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DOPAMINERGIC STIMULATION AND β-ADRENERGIC BLOCKADE ATTENUATE DMBA-INDUCED MAMMARY TUMOUR GROWTH

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ABSTRACT

The global burden of breast cancer necessitates innovative adjunct therapies to address conventional treatment limitations. This study investigated the antitumour potential of repurposing propranolol (β-adrenergic blocker) and dopamine (dopaminergic agonist) in comparison with tamoxifen, utilizing a DMBA-induced mammary carcinoma rat model. In this preclinical *in vivo* study, sixty female albino rats were enrolled; fifty rats underwent mammary tumour induction and, upon developing palpable tumours, were allocated into five treatment groups (n=10 per group), while the remaining ten rats served as plain controls. Assessment parameters include tumour volume, proliferative (Ki-67) and apoptotic (Caspase-3) markers, serum VEGF, and hepatic oxidative stress (MDA and GSH). Findings revealed that dopamine and tamoxifen significantly suppressed tumour growth by week four, while propranolol exhibited a moderate effect. Tamoxifen and dopamine effectively suppressed cellular proliferation, as indicated by reduced Ki-67 expression. Furthermore, all interventions successfully triggered apoptosis, evidenced by significantly elevated Caspase-3 levels. Notably, dopamine exhibited the most potent anti-angiogenic profile, restoring serum VEGF to near-normal

concentrations. While tamoxifen and propranolol effectively mitigated hepatic oxidative stress, dopamine displayed limited antioxidant efficacy. In conclusion, the present findings indicate that the pharmacological targeting of β-adrenergic and dopaminergic signalling cascades yields significant antitumour efficacy. These pathways represent highly promising candidates for adjunct breast cancer management, justifying more extensive clinical evaluations of these repurposed therapeutic agents.

KEYWORDS: Breast cancer; Drug repositioning; Angiogenesis; Apoptosis; Propranolol; Dopamine.

1. INTRODUCTION

Breast cancer remains a global health challenge, with hormone receptor-positive tumours comprising approximately two-thirds of cases. Although tamoxifen—a selective oestrogen receptor modulator (SERM)—remains a fundamental component of endocrine therapy, its long-term use is frequently limited by acquired resistance and clinically significant adverse effects, including thromboembolism and endometrial carcinoma.[1-3] These limitations highlight the need for adjunctive therapeutic approaches that target additional pathways implicated in tumour development and treatment resistance.

Mammary tumour progression arises from the convergence of uncontrolled proliferation, impaired apoptotic mechanisms, and persistent angiogenesis. Therefore, comprehensive evaluation of potential therapeutic agents requires simultaneous assessment of key biological markers such as Ki-67 (cell proliferation), caspase-3 (apoptotic executioner), and VEGF (a principal regulator of tumour-associated angiogenesis).[4, 5] Moreover, oxidative stress—reflected by increased malondialdehyde (MDA) and diminished glutathione (GSH)—contributes to genomic instability and facilitates carcinogenic signalling in breast tissue.[6, 7]

Recent evidence underscores the influence of neurohumoral pathways on the tumour microenvironment. β-adrenergic receptor (β-AR) activation has been linked to enhanced tumour growth and angiogenesis through cAMP/PKA-mediated mechanisms and VEGF induction[8], while β-AR blockade with propranolol has demonstrated antitumour potential in both experimental and clinical contexts.[9,10] Dopaminergic signalling also plays a modulatory role; dopamine can suppress angiogenesis by interfering with VEGF-driven pathways and reducing vascular permeability.[11, 12]

Building on these observations, the present study incorporates dopaminergic stimulation and β-adrenergic blockade within the same experimental platform to provide a direct comparison with tamoxifen. Despite increasing interest in neurohumoral modulation, few studies have systematically evaluated these mechanisms side-by-side in a 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma model. By examining tumour size, apoptosis, proliferation, and angiogenesis while controlling for route-of-administration differences, this work aims to characterise the pharmacodynamic profiles of dopamine and propranolol as potential non-conventional adjuncts to endocrine therapy. Consequently, the primary aim of this research was to evaluate whether these pharmacological agents could elicit antitumour effects comparable to those of tamoxifen through coordinated modulation of angiogenic, proliferative, and apoptotic pathways.

2. MATERIALS AND METHODS

2.1 Animals

Sixty healthy female albino rats (55 days old; 110 and 130 g) were obtained from the animal house of the Medical Research Institute, Alexandria University. Animals were housed under standard laboratory conditions (controlled light–dark cycle and controlled temperature), with free access to standard chow and water, and were allowed to acclimatize for at least one week before experimentation. The study protocol was reviewed and approved by the relevant institutional committees, including the Research Ethics Committee of the Medical Research Institute, Alexandria University, Egypt. All experimental procedures were conducted in compliance with the approved protocol throughout the study period and in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition).[13]

2.2 Drugs and chemicals

The drugs used in this study included tamoxifen citrate (Tamoxifen; Amrya Pharmaceutical Industries, Alexandria, Egypt), propranolol hydrochloride (Mayestrotense; Egypharma, Egypt), and dopamine hydrochloride (Dopamine Fresenius; Fresenius Kabi GmbH, Hamburg, Germany). 7,12-Dimethylbenz(a)anthracene (DMBA; Sigma, St. Louis, MO, USA) was suspended in sesame oil for tumour induction. Unless otherwise specified, drugs were freshly prepared in 0.9% saline on the day of administration.

