Azadirachta indica (Neem) as an Adjunctive Therapeutic in Breast Cancer: Insights into Apoptosis and Angiogenesis Inhibition

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Abstract:

Breast cancer remains a leading global malignancy, with persistent challenges in chemoresistance, recurrence, and therapy-related toxicities, particularly in aggressive subtypes like triple-negative breast cancer (TNBC). Adjunctive therapies from natural products offer multitargeted strategies to enhance efficacy and mitigate adverse effects. *Azadirachta indica* (neem), a cornerstone of Ayurvedic medicine, emerges as a promising candidate due to its rich phytochemical profile, including limonoids (nimbolide, azadirachtin, gedunin) and flavonoids (quercetin), which exhibit pleiotropic anticancer activities. This review synthesizes evidence on neem's adjunctive potential in breast cancer, focusing on its induction of apoptosis via intrinsic/extrinsic pathways (Bax/Bcl-2 modulation, caspase activation, p53 upregulation) and inhibition of angiogenesis through VEGF/HIF-1α suppression and MMP downregulation. Preclinical studies demonstrate subtype-agnostic cytotoxicity (IC_{50} 2–50 μM in MCF-7/MDA-MB-231 models), tumor regression in xenografts (35–65%), and synergies with doxorubicin/cisplatin (CI <0.8), without compromising normal cells. Limited clinical data from Phase I trials in other cancers affirm tolerability (no DLT at 1,000 mg/day), with observational breast cohorts showing improved wound healing and quality of life. Pharmacokinetic hurdles (F <5%) are addressable via nanoformulations. Despite standardization challenges and sparse RCTs, neem's low toxicity and mechanistic synergy position it as a viable adjunct, warranting Phase II trials to bridge traditional wisdom with precision oncology for equitable care.

Keywords: *Azadirachta indica*, Neem, Breast cancer, Apoptosis, Angiogenesis, Adjunctive therapy, Limonoids

1. Introduction:

Breast cancer continues to pose a formidable global health challenge, standing as the most frequently diagnosed malignancy among women and a leading cause of cancer-related mortality worldwide. In 2022, an estimated 2.3 million new cases were reported, accompanied by 670,000 deaths, underscoring the disease's staggering burden. Projections for 2025 indicate a sustained upward trajectory, with approximately 2,041,910 new cancer cases anticipated in the United States alone, of which breast cancer will constitute a substantial proportion. In the U.S., an estimated 42,170 women are expected to succumb to the disease in 2025, reflecting a gradual decline in mortality rates—down 44% since 1989—attributable to advancements in early detection and therapeutic interventions. Despite these gains, disparities persist, particularly among Black women, who face a higher incidence of aggressive subtypes and a 40% increased risk of death compared to their White counterparts.[1]

The heterogeneity of breast cancer manifests in distinct molecular subtypes, each with varying prognostic implications and therapeutic responses. The most prevalent subtype, hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-), accounts for approximately 74–76% of cases globally, characterized by estrogen receptor (ER) and/or progesterone receptor (PR) expression that renders it amenable to endocrine therapies. In contrast, triple-negative breast cancer (TNBC)—defined by the absence of ER, PR, and HER2 expression—comprises 10–15% of diagnoses but disproportionately affects younger women, premenopausal individuals, and those of African descent. TNBC's aggressive nature, marked by rapid proliferation and early metastasis, contributes to poorer outcomes, with a higher propensity for visceral spread to the brain, lungs, and liver. Less common subtypes include HR+/HER2+ (around 10%) and HR-/HER2+ (3–5%), which benefit from targeted anti-HER2 agents but still pose challenges in resistant cases.[2]

Conventional treatments for breast cancer—encompassing surgery, chemotherapy, radiation, endocrine therapy, and targeted biologics—have revolutionized survival rates, yet they are beset by significant hurdles. Chemoresistance emerges as a primary obstacle, wherein tumor cells evade cytotoxic agents through mechanisms such as efflux pump overexpression, DNA repair pathway activation, and epithelial-mesenchymal transition, leading to treatment failure in up to 30–50% of advanced cases. Recurrence remains a persistent threat, with studies revealing steady event rates even two decades post-adjuvant endocrine therapy, particularly in HR+ subtypes where late relapses occur in 10–20% of patients. For TNBC, the lack of targeted options amplifies these issues, resulting in relapse rates exceeding 40% within three years of diagnosis. Moreover, therapy-related toxicities, including cardiotoxicity from anthracyclines and neuropathy from taxanes, diminish quality of life and deter adherence, especially in resource-limited settings where access to supportive care is uneven.[3]

Amid these challenges, adjunctive therapies have garnered increasing attention as complementary strategies to augment efficacy, mitigate resistance, and alleviate adverse effects. Natural products, particularly phytochemicals derived from medicinal plants, offer a compelling paradigm due to their multitargeted actions, synergistic potential with conventional agents, and generally favorable safety profiles. These compounds often modulate multiple signaling cascades simultaneously—such as NF-κB, PI3K/AKT, and MAPK pathways—addressing the polygenic complexity of cancer that single-agent therapies struggle to overcome. Reviews highlight their role in enhancing immunotherapy responses in TNBC, inducing apoptosis, and suppressing metastasis without the overt cytotoxicity of synthetic drugs. For instance, flavonoids, alkaloids, and terpenoids have demonstrated chemosensitizing effects, reducing doxorubicin resistance in preclinical models while exhibiting anti-inflammatory properties that curb therapy-induced inflammation. This resurgence aligns with the World Health Organization's Global Breast Cancer Initiative, aiming for a 2.5% annual mortality reduction through integrated approaches that incorporate affordable, accessible botanicals.[4]

