.

Groups	Tumor incidence (%)	Number of tumors (N)	Tumor volume (mm3 /rat)
I	0(%)	(0)/6	-
II	100(%)	(6)/6	24.31 ± 3.09
III	50(%)	(3)/6	17.29 ± 3.93*
IV	16(%)	(2)/6	13.87±3.54**
V	14(%)	(1)/6	11.80 ± 3.12**
VI	0(%)	(0)/6	-

Tumor volume was measured using the formula V = 4/3π (D1/2) (D2/2) (D3/2), where D1, D2 and D3 are the three diameters (in mm) of the tumor; () indicates total number of rats bearing tumors. Values are expressed as mean ± SD for six rats in each group. Significant levels are ***P < 0.001 when compared with DMBA group.

Figure.2 presented the concentrations of lipid peroxidative marker (TBARS) in plasma, liver, and mammary tissues of both the control and experimental rats. DMBA administered rats (Group II) displays significantly elevated levels of TBARS than control rats (Group I). Oral administration of Fish oil 0.5ml (Group III) led to a modest, yet significant, decrease in TBARS levels when compared to the DMBA-induced group. In stark contrast, the combination of fish oil and tamoxifen at 50µg/kg b.wt (Group IV) and 100µg/kg b.wt (Group V) resulted a dramatic and significant drop in TBARS levels when compared with DMBA-induced rats. However, no

significant alteration was observed in fish oil (Group VI) alone treated rats when compared with control rats.

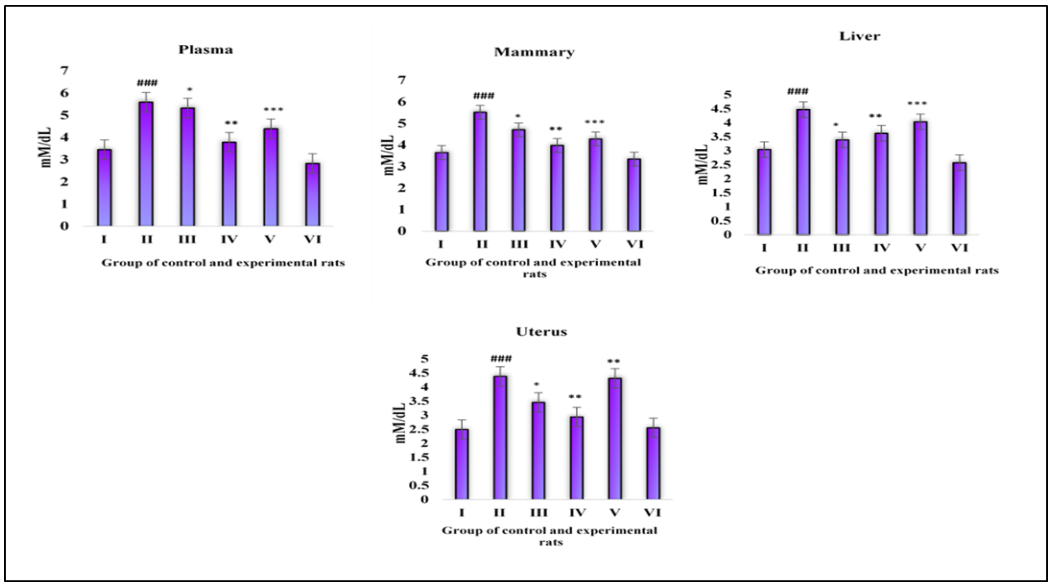

Figure 2. Effect of fish oil and tamoxifen on lipid peroxidation (TBARS) in the plasma, liver, uterus, and mammary tissue of control and experimental rats. Values are expressed as mean ± SD for six rats in each group. Significant levels are ###P < 0.001 when compared with a control group and *P < 0.05, **P < 0.01, ***P < 0.001 when compared with the DMBA group.