2.3 Induction of mammary carcinoma

Mammary tumours were induced in fifty rats through a single oral dose of DMBA (20 mg/rat), which was prepared as a suspension in 1 mL of sesame oil and administered via

gastric gavage. Ten rats served as plain controls and received 1 mL sesame oil only. Starting from week 5 post-DMBA, rats were palpated weekly for tumour detection, and palpable measurable tumours developed within 24–36 weeks. Animals presenting skin lesions unrelated to mammary tumours were excluded prior to group allocation.

2.4 Experimental design and treatment groups

After the development of palpable mammary tumours, tumour-bearing rats were allocated into experimental groups (n = 10 per group). Due to the asynchronous nature of tumour induction in the DMBA model, animals were enrolled upon developing palpable tumours of comparable size. At enrolment, eligible animals were randomly assigned using a manual randomization procedure into five tumour-bearing groups (two route-specific controls and three treatment groups) to ensure comparable baseline tumour burden across groups.

- Group Ia (Control, p.o.): Received 1 mL of 0.9% saline p.o. once daily.
- Group Ib (Control, i.p.): Received 1 mL of 0.9% saline i.p. once daily.
- Group II (Tamoxifen): Received tamoxifen citrate (0.6 mg/kg/day) p.o. via gastric gavage.
- Group III (Propranolol): Received propranolol hydrochloride (3 mg/kg/day) i.p.
- Group IV (Dopamine): Received dopamine hydrochloride (50 mg/kg/day) i.p.

All drugs were dissolved in 0.9% saline and administered once daily for 28 consecutive days. The dopamine dosage was determined in accordance with prior literature demonstrating its safety and potent anti-angiogenic activity in rodent tumour models, without systemic toxicity.[14] To avoid potential confounding effects related to different administration routes, separate oral and intraperitoneal control groups were included. Accordingly, each treatment group was compared with its route-specific control to ensure that observed effects reflected true pharmacological activity rather than administration-related variability.

2.5 Tumour volume assessment

Tumour volumes were measured at baseline (day 0; treatment initiation) and weekly thereafter. For each palpable tumour, the shortest (d_1) and longest (d_2) diameters were measured using a micrometer caliper, and radii were calculated as $r_1 = d_1/2$ and $r_2 = d_2/2$. Tumour volume (V) was estimated assuming a prolate spheroid using the following formula:

$$V = (4/3) \times \pi \times r_1^2 \times r_2$$

In this formula, r_1 and r_2 represent the minor and major radii, respectively, as previously described.[15]

In rats bearing multiple tumours, total tumour volume per rat was calculated by summing the volumes of all individual tumours. The progression of tumours was quantified as the percentage variation in total volume compared to the initial baseline measurements.

$\%\Delta V = [(V_t - V_0)/V_0] \times 100$

Where V_t is the tumour volume at time t and V_0 is the baseline tumour volume.

2.6 Sampling

Upon completion of the 28-day treatment period, rats were subjected to an overnight fast with free access to water. Blood sampling from the retro-orbital plexus using glass capillary tubes was performed rapidly by experienced personnel to minimize distress. The collected blood was allowed to clot, and serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum samples were stored in aliquots at −20 °C for subsequent VEGF assay. Immediately following blood collection, animals were humanely euthanized by cervical dislocation, ensuring a rapid termination of life in accordance with the approved institutional laboratory practices at the time. Mammary tumours and livers were then excised, washed in ice-cold saline, and blotted dry. Tumour tissues were divided as follows.

(i) portions fixed in 10% neutral buffered formalin for histopathology and Ki-67 immunohistochemistry; and

(ii) portions immediately homogenised for caspase-3 determination.

Liver samples were homogenised in ice-cold buffer for the assessment of MDA and reduced glutathione (GSH)

2.7 Histopathological examination and Ki-67 immunohistochemistry

Formalin-fixed tumour specimens were embedded in paraffin, sectioned at 3–4 μm, and stained with haematoxylin and eosin (H&E) for histopathological evaluation and grading. Additional sections were processed for Ki-67 immunohistochemistry using a rabbit monoclonal anti-Ki-67 antibody (Clone SP6; Lab Vision, Fremont, CA, USA) and the standard streptavidin–biotin peroxidase method. Nuclear staining in tumour cells was considered positive.