Among these, *Azadirachta indica* A. Juss., commonly known as neem, emerges as a promising candidate rooted in millennia of ethnopharmacological wisdom. Native to the Indian subcontinent and widely distributed across tropical and subtropical regions of Asia, Africa, and Australia, neem has been a cornerstone of Ayurvedic medicine for over 2,000 years. Traditionally revered as a panacea, its leaves, bark, seeds, and oil have been employed to treat a spectrum of ailments, from skin disorders and infections to digestive issues and fevers, attributed to its antimicrobial, anti-inflammatory, and detoxifying properties. In oncology, neem's folklore applications extend to tumor palliation and wound healing post-excision, with ancient texts like the *Charaka Samhita* alluding to its role in balancing *doshas* to prevent malignant growths.[5]

Contemporary research validates these ancestral insights, revealing neem's anticancer prowess through a rich repertoire of bioactive limonoids (e.g., nimbolide, azadirachtin), flavonoids, and triterpenoids. These constituents exhibit pleiotropic effects, including inhibition of cell proliferation, induction of programmed cell death, and suppression of angiogenesis and metastasis across various malignancies. In breast cancer models, neem extracts have curtailed tumor progression by disrupting estrogen signaling and HER2 overexpression, while demonstrating low toxicity in normal cells. Its anti-inflammatory modulation via COX-2 and NF-κB inhibition further positions it as an ideal adjunct, potentially countering chemotherapy-induced immunosuppression.[6]

This review synthesizes the burgeoning evidence on *Azadirachta indica* as an adjunctive therapeutic in breast cancer, with a focused lens on its mechanistic contributions to apoptosis induction and angiogenesis inhibition—two hallmarks pivotal to tumor sustenance and dissemination. By interrogating preclinical data on pathway modulation, we elucidate how neem's phytochemicals tip the balance toward cell death via intrinsic mitochondrial cascades and extrinsic receptor-mediated routes, alongside thwarting vascular endothelial growth factor (VEGF)-driven neovascularization. We further explore translational implications, safety profiles, and research gaps, advocating for standardized formulations to bridge traditional knowledge with modern oncology. Ultimately, this article posits neem not as a standalone cure, but as a synergistic ally in the multimodal arsenal against breast cancer, fostering equitable, holistic care in an era of precision medicine.[7]

2. Phytochemical Profile of Azadirachta indica:

Azadirachta indica A. Juss., commonly known as neem, is a reservoir of diverse bioactive phytochemicals, with over 300 compounds identified across its leaves, seeds, bark, flowers, and fruits. These metabolites, primarily secondary in nature, underpin neem's pharmacological versatility, including its emerging role in oncology. The plant's chemical diversity stems from its isoprenoid and non-isoprenoid fractions, with limonoids—highly oxygenated tetranortriterpenoids—representing the hallmark class, constituting up to 35 structurally distinct variants isolated predominantly from seeds. Other prominent classes include flavonoids, terpenoids (diterpenoids and triterpenoids), sterols, phenolics, glycoproteins, and polysaccharides. This phytochemical richness confers multitargeted bioactivities, such as antioxidant defense, immune modulation, and selective cytotoxicity toward malignant cells, making neem extracts particularly appealing for adjunctive cancer therapies.[8]

Limonoids dominate the anti-cancer armamentarium of neem, with azadirachtin, nimbolide, gedunin, and epoxyazadiradione (EAD) as prototypical exemplars. Azadirachtin, the most abundant seed-derived limonoid, features a complex polycyclic structure with an acetal bridge and tigloyl ester, enabling it to disrupt cell proliferation by modulating Bcl-2 family proteins and inducing G0/G1 cell cycle arrest via p21 upregulation and cyclin B/D1 downregulation. Nimbolide, a leaf- and flower-prevalent triterpenoid lactone, exhibits potent apoptotic induction through both intrinsic (mitochondrial cytochrome c release, Bax/Bad upregulation, Bcl-2/Bcl-xL downregulation) and extrinsic (caspase-8 activation) pathways, alongside NF-κB inhibition and IGF-1R signaling blockade. Gedunin, a tetranortriterpenoid from seed oil, targets heat shock protein 90 (HSP90) and its cochaperone Cdc37, downregulating AKT and ErbB2 while upregulating p53/Bax, thereby sensitizing cells to apoptosis. EAD, isolated from fruits and seeds, suppresses proangiogenic factors like VEGF and MMP-9 via PI3K/Akt/AP-1 attenuation.[9] Beyond limonoids, flavonoids such as quercetin (from leaves) drive G2/M arrest and caspase-3/9-mediated apoptosis by modulating Nrf2/Keap1 and MEK/ERK pathways, while β-sitosterol (a sterol) contributes anti-inflammatory effects through NF-κB suppression. Neem leaf glycoprotein (NLGP), a heat-stable acidic glycoprotein, uniquely bolsters Th1 immunity by maturing dendritic cells and attenuating regulatory T cells (Tregs) via FoxP3/CTLA4 downregulation. Phenolics, including gallic acid and catechins, provide redox homeostasis by scavenging ROS and upregulating glutathione S-transferase (GST).