The present study evaluated the status of enzymatic antioxidants (SOD, CAT, and GPx) and non-enzymatic antioxidants (GSH, vitamin C, and vitamin E) in the plasma, liver, uterus, and mammary tissues of control and experimental rats. A significant decline in both enzymatic and non-enzymatic antioxidant levels was observed in DMBA-induced rats (Group II) when compared to control rats (Group I), indicating enhanced oxidative stress. Oral administration of FO (0.5 mL) to DMBA-treated rats (Group III) resulted in a modest yet significant restoration of antioxidant levels relative to Group II. No significant alterations were observed in rats treated with fish oil alone (Group VI) compared to the control group, suggesting that fish oil does not disrupt the normal antioxidant status. Notably, combined treatment with FO and TAM at doses of 50 µg/kg b.wt (Group IV) and 100 µg/kg b.wt (Group V) significantly elevated the levels of SOD, CAT, GPx, GSH, vitamin C, and vitamin E. This therapeutic regimen was more effective than fish oil alone in tumor-bearing rats in attenuating DMBA-induced oxidative stress.

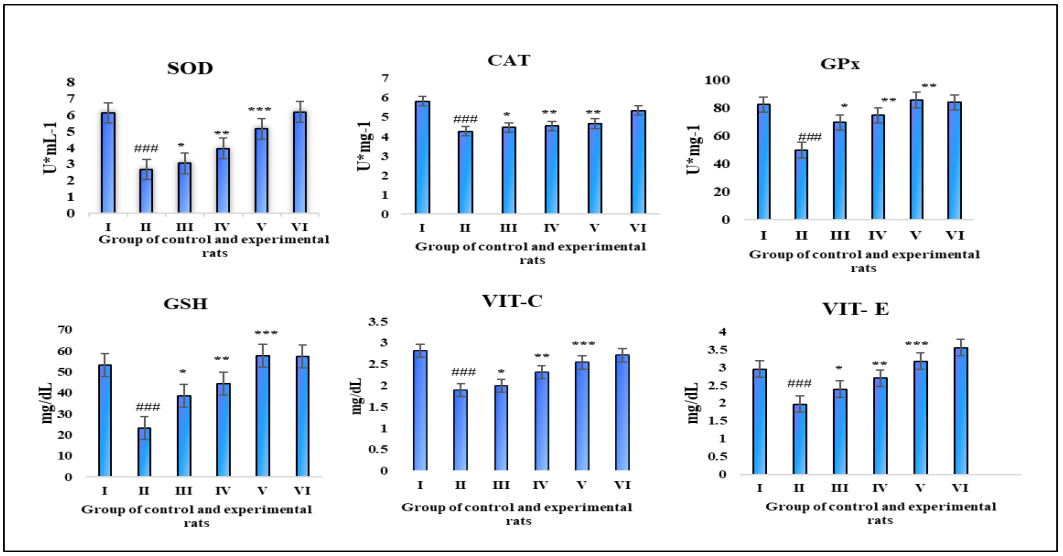

Figure 3. Effect of fish oil and tamoxifen on enzymatic and non-enzymatic antioxidants in plasma of control and experimental rats G1 - CONTROL; G2 - DMBA; G3 - DMBA+FO; G4 - DMBA+FO+ TAM(Low); G5 - DMBA+FO+TAM(High); G6 –FO(Alone).

UA: Amount of enzyme to inhibit 50% NBT reduction/min; UB: µmol of H2O2 consumed/min; UC: µg of GSH consumed/min; Values are expressed as mean ± SD for six rats in each group. Significant levels are ###p < 0.001 when compared with control group and **p < 0.01, ***p < 0.001 when compared with DMBA group.

