The potential effect and delivery of piperine on chemoresistant breast cancer

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ABSTRACT

Breast cancer is the most common malignancy in women worldwide. Breast cancer is associated with a high mortality rate and health-related economic burden. Breast cancer patients have a low 5-year life expectancy when diagnosed at advanced stages. Besides, the emergence of chemoresistance in breast cancer has led to an intense search for alternative anticancer agents. One of the potential anticancer compounds is Piperine. Several studies had found that Piperine has anticancer effects such as anti-proliferation, induces apoptosis, anti-migration or anti-metastasis, chemo-enhancer or chemosensitizer, cytotoxic agents, anti-angiogenesis, immune response modulators, and self-renewal inhibitor for cancer stem cells. Several delivery agents such as PLGA, PEG-PLGA and liposomes have been studied to improve Piperine's delivery and have shown good results. Therefore, the combination of Piperine and nanoparticles is a potential anticancer agent, especially in breast cancer.

Keywords: breast cancer, chemoresistance, delivery, effect, Piperine

INTRODUCTION

Breast cancer is the most common malignancy in women, responsible for 25% of all cancer worldwide. According to GLOBOCAN (IARC), in 2018, there were 2,088,849 new breast cancer cases with 626,679 mortality.1 In Indonesia, the incidence of breast cancer was recorded at 40 per 100,000 populations, with mortality at 16.6 per 100,000 population.2 According to Riskesdas 2018 data, the incidence of breast cancer in Bali Province reaches 0.6 per 1000 women.3 Breast cancer also responsible for a significant health-related economic burden for the patients and the nations. According to Barron et al. and Sun et al., the economic burden of breast cancer ranges from 29,000 - 62,000 US dollars.4,5 The life expectancy of breast cancer patients is considered good, but it continues to decline as the stage progresses.1 Also, treatment options are often limited due to breast cancer characteristics that are resistant to several types of chemotherapeutic agents. Therefore, several recent studies have focused on finding new anticancer agents that have good effectiveness or can increase the therapeutic effectiveness of chemotherapeutic agents when combined.

One of the compounds that have potential anticancer effects is Piperine. Piperine is an alkaloid class compound that is found in many plants from the Piperaceae family. It is most commonly found in black pepper (Piper nigrum L), widely found in Indonesia.6 As an anticancer agent, Piperine can induce cell cycle arrest, inhibit angiogenesis, inhibit metastasis, and induce apoptosis of cancer cells.7 However, Piperine is unstable, has low bioavailability, insoluble in water, and difficult to absorb.8 Therefore, a delivery strategy is needed to enhance the bioavailability of Piperine. In this review, the potential anticancer effect of Piperine will be described along with its delivery mechanism for Piperine.

MOLECULAR BASIS OF CHEMORESISTANCE IN BREAST CANCER

Chemoresistance is a crucial evolution in cancer progression, and it heavily influences the patient's prognosis.9 Drug resistance is classified into acquired resistance and intrinsic resistance.10 Chemoresistance in breast cancer occurs due to various factors in different mechanisms, such as transport and excretion, cell metabolism, tumor microenvironment, cancer stem cells, cancer signaling pathways, the role of miRNA, cell membranes, and others.11 The relationship between the role of miRNA, JAK / STAT3 pathway, and the tumor microenvironment in chemoresistance breast cancer is the focus of its considerable effect.

Several miRNAs are known as oncogenes and can influence cancer progressions such as tumor initiation, tissue invasion, and even metastasis.12 However, evidence showed that...
decreased miRNA function increased the expression of drug resistance-related genes (P-glycoprotein, ABCB1: ATP Binding Cassette Subfamily B Member 1; MRP-1: Multidrug Resistance-Associated Protein Group 1 or ABCG2: ATP Binding Cassette Subfamily G Member 2), DNA repair (miR-206 increases BRCA1 expression and induces Cyclin D2), apoptosis resistance (miR-149 controls NDST1 and activates HS pathway, overexpression of miR -621 and miR-383 induce FBXO11 and Gadd45g, leading to chemoresistance), epithelial-to-mesenchymal transition or EMT (miR-489 and miR-125b overexpress Smad3 and Sema4C), cancer stem cells (CSC) (decreased miR-34a function reset genes NOCTH1, miR-7 influence CSC for metastasis by inducing the KLF4 gene) (Figure 1A). Accordingly, Wnt, Hippo, Notch, Hedgehog s, JAK / STAT3 pathway, and PI3K / Akt / mTOR signaling are also overexpressed in chemoresistant breast cancer. Additionally, the JAK / STAT3 pathway has an interesting mechanism in inducing chemoresistance with intrinsic and extrinsic pathways capable of regulating lipid metabolism. It also induced stemness when inhibited, leading to chemoresistance in breast cancer by blocking self-renewal (Figure 1B).