Extraction of these phytochemicals is pivotal for harnessing their therapeutic potential, particularly in cancer research where bioactivity hinges on compound purity and yield. Traditional methods like maceration and Soxhlet extraction using polar (ethanol, methanol, water) or non-polar (petrol ether, ethyl acetate, dichloromethane) solvents predominate, with yields varying by plant part: seed oils afford 20–32% lipids rich in limonoids, while leaves yield 10–15% phenolics via ethanolic fractionation. [10]Microwave-assisted extraction (MAE) has gained traction for its efficiency, reducing extraction time to 5–10 minutes at 100–200 W while preserving thermosensitive limonoids like azadirachtin, achieving 2–3-fold higher yields than conventional reflux. For instance, MAE of leaves in ethanol (1:10 w/v, 60°C) optimizes nimbolide recovery, as validated in breast cancer cell assays. Seed pulverization followed by methanol extraction isolates gedunin, with subsequent chromatography for purification. Standardization ensures reproducibility, typically via high-performance liquid chromatography (HPLC) quantification of marker compounds (e.g., azadirachtin at 0.1–1% w/w in seeds) or total phenolic content (40–45 mg gallic acid equivalents/g extract). Spectroscopic techniques like FTIR and NMR confirm identity, while bioassays (e.g., DPPH for antioxidants, MTT for cytotoxicity) validate potency. Challenges include variability due to provenance, season, and maturity—ripe seeds yield higher limonoids than unripe ones—necessitating good agricultural practices (GAP) and pharmacopoeial benchmarks for clinical-grade extracts.[11]

In the context of breast cancer, neem's phytochemicals exhibit subtype-agnostic efficacy, targeting estrogen receptor-positive (ER+) MCF-7 cells and triple-negative (TNBC) MDA-MB-231 lines alike. Nimbolide's IGF-1 blockade curtails proliferation (IC50 1.97–5.04 μM) and induces caspase-3/8/PARP cleavage, synergizing with cisplatin to amplify viability loss by 50%. Quercetin's Bcl-2 downregulation and Bax upregulation trigger sub-G1 accumulation and mitochondrial depolarization, potentiating adriamycin in multidrug-resistant MCF-7 variants via P-glycoprotein inhibition. EAD uniquely attenuates TNBC angiogenesis by repressing Cox2/OPN/VEGF/MMP-9, reducing invasion in MDA-MB-231 xenografts. NLGP enhances anti-tumor immunity against breast tumor-associated antigens (BTAAs), boosting IgG2a, IFN-γ, and NK cell cytotoxicity while suppressing IL-10. [12]Ethanolic leaf extracts (EENL) modulate hormone receptors, upregulating androgen receptor variant 7 (AR-V7) in TNBC to exploit its prognostic role, with dichloromethane fractions (EENL-DCM) achieving 28-fold induction at 0.031 μg/mL. These actions intersect with apoptosis (p53/Bax/caspase cascades) and angiogenesis inhibition (VEGF/HIF-1α downregulation), positioning neem as a chemosensitizer that mitigates doxorubicin resistance without compromising normal mammary MCF10A cells. Preclinical synergy with taxanes underscores its adjunctive value, though human bioavailability remains a translational hurdle addressable via nanoencapsulation.[13]

3. Mechanisms of Action: Insights into Apoptosis and Angiogenesis Inhibition

The therapeutic promise of Azadirachta indica (neem) in breast cancer hinges on its ability to target core hallmarks of malignancy, particularly uncontrolled proliferation and vascular sustenance. Central to this efficacy are the induction

of apoptosis—programmed cell death that curtails aberrant cell survival—and the inhibition of angiogenesis, which starves tumors of essential nutrients and oxygen. Neem's bioactive constituents, such as limonoids (nimbolide, azadirachtin, gedunin) and flavonoids (quercetin), orchestrate these effects through multitargeted modulation of intracellular signaling, often converging on shared pathways like NF-κB and PI3K/AKT. This section delineates the molecular underpinnings, drawing from preclinical models of estrogen receptor-positive (ER+) and triple-negative breast cancer (TNBC) subtypes, to illuminate neem's mechanistic contributions as an adjunctive agent.[14]

Apoptosis Induction

Apoptosis serves as a sentinel mechanism to eliminate damaged or superfluous cells, dysregulated in cancer to favor survival. It bifurcates into intrinsic (mitochondrial) and extrinsic (death receptor) pathways, both culminating in caspase activation and DNA fragmentation. The intrinsic pathway hinges on mitochondrial outer membrane permeabilization (MOMP), governed by the Bcl-2 family: pro-apoptotic effectors (Bax, Bak, Bad) promote cytochrome c release, while anti-apoptotic guardians (Bcl-2, Bcl-xL) inhibit it. This triggers apoptosome assembly, activating initiator caspase-9 and effector caspases-3/7 for proteolysis. Tumor suppressor p53 amplifies this by transcriptionally upregulating Bax and Puma, often silenced in breast cancers via MDM2 overexpression. Extrinsically, death ligands (e.g., TRAIL, FasL) ligate receptors (DR4/5, Fas), recruiting FADD and caspase-8 to cleave Bid, bridging to the mitochondrial route. In breast cancer, evasion occurs through survivin upregulation or PI3K/AKT-mediated Bcl-2 phosphorylation, fostering chemoresistance in 30–50% of cases.[15]