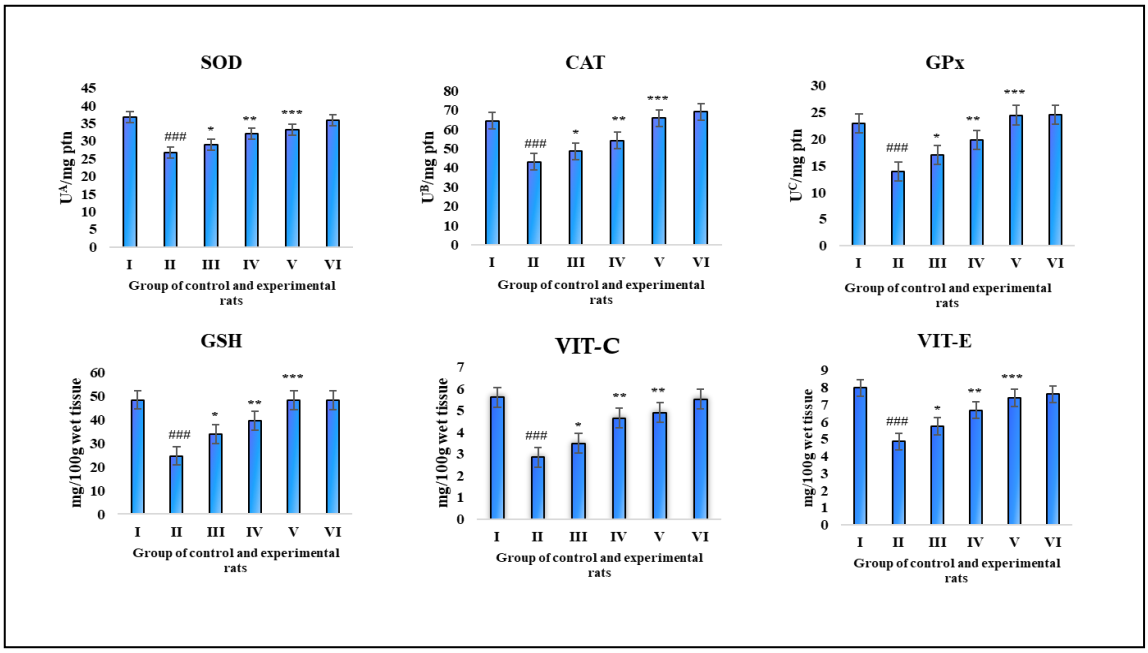

Figure 4. Effect of fish oil and tamoxifen on enzymatic and non-enzymatic antioxidants in mammary of control and experimental rats. **G1 - Control; G2 - DMBA; G3 - DMBA+FO; G4 - DMBA+FO+ TAM(Low); G5 - DMBA+FO+TAM(High); G6 –FO(Alone).** UA: Amount of enzyme to inhibit 50% NBT reduction/min; UB: µmol of H2O2 consumed/min; UC: µg of GSH consumed/min; Values are expressed as mean ± SD for six rats in each group. Significant levels are ###p < 0.001 when compared with control group and **p < 0.01, ***p < 0.001 when compared with DMBA group