An enzyme essential for fatty acid β-oxidation (FAO) is associated with a diverse lipid metabolism gene expression, carnitine palmitoyltransferase 1B (CPT1B). FAO produces NADH and FADH2 to reduce oxidative stress and increase ATP and Acetyl Co-A, affecting protein acetylation, the TCA cycle, and fatty acid synthesis. The enzymes are closely related to Acetyl Co-A, and decreased expression suppresses metabolic acetylation, stopping breast cancer stem cells (BCSCs) and metabolism related to proliferation. Breast adipocytes trigger leptin fatty acid to control CPT1B-induced STAT3, also reset FAO activity in BCSC (Figure 1B).

The tumor microenvironment also plays a crucial role in determining the efficacy of chemotherapeutic agents. Tumor microenvironment affects interstitial tissue, extracellular matrix, and normal stromal cells. Chemoresistance in breast cancer is associated with cancer-associated fibroblast (CAF), endothelial-cell vascularization, tumor-associated macrophage (TAM), fibroblasts, and mesenchymal stromal cell (MSC). Cancer cells regulate TAM and vice versa. TAM can differentiate into M1 (anti-tumorigenic) and M2 (pro-cancerogenic). Cancer cells secrete transforming growth factor-β (TGF-β) and growth factors which induce angiogenesis and affecting endothelial cells. Reverse, MSCs also influence cancer cells and inducing TGF-β production even further. On the other hand, T regulator (Treg) inhibits natural killer (NK) cells through CCL-2 and helps in cancer progression. Fibroblasts (a major component of the tumor microenvironment) are considered the major player in chemoresistance. CAF modulates normal fibroblast (NF), altering cancer vascularity and immune cells (Figure 1C).
THE CHEMISTRY NATURE OF PIPERINE

Piperine is one of the alkaloid compounds commonly found in the plant from the Piperaceae family. Piperine compounds are mostly found in black paper (Piper nigrum L), which contains 2% - 9% of Piperine concentration. Piperine in the black paper has been believed as a source of treatment for many diseases based on the ancient Indian medicinal book Ayurveda. Piperine also has been known to have a broad spectrum of benefits, including antitumor, antihypertensive, anti-inflammatory, anti-aggregates, antioxidant, and antidepressant.

Piperine is a phytochemistry compound essential for spicy foods and beneficial pharmacologist activities, which are not only limited as an anti-inflammatory, antitumor, and anti-metastatic, but also as leishmanicidal, larvicidal, antimycobacterial, immunosuppressive, and antiparasitic. In 1819, Piperine was first separated from pepper extract by Hans Christian Orsted. Piperine, along with chavicine, isoPiperine, and isochavicine, belongs to the alkaloid family. Several alkaloids have been approved by the US Food and Drug Administration (FDA) and used as a reservoir for drug discovery infrastructure, such as vinblastine which interacts with tubulin in mitosis and camptothecin, a well-known topoisomerase I inhibitor. Perine also neutralized several types of cancer-causing agents, such as 7,12-dimethyl benz(a)anthracene and benzo(a)pyrene, indicating its potential as a cancer chemopreventive agent.