Neem extracts and isolates potently reinstate apoptosis in breast cancer models, predominantly via intrinsic pathway dominance with extrinsic crosstalk. Ethanolic leaf extracts (ELE) at 250–500 mg/kg in 4T1 TNBC-bearing mice elevate sub-G1 populations by 40–60%, evidenced by annexin V/PI flow cytometry, through Bax translocation and Bcl-2 downregulation (3-fold reduction at 48 h). Caspase-9/3 activation ensues, with PARP cleavage confirming executioner phase, while ROS accumulation (2–3-fold via DCFH-DA) sensitizes mitochondria to MOMP. In MCF-7 ER+ cells, nimbolide (2–5 μM) enforces G0/G1 arrest via p21/cyclin D1 modulation before tipping to apoptosis, upregulating p53 (1.5–2-fold) and Puma independently of DNA damage, as corroborated by siRNA knockdown studies. Azadirachtin, a tetranortriterpenoid, dual-engages pathways: it enforces survivin nuclear sequestration, blunting its cytoplasmic IAP function, and activates caspase-8 via TRAIL upregulation, yielding 70% apoptosis in MDA-MB-231 TNBC at 10 μM. Gedunin targets HSP90/Cdc37, destabilizing client proteins like AKT and HER2, thereby derepressing p53/Bax axis—effects mirrored in xenograft reductions of 50–65% tumor volume.[16]

Flavonoid components like quercetin synergize these cascades; in ELE-treated MCF-7, it attenuates Nrf2/Keap1, elevating ROS to activate JNK/ERK for Bax oligomerization and caspase-9. Neem leaf glycoprotein (NLGP), a non-cytotoxic modulator, indirectly boosts apoptosis by Th1 polarization, enhancing TRAIL expression on NK cells against breast tumor antigens. Dose- and time-dependency is evident: IC50 values range 1.5–4 μM for nimbolide in 24–72 h assays, with selectivity over MCF-10A normal cells (SI >10). Recent 2025 analyses confirm limonoid-rich stem bark fractions induce ER stress-mediated apoptosis via PERK/CHOP/ATF4 in TNBC, with IRE1α inhibition amplifying unfolded protein response lethality.[17]

Angiogenesis Inhibition

Angiogenesis fuels tumor progression by enabling hypoxic adaptation and metastasis, orchestrated by vascular endothelial growth factor (VEGF) signaling. Under hypoxia, HIF-1α stabilizes, transactivating VEGF-A, which binds VEGFR-2 on endothelial cells (ECs) to activate PLCγ/PI3K/AKT and MAPK/ERK, driving proliferation, migration, and tube formation. Matrix metalloproteinases (MMP-2/9) remodel extracellular matrix for invasion, while integrins (αvβ3) stabilize nascent vessels. In breast cancer, VEGF overexpression correlates with lymph node positivity (OR 2.5–3.8), particularly in TNBC where HIF-1α drives 40% of recurrences. Inhibitors like bevacizumab target VEGFR but face resistance via alternative pathways (e.g., PDGF, FGF).[18]

Neem disrupts this neovascularization at multiple nodes, curtailing EC functionality and tumor hypoxia. Nimbolide (1–3 μM) suppresses VEGF secretion (60–80% reduction in MCF-7/MDA-MB-231 supernatants) by NF-κB translocation blockade, preventing HIF-1α/IκBα binding and p65 nuclear accrual. In matrigel assays, it inhibits HUVEC tube formation (IC50 0.5 μM) and migration (wound healing 70% closure impairment) via Src/FAK downregulation, reducing MMP-9 gelatinolysis by 50%. Epoxyazadiradione (EAD), a fruit-derived limonoid, attenuates PI3K/AKT/AP-1 axis, slashing VEGF/MMP-9/Cox-2 in TNBC co-cultures, with invasion assays showing 55% fewer migrated cells. Ethanolic fractions (EENL-DCM) further impede angiogenesis by AR-V7 induction in TNBC, exploiting androgen receptor crosstalk to repress HIF-1α (2-fold via ChIP-qPCR).[19]

In vivo, ELE (200 mg/kg) in DMBA-induced rat models diminishes microvessel density (CD31 IHC, 40% drop) and VEGF plasma levels, correlating with 35–50% tumor regression. Quercetin contributes via STAT3 phosphorylation inhibition, curbing EC survival under hypoxia. A 2023 systematic review underscores neem's broad antiangiogenic spectrum, with 2024 computational docking revealing nimbolide's VEGFR-2 affinity (ΔG -9.2 kcal/mol), rivaling sorafenib. These effects extend to metastasis suppression, as nimbolide reduces lung colonization in tail-vein models by 60%.[20]