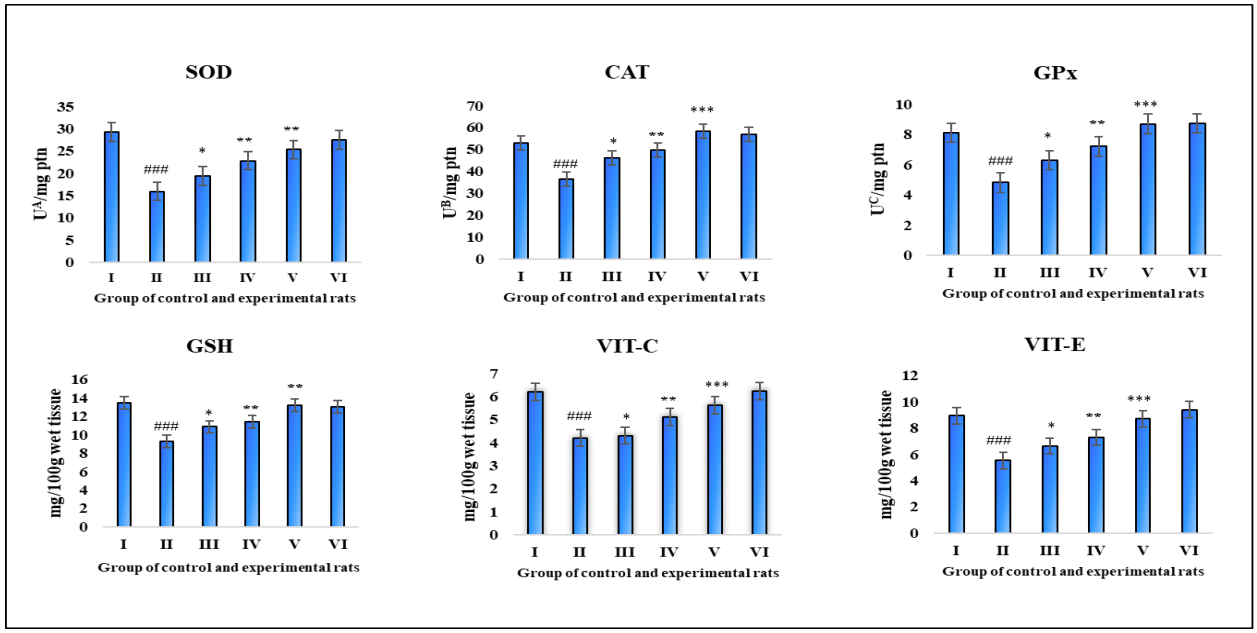

Figure 5. Effect of fish oil and tamoxifen on enzymatic and non-enzymatic antioxidants in liver of control and experimental rats. **G1 - Control; G2 - DMBA; G3 - DMBA+FO; G4 - DMBA+FO+ TAM(Low); G5 - DMBA+FO+TAM(High); G6 –FO(Alone)** UA: Amount of enzyme to inhibit 50% NBT reduction/min; UB: µmol of H2O2 consumed/min; UC: µg of GSH consumed/min; Values are expressed as mean ± SD for six rats in

each group. Significant levels are ###p < 0.001 when compared with control group and **p < 0.01, ***p < 0.001 when compared with DMBA group

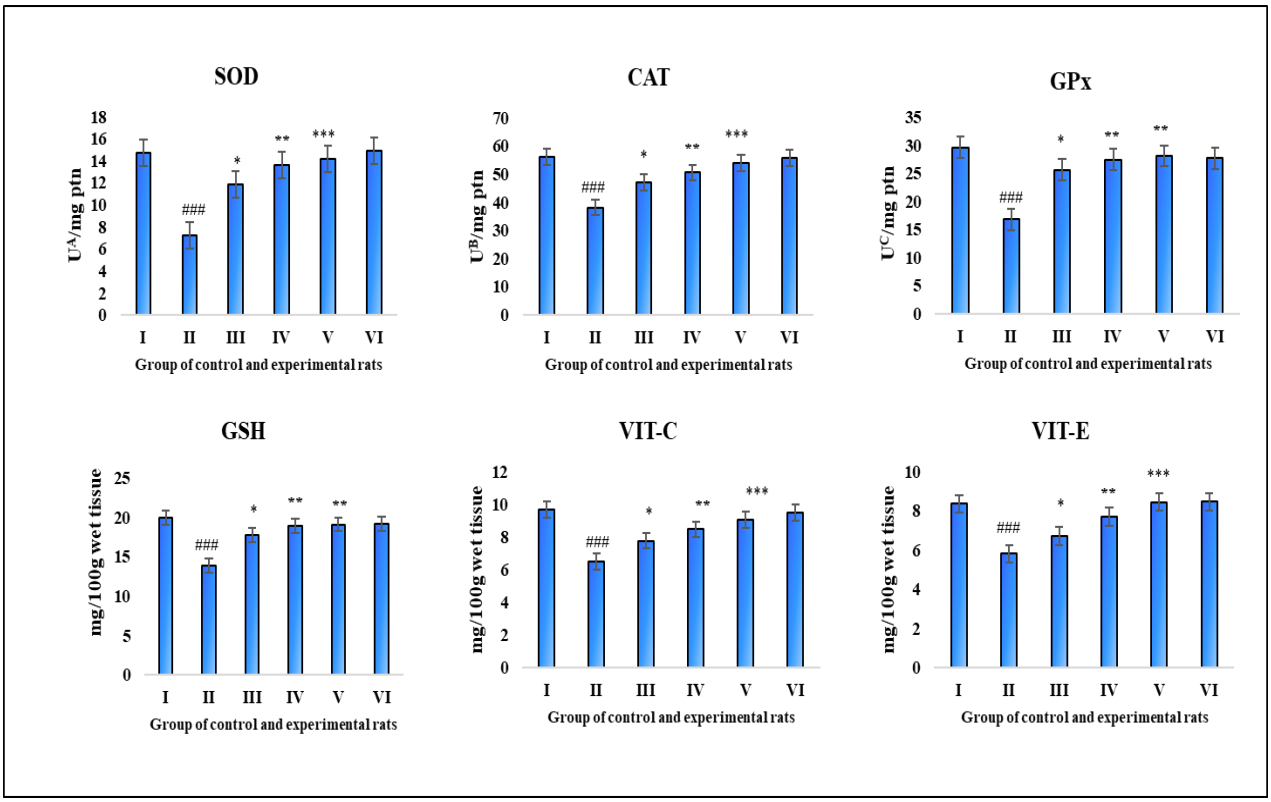

Figure 6. Effect of fish oil and tamoxifen on enzymatic and non-enzymatic antioxidants in uterus of control and experimental rats. **G1 - Control; G2 - DMBA; G3 - DMBA+FO; G4 - DMBA+FO+ TAM(Low); G5 - DMBA+FO+TAM(High); G6 –FO(Alone).** UA: Amount of enzyme to inhibit 50% NBT reduction/min; UB: µmol of H2O2 consumed/min; UC: µg of GSH consumed/min; Values are expressed as mean ± SD for six rats in each group. Significant levels are ###p < 0.001 when compared with control group and **p < 0.01, ***p < 0.001 when compared with DMBA group.