To ensure objective evaluation and minimize observer bias, all slides were coded and examined by a pathologist blinded to the treatment groups. For each specimen, Ki-67

immunoreactivity was evaluated in at least five representative, non-overlapping high-power fields (×400). The proliferation index was determined by calculating the percentage of positive cells and semiquantitatively categorized as: negative (<5%), mild (+, 5–10%), moderate (++, 10–20%), and high (+++, ≥20%).

2.8 Biochemical assays

Tumour caspase-3 and serum vascular endothelial growth factor (VEGF) levels were quantified using commercial ELISA kits according to the manufacturers' instructions. Tumour tissues were homogenised in ice-cold buffer and centrifuged, and caspase-3 levels were determined in the supernatants using the Caspase-3 Instant ELISA kit (Cat. No. BMS2012INST; Bender MedSystems, Vienna, Austria). Serum VEGF concentrations were measured using the RayBio Rat VEGF ELISA kit (RayBiotech, Norcross, GA, USA). Standards and samples were assayed in duplicate, absorbance was read at 450 nm, and concentrations were calculated from the standard curves. Results were expressed as ng/mL for tumour homogenate supernatants (caspase-3) and pg/mL for serum (VEGF). To maintain objectivity, all biochemical assays were performed by investigators blinded to treatment allocation, and treatment codes were disclosed only after the completion of all assessments.

2.9 Hepatic oxidative stress markers

Hepatic MDA levels were determined using the thiobarbituric acid (TBA) reaction.[16] Absorbance of the organic phase was measured at 532 nm, and MDA concentrations were obtained from a standard curve constructed using 1,1,3,3-tetramethoxypropane. Results were normalised to tissue weight and expressed as nmol/100 mg wet tissue. Reduced glutathione (GSH) was determined spectrophotometrically using the 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) method.[17] Absorbance was measured at 412 nm, and GSH concentrations were calculated from a preconstructed standard curve. Results were expressed as mg/g wet tissue.

2.10 Statistical analysis

Statistical evaluations were conducted using the PASW Statistics software package, version 18.0. Quantitative data are presented as mean ± standard deviation (SD), with n = 10 animals per group.

The Shapiro–Wilk test was applied to assess the normality of data distribution. Serum VEGF, tissue caspase-3, and hepatic MDA levels demonstrated approximately normal distributions and were compared among groups using one-way analysis of variance (ANOVA), followed

by Scheffé's post hoc test for pairwise comparisons. Hepatic GSH concentrations and other non-normally distributed variables (notably the percentage change in tumour volumes) were analysed using the Kruskal–Wallis test, with subsequent Mann–Whitney U tests for pairwise comparisons between treatment groups and their respective route-matched controls, as well as for secondary comparisons among active treatment arms.

Qualitative variables, such as Ki-67 immunoreactivity grades, were analysed using the Chi-square test with Monte Carlo correction applied to all comparisons. All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Tumour growth and volume changes

Baseline tumour volumes were comparable across all groups at treatment initiation. In untreated controls, mammary tumours showed aggressive progressive growth, reaching a striking 1746.66–1916.45% increase by week 4 (Fig. 1). All pharmacological interventions significantly attenuated this expansion ($p \leq 0.05$). By the end of the treatment period, tumour volume had increased by only 117.50% in the tamoxifen group ($p \leq 0.05$ vs oral control), 223.41% in the dopamine group ($p \leq 0.05$ vs i.p. control), and 661.50% in the propranolol group ($p > 0.05$ vs i.p. control) (Fig. 1).

Tamoxifen demonstrated the strongest and most sustained suppression of tumour growth, consistent with its established potency in inhibiting oestrogen‑driven proliferation in rat mammary carcinoma models.[18] Dopamine also produced marked antitumour activity, particularly during the first two weeks, resulting in tumour control approaching that of tamoxifen. This early and robust response suggests that dopaminergic stimulation may disrupt key metabolic and angiogenic requirements essential for initial tumour expansion.[11] In contrast, propranolol produced a moderate and less durable effect, implying that β‑adrenergic blockade alone may be insufficient to exert sustained tumour control in advanced or rapidly proliferating lesions. This pattern aligns with previous reports suggesting that while β‑blockers can interfere with early angiogenic and inflammatory signalling, they often show limited efficacy as a monotherapy in established solid tumours compared to combined regimens.[19]