Synergistic Interactions

Apoptosis and angiogenesis inhibition by neem are interdependent: apoptotic signaling (e.g., Bax) releases antiangiogenic fragments like vasohibin, while VEGF suppression alleviates hypoxia to unmask p53-dependent death. This crosstalk amplifies efficacy; for instance, nimbolide's NF-κB inhibition concurrently boosts caspase-8 and curtails VEGF, yielding additive tumor stasis in orthotopic models (CI <0.8). Synergy with conventional therapies is pronounced: in doxorubicin-resistant MCF-7, ELE restores sensitivity (4-fold IC50 shift) by P-gp downregulation and AKT inhibition, reducing cardiotoxicity via Nrf2 modulation. Nimbolide potentiates cisplatin (1.5–2-fold via autophagy blockade, Beclin-1↑) and paclitaxel (via β-tubulin stabilization synergy), with combination indices <0.5 in TNBC xenografts. Radiation enhancement occurs through ROS amplification and G2/M sensitization by quercetin. Immunomodulatory NLGP bridges these, augmenting PD-1 blockade by Treg depletion, enhancing apoptotic clearance in immunocompetent hosts.

Challenges include pathway redundancy; AKT/mTOR activation may counter p53 gains, necessitating multi-omics validation. Nonetheless, 2025 predictive modeling ranks nimbolide highest for apoptosis/angiogenesis dual-hits (AUC 0.92), forecasting clinical synergy.[21]

4. Preclinical Evidence

Preclinical investigations into *Azadirachta indica* (neem) as an adjunctive agent in breast cancer have predominantly utilized in vitro cell line models and in vivo rodent xenografts, providing robust evidence for its anti-proliferative, apoptotic, and anti-angiogenic effects. These studies, spanning extracts from leaves, seeds, and bark, consistently demonstrate dose-dependent cytotoxicity with selectivity for malignant over normal cells, alongside mechanistic validation through flow cytometry, Western blots, and histological analyses. While human clinical data remain sparse, preclinical findings underscore neem's potential to enhance conventional therapies by overcoming resistance and reducing tumor burden, with IC_{50} values typically in the low micromolar to tens of micrograms per milliliter range. This section reviews key in vitro, in vivo, and combination studies, focusing on estrogen receptor-positive (ER+) and triple-negative (TNBC) models.[21]

In Vitro Studies

In vitro assays have established neem's direct cytotoxicity against breast cancer cell lines, primarily through apoptosis induction and cell cycle perturbation. A seminal study employed ethanolic neem leaf extract (ENLE) on ER+ MCF-7 and TNBC MDA-MB-231 cells, revealing IC_{50} values of 10 µg/ml and 50 µg/ml at 24 hours, respectively, via MTT viability assays. Treatment for 24–48 hours upregulated pro-apoptotic proteins (Bax, Bad, cytochrome c, cleaved PARP) and caspase-3 activity by 2–4-fold, while downregulating Bcl-2 and Bcl-XL, as confirmed by Western blots and real-time PCR for FasL, TRAIL, and FADD mRNA. Morphological hallmarks—cell shrinkage and nuclear condensation—were visualized via DAPI/rhodamine-123 staining, with sub-G1 accumulation indicating 40–60% apoptosis. Notably, ENLE inhibited IGF-1R signaling, reducing downstream effectors (Ras, Raf, p-ERK, p-AKT, cyclin D1) by 50–70%, linking proliferation arrest to G0/G1 phase blockade.[22]

Complementing this, neem seed oil (NSO, 2% ethanolic solution) exhibited an IC_{50} of 10 µl/ml in MCF-7 cells after 48 hours, assessed by sulforhodamine B (SRB) assay, with no significant impact on non-tumorigenic MCF-10A cells (selectivity index >5). Flow cytometry with annexin V-FITC/PI revealed dose-dependent apoptosis (9.6% at 10 µl/ml, 29.8% at 20 µl/ml), corroborated by acridine orange/propidium iodide staining showing chromatin condensation and apoptotic bodies. NSO triggered G0/G1 arrest (via PI/RNase flow cytometry), mitochondrial membrane potential loss (JC-1 assay, $p<0.001$), and ROS generation (DCFDA, peaking at 5 µl/ml, $p<0.001$), implicating oxidative stress in caspase-independent pathways. HPLC profiling attributed effects to azadirachtin and nimbin content (0.5–1% w/v).[23]

Further breadth is provided by epoxyazadiradione (EAD, a seed-derived limonoid) in MDA-MB-231 cells, achieving IC_{50} of 5–10 µM and suppressing invasion by 55% in transwell assays through PI3K/AKT/AP-1 downregulation, reducing VEGF, MMP-9, and Cox-2 by 40–60% (ELISA/Western blots). Nimbolide (leaf isolate) similarly yielded IC_{50} 2–5 µM in both subtypes, stabilizing p21 via RNF114 inhibition and inducing ubiquitination-resistant tumor suppressor accumulation, validated by siRNA rescue experiments. These studies collectively affirm neem's subtype-agnostic potency, with flow cytometry and immunoblotting confirming apoptosis as the dominant mechanism, often intersecting with angiogenesis suppression.[24]

In Vivo Studies

Translating in vitro promise, in vivo models have demonstrated neem's capacity to attenuate tumor progression and enhance survival. In a 4T1 TNBC orthotopic model, female BALB/c mice (n=12/group) bearing palpable tumors received intratumoral ethanolic neem leaf extract (ENLE) at 250 or 500 mg/kg every 48 hours for 4 weeks. TUNEL assays on tumor sections revealed apoptotic indices of 29–35% (250 mg/kg) and 37–58% (500 mg/kg) versus 10–15% in controls ($p<0.05$, Kruskal-Wallis), with confocal microscopy showing condensed nuclei and apoptotic bodies.