Figure-7 Representative photomicrographs illustrating the histopathological alterations in the liver of control and experimental rats. Group I (A), the control group, together with Group V (E) FO + TAM, 50 µg/kg b.wt and Group VI (F) FO + TAM, 100 µg/kg b.wt, exhibited preserved hepatic architecture with well-organized hepatocyte cords and normal sinusoidal spaces. In contrast, Group II (B), the DMBA-induced tumor-bearing rats, demonstrated marked architectural distortion characterized by nuclear pleomorphism, sinusoidal dilatation, and prominent feathery degeneration. Group III (C), treated with FO (0.5 mL), showed portal triad with surrounding hepatocytes exhibiting feathery degeneration, dilated sinusoids, and mild necrosis. Group IV (D), treated FO + TAM, 50 µg/kg b.wt, exhibited mild sinusoidal dilatation, inflammatory cell infiltration, and a near-normal hepatic architecture. The hepatoprotective effects of the tamoxifen combinations were comparable between the low- and high-dose groups; however, the high-dose regimen produced marginally greater protection than fish oil alone.

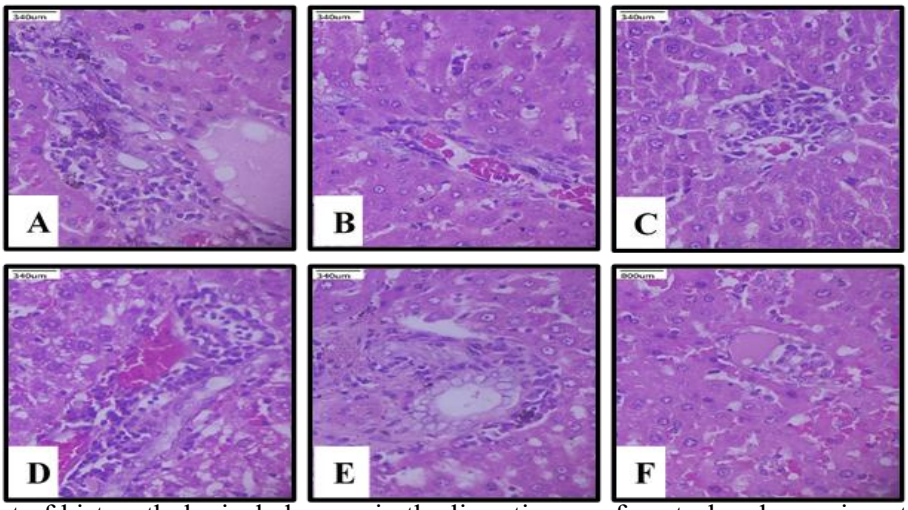

Figure 7. Effect of histopathological changes in the liver tissues of control and experimental rats (hematoxylin and eosin staining showed at 40x).

Control (A, E and F); Normal architecture of hepatocytes, (B) DMBA; Loss of architecture with nuclear pleomorphism and dilated sinusoids along with feathery degeneration, (C) treated Fish oil; Portal triad with surrounding hepatocytes showing feathery degeneration with dilated sinusoids and mild necrosis, (D) treated Fish oil and tamoxifen 50µg/kg b.wt; Portal triad with mild inflammation and markedly sinusoidal dilatation almost near normal architecture of hepatocytes.