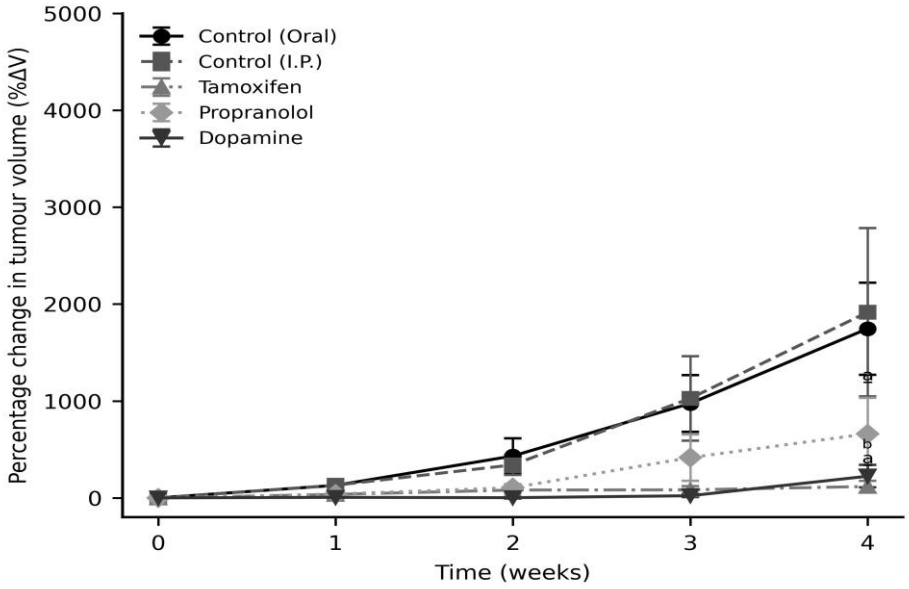

Fig. 1: Effect of different treatment regimens on the percentage change in total mammary tumour volume relative to baseline (day 0) over four weeks in rats bearing DMBA-induced mammary carcinoma. Data are expressed as mean ± SD (n = 10 per group).

3.2. Tumour proliferative activity (Ki‑67 expression)

Untreated tumour‑bearing rats exhibited high proliferative activity, with most tumours showing moderate to strong Ki‑67 immunoreactivity (Table 1; Fig. 2A, E). The distribution of Ki‑67 expression did not differ significantly between the oral and intraperitoneal control subgroups ($p > 0.05$). Tamoxifen and dopamine were each associated with a significant reduction in tumour proliferative activity compared with their corresponding route‑matched controls ($p \leq 0.05$), reflected by a shift toward negative or mild staining categories and the absence of strongly positive cases (Table 1; Fig. 2B, D, E).

The pronounced anti‑proliferative effect of tamoxifen is consistent with its established ER‑mediated suppression of mammary tumour growth in rodent models.[20] The comparable reduction produced by dopamine is particularly noteworthy, supporting the concept that dopaminergic stimulation can arrest the cell cycle in malignant mammary epithelial cells, possibly through D2‑receptor–dependent pathways that modulate growth factor signaling.[21] Propranolol produced an intermediate effect that did not reach statistical significance relative to the intraperitoneal control ($p = 0.094$) (Table 1; Fig. 2C, E), suggesting that β‑adrenergic blockade may exert its antitumour effect primarily by altering the tumour

microenvironment—such as suppressing angiogenesis or stress‑related signalling—rather than by directly inhibiting tumour cell proliferation.

Table 1: Effect of different treatment regimens on Ki-67 immunoreactivity in tumour tissues of rats bearing DMBA-induced mammary carcinoma (n = 10 per group).

Ki-67 grade	Oral control (Ia) n (%)	i.p. control (Ib) n (%)	Tamoxifen (II) n (%)	Propranolol (III) n (%)	Dopamine (IV) n (%)
Negative	0 (0.0)	0 (0.0)	3 (30.0)	2 (20.0)	2 (20.0)
Mild (+)	2 (20.0)	2 (20.0)	7 (70.0)	5 (50.0)	7 (70.0)
Moderate (++)	6 (60.0)	7 (70.0)	0 (0.0)	3 (30.0)	1 (10.0)
Strong (+++)	2 (20.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)

Data are expressed as n (%). Overall comparisons among groups were performed using the Chi-square test with Monte Carlo correction. Statistical significance was set at $p \leq 0.05$. Ki-67 grades were defined as: negative (<5%), mild (+, 5–10%), moderate (++, 10–20%), and strong (+++, >20%). DMBA: 7,12-dimethylbenz(a)anthracene; i.p.: intraperitoneal; p.o.: oral