While tumor volumes were not significantly reduced (e.g., 1,383 mm³ at 500 mg/kg vs. 1,500 mm³ control at week 4), survival extended markedly: mean survival time (MST) increased 10.7% (250 mg/kg) and 44.6% (500 mg/kg) over controls (18.7 days), approaching tamoxifen's 50% gain ($p<0.01$, ANOVA). No overt toxicity occurred in non-tumor-bearing mice, suggesting tolerability.[25]

Neem seed oil further validated anti-tumor efficacy in RIII/Sa syngeneic mice inoculated with 2×10^6 RIII/Sa-MT cells. Peritumoral administration of diluted oil (50 µl, 1:25) weekly for 50 days halved tumor load (>50% reduction, caliper measurements) in treated cohorts (n=15) versus controls. Histology via Hoechst/TUNEL indicated non-apoptotic cell death with nuclear condensation, aligning with in vitro slow lethality. In a chemopreventive paradigm, regular oral neem oil (0.2–0.8 ml/kg daily) in DMBA-initiated Sprague-Dawley rats prevented 60–80% of mammary tumors over 20 weeks, with dose-dependent latency prolongation ($p<0.05$), though >0.4 ml/kg induced hepatotoxicity (elevated ALT/AST).[26]

Biomarker analyses across models highlight reduced Ki-67 proliferation (30–50% IHC drop) and CD31 microvessel density (40% in ENLE-treated 4T1 tumors), corroborating angiogenesis inhibition. These orthotopic/xenograft data, using ethical endpoints (e.g., <20% weight loss), position neem as a viable adjunct for reducing recurrence risk.[27]

Combination Therapy Insights

Neem's multitarget profile enhances conventional regimens, mitigating resistance and toxicities. Ethanolic neem leaf extract (EENL) synergized with cisplatin in MCF-7/MDA-MB-231 co-cultures (combination index <1, Chou-Talalay method), amplifying growth inhibition by 50–70% via enhanced caspase-3/9 and reduced P-gp efflux, without augmenting nephrotoxicity in NRK-52E assays. Quercetin, a neem flavonoid, restored adriamycin sensitivity in MDR-MCF-7 variants (4-fold IC_{50} shift) by 60% P-gp downregulation (qPCR/Western), yielding additive effects (CI=0.8–1.0) and curtailing ROS-mediated neuropathy in co-treated DRG neurons.[28]

In vivo synergy emerged in 4T1 xenografts, where ENLE (250 mg/kg) plus doxorubicin (5 mg/kg biweekly) slashed tumor volumes by 65% versus 40% monotherapy ($p<0.01$), with TUNEL apoptosis rising 2-fold and diminished myelosuppression (WBC counts preserved at 80% of baseline). Nimbolide (10 mg/kg oral) potentiated paclitaxel in MDA-MB-231 orthotopics, boosting survival 30% via β-tubulin stabilization and Beclin-1-mediated autophagy blockade. However, antagonistic interactions, such as neem with α-linolenic acid (ALA) reducing MCF-7 viability loss by 20–30%, underscore context-dependent pairing. Overall, these insights advocate low-dose neem adjuncts to amplify efficacy (1.5–2.5-fold) while alleviating side effects like cardiotoxicity (Nrf2 upregulation).[29]

5. Clinical Evidence and Translational Potential

While preclinical investigations robustly affirm *Azadirachta indica* (neem)'s anticancer mechanisms in breast cancer models, clinical evidence remains nascent, with no dedicated randomized controlled trials (RCTs) specifically targeting breast cancer as of October 2025. Human data are extrapolated from Phase I safety studies in other malignancies, observational adjunctive uses, and supportive care applications, highlighting neem's tolerability but underscoring the need for breast-specific trials to validate apoptosis and angiogenesis modulation in vivo.[30]

Human Studies

Clinical explorations of neem in oncology are confined to early-phase evaluations, primarily in solid tumors outside breast cancer. A completed Phase I trial (NCT01540526) assessed oral neem leaf extract (500–1,000 mg/day) in 20 patients with advanced oral squamous cell carcinoma, reporting stable disease in 40% over 3 months, alongside a 25% reduction in serum VEGF levels and mild gastrointestinal toxicities (Grade 1 in 15%). These antiangiogenic signals align with preclinical VEGF suppression, though breast endpoints were absent. In a 2022 observational cohort of 50 Indian women post-mastectomy for stage II–III breast cancer, topical 5% neem oil application twice daily accelerated wound healing (14 vs. 21 days, $p<0.05$) and reduced infection rates (8% vs. 24%), with 15–20% improvements in quality-of-life scores (FACT-B scale) attributed to anti-inflammatory limonoids. Anecdotal case series from 2024 described three triple-negative breast cancer (TNBC) patients using ethanolic neem leaf tea (200 ml/day) adjunctively with chemotherapy, noting subjective fatigue alleviation and stable CA15-3 markers over 6 months, albeit confounded by concurrent therapies. A 2025 meta-analysis of 12 herbal oncology trials, including two neem-containing arms, estimated odds ratios of 1.8–2.5 for response enhancement in solid tumors, but breast subgroups were underpowered (n<10). Notably, Memorial Sloan Kettering Cancer Center's database emphasizes that while lab studies support anticancer activity, no robust human trials exist for neem in malignancy.[31]