The histopathological changes in the mammary tissues of control and experimental rats are shown in Figure 8 Group I (A), the control group, exhibited normal mammary gland architecture with intact ductal epithelial lining and well-organized terminal ductal–lobular units. In contrast, the mammary tissues of DMBA-induced rats (Group II, B) showed marked pathological alterations characterized by invasive ductal carcinoma with irregular cellular proliferation, distorted ductal structures, and dense infiltration of malignant cells. Tumor-bearing rats treated with FO alone (Group III, C) demonstrated moderate improvement with reduced ductal hyperplasia and partial restoration of normal ductal architecture. Mammary tissues of tumor-bearing rats treated with the combined administration of FO + TAM at 50 µg/kg body weight (Group IV, D) showed substantial regression of neoplastic changes with near-normal ductal organization. Furthermore, rats treated with FO + TAM at 100 µg/kg body weight (Group V, E) exhibited almost complete restoration of normal mammary tissue architecture with minimal residual pathological alterations. Comparatively, the combined treatment of fish oil with high-dose tamoxifen was found to be more effective in reversing DMBA-induced histopathological changes than fish oil alone or the low-dose combination.

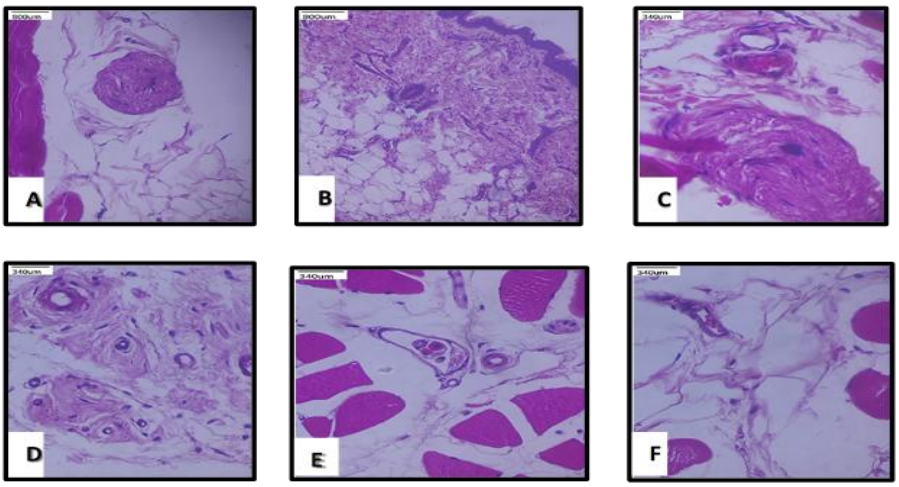

Figure 8. Effect of histopathological changes in the mammary tissues of control and experimental rats (hematoxylin and eosin staining showed at 40x).

Histology on mammary tissues of control (A), and fish oil (alone) (F) alone treated rats showed normal architecture of mammary tissue; In contrast, the DMBA-induced group (B) exhibited marked pathological alterations, including prominent ductal hyperplasia and tumor cell infiltration. Mammary tissues of Fish oil (C) treated rats showed moderate ductal hyperplasia and mild tumor infiltration; Mammary tissues Fish oil and tamoxifen 50µg/kg b.wt (D) treated and Fish oil and tamoxifen 100µg/kg b.wt (E) rats showed almost near normal ductal architecture

Figure-9 illustrates the histopathological changes observed in the uterine tissues of control and experimental rats. Group I (A), the control group, exhibited normal uterine architecture with an intact endometrium containing numerous well-developed uterine glands and a well-organized myometrium composed of longitudinal and circular smooth muscle layers. In contrast, uterine tissues of DMBA-induced tumor-bearing rats Group II (B), showed marked pathological alterations, including disruption of the luminal epithelium, abnormal simple columnar epithelial lining, and structural disorganization of the endometrial glands. Tumor-bearing rats treated with FO alone Group III (C), showed partial restoration of uterine architecture with a moderate reduction in glandular distortion and improved cellular arrangement. Uterine tissues of rats treated with FO in combination with TAM at 50 µg/kg b.wt Group IV (D), exhibited near-normal endometrial structure with rounded nuclei, normal mitotic activity, and mildly dilated blood vessels. Furthermore, rats treated with FO + TAM at 100 µg/kg b.wt Group V (E), demonstrated almost complete restoration of normal uterine histoarchitecture with well-preserved endometrial glands and myometrial layers. Comparatively, the combined treatment of fish oil with high-dose tamoxifen was found to be more effective in reversing DMBA-induced uterine histopathological alterations than fish oil alone or the low-dose combination.