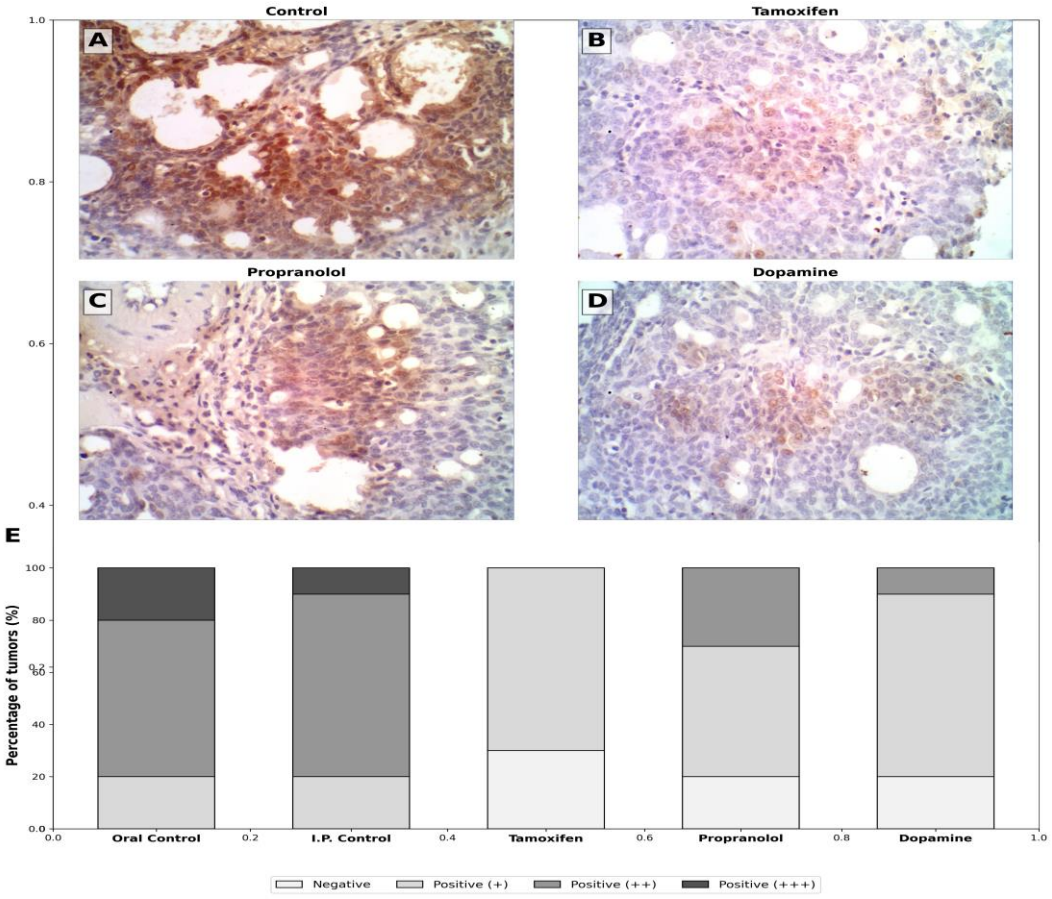

Fig. 2: Effect of tamoxifen, propranolol, and dopamine on Ki-67 expression in DMBA-induced mammary tumours. (A–D) *Representative photomicrographs of Ki-67*

immunohistochemical staining (×400) in: (A) tumour-bearing control, (B) tamoxifen-treated, (C) propranolol-treated, and (D) dopamine-treated groups. (E) Quantitative distribution of Ki-67 immunoreactivity grades (negative, +, ++, +++) expressed as percentage of tumours per group (n = 10). Statistical significance was determined using the Chi-square test with Monte Carlo correction ($p \leq 0.05$).

3.3. Histopathological grading

Histopathological examination of H&E‑stained tumour sections revealed high‑grade invasive ductal carcinomas (Grade III) in both tumour‑bearing control groups, characterised by marked nuclear pleomorphism, high mitotic activity, and poor glandular differentiation (Fig. 3A). These features are consistent with aggressive tumour behaviour and align with the high proliferative activity observed by Ki‑67 immunostaining in the same groups.

Tamoxifen‑treated tumours showed a pronounced shift toward lower histological grades (I–II), with improved glandular differentiation and reduced nuclear atypia (Fig. 3B). This downgrading is in agreement with the marked reduction in Ki-67 expression observed in this study, supporting the notion that tamoxifen suppresses proliferation within tumour tissue.[20] This is further corroborated by the pro-apoptotic effects of tamoxifen, as evidenced by the caspase-3 results discussed later in this study.[22] Dopamine-treated tumours were predominantly Grade II and frequently exhibited apoptotic bodies and stromal inflammatory infiltration; intraductal papillary carcinoma patterns were also observed in some cases (Fig. 3D). The presence of prominent apoptosis and stromal remodelling, together with the observed reduction in Ki-67 expression, suggests that dopaminergic modulation induces both cytotoxic and microenvironmental alterations that contribute to a less aggressive tumour phenotype.[11, 12, 21] In contrast, propranolol‑treated tumours displayed mixed features ranging from Grades II to III, without a consistent pattern of downgrading (Fig. 3C). This heterogeneity is consistent with the modest, non‑significant reduction in Ki‑67 expression and the intermediate effects on tumour volume, indicating that β‑adrenergic blockade alone may be insufficient to drive robust histological regression despite its measurable impact on angiogenic signaling.[9]