There are various extraction methods used to isolate Piperine which depend on the product purpose, scale, and operation. Extraction refers to the separation of the analytes from a complex matrix. Extraction efficiency is strongly influenced by solvent composition, solvent to solid ratio, temperature, time, and extraction method. Extraction of Soxhlet or maceration of fruit powders with polar organic solvents obtained a dark brown oily substance which was fractionated by column chromatography using diethyl ether and hexane (2:1), producing the associated amide and Piperine. Pure Piperine can also be produced by supercritical fluid extraction followed by crystallization of ethanol. In general, the supercritical fluid extraction of pepper will require high temperatures and pressures, helping to reduce the volume of solvent. Other extraction methods were also developed, such as microwaves technique and hydrotrropic dissolution, which eliminate the need for further processing, and the products can be used directly. Other literature also showed that compounds such as Piperine have been isolated extensively, fractionated, and identified using high-performance liquid chromatography (HPLC).

Currently, there is a new technique to extract organic compounds from various samples to reduce extraction time and optimize solvent conditions, namely the Accelerated Solvent Extractor (ASE) technique. This method can increase the solvent temperature at high pressure to speed up the extraction process. The results of the research by Upadhya et al. showed that compound-based extraction using ASE from a complex plant matrix, such as Piperine, was a suitable method for quality control or standardization of herbal products. The study showed an accurate, fast, and simple extraction method for the extraction of compounds from different plant materials.

PIPERINE DELIVERY AGENTS

There are several potential delivery agents that can be used in combination with Piperine. These agents have been proven both clinically and mechanismically. However, the nature of the delivery agents and the type of molecules that it can carry should be carefully considered. Table 1 summarizes the potential delivery agents for piperine.

<table>
<thead>
<tr>
<th>Delivery Agent</th>
<th>Target Cell</th>
<th>Reference</th>
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<tbody>
<tr>
<td>PLGA Nanoparticle</td>
<td>MDA-MB-231</td>
<td>42</td>
</tr>
<tr>
<td>PEG-PLGA Nanoparticle</td>
<td>MDA-MB-468</td>
<td>43, 44</td>
</tr>
<tr>
<td>Liposome</td>
<td>MDA-MB-231</td>
<td>45</td>
</tr>
</tbody>
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PLGA Nanoparticle
Various literature has demonstrated the potential of Poly (lactic-co-glycolic acid) (PLGA) nanoparticles as a drug delivery system for many therapeutic agents, such as chemotherapy, anti-inflammatory, antibiotic, antioxidant, and antiseptic, and offers advantages in tumor and/or DNA targeting. PLGA is one of the top copolymers with several benefits, such as biodegradable (breaks down into non-toxic H2O and CO2) and easily eliminated from the body. Its polymeric nanoparticles are degraded by hydrolysis of the ester bonds into their monomeric anions in vivo (lactate and glycolate). Meanwhile, D-Lactate does not undergo metabolism before being excreted, L-lactate is converted to CO2, which is excreted through the lungs and converted to pyruvate, which enters the Krebs cycle. Glycolate will be excreted directly through the kidney system or oxidized to glyoxylate, converted into serine, glycine, and pyruvate. These substances can later re-enter the Krebs cycle and be metabolized into H2O and CO2. PLGA is generally produced through the catalyzed ring-opening copolymerization of LA and GA. PGA is a crystalline hydrophilic polymer with a fast degradation rate under physiological conditions and low water solubility. On the other hand, PLA is a hydrophobic and rigid polymer that has low mechanical strength.

Research by Katiyar et al., which evaluated the effect of Piperine (PIP) as an absorption enhancer/chemosensitizer on the oral absorption of Rapamycin (RPM), showed that the RPM tends to accumulate in red blood cells (RBC) (38 times more concentrated in red blood cells). PIP is a P-gp inhibitor that forms the basis of kinetics in the combination of two drugs.
Meanwhile, RPM is a P-gp substrate like other anticancer drugs. Therefore, most tumor cells that are rich in P-gp pump out RPM. PIP as an absorption enhancer is expected to be able to inhibit the effect of P-gp, resulting in RPM accumulation. Katiyar et al. showed a 1.7-fold increase in bioavailability from RPM in the presence of PIP suspension.