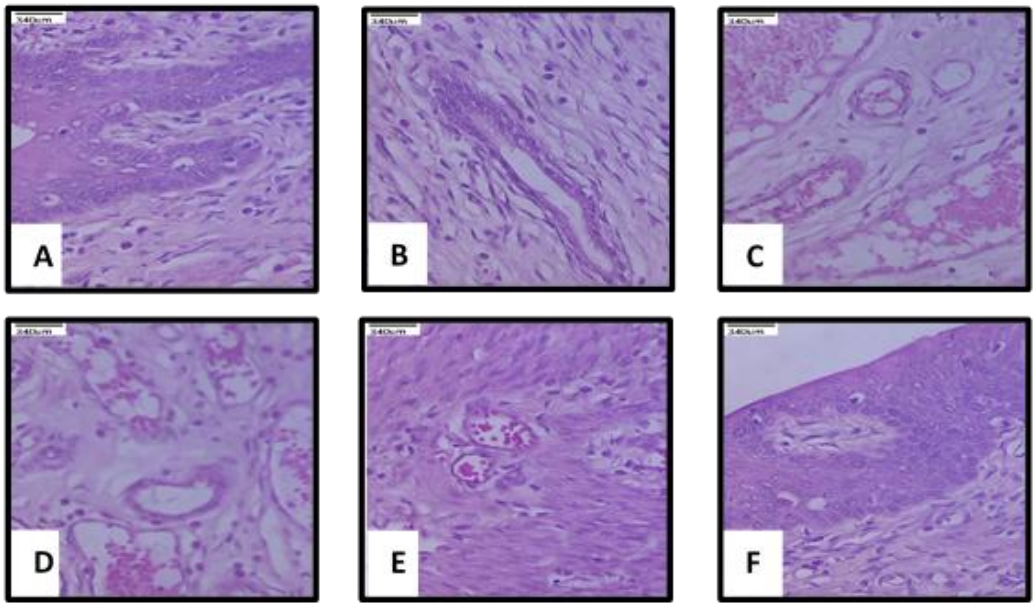

Figure 9. Effect of histopathological changes in the uterus tissues of control and experimental rats (hematoxylin and eosin staining showed at 40x).

Histology on uterus tissues of control (A, E and F); showed endometrium contains large number of uterine glands, (B) DMBA; showed luminal epithelial from outside simple of columnar cells, (C) treated Fish oil; lower significant in the uterine glands, (D) and (E) treated Fish oil and Tamoxifen; the cells with normal nucleuses and mitosis. Breast cancer remains a major global public health challenge and a leading cause of cancer-related mortality among women. Although tamoxifen (TAM), a selective estrogen receptor modulator (SERM), is widely used for the treatment of hormone-responsive breast cancer, its long-term administration is frequently associated with hepatotoxicity, oxidative stress, and metabolic disturbances (Chapkin *et al*. 2007). Therefore, the identification of safe and effective adjuvants that can enhance tamoxifen efficacy while reducing systemic toxicity is of considerable therapeutic importance. Fish oil (FO), rich in omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has emerged as a promising chemotherapeutic agent due to its antioxidant,

anti-inflammatory, and antiproliferative properties. DMBA, a polycyclic aromatic hydrocarbon, initiates mammary carcinogenesis primarily through metabolic activation into highly reactive epoxide intermediates that form DNA adducts and generate excessive reactive oxygen species (ROS). In the present study, DMBA-treated rats exhibited significant body-weight loss, reflecting tumor-associated cachexia and metabolic impairment. In contrast, FO and TAM co-administration markedly improved body weight, suggesting restoration of metabolic homeostasis and attenuation of tumor burden.

Oxidative stress is a central mediator of DMBA-induced carcinogenesis, triggering lipid peroxidation, DNA damage, and redox-sensitive oncogenic signalling cascades. The elevated TBARS levels observed in plasma and tissues of DMBA-treated rats indicate extensive membrane lipid damage. Treatment with FO and TAM significantly reduced TBARS levels, which may be attributed to the ability of omega-3 PUFAs to scavenge free radicals, suppress NADPH-oxidase activity, and enhance membrane fluidity, thereby limiting oxidative injury (Calviello & Serini 2010).At the molecular level, omega-3 PUFAs are known to downregulate pro-oncogenic transcription factors such as NF-κB and AP-1, which are activated under oxidative stress and promote inflammation, angiogenesis, and tumor progression.