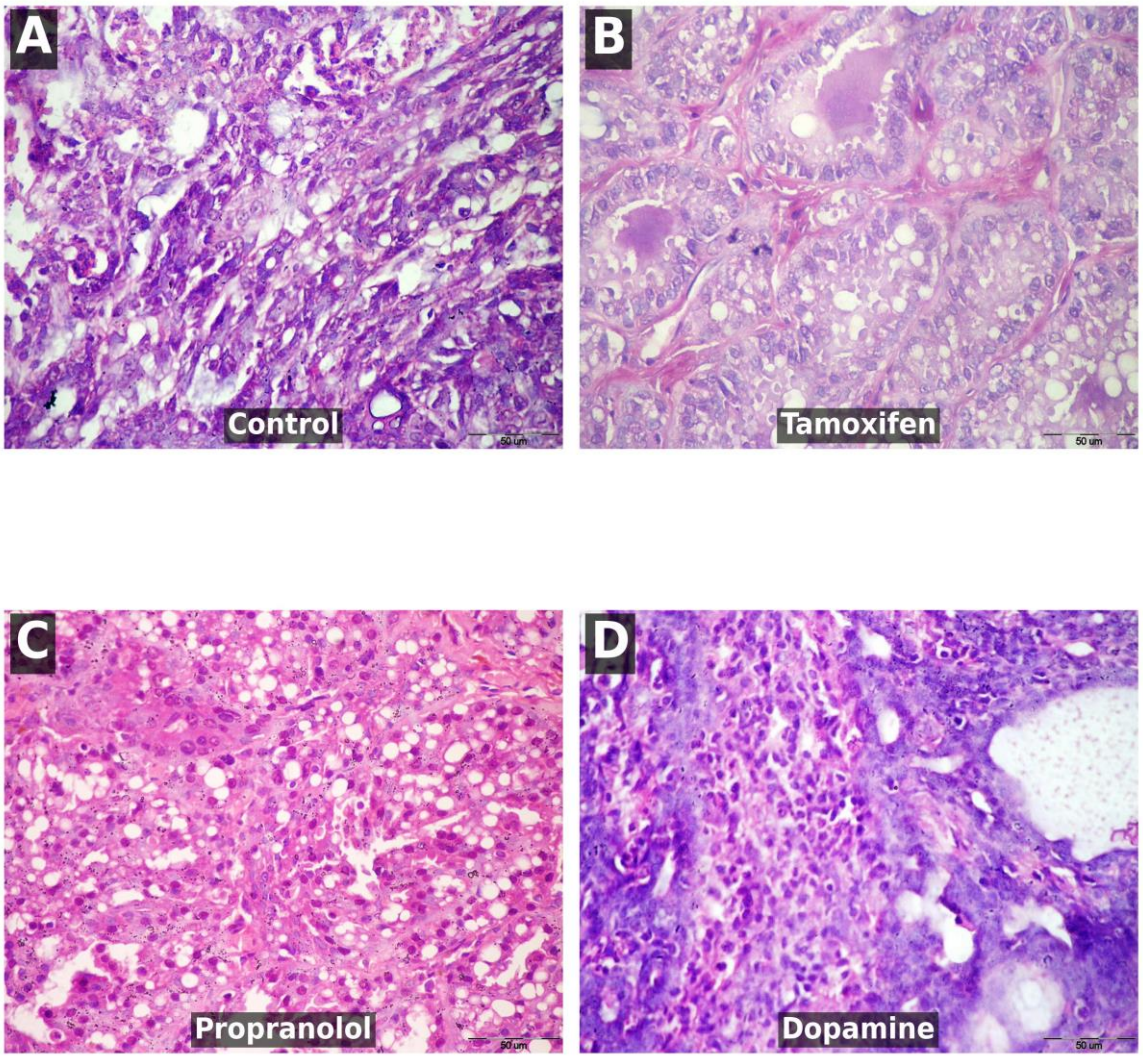

Fig. 3: Histopathological features of DMBA-induced mammary tumours following treatment. *Representative haematoxylin and eosin (H&E)-stained sections from tumour-bearing control and treated groups. (A) Tumour-bearing control showing invasive ductal carcinoma (IDC) grade III with marked nuclear pleomorphism and poor differentiation. (B) Tamoxifen-treated tumour demonstrating histological downgrading to IDC grades I–II with improved glandular differentiation. (C) Propranolol-treated tumour showing an intermediate pattern (IDC grades II–III) with moderate glandular differentiation and inflammatory stroma. (D) Dopamine-treated tumour, predominantly IDC grade II, with prominent apoptotic figures and inflammatory stromal infiltration. (H&E staining, ×400 magnification).*

3.4. Apoptotic marker (Tumour caspase‑3)

Tumour caspase‑3 levels were comparable between the oral and intraperitoneal tumour‑bearing controls (14.76 ± 1.30 and 14.91 ± 1.01 ng/mL, respectively). Daily treatment with tamoxifen, propranolol, or dopamine resulted in a significant elevation of tumour caspase‑3

compared with their corresponding route-matched controls ($p < 0.05$; Fig. 4), indicating enhanced apoptotic activity across all active treatments. The highest caspase-3 level was observed in the dopamine group (18.58 ± 1.42 ng/mL), followed by propranolol (17.44 ± 1.62 ng/mL) and tamoxifen (16.79 ± 1.42 ng/mL); however, the differences among the three active treatments were not statistically significant ($p > 0.05$; Fig. 4).

This pro-apoptotic shift, as evidenced by elevated caspase-3, aligns with the observed reduction in tumour volumes across all treated groups. For tamoxifen, this activation is likely mediated through both genomic and non-genomic apoptotic triggers, as caspase-3 expression in ER-positive breast cancer has been shown to predict and support the therapeutic response to tamoxifen.[22, 23] In the case of dopamine and propranolol, the induction of apoptosis may be secondary to the withdrawal of neurohumoral survival signals that ordinarily support tumour persistence. This is consistent with evidence that dopaminergic and β-adrenergic pathways significantly modulate tumour growth, angiogenesis, and cell survival mechanisms.[8, 9, 11, 21] Notably, the absence of a significant difference between the three treatments suggests that neurohumoral modulators can be as effective as standard endocrine therapy in restoring apoptotic sensitivity in DMBA-induced mammary carcinoma.

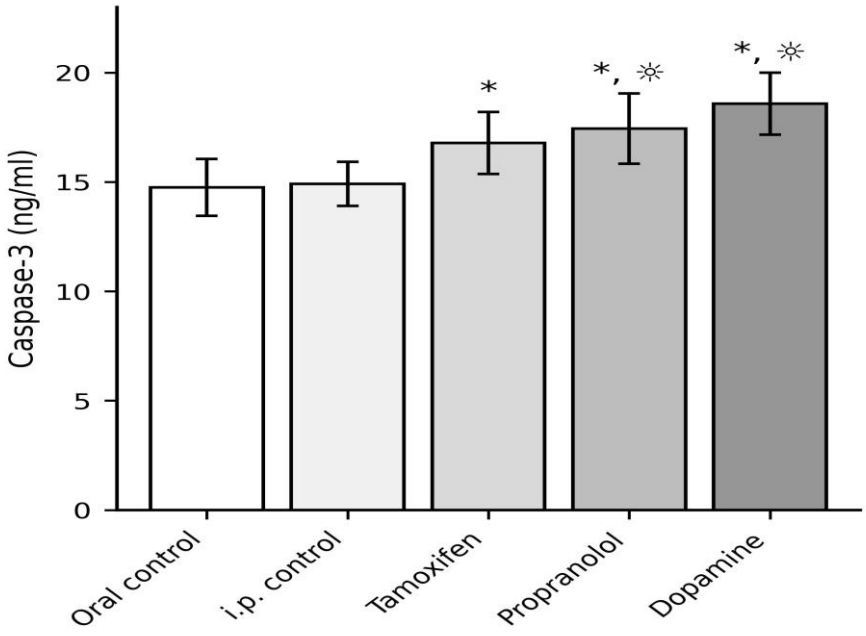

Fig. 4: Effect of different therapeutic regimens on caspase-3 level (ng/mL) as a marker of apoptosis after 4 weeks of treatment in the tumour tissues of rats bearing DMBA-induced mammary carcinoma. *Data are expressed as mean ± SD (n = 10 per group).*

**: Statistically significant difference compared with the oral control group ($p \leq 0.05$).*

☼: Statistically significant difference compared with the intraperitoneal control group ($p \leq 0.05$).

3.5. Angiogenic marker (Serum VEGF)

DMBA-induced mammary carcinoma significantly elevated serum VEGF levels in tumour-bearing controls compared with plain controls ($p \leq 0.05$; Table 2), confirming that angiogenesis is a critical driver of sustained tumour growth in this model. Treatment with dopamine or propranolol significantly reduced VEGF concentrations versus the intraperitoneal control ($p \leq 0.05$), whereas tamoxifen produced only a modest, non-significant decrease compared with the oral control ($p > 0.05$). Notably, dopamine treatment resulted in the lowest serum VEGF levels, which were comparable to those observed in the plain control group (Table 2).

This superior anti-angiogenic profile of dopamine highlights a distinct mechanistic advantage, consistent with its known ability to inhibit VEGF-mediated angiogenesis by inducing VEGFR2 internalisation and inhibiting its phosphorylation and downstream signaling.[11, 14, 21] Propranolol also significantly reduced VEGF, supporting the role of β-adrenergic signalling in promoting a pro-angiogenic microenvironment via stress-linked cAMP/PKA pathways.[8, 9, 19] In contrast, the failure of tamoxifen to significantly lower VEGF suggests that its primary antitumour mechanism in this model is anti-proliferative and pro-apoptotic rather than predominantly anti-angiogenic.[7, 19, 21] These observations indicate that dopaminergic modulation could serve as a powerful adjunct to standard endocrine therapy by providing the robust anti-angiogenic coverage that tamoxifen appears to lack in this experimental setting.

Table 2: Effect of different treatment regimens on serum VEGF, hepatic MDA, and hepatic GSH levels in rats bearing DMBA-induced mammary carcinoma.

Treatment group (Dose; Route)	VEGF (pg/mL)	MDA (nmol/100 mg)	GSH (mg/g)
Plain control	53.80 ± 19.32	7.53 ± 0.95	0.50 ± 0.06
Oral control (Ia) (1 mL 0.9% saline; p.o.)	145.64 ± 26.78[a]	11.48 ± 1.01[a]	0.12 ± 0.03[a]
i.p. control (Ib) (1 mL 0.9% saline; i.p.)	146.84 ± 14.07[a]	11.75 ± 0.92[a]	0.12 ± 0.03[a]
Tamoxifen (II)	128.14 ± 24.73[ab]	9.22 ± 0.76[abc]	0.24 ± 0.06[abc]

(0.6 mg/kg; p.o.)			
Propranolol (III) (3 mg/kg; i.p.)	109.93 ± 20.18^{abc}	9.18 ± 0.79^{abc}	0.28 ± 0.06^{abc}
Dopamine (IV) (50 mg/kg; i.p.)	63.32 ± 19.04^{bc}	11.21 ± 1.61^{a}	0.22 ± 0.06^{abc}
Overall p-value (among groups)	< 0.001		

Data are expressed as mean ± SD (n = 10 per group). Overall comparisons for VEGF and MDA were performed using one-way ANOVA followed by Scheffé's post hoc test. For GSH, overall comparisons were conducted using the Kruskal–Wallis test, followed by Mann–Whitney U tests for pairwise comparisons. Superscripts denote statistically significant differences at $p \leq 0.05$: ^{a} vs. plain control; ^{b} vs. oral control (Ia); ^{c} vs. i.p. control (Ib).

3.6. Oxidative stress indices (Hepatic MDA and GSH)

Mammary carcinogenesis is associated with systemic redox imbalance, as evidenced by the significant hepatic lipid peroxidation (high MDA) and antioxidant depletion (low GSH) observed in untreated tumour‑bearing rats compared with plain controls (Table 2). Tamoxifen and propranolol effectively restored this balance, significantly improving both indices relative to their respective route‑matched controls ($p \leq 0.05$; Table 2). This systemic protective effect is consistent with the known antioxidant and free radical‑scavenging properties of tamoxifen in DMBA‑induced models[6], and with reports that β‑blockers can modulate oxidative injury and stabilise cellular redox status.[24] Such antioxidant capacity may complement antitumour activity by limiting further genomic instability and pro‑tumour inflammatory signaling.[6]

In contrast, dopamine's antioxidant effect was only partial; while it significantly improved hepatic GSH ($p \leq 0.05$), it did not produce a significant reduction in MDA (Table 2). Consequently, MDA levels remained significantly higher in the dopamine group than in the tamoxifen and propranolol groups. This indicates that while dopamine is a potent local antitumour and anti‑angiogenic agent, its systemic antioxidant capacity may be more limited compared to propranolol or tamoxifen in this experimental setting.

3.7 Study limitations

Several limitations of the present study should be acknowledged. First, the work was performed in a single DMBA-induced mammary carcinoma model with a relatively modest sample size (n = 10 per group), which may limit the generalisability of the findings;

confirmation in additional settings, such as transgenic or orthotopic xenograft models, would therefore be valuable. Second, although Ki-67 expression was assessed by an experienced pathologist who was blinded to group allocation, reliance on semi-quantitative grading inherently introduces a degree of subjectivity when compared with fully automated digital image analysis.

Third, only single fixed doses of each agent were employed, and formal dose–response evaluation was not undertaken; future studies are needed to define the optimal therapeutic window for both dopamine and propranolol in this context. In addition, oxidative stress was evaluated in hepatic tissue rather than directly within the tumour microenvironment, which may not fully reflect local redox dynamics. Finally, angiogenic activity was inferred from circulating VEGF levels, without direct assessment of intratumoural microvessel density or downstream signalling events (such as VEGFR2 phosphorylation or D1/D2 receptor involvement). Incorporation of these mechanistic and translational endpoints in future studies would help to more precisely delineate the clinical potential of these neurohumoral modulators.

4. CONCLUSION

In conclusion, the present study provides compelling preclinical evidence that pharmacological modulation of neurohumoral pathways—specifically through β-adrenergic blockade and dopaminergic stimulation—exerts significant antitumour activity in a DMBA-induced mammary carcinoma model. These interventions effectively suppressed tumour growth, inhibited cellular proliferation, and enhanced apoptotic engagement, with dopamine demonstrating a particularly potent anti-angiogenic profile. These findings highlight the potential of β-adrenergic and dopaminergic signalling as promising non-conventional therapeutic targets in breast cancer management. Such results justify further mechanistic exploration and support the initiation of early-phase clinical trials to evaluate the efficacy of these agents as adjuncts to standard endocrine therapies, potentially offering a multimodal approach to improving clinical outcomes.

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