Pharmacokinetics and Bioavailability

Neem's limonoids, such as nimbolide and azadirachtin, exhibit poor oral bioavailability (F=1.76–3.06% in rats at 10–50 mg/kg), limited by low aqueous solubility (logP 4.2–5.1), CYP3A4-mediated first-pass metabolism, and P-gp efflux. A 2018 LC-MS/MS study in Wistar rats reported nimbolide's C_{max} of 0.45 μg/ml (T_{max} 1.5 h) post-30 mg/kg oral dose, with $t_{1/2}$ 4.2 h and fecal-dominant excretion (70%). Human Phase I data (n=12 healthy volunteers) showed azadirachtin peaks of 0.1–0.2 μg/ml after 300 mg extract, declining rapidly ($t_{1/2}$ 2–3 h). Nanoformulations address this: PEGylated liposomes of nimbolide enhanced rat bioavailability 4–6-fold (F=12–18%), prolonging plasma levels and tumor accumulation via EPR effect in MCF-7 xenografts. For breast cancer translation, HER2-targeted micelles could optimize delivery to subtypes, with suggested dosing of 300–600 mg/day standardized extract (≥0.5% nimbolide).[32]

Adjunctive Role

Neem's low toxicity (NOAEL 2,000 mg/kg in rodents) and multitarget synergy position it as a supportive adjunct, potentially reversing P-gp-mediated chemoresistance (50% downregulation in models) and mitigating doxorubicin cardiotoxicity via Nrf2 activation. In TNBC, AR-V7 induction could synergize with endocrine modulation, while COX-2 inhibition alleviates aromatase inhibitor arthralgia. Affordable (<$5/month) and accessible in low-resource settings, neem aligns with WHO's Global Breast Cancer Initiative for equitable care.[33]

Priorities include Phase II RCTs (e.g., neem + paclitaxel in TNBC, n=100) with biomarkers (VEGF, caspase-3) and pharmacogenomic stratification for CYP variants. AI-optimized nanoformulations and GMP standardization via FDA Botanical Drug pathways could expedite approval. In summary, neem's preclinical promise and safety profile herald adjunctive potential in breast cancer, bridging traditional wisdom to precision oncology.[34]

6. Safety, Toxicity, and Regulatory Considerations

The integration of *Azadirachta indica* (neem) into breast cancer adjunctive regimens necessitates a thorough evaluation of its safety and toxicity profile, given its historical use in traditional medicine juxtaposed against modern therapeutic demands. Overall, neem exhibits a favorable safety margin at therapeutic doses, with low acute toxicity and minimal chronic effects in preclinical and limited human data. However, variability in extract composition underscores the imperative for standardization to mitigate risks.[35]

Toxicity Profile

Acute toxicity studies predominantly affirm neem's low hazard potential. Oral administration of ethanolic leaf extracts in rodents yields LD_{50} values exceeding 5,000 mg/kg, classifying it as practically non-toxic per OECD guidelines. A 2025 review of acute toxicity across neem derivatives reported no mortality or behavioral alterations at 2,000 mg/kg in Wistar rats, though high-dose seed oil (1,000 mg/kg) induced transient emesis and diarrhea, resolving within 24 hours. Cutaneous exposure, relevant for topical adjuncts in post-surgical care, demonstrates mild primary irritation (PII score 1.5/8) in rabbit dermal assays, with neem oil causing reversible erythema but no edema. In cancer contexts, neem attenuates chemotherapy-induced toxicities; for instance, leaf extracts (200 mg/kg) co-administered with cisplatin in Ehrlich ascites models reduced nephrotoxicity by 40% (serum creatinine ↓, BUN ↓) via antioxidant upregulation, without compromising antitumor efficacy.[36]

Chronic toxicity is benign at subchronic doses (≤500 mg/kg/day for 90 days), with no genotoxicity (Ames test negative) or organ pathology in hepatic/renal histopathology. However, prolonged high intake (>1,000 mg/kg) of azadirachtin-rich fractions elevates ALT/AST (2–3-fold) and sperm motility suppression in male rats, signaling potential fertility impacts. Human data, derived from Ayurvedic cohorts (n>1,000, doses 300–600 mg/day for 6–12 months), report adverse events in <5%: predominantly mild gastrointestinal (nausea, 2.1%) and dermatological (pruritus, 1.3%). In oncology patients, Phase I trials (e.g., oral SCC, n=20) noted Grade 1 fatigue (10%) but no dose-limiting toxicities up to 1,000 mg/day. Contraindications include pregnancy (teratogenic in rabbits at 100 mg/kg, causing resorptions) and lactation, due to azadirachtin bioaccumulation in milk; immunocompromised states warrant caution owing to immunomodulatory effects. Hypersensitivity, rare (0.5%), manifests as urticaria.[37]

Drug Interactions

Neem's CYP3A4 induction potential (1.5–2-fold in vitro) may accelerate metabolism of tamoxifen and aromatase inhibitors (e.g., letrozole), reducing their AUC by 20–30% and potentially attenuating endocrine efficacy. In silico docking reveals deacetylnimbin's competitive binding to ERα (ΔG -8.7 kcal/mol), akin to tamoxifen, suggesting additive antiestrogenic effects but risk of over-suppression in HR+ subtypes. Preclinical synergy with doxorubicin (CI<0.7) contrasts potential antagonism with CYP2D6 substrates like tamoxifen, where neem flavonoids inhibit P-gp, paradoxically enhancing bioavailability. Clinical monitoring of INR and hormone levels is advised for co-administration.[38]

Quality Control

Standardization mitigates batch variability, targeting ≥0.5% nimbolide via HPLC and microbial limits (<10^3 CFU/g). Adulteration with *Melia azedarach* poses toxicity risks, necessitating DNA barcoding.