The antioxidant defense system, comprising enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), constitutes the primary barrier against ROS-mediated cellular injury. The marked depletion of these enzymes in DMBA-treated rats reflects severe oxidative imbalance. Restoration of SOD, CAT, and GPx activities following FO and TAM treatment suggests enhanced detoxification of superoxide radicals and hydrogen peroxide, thereby preventing mitochondrial dysfunction and apoptotic resistance. Concurrently, the replenishment of non-enzymatic antioxidants including reduced glutathione (GSH), vitamin C, and vitamin E further strengthens intracellular redox buffering and protects membrane lipids from peroxidative damage. GSH also plays a critical role in conjugation and detoxification of DMBA-derived electrophilic metabolites through glutathione-S-transferase-mediated pathways.

Histopathological evaluation provided substantial morphological evidence of these biochemical and molecular events. Severe hepatocellular degeneration, sinusoidal dilatation, and nuclear pleomorphism observed in DMBA-treated rats are indicative of oxidative and xenobiotic-induced liver injury. Concurrent administration of FO and TAM preserved hepatic cytoarchitecture, possibly via modulation of hepatic antioxidant enzymes and inhibition of lipid peroxidation-mediated membrane disruption (Fabian *et al*.2015). Uterine tissues also demonstrated substantial structural recovery following combination therapy, highlighting the systemic cytoprotective effects of FO-mediated redox regulation. Mammary tissues of DMBA-induced rats exhibited hallmark features of invasive ductal carcinoma, including ductal distortion, nuclear atypia, hyperchromatic, and necrosis. FO monotherapy produced only partial tumor regression; however, the combined treatment with FO and TAM, particularly at the higher tamoxifen dose, resulted in near-complete restoration of normal mammary architecture.

This pronounced therapeutic response may be mechanistically linked to synergistic induction of apoptosis via mitochondrial (intrinsic) pathways, enhanced activation of caspase-3, and suppression of proliferative signalling pathways such as PI3K/Akt and MAPK (Manni *et al*. 2010). Omega-3 fatty acids are also known to alter lipid raft composition, thereby enhancing tamoxifen-mediated Estrogen receptor antagonism and improving drug sensitivity in breast cancer cells. Collectively, the present study provides significant biochemical evidences, histopathological, and mechanistic insights that fish oil significantly potentiates the anticancer and antioxidant efficacy of tamoxifen against DMBA-induced mammary carcinogenesis (Wu *et al*. 2015). The combination therapy not only suppresses tumor progression through redox modulation, apoptotic activation, and inhibition of oncogenic signalling but also confers substantial protection to vital organs including the liver, mammary and uterus. These findings provide strong evidence that fish oil serves as a safe and mechanism-driven adjuvant to tamoxifen, enhancing therapeutic efficacy while concurrently reducing systemic toxicity.

CONCLUSION

The present investigation unequivocally demonstrates that dietary fish oil markedly potentiates the anticancer therapeutic effectiveness of tamoxifen in DMBA-induced mammary carcinogenesis in female Sprague–Dawley rats. The combinatorial regimen profoundly attenuated oxidative burden, re-established endogenous antioxidant defense systems, and substantially ameliorated the histoarchitectural integrity of mammary, hepatic, renal, and uterine tissues. These coordinated effects signify a true pharmacological synergy rather than a simple additive therapeutic outcome. At the mechanistic level, the augmented therapeutic response is plausibly mediated through omega-3 fatty acid–driven modulation of cellular redox equilibrium, suppression of pro-tumorigenic signalling cascades, and facilitation of programmed apoptotic cell death within neoplastic tissues, thereby enhancing tumor susceptibility to tamoxifen. Notably, the preservation of vital organ microarchitecture further underscores the capacity of fish oil to abrogate chemotherapy-associated off-target cytotoxicity. Collectively, the evidence from the present investigation positions fish oil as a biologically active, pharmacologically compatible, and clinically translatable adjuvant that amplifies tamoxifen responsiveness while concurrently conferring systemic cryoprotection. This integrative regimen offers a scientifically grounded, low-toxicity strategy with substantial potential to improve outcomes in endocrine-driven breast cancer interventions.

ACKNOWLEDGMENT

The authors express sincere thanks to the Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Chidambaram, Tamil Nadu, India for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

This study was approved by the Institutional Animal Ethics Committee (IAEC), regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Registration number AU-IAEC/1309/12/21).

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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