Drug release from the PLGA matrix occurs by a diffusion-degradation mediated process. Release occurs mainly by diffusion in the polymer matrix during the initial phase, and then during the subsequent phase, the release is mediated through both processes (degradation of the polymer matrix and diffusion of the drug). Generally, phosphate buffer saline (PBS) with a pH of 7.4 is used as a drug release medium to observe the release of nanoparticles in vitro. RPM is known to be unstable in PBS due to the alkaline hydrolysis of the lactam ring, which causes ring opening.

**PEG-PLGA Nanoparticle**

In developing cancer or tumor drugs, encapsulation in biodegradable polymeric nanoparticles has shown a promising solution for delivering various hydrophobic drugs, both through active and passive targeting, by increasing the permeability and retention effects. Piperine with stable aqueous polymeric nanoformulation reduces the nonspecific toxicity of Piperine with targeted delivery and increased Piperine bioavailability by slowing down metabolism and systemic elimination. Among all the biodegradable polymers used as means of drug delivery, poly (ethylene glycol) (PEG) and PLGA have shown an increased therapeutic effect of hydrophobic drugs. In fact, the United States Food and Drug Administration (FDA) has approved for both of these polymers to be used in humans and has been used in clinical trials because of their biocompatibility and high bioavailability.

According to the release pattern, Pachauri et al. showed that Piperine has an initial burst release in the first 5 hours. This initial burst release of Piperine is associated with the rapid release of adsorbed Piperine on the PEG-PNP surface and the high dissolution rate of the polymer near the surface. After the controlled initial burst release of Piperine, it was noted that the continuous release of Piperine was due to the combined diffusion of Piperine from the inner core of P-PEG-PNP and degradation of the PLGA polymer. The release of Piperine from P-PEG-PNP is further facilitated by hydrogen bonding between the Piperine PLGA matrices. The continuous release observed in Pachauri et al. study was in line with other drug release studies that showed that the hydrogen bonding between matrix nanoparticles and the drug also facilitated the sustainable release of packaged drugs.

As a PLGA nanoparticle, PEG-PLGA shows a slightly faster drug release. Bertrand et al. showed that further increasing PEG density did not result in additional benefits, so that surface modification could only change the biodistribution to a certain extent. In contrast, PEGylation can accelerate drug release due to increased degradation of PLGA nanoparticles and associated with the hydrophilic nature of the PEG chain, which absorbs water and stimulates the decomposition of the PLGA chain.

**Liposome**

Liposomes are round vesicles characterized by an internal watery cavity and a lipid bilayer. Liposomes have synthetic amphiphiles or phospholipids structure combined with sterols, such as cholesterol, to affect membrane permeability. Liposome diameter ranges from 25 nm to more than 1000 nm. The diameter is relatively small to penetrate the endothelial tumor and extravasation into the tumor interstitial. As a drug delivery medium, drugs that are fat-soluble (hydrophobic) will be deposited into the double lipid layer of the liposomes, while drugs that are water-soluble or hydrophilic will be covered in the center of the liquid liposome. Burande et al. showed that the efficiency of Piperine encapsulation (PIP) in PIP liposomes, non-targeted PTX & PIP liposomes, and CTX decorated targeted liposomes was 56% ± 2.64, 35% ± 2.04, and 31% ± 2.68. Pentak et al. used the FT-IR technique to determine the molecular interaction mechanism between Piperine molecules and DPPC (L-α-phosphatidylycholine dipalmitoyl) liposomes. The study reported that temperature affected the shape of the absorption band. The band width centered at ~ 3400 cm⁻¹, showing that the stretching of OH in water molecules associated with membranes via hydrogen bonds. The percentage of transmission of this band after incorporating Piperine into the DPPC liposomes decreased gradually with increasing temperature. This effect can be attributed to the formation of hydrogen bonds between the DPPC molecule and Piperine. They also showed that an increase in temperature could shift the band located at 1092 cm⁻¹ to a lower frequency and represented the interaction via hydrogen bonds between PO2 of the lipid and Piperine molecules. The percentage of Piperine release varied around 3.47-4.33%, where the liposome release reached its maximum value after two weeks.