Regulatory Considerations

In the U.S., neem qualifies as a dietary supplement under DSHEA 1994, exempt from premarket approval but subject to GMP (21 CFR 111) and adverse event reporting; FDA warnings (2024) target unapproved anticancer claims. EMA classifies neem preparations as herbal medicinal products, with HMPC monographs establishing traditional use for oral health but requiring THMPD authorization for novel claims. In India, AYUSH regulates via Schedule T, mandating pharmacopoeial standards. Harmonization via ICH Q3D for elemental impurities is evolving.[39]

7. Challenges and Future Perspectives

The burgeoning interest in *Azadirachta indica* (neem) as an adjunctive therapeutic in breast cancer is tempered by several translational challenges that must be addressed to realize its clinical potential. These hurdles span methodological, biological, and systemic domains, impeding the seamless progression from bench to bedside. Concurrently, innovative strategies offer pathways forward, emphasizing rigorous validation and interdisciplinary collaboration to harness neem's apoptosis- and angiogenesis-modulating prowess.[40]

Challenges

Foremost among challenges is the standardization of neem extracts, a perennial issue in phytomedicine. Variability in phytochemical profiles—driven by factors such as geographic provenance, harvest timing, and extraction solvent—yields inconsistent bioactivity; for instance, nimbolide content fluctuates 5–20-fold across Indian vs. African neem cultivars, confounding reproducibility in preclinical assays. This heterogeneity undermines mechanistic interpretations, as studies often attribute effects to "neem extract" without quantifying markers like azadirachtin (>0.5% w/w). Compounding this, limited mechanistic depth persists: while apoptosis (Bax/caspase upregulation) and angiogenesis inhibition (VEGF↓) are well-documented in 2D models, 3D organoids and patient-derived xenografts reveal off-target effects, such as unintended ER stress in normal epithelia, necessitating advanced proteomics to delineate subtype-specific impacts in ER+ vs. TNBC.[41]

Bioavailability remains a translational bottleneck, with oral F<5% for limonoids due to CYP3A4 metabolism and P-gp efflux, restricting systemic exposure in vivo. Large-scale clinical trials are scarce, with only Phase I data in non-breast cancers, highlighting funding biases toward allopathic agents and regulatory skepticism toward botanicals. Ethical concerns arise in vulnerable populations, where cultural reliance on neem in low-resource settings risks unmonitored polypharmacy, potentially exacerbating interactions with tamoxifen (CYP2D6 inhibition). Finally, publication bias favors positive preclinical outcomes, with <10% of studies reporting null effects, skewing meta-analyses.[42]

Future Perspectives

Overcoming these challenges demands multifaceted innovation. Proposed clinical trials include Phase II RCTs (e.g., neem nanoformulations + paclitaxel in TNBC, n=120, endpoints: PFS, VEGF biomarkers) registered via NCT, incorporating pharmacogenomic stratification for CYP variants to personalize dosing. Novel delivery systems—such as HER2-targeted liposomes enhancing nimbolide AUC 5-fold—could bypass bioavailability issues, validated in PDX models. Multi-omics integration (transcriptomics, metabolomics) will elucidate synergies, e.g., NF-κB crosstalk with immunotherapy, while AI-driven predictive modeling (e.g., docking for limonoid-VEGFR affinity) accelerates hit optimization.Interdisciplinary consortia, blending Ayurvedic expertise with oncology, could standardize GAP-compliant cultivation, targeting uniform limonoid yields. Equity-focused designs should prioritize diverse cohorts, addressing disparities in Black women's TNBC outcomes. Long-term, neem's scaffold could inspire semi-synthetic derivatives, evading natural variability.[43]

8. Conclusion:

Azadirachta indica (neem) holds substantial promise as an adjunctive therapeutic in breast cancer, leveraging its phytochemicals to induce apoptosis and inhibit angiogenesis, thereby addressing key resistance and recurrence drivers. While preclinical evidence is compelling, clinical translation demands standardized formulations, bioavailability enhancements, and rigorous trials to fully integrate this ancient remedy into modern oncology. Ultimately, neem exemplifies integrative medicine's potential to deliver equitable, multitargeted care, bridging traditional wisdom with precision strategies to improve outcomes for diverse patient populations.

Declarations

Ethics approval and consent to participate

This article is a review and does not contain any studies with human participants or animals performed by the authors. Therefore, ethical approval and consent to participate were not required.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable. This is a review article, and all data analyzed or discussed are available in the published literature cited within the manuscript.

Competing interests

The author declares that there are no competing interests related to this manuscript.

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