Molecular Mechanisms and Nanotechnological Advancements Establishing Piperine as a Promising Anticancer Therapeutic

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***Abstract*—Breast cancer is the most common malignancy in the world among women with high mortality rate and much influence on health economic burden. Emergence of chemoresistance to breast cancer requires the need to discover other anticancer agents, and piperine is a good option. Piperine is a good option in fighting cancer cells because it has several signaling pathways. It does this by stopping cell cycle and encouraging apoptosis, altering signaling protein expression, decreasing transcription factors and blocking tumor growth. Piperine along with other phytochemicals like paclitaxel, thymoquinone, hesperidin, bee venom, tamoxifen, mitoxantrone, piperlongumin and curcumin have shown a strong resistance in cancer to overcome drug resistance. The incorporation of piperile into the nanotechnology of carbon nanotube, PLG /PEG nanoparticle and liposome, greatly enhances the release and activity. This review summarizes the data on the chemical properties of piperine and its bioavailability, molecular targets in cancer, and the use of nanotechnological methods. Piperile has been demonstrated to have anticancer effects such as anti-proliferative, pro-apoptotic, anti-migratory, and anti-metastatic. It can also help regulate the immune response, prevent the self-renewal of cancer stem cells, act as a chemo-enhancer, cytotoxic agent and an anti-**

***Index Terms*—Piperine, Breast cancer, Apoptosis, Cell cycle arrest, Nanoparticles**

1. INTRODUCTION

Cancer remains one of the leading the world health challenges and ranks second in most fatal diseases currently. Therapeutic strategies against various forms of cancer face significant obstacles, including chemoresistance, severe toxicity, and the pervasive issues of relapse and metastasis [1]. The World Health Organization figures of 2018 denote that newly detected cases of cancer were about 1.73 million and over 609,000 deaths that were attributed to cancer in the United States alone. Despite significant advancements in cancer treatment, the incidence and mortality rates have not seen a substantial decrease over the last three decades. Therefore, a deeper understanding of the molecular mechanisms driving tumor initiation and progression is crucial for effective cancer prevention and treatment. Current therapeutic interventions aim to selectively inhibit or halt tumor growth, prevent metastasis, and minimize adverse effects.[2], [3] It is widely acknowledged within the scientific community that while synthetic anticancer agents form the cornerstone of modern oncological practice, numerous compounds derived from plants also exhibit potent antitumor activities and engage diverse mechanisms of action.[4]The affected plant species are wide in phytogeographic scope and include Taxus brevifolia, Catharanthus roseus, Betula alba, some species of Cephalotaxus, Erythroxylum previllei, Curcuma longa, and many others.[5] Among the diverse array of botanical compounds, piperine, the principal constituent isolated from Piper nigrum, serves as a prime illustration. This spice, revered as 'The King of Spices,' is known as Kali Mirch in Hindi and Black Pepper in English[6]. Though originating from exotic locales, black pepper has become a global staple, transcending its role as a mere spice to

function as a preservative and a key ingredient in the fragrance industry.[7] Both Piper nigrum and its constituent compounds are integral to a wide array of culinary applications and therapeutic regimens, utilized either in their whole, unprocessed form or as purified piperine. Piperine, recognized as the principal pungent alkaloid in black or long pepper, plays a crucial role in traditional medicinal systems, particularly Ayurveda and Unani medicine.[8], [9] Chemically, it is a piperidine ester with 1-piperoyl. Its pharmacological profile has recently been re-examined, revealing a remarkably broad therapeutic potential.[10] Relevant targets are antihypertensive and antiplatelet, antioxidant, antitumor, antiasthmatics, antipyretics, analgesics, anti-inflammatory, antidiarry, antispasmodic, anxiolytes, antidepressant, hepato-protective, immuno-modulatory, antibacterial, antifungal, anti-thyroid, anti-apoptotic, anti-metastatic, antimutagenic, antispermatogenic, anti-Col It is also significant that it allows increasing drug bioavailability by inhibiting metabolising enzymes.[11], [12] The digestive activity is also sustained through stimulation of pancreatic and intestinal enzymes.

Given such multifactored characteristics, the present review offers a recent evaluation of the structural-activity relationships (SAR) between piperine and its analogues, the reported in vitro and in animal models and clinical studies of the anticancer activity of the compounds, and the different delivery systems investigated against cancer.

2. MATERIALS AND METHODS

We conducted a comprehensive literature search across PubMed, Springer Link, ScienceDirect, and Google Scholar using keywords such as “piperine,” “pharmacological activities,” “piperine plus anticancer,” “piperine plus anti-inflammatory,” “piperine plus hepatoprotective,” and “nanotechnology,” covering publications from 2010 to 2025. To ensure thoroughness, reference lists of relevant papers were also screened manually. Only English-language studies highlighting piperine biological potential, particularly its anticancer mechanisms, antibacterial, antioxidant, and anti-inflammatory roles, were included. Articles focusing on extraction processes, food packaging, cardioprotective, or neuroprotective effects were excluded. Eligible studies were systematically analyzed and synthesized in this review.

3. PIPERINE: CHEMISTRY, BIOAVAILABILITY, AND AMINO ACID DERIVATIVES IN ANTICANCER THERAPY

Natural products have long been recognized as valuable sources of therapeutic agents, either used directly as medicines or serving as templates for semi-synthetic derivatives. Despite their importance, the clinical translation of most natural products is limited due to their low abundance and difficulties in large-scale extraction. An exception to this trend is piperine, the principal alkaloid of Piper nigrum (black pepper), which is present in relatively high concentrations ranging from 3–7%. This abundance makes piperine feasible for extraction in bulk quantities for commercial and pharmacological use. Chemically, it belongs to the N-acylpiperidine family [9], characterized by a (1E, 3E)-1-(1,3-benzodioxol-5-yl)-5-oxo penta-1,3-dien-5-yl group attached to a nitrogen atom. Piperine is classified within several chemical categories, including benzodioxoles, piperidine alkaloids, tertiary carboxamides, and N-acylpiperidines. Its biosynthetic precursor is piperic acid, typically found in the (E,E) configuration. The compound crystallizes in a monoclinic form as needle-like structures [13]. In terms of solubility, piperine is sparingly soluble in water (approximately 40 mg/L at 18 °C, equivalent to 1 g in 25 L) but dissolves more readily in organic solvents. For instance, 1 g of piperine dissolves in 15 mL alcohol, 36 mL ether, and 1.7 mL chloroform under similar conditions. It is only slightly soluble in aqueous acids. Piperine salt, prepared with B4H2PtCl6, yields orange-red crystalline needles. When subjected to oxidative iodination using iodine and potassium iodide, it produces crystalline derivatives that melt at 145 °C. Early chemical studies, notably by Anderson, demonstrated that alkaline treatment of piperine cleaves it into two components: piperidine (a base) and piperic acid (an acid). Later synthesis involved reacting piperoyl chloride with piperidine to regenerate the parent alkaloid. Biologically, piperine exhibits diverse activities, functioning as an NF-κB inhibitor, a plant metabolite, and a dietary component detectable in human serum [13]. Beyond its intrinsic properties, research has focused on derivatization to

enhance its bioactivity. Expanding this line of investigation, piperic acid derivatives linked with a broader spectrum of amino acids and substituted anilines demonstrated consistently higher cytotoxicity compared to native piperine across all tested cancer cell lines.

4. BIOAVALIBILITY: TOXICITY, ABSORPTION AND METABOLISM OF PIPERINE

The main limitation for using PIP in clinical applications is its lipophilicity and insolubility in water. This affects its metabolism, absorption, and toxicity depending on how it is administered. [14]. Human consumption of piperine (5 mg kg/day) in black pepper has been established as having no observed adverse effects. [15]. Furthermore, piperine administered at a dose of 100 mg/kg demonstrated no toxicity in mice. The median lethal dose for a single intravenous administration was determined to be 15.1 mg/kg, while for oral administration, the LD50 values were 330 mg/kg in mice and 514 mg/kg in rats. [16]. Remarkably, PIP has demonstrated a notable absence of adverse effects on reproductive functions in rat testes, including germ cells, spermatogenesis, and epididymal enzymes, even at low doses [17]. Recent In vivo and in vitro investigations have demonstrated that PIP does not exhibit genotoxicity.[18] However, the administration of PIP (5 and 10 mg/kg) for 30 days showed severe degeneration in the germ cells[19]. Research involving male mice examined piperine impact on the immune system. Daily administration of piperine at doses of 1.12, 2.25, or 4.5 mg/kg for five days did not produce overt toxic effects. However, the highest dose significantly reduced spleen, thymus, and mesenteric lymph node weights, though not peripheral lymph nodes. Piperine also decreased cell counts in lymphoid organs, with lower doses increasing spleen cell numbers. Higher doses diminished B-lymphocyte responsiveness and the quantity of antibody-producing cells in the spleen, alongside lower blood antibody levels. T-lymphocyte immune responses were also impaired by doses of 1.12 and 2.25 mg/kg. Given that the lowest dose of 1.12 mg/kg showed no adverse immune effects, it is deemed safe "no observed adverse effect level (NOAEL)" dose.[20] While extensive research into the pharmaceutical applications and daily consumption of piperine is ongoing, comprehensive safety evaluations are still pending.

The metabolic absorption of piperine has been recently investigated. Following intraperitoneal administration in mice, PIP was detected in the liver at concentrations of 1-2.5%. After oral administration, between 8 and 15% of this alkaloid was found in the spleen, kidney, serum, and intestine in mice. Another study indicated that PIP administration in rats led to a hepatic concentration of 50%. Regardless of the administration route, only 3% of the administered dose was excreted in the feces as PIP. Additionally, PIP disappeared at a concentration of approximately 60% in the intestinal mucosa and serous fluid, potentially due to its apolar nature facilitating crossing of the intestinal barrier.[21] Piperine shares structural similarities with compounds like piperidide, pipididine, and piperidine, which have been identified in human urine. Metabolic studies indicate that PIP can be converted by amidase into peptic acid, which is then further metabolized through oxidation into vanillic acid, piperonal, and piperonyl acid.[22] In mice, a total of 148 excreted metabolites of piperine were identified, primarily resulting from glucuronic conjugation, sulfate conjugation, ring cleavage, dehydrogenation, hydrogenation, methylation, demethylation, hydroxylation, methoxylation, oxidation, glucuronidation, and their combined metabolic transformations.[23] This alkaloid can provide significant benefits only when it is better absorbed and transported to host cell.

5. TARGET SITE OF PIPERINE ON CANCER CELLS

To provide a fundamental understanding of carcinogenesis, it is essential to recognize that cancer development, or neoplasm expansion, primarily arises from a disruption in normal cell cycle regulation. This disruption impairs the tightly controlled sequence of cellular growth, DNA replication, and division. In breast cancer cells, proliferation is influenced by several interconnected mechanisms, including the induction of cell cycle arrest and apoptosis, alterations in signaling protein expression, reduction in transcription factors, and inhibition of tumor growth [24].

Piperine Inhibits P-glycoprotein Activity in Breast Cancer

One of the major challenges in cancer therapy is multidrug resistance (MDR), which is frequently linked to the overexpression of transmembrane proteins called ATP-binding cassette (ABC) transporters. These proteins actively expel chemotherapeutic drugs from cancer cells, thereby lowering intracellular drug concentrations and contributing to therapy failure. The human ABC transporter family consists of seven subfamilies, among which ABCB1 encodes P-glycoprotein (P-gp), ABCC1 encodes multidrug resistance-associated protein 1 (MRP1), and ABCG2 encodes the breast cancer resistance protein (BCRP) [25]. Overexpression of any of these transporters—P-gp, MRP1, or BCRP—can significantly reduce the cytotoxic effects of chemotherapeutic agents, effectively protecting tumor cells from treatment [26, 27].

Piperine (PIP) has been shown to reverse MDR in cancer cells by reducing the expression levels of ABCB1, ABCC1, and ABCG2, which encode P-gp, MRP1, and BCRP, respectively [28]. Interestingly, PIP exerts a concentration-dependent effect on P-gp ATPase activity: at low concentrations, it inhibits ATPase activity, while at higher concentrations (50–100 μM), it enhances it [29]. This ability to modulate P-gp function highlights the potential of PIP as a complementary agent in breast cancer therapy, where overcoming transporter-mediated drug resistance remains a critical goal. Notably, MRP1 and BCRP are crucial in preventing drug accumulation in resistant cancer cells, and their modulation by PIP can enhance chemotherapeutic efficacy.

Piperine Induces Cell Cycle Arrest in Cancer Cells

The normal progression of the cell cycle is governed by cyclin/cyclin-dependent kinase (CDK) complexes and cyclin-dependent kinase inhibitors (CKIs). For instance, cyclin D1, cyclin D3, CDK4, and CDK6 regulate the G1 phase progression, while cyclin B1 controls the transition into mitosis [30]. Piperine interferes with these regulatory pathways, making it a valuable molecular probe in cancer research. Specifically, PIP reduces cyclin D1 expression, leading to inhibition of cyclin D1-CDK4/6 interactions. This suppression halts cells in the G1/S phase, preventing their entry into the S phase, and effectively slows down proliferation [24]. Furthermore, PIP can induce accumulation of cells in the G2 phase by decreasing cyclin B1 levels, thereby blocking entry into mitosis. Another mechanism through which PIP exerts cell cycle control is the upregulation of CKIs such as P21 and P27, which inhibit the activities of CDK1, CDK2, and CDK4, further reinforcing cell cycle arrest at multiple checkpoints [31]. These effects underline the therapeutic potential of PIP in controlling abnormal cell proliferation in breast cancer and potentially other malignancies.

Piperine Induces Apoptosis

Oncogenesis often arises from genetic alterations that disrupt the balance between cell survival and programmed cell death. Growth factors and cytokines, such as epidermal growth factor (EGF) and members of the EGF receptor family, trigger intracellular signaling cascades that promote proliferation. Piperine intervenes in these pathways by reducing EGF production and downregulating HER2 expression, thereby attenuating hyperproliferative signals that drive neoplastic growth [32].

In breast cancer cells expressing SREBP-1, PIP decreases the expression of the fatty acid synthase (FAS) gene, inhibits ERK1/2 signaling, and lowers SREBP-1 levels. This cascade ultimately leads to apoptosis and reduced cell migration and proliferation [33]. The underlying principle is that cancer develops when cells evade apoptosis; thus, triggering programmed cell death is a central strategy in cancer therapy. Piperine contributes to this process by downregulating Bcl-2, an anti-apoptotic protein. The loss of Bcl-2 allows pro-apoptotic effectors, Bak and Bax, to form pores in the mitochondrial membrane. When cytochrome c is released from mitochondria, it associates with Apaf-1, activating caspase-3 and initiating the apoptotic cascade, culminating in cancer cell death [34]. Empirical evidence has shown that PIP can provoke apoptosis in diverse cancer cell types by modulating the activity of critical intracellular proteins, including NF-κB, AP-1, and STAT3 [35]. This multi-targeted modulation of signaling pathways emphasizes Piperine potential as a versatile therapeutic agent, capable of influencing both the proliferation and survival of cancer cells.

6. PIPERINE DELIVERY AGENTS AND COMBINATION DRUGS

Piperine, a bioactive compound found in black pepper, offers multiple possibilities for drug delivery due to its inherent bioavailability-enhancing and chemosensitizing properties. The clinical and mechanistic effectiveness of various delivery systems for Piperine has been demonstrated. However, the selection of an appropriate delivery agent depends on the chemical nature of Piperine and the type of drug molecules to be co-administered. Piperine has been primarily explored as a chemosensitizer in histiocancer cells to enhance the efficacy of chemotherapeutic agents. This strategy of combining Piperine with standard chemotherapeutics addresses chemoresistance, which is a significant challenge in cancer therapy. Combination therapy is widely accepted as safe and effective, often resulting in synergistic therapeutic effects while reducing drug doses, toxicity, and the development of chemotherapy resistance [36].

PLGA Nanoparticles

Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable copolymer widely used as a drug delivery matrix due to its favorable safety profile and degradability. PLGA degrades into non-toxic byproducts such as water and carbon dioxide, which are easily eliminated by the body. Its nanoparticles are broken down *In vivo* through hydrolysis of ester bonds into lactate and glycolate monomers. D-lactate is excreted directly, while L-lactate is converted into pyruvate and enters the Krebs cycle, ultimately producing CO2. Glycolate is either excreted through the kidneys or metabolized into glyoxylate, serine, glycine, and pyruvate, which also enter the Krebs cycle. Drug release from PLGA occurs through a diffusion-coupled degradation process. Initially, drug release is governed by diffusion from the polymer matrix, and as degradation progresses, both polymer breakdown and diffusion contribute to sustained drug release. Typically, phosphate-buffered saline (PBS) at pH 7.4 is used to monitor in vitro nanoparticle release [37, 38].

PEG-PLGA Nanoparticles

PEGylation of PLGA nanoparticles improves their delivery efficiency for hydrophobic anticancer drugs. Encapsulation in biodegradable polymeric nanoparticles enhances both active and passive targeting, increasing drug permeability and retention in tumors [39]. Pachauri et al. reported that Piperine encapsulated in PEG-PLGA nanoparticles exhibited an initial burst release within the first five hours, attributed to the rapid release of surface-associated drug and the polymer's near-surface degradation. This was followed by a sustained, controlled release pattern linked to the gradual degradation of the PLGA polymer matrix [40].

Liposomes

Liposomes are lipid-based nanoparticles ranging from 25 nm to over 1000 nm in diameter. Their small size allows efficient penetration of tumor endothelium and accumulation in the tumor interstitial space. Liposomes can encapsulate hydrophobic drugs within their lipid bilayer or hydrophilic drugs in their aqueous core, providing versatile drug delivery options [41]. Burande et al. reported encapsulation efficiencies of Piperine in various liposomal formulations, with non-targeted Piperine liposomes at 56% ± 2.64, PTX and Piperine liposomes at 35% ± 2.04, and CTX-decorated targeted liposomes at 31% ± 2.68 [42].

Multiwalled Carbon Nanotubes (MWCNTs)

Carbon nanotubes are cylindrical nanostructures made of hexagonal carbon lattices, offering high surface area and porosity. Their surfaces can be functionalized to interact with proteins, peptides, or nucleic acids, making them useful for drug delivery, diagnostics, and biosensing. Multiwalled carbon nanotubes (MWCNTs) provide significant drug-loading capacity due to their cylindrical geometry and pore size range of 4–30 nm [43]. Reza et al. demonstrated that co-administration of Piperine with docetaxel-loaded MWCNTs enhanced tissue permeability, bioavailability, and anticancer efficacy. In MCF-7 cells, anticancer activity increased 2.7-fold, and in MDA-MB-231 cells, efficacy increased 4.2-fold compared to the free drug [44].

Combination Drugs

Chemoresistance remains a major obstacle in effective cancer therapy. Piperine is commonly used to sensitize cancer cells to chemotherapy, enhancing drug efficacy while reducing dosage, toxicity, and resistance [45, 46]. Talib et al. observed synergistic effects when Piperine was combined with Thymoquinone (TQ) in breast cancer models, inducing apoptosis through VEGF inhibition and modulating Th1 anticancer responses. *In vivo,* this

combination significantly reduced tumor size and promoted necrosis [47]. Tamoxifen, a hormone-suppressing drug, often causes adverse effects such as endometrial hyperplasia and thromboembolic events. Combining Piperine with Tamoxifen enhances cytotoxicity by reducing P-gp expression [48]. Additionally, co-treatment with Piperine, Tamoxifen, Bee venom, and Hesperidine demonstrated potent apoptosis induction in breast cancer cells, decreasing Bcl2 expression and increasing Bax levels, along with reduced ER and EGFR expression. This combination highlights the complementary potential of Piperine in enhancing Tamoxifen efficacy [49].

7. ANTICANCER ACTIVITY OF PIPERINE

Breast cancer

Piperine, a biologically active alkaloid present in the fruits of Piper nigrum, has been demonstrated to modulate the proliferation and migration of MCF-7 breast cancer cells in a dose-dependent manner. Specifically, piperine impedes the progression of the cell cycle, suppresses cellular proliferation, and reduces metastatic potential. These effects correlate with the downregulation of Rac1 gene expression and the levels of Rac1 and RhoA proteins, along with reduced concentrations of cyclin D1, NF-κB, MMP-2, MMP-9, VEGFA, and ICAM1. Concurrently, piperine induces oxidative stress by enhancing reactive oxygen species (ROS) production and activating caspase-3, which is reflected in an increase in its activity and protein levels. Collectively, these findings suggest that piperine exerts substantial chemopreventive and therapeutic effects in breast cancer models [50].

In cancer pharmacology, piperine is widely recognized for its anti-tumor properties, particularly through the induction of apoptosis. The mechanism involves caspase-3 activation followed by the cleavage of poly(ADP-ribose) polymerase (PARP), a hallmark of apoptotic cell death. Additionally, piperine suppresses HER2 gene expression at the transcriptional level, modifying the malignant cell phenotype. It further inhibits ERK1/2 signaling, which downregulates sterol regulatory element-binding protein-1 (SREBP-1) and fatty acid synthase (FAS), both critical for cell growth. Consequently, piperine reduces epidermal growth factor (EGF)-induced MMP-9 expression by modulating AP-1 and NF-κB transcriptional activity, limiting cell migration. Pre-treatment with piperine enhances sensitivity to paclitaxel, especially in HER2-overexpressing cancers that typically exhibit drug resistance. These observations indicate that piperine can function either as a monotherapy or as an adjuvant in standard chemotherapy regimens [33].

Studies have shown that piperine effectively inhibits the proliferation of triple-negative breast cancer (TNBC) and estrogen receptor-positive breast cancer cell lines, while minimally affecting non-cancerous mammary epithelial cells [24]. Piperine treatment reduced the number of TNBC cells in the G2 phase, with Western blot analyses revealing decreased levels of cell cycle-specific proteins and increased expression of the cyclin-dependent kinase inhibitor p21(Waf1/Cip1). Another mechanism of cell death involved the downregulation of phosphorylated Akt, leading to mitochondrial-mediated apoptosis. Moreover, piperine synergized with gamma radiation, enhancing cytotoxicity in TNBC cells. Microscopy and quantitative real-time PCR studies further indicated that piperine decreased cellular motility and downregulated genes associated with migration, suggesting anti-metastatic potential. These findings support exploring piperine as a therapeutic agent for endocrine-independent breast cancer [24]. To evaluate combinatorial approaches, piperine supplementation was tested alongside TRAIL-based therapy in TNBC cells [51].

The combination amplified apoptosis, with higher piperine concentrations (70–280 μM) on 4T1 cells triggering increased caspase-3 activation. Cells exposed to 140–280 μM remained in prolonged G2/M phases, exhibited lower cyclin-B1 expression, and reduced MMP-9 and MMP-13 mRNA levels. Piperine also inhibited 4T1 cell motility in vitro. Animal studies confirmed dose-dependent tumor growth inhibition in mice treated with 2.5 and 5 mg/kg piperine. Collectively, these results highlight the potential of piperine as an adjunct in TRAIL-based TNBC therapy. Comparative studies of anticancer efficacy revealed that piperine enhances the effects of curcumin in nanoparticle formulations against MDA-MB-231 breast cancer cells, surpassing the activity of docetaxel alone [52]. Genes involved in tumor growth, invasion, and progression, such as AKT1, MYC, NOTCH1, IL6, JUN, EGFR, MAPK1,

RARB, BCL2, CCND1, MAPK8, BIRC5, and ESR1, were downregulated more significantly in nanoparticle-treated cells than in docetaxel-treated groups. These results suggest that co-delivery of piperine and curcumin in nanoparticles may be an effective approach to inhibit breast cancer progression. Piperine-loaded metal-organic frameworks coated with macrophage membranes were developed to enhance delivery to breast cancer cells [53]. These MM@PIP@MIL-100 nanoparticles demonstrated 4–17 times greater cytotoxicity against MCF-7, BT-549, SKBR-3, and MDA cell lines compared to free piperine, indicating a promising strategy for more efficient breast cancer therapy. Investigations into piperine effect on radioresistance revealed that it enhances radiation-induced cell death by modulating estrogen receptor signaling, promoting ERβ activation while reducing ERα expression [54].

Piperine appears to function as a selective estrogen receptor modulator and influences DNA-PK complex components and DNA damage response proteins, suggesting a novel approach for overcoming radioresistance in breast cancer cells. Interaction studies between piperine and calf thymus DNA (ctDNA) using spectroscopic, thermal, and viscosity analyses indicated that hydrophobic forces predominantly drive the binding [55]. Fluorescence quenching assays confirmed piperine as a quencher, with dynamic quenching mechanisms at 298, 303, and 308 K. Piperine treatment also increased ROS production, lipid peroxidation, and caspase-3 activity, while decreasing superoxide dismutase activity, leading to cytotoxic effects in MDA-MB-231 cells. Piperine effect on leptin-induced breast cancer was examined to explore its potential in obesity-associated cancer [56]. Piperine reduced proliferation, colony formation, migration, and invasion in leptin-stimulated breast cancer cells, downregulating PPARα expression while enhancing miR-181c-3p-mediated anticancer effects. Oral administration in obese mice suppressed tumor growth, indicating a therapeutic role in obesity-linked breast cancer. Clinical application of piperine is limited by its poor solubility and high lipophilicity. Encapsulation in polycaprolactone nanoparticles (PIP-PCL-NPs) enhanced stability and cytotoxicity against MCF-7 cells, suggesting that nanoformulations can improve therapeutic potential [57].

Dual-drug delivery systems combining curcumin and piperine using zein/chitosan-coated iron oxide nanoparticles demonstrated synergistic anticancer effects against MCF-7 cells [58]. These pH-sensitive nanoparticles released drugs faster in acidic tumor environments, improving cytotoxicity and offering a targeted therapeutic strategy. Piperine was also shown to inhibit proliferation and migration in MCF-7 and MDA-MB-231 cells using colony formation, wound healing, Matrigel migration, flow cytometry, RT-qPCR, and Western blotting [59]. G0/G1 phase reduction and G2/M arrest were observed, alongside decreased Rac1 expression, particularly in MDA-MB-231 cells. Piperine-loaded nanoemulsions were developed to overcome poor solubility and enhance cytotoxic activity against 4T1 and MCF-7 cells [60]. These nanoemulsions exhibited sustained release and maintained piperine cytotoxicity, with no adverse effects observed in Hen's Egg Test on Chorioallantoic Membrane assay.

Genital cancer

Piperine, in combination with mitomycin-C, inhibits the JNK-NF-κB-STAT3 signaling cascade, downregulating the Bcl-2 survival pathway in cervical tumor cells, including HeLa cells [61]. Piperine at 8–20 μM also inhibited ovarian A2780 cell viability by inducing apoptosis via a JNK/p38 MAPK-dependent pathway [62]. This involved cytochrome c release, caspase-3 and caspase-9 activation, and decreased JNK/p38 MAPK activity, highlighting piperine therapeutic potential in reproductive cancers. In prostate cancer, piperine was shown to suppress proliferation of DU145, PC-3, and LNCaP cell lines, arresting cells in the G0/G1 phase while downregulating cyclin D1 and cyclin A [63]. Upregulation of p21Cip1 and p27Kip1 further inhibited cell division. Autophagy markers LC3B-II and puncta formation indicated autophagic activation in LNCaP and PC-3 cells, which was verified using chloroquine inhibition. These results indicate piperine mitigates prostate cancer growth through cell cycle arrest and autophagy induction.

Piperine affects voltage-gated potassium channels (IK) in LNCaP and PC-3 cells, leading to depolarization and reduced G1 phase progression, inducing apoptosis [64][65]. These findings suggest potassium current blockade as a mechanism of action. Further studies demonstrated piperine inhibits prostate cancer cell growth and migration via the

Akt/mTOR/MMP9 pathway [66]. Piperine effects were similar to those of the Akt inhibitor LY294002, indicating its potential in preventing prostate cancer metastasis. Piperine induces apoptosis in LNCaP, PC-3, and DU-145 cells by activating caspase-3 and cleaving PARP-1, while decreasing STAT-3 and NF-κB phosphorylation [67].

Gastro-intestinal tract cancer

Piperine induces lipid peroxidation in hamster buccal pouch models treated with DMBA, reducing protein and nucleic acid levels in cancerous tissues, supporting a redox-mediated mechanism of anticancer activity [68]. In AGS gastric cancer cells, piperine decreased anti-apoptotic proteins Bcl-2, XIAP, and Akt, while increasing p53, Bax, cleaved caspase-9, and cleaved-PARP [32]. Piperine suppressed IL-1-induced p38 MAPK and STAT3 activation, reducing IL-6 expression in TMK-1 gastric cancer cells, demonstrating dose-dependent inhibition of inflammatory signaling [32][69]. In HT-29 colon cancer cells, piperine downregulated pro-survival proteins Bcl-2, Mcl-1, and survivin, upregulated Fas, and modulated cyclins and CDK inhibitors, demonstrating a complex antiproliferative and pro-apoptotic effect [34]. Piperine exposure enhanced ROS production and modulated mTORC1 signaling in HRT-18 rectal cancer cells, inhibiting proliferation and providing mechanistic insights into its protective effects against colorectal carcinogenesis [70].

Lung cancer

Piperine demonstrated cytotoxic effects against A549 lung cancer cells by inducing G2/M cell cycle arrest and apoptosis, mediated through caspase-3 and caspase-9 activation and modulation of Bax/Bcl-2 levels [71]. Piperine mitigated benzo(a)pyrene-induced lung tumorigenesis in Swiss albino mice by reducing lipid peroxidation, protein carbonylation, nucleic acid degradation, and polyamine synthesis [72]. Piperine improved mitochondrial enzyme activity and enhanced NADPH-related protein expression, suggesting protective effects against lung cancer-related genomic instability [73]. Piperine modulated ATPase enzyme activity in erythrocytes and tissues, further supporting its chemopreventive role [74]. In C57BL/6 mice, piperine reduced lung metastasis initiated by B16F-10 melanoma cells, lowering tumor burden and biochemical markers, indicating its anti-metastatic potential [75].

Other cancers

In human fibrosarcoma HT-1080 cells, piperine inhibited PKCα and ERK phosphorylation, reducing NF-κB and AP-1 activation and subsequently downregulating MMP-9 expression [76]. Piperine inhibited multiple transcription factors in B16F10 melanoma cells, reducing pro-inflammatory cytokines and promoting cell death via ROS generation, calcium imbalance, and mitochondrial depolarization [77]. Synthetic piperine-amino acid ester conjugates exhibited cytotoxic effects against several human cancer cell lines, including IMR-32, MCF-7, PC-3, DU-145, Colo-205, and Hep-2 [78]. Piperine reduced KB cell viability in a dose-dependent manner, inducing ROS production, nuclear condensation, MMP loss, caspase-3 activation, and G2/M cell cycle arrest [79]. These findings reinforce piperine potential as a chemotherapeutic agent, warranting further research for anticancer drug development.

8. ANTI-INFLAMMATORY STUDY

Inflammation is a common physiological response that primarily serves as a protective mechanism, preventing the spread of infection and assisting the body in restoring its normal structure and function. However, excessive or uncontrolled inflammation can lead to pathological outcomes, including immune system dysfunction, rheumatoid arthritis, sepsis, atherosclerosis, organ damage, and even mortality [80]. Piperine, a bioactive alkaloid found in black pepper, was first recognized for its anti-inflammatory properties in 1990 through its ability to stimulate the pituitary-adrenal axis [81]. The present study explores the anti-inflammatory potential of piperine extracted from pepper. The compound was isolated using various solvents, and the ethanolic extract showed a positive reaction for piperine, with its concentration estimated at approximately 1.207 mg/mL [82].

In assessing its anti-inflammatory effects, piperine at a concentration of 10 mg/mL demonstrated activity comparable to conventional anti-inflammatory agents such as diclofenac sodium and ascorbic acid, a result further supported by molecular docking studies. The antioxidant properties of piperine were evaluated by examining its interaction with Cytochrome P450 family 2 subfamily C member 9 (CYP2C9) and

Nicotinamide adenine dinucleotide phosphate (NADPH) Oxidase, showing binding energies of -7.9 kcal/mol and -6.2 kcal/mol, respectively. Additionally, piperine displayed a binding energy of -6.2 kcal/mol with Cyclooxygenase-2 (COX-2), highlighting its anti-inflammatory potential [83].

Mechanistically, piperine regulates inflammatory responses by modulating cytokine production and reducing oxidative stress through the inhibition of Nuclear Factor-κB (NF-κB) signaling and MAPK pathways. In RAW264.7 cells, it effectively decreased Nitric Oxide (NO) and reactive oxygen species (ROS) expression induced by lipopolysaccharide, while downregulating the mRNA and protein levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. Concurrently, piperine enhanced the production of the anti-inflammatory cytokine IL-10 [76].

Further studies demonstrated that piperine mitigates inflammation in various disease models. In gout induced by monosodium urate, it reduced leukocyte infiltration, cartilage damage, lipid peroxidation, and C-reactive protein production while preventing tophi formation through NETosis [84]. Piperine also decreased pulmonary edema, inflammatory cytokine levels, and lung tissue damage. In a mouse model of acetate-induced ulcerative colitis, it effectively alleviated colon shortening and splenic enlargement [85]. Additionally, a combination therapy comprising curcumin (200 mg/kg), piperine (10 mg/kg), and ferrous sulfate (0.1 mg/kg) exhibited synergistic anti-inflammatory and anti-arthritic effects, suggesting potential as an adjunct treatment in rheumatoid arthritis and reducing reliance on long-term steroid use [86].

9. LIMITATIONS IN CLINICAL USE OF PIPERINE

At high doses, piperine is acutely toxic to mice, rats, and hamsters. The LD50 values for a single i.v., i.p., s.c., i.g. and i.m. administration of piperine to adult male mice were 15.1, 43, 200, 330, and 400 mg/kg body wt, respectively[16] . Considering oral application, LD50 values were shown to be 330 mg/kg in mice and 514 mg/kg in rats . Piperine increases serum aspartate minotransferase and ALP, while total serum protein decreased, which results in considerable damage to the liver in CF-1 albino mice. Administration of piperine enhances the aflatoxin B1 binding to calf thymus DNA *In vivo* in rat tissues[87] .In a study by D'cruz and Mathur focused on the impact of piperine on the epididymal antioxidant system in adult male rats, a detrimental effect was observed, characterized by reduced weights in the caput, corpus, and cauda epididymis regions. The findings also indicated a decline in sperm count, motility, and viability, along with decreased sialic acid levels and reduced activity of antioxidant enzymes at a 100 mg/kg dosage. Consequently, the elevated levels of reactive oxygen species in the epididymis suggest that piperine intake can impair sperm function.[88]. Similar effects of piperine were recently reported for pubertal rats administered with piperine (5 and 10 mg/kg) for 30 days. In the mentioned study, piperine increased testosterone (T) and follicle-stimulating hormone (FSH) levels, number and size of Leydig cells, but negatively affected spermatogenesis. This was partially opposite to the results of the earlier study, which reported only negative effects of piperine to testes, and performed the same treatment of mature male albino rats (administered for 30 days at the same doses of (5 and 10 mg/kg)[89]. The results of this study showed that lower dose caused partial degeneration of germ cell types, while a higher dose, on the other hand, caused severe damage to the seminiferous tubule, a fall in caput and cauda epididymal sperm concentrations, decrease in seminiferous tubular and Leydig cell nuclear diameter and desquamation of spermatocytes and spermatids. In these, piperine treated rats, an increase in serum gonadotropins, and a decrease of intratesticular concentration were reported as well.

10. CONCLUSION

Many alkaloids are effective at slowing cancer cell growth, and the FDA has approved several naturally occurring alkaloid-based drugs for cancer treatment. Piperine is increasingly being studied for its biological activities and potential medical uses, showing promise as a therapeutic agent. Researchers have gained a deeper understanding of piperine pharmacological effects and mechanisms, leading to significant advancements. Its structure contains features like hydrogen bond acceptors, hydrophobic centers, and aromatic rings that facilitate receptor binding. Studies have shown piperine can reduce cell

cycles, increase apoptosis, inhibit multidrug resistance, block signaling pathways, and decrease metastasis promoters. The research findings indicate that piperine demonstrates the capacity to selectively target various cancer cell types, employing distinct mechanisms of action that are contingent upon the specific cancer identified. However, the precise mechanisms behind its chemotherapeutic and antitumor effects are not yet fully understood. Piperine has also been combined with other chemotherapy drugs, showing improved antitumor activity and reduced toxicity due to synergistic effects against breast cancer cells.

Given that piperine can be toxic to rodents at high dosages, it is crucial to determine if it poses a greater toxicity risk to other bodily systems. Piperine poor water solubility, attributed to its hydrophobic nature, currently restricts its clinical application. Nevertheless, research is actively exploring nanotechnology-based delivery systems to mitigate human risk and enhance piperine chemotherapeutic efficacy. This review examines various nanoparticles, including polymeric nanoparticles, carbon nanotubes, and liposomes, suitable for piperine delivery.To support the therapeutic application of piperine, comprehensive elucidation of its precise mechanism of action is essential for researchers. There is a promising and encouraging potential for piperine, considered safe for human consumption, to exhibit significant chemopreventive effects.

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