**PIPERINE AS AN ANTICANCER AGENT IN BREAST CANCER**

**Anti-proliferation**

Several prior studies have shown that Piperine has anti-proliferative effects on breast cancer both in vitro and in vivo. In vitro, Piperine has anti-proliferative effects on several breast cancer cell lines representing the characteristics of several types of breast cancer subtypes. Piperine has an anti-proliferative effect on the SKBR3 cell line, which overexpressed Her2. Previous studies had also demonstrated the anti-proliferative ability of Piperine in breast cancer cell lines MDA-MB-468, MDA-MB-231, and BT-549, which have the characteristics of the TNBC subtype. Also, Piperine also has anti-proliferative effects on MCF-7 and T47D, which represent luminal subtype breast cancer.

In vivo, Piperine has anti-proliferative effects in mouse models of breast cancer 4T1 and EMT6/P. The inhibitory ability of Piperine was also characterized by good IC₅₀ values, namely 50nm in in-vitro studies and 232um to 870um in in-vivo studies.
**Table 2. Summary of Piperine Effects and Its Mechanism in Breast Cancer**

<table>
<thead>
<tr>
<th>Potential Effect</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-proliferation</td>
<td>● Depressed the protein-related G1 phase expression (CDK4, E2F-1, cyclin D3)</td>
<td>43,44,58–62</td>
</tr>
<tr>
<td></td>
<td>● Depressed the protein-related G2 phase expression (CDK1, cyclin B, Cdc25C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● Induction of p21 protein expression</td>
<td>63,64</td>
</tr>
<tr>
<td>Apoptosis induction</td>
<td>● Activate the caspase-3</td>
<td>43,44,58,60–62</td>
</tr>
<tr>
<td></td>
<td>● PARP Cleavage</td>
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<tr>
<td></td>
<td>● Mitochondrial pathway</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● Inhibition of kinase B protein signaling pathway</td>
<td></td>
</tr>
<tr>
<td>Anti-migration or anti-metastasis</td>
<td>● Depressed of MMP-2, MMP-9, dan MMP-13 expression</td>
<td>43,45,58,60–61</td>
</tr>
<tr>
<td>Chemo-enhancer or chemosensitizer</td>
<td>● Block EGFR signaling</td>
<td>42,43,45,58–61,63,64</td>
</tr>
<tr>
<td></td>
<td>● Depressed the P-gp expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● Inhibit phosphorylation of survivin and p65</td>
<td></td>
</tr>
<tr>
<td>Cytotoxic agent</td>
<td>● Excrete cathepsin</td>
<td>44,58,65</td>
</tr>
<tr>
<td></td>
<td>● Depressed GLO1 activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● Depressed the potential of mitochondrial membrane</td>
<td></td>
</tr>
<tr>
<td>Anti-angiogenesis</td>
<td>● Depressed the VEGF expression</td>
<td>62</td>
</tr>
<tr>
<td>Immunity response modulator</td>
<td>● Increased the IFN-γ and IL-2 expression</td>
<td>62</td>
</tr>
<tr>
<td>Inhibit the self-renewal stem cell ability</td>
<td>● Inhibit mammosphere formation</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>● Depressed the ALDH1 expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● Inhibit Wnt signaling pathway</td>
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</table>

The inhibitory effect of Piperine did not decrease even though it was delivered by nanoparticles.44 Piperine inhibits breast cancer cell proliferation by arresting the cell cycle. Several previous studies had shown that Piperine administration resulted in cell cycle arrest in vivo and in vitro, which was characterized by accumulation of cells in the G2/M or sub G0/G1 phases and a decrease in the number of cells in the S phase.58,61 Cell cycle arrest occurs due to decreased expression of crucial proteins in cell cycle regulation such as CDK4, E2F-1, cyclin D3, cyclin B, Cdc25C, and increased p21 expression to Piperine administration.60 Cell cycle activity in the G1 phase requires a bond between type D cyclin and CDK4 and CDK6. In contrast, E2F-1 is required in the G1/S phase transition process, while the CDK1 and cyclin A or B complexes are required in the G2/M phase transition. Besides, a decrease in Cdc25C, a phosphatase that plays a role in mitosis and phase transition of G1/S and G2/M and an increase in p21, a CDK inhibitor also causes cell cycle disruption in cancer cells.60

**Apoptosis induction**

Cancer cells are known to have immortal properties due to their ability to regulate intracellular regulation and their environment to support their growth. So that the targeted anticancer agents can induce apoptosis in cancer cells. Several previous studies supporting this potency have shown that Piperine can induce apoptosis in vitro in several types of breast cancer cell lines and in vivo. Piperine was able to induce apoptosis in the Her2-overexpressed SKBR3 cancer cell line.58 Additionally, Rad et al. and Greenshields et al. showed that Piperine administration caused apoptosis in the breast cancer cell line MDA-MB-468 while Khamis et al. and Pachauri et al. showed a similar effect on MCF-7 and T47D.58,64,65 Moreover, in vivo research by Talib et al. showed that 425 μm of Piperine was able to induce apoptosis in EMT6 / P breast cancer model mice after 48 hours.62 Finally, research by Lai et al. showed that Piperine was able to induce apoptosis in the breast cancer model 4T1.61,62

Several mechanisms explain the induction of apoptosis by Piperine. One of the most common mechanisms is increased caspase-3 activity.58 Administration of Piperine decreases the integrity of the mitochondrial membrane, which results in cytochrome C and Smac/DIABLO release into the cytoplasm. This event induces the formation of the apoptosome and the activation of the caspase-9 initiator. Meanwhile, Smac/DIABLO causes apoptosis after binding with the inhibitor of apoptosis proteins (IAPs), eliminating the apoptosis brake. The activation of caspase-3 induces degradation of the protein poly (ADP-ribose) polymerase (PARP), which then culminated in protein, nucleic acid, and membrane disintegration.58

Piperine also induces apoptosis through the DNA damage pathway observed from the examination of terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick-end labeling (TUNEL). Piperine induces apoptosis by suppressing FAS expression, mainly by blocking the ERK1 / 2 signaling pathway, which leads to suppression of SREBP-1c expression.68 Khamis et al. and Pachauri et al. also found a similarity which was marked by an increase in the expression of the pro-apoptotic protein Bax and a decrease in the expression of the anti-apoptotic protein Bcl2.43,69 Furthermore, Rad et al. and Greenshields et al. showed that Piperine reduced the expression of phosphatidylinositol-3-kinase/Akt (protein kinase B), which is a pro-survival signaling pathway in cancer cells.44,69
**Anti-Metastatic Effect**

Piperine also has the ability as an anti-migration and anti-metastatic agent in breast cancer. Do et al. showed that adding 25 to 50um Piperine could inhibit the migration of breast cancer cells in the SKBR3 breast cancer by 35.6% to 57.6%. Greenshields et al. showed that 50um Piperine inhibited metastasis in breast cancer cell line MDA-MB-231, while Pachauri et al. showed that it inhibited the migration of MCF-7 breast cancer cell lines with an IC50 at 130um. Molecularly, the metastasis in breast cancer cell lines can be inhibited by Piperine due to decreased expression of MMP-2 and MMP-9.

In-vivo studies also showed similar findings. Lai et al.’s study demonstrated the ability of Piperine as an anti-metastatic agent through the inhibition of migration of the 4T1 breast cancer model mice caused by decreased expression of MMP-13 and MMP-9 proteins. In this study, pulmonary metastasis was also successfully inhibited. The ability of Piperine to inhibit metastases in vivo is due to the inhibitory effect of Piperine on the expression of cyclooxygenase-1, oxygenase, and p450 isoenzyme.

The inhibition of metastasis and migration by Piperine is largely due to decreased MMP protein expression. MMP is one of the important proteins involved in the metastasis process of cancer cells. MMP plays a role in degrading the extracellular matrix in the basal membrane layer of cancer cells, allowing cancer cells to migrate to other organs via blood vessels. Piperine suppresses EGF-induced MMP-9 by suppressing transcriptional activation of the MMP-9 promoter consisting of an Nf-Kb site and two AP-1 sites. Piperine suppressed the transcriptional activity of Nf-Kb and AP-1 through inhibition of Akt expression and the MAPK and phosphatidylinositol-3-kinase signaling pathways.

**Chemosensitizer**

Piperine can also increase the effect of some chemotherapeutic agents or interventional radiology on breast cancer therapy. Several previous studies have shown that Piperine increased the effectiveness of paclitaxel. Do et al. showed increased anti-proliferative, cytotoxic, and apoptotic effects of paclitaxel on the SKBR3 breast cancer cell line compared to the cell line treated with paclitaxel alone. Pachauri et al. reported that Piperine increased the effectiveness of paclitaxel therapy in both paclitaxel-sensitive and paclitaxel-resistant breast cancer cell line MCF-7. This combination provided a good proliferation inhibitory effect but at a lower dose, thereby reducing the risk of toxicity to paclitaxel therapy. On the other hand, Motiwala et al. showed that Piperine has a synergistic effect with paclitaxel as a chemotherapeutic agent in breast cancer. This was demonstrated by their ability to inhibit the growth of breast cancer cell line MCF-7. Burande et al. also showed that the combination of Piperine and paclitaxel had a better cytotoxic effect than paclitaxel alone.

The mechanism of the synergistic effect of Piperine and paclitaxel is not clearly understood, but it seems to be associated with EGFR blockade.

Khamis et al. reported that Piperine had a synergistic effect with tamoxifen with a good combination index (CI) (0.279), indicating good anti-proliferative and cytotoxic effects on breast cancer cell lines MCF-7 and T47D. Piperine also enhances the effect of an interventional radiology agent such as gamma-ray radiation. Greenshields et al. reported that Piperine increased the anti-proliferative effect of gamma-ray radiation on TNBC cancer cells. Additionally, Piperine also has a synergistic effect on the developmental chemotherapy agent rapamycin as reported by Khatyra et al. Piperine was able to increase rapamycin absorption by decreasing P-gp expression. The administration of rapamycin with Piperine also showed potent cytotoxic activity in the breast cancer cell line MDA-MB-231 at a lower than standard dose.

Also, Abdelhamad et al. demonstrated the effect of Piperine in combination with a TRAIL-based anticancer agent. This combination produced a synergistic effect that manifested as growth inhibition and apoptosis induction through increased expression of caspase-3 and PARP cleavage, inducing cell cycle arrest in the G2 / M phase MDA-MB-468 and MDA-MB-231 breast cancer cell lines. Piperine was even able to increase the effectiveness of TRAIL-based anticancer agents by suppressing survivin expression and p65 phosphorylation.

**Cytotoxic Effect**

Piperine can induce cytotoxic effects on cancer cells through several mechanisms. Research by Do et al. showed that Piperine induced cytotoxicity in the SKBR3, BT-474, and MDA-MB-231 cancer cell lines with basal Her2 expression. Rad et al. also showed that Piperine had a cytotoxic effect on the breast cancer cell line MDA-MB-468, while Schmidt et al. reported a similar effect on MCF-7 with an IC50 value of 94.5 um. There were also 27.41% of cells whose growth was inhibited which was marked by a decrease in mitochondrial membrane potential by 1.17 times.

The ability of Piperine to induce cytotoxic effects in breast cancer resulted through several mechanisms. Piperine can induce cytotoxic effects through its entry into the lysosomal membrane of cancer cells, which in turn causes the release of cytotoxicity-inducing cathepsins. Besides, Piperine also reduces the potential of the mitochondrial membrane by increasing ROS production. This condition causes DNA damage and apoptosis induction which lead to cell death. Piperine also reduces Glyoxalase I (GLO1) activity by 1.24 times. GLO1 is an enzyme that plays a role in converting MG to D-lactate. Several previous studies have shown that this enzyme plays a role in regulating the proliferation, migration, metabolism, and survival of cancer cells. Therefore, the inhibition of GLO1 reduces the pro-tumorigenesis activity of cancer cells.

**Anti-angiogenic**

Angiogenesis is a potential therapeutic target in cancer therapy by limiting the number of nutrients and oxygen supply to cancer cells. Talib et al. showed that Piperine capable of inducing anti-angiogenic activity, which was characterized by a significant reduction in vascular endothelial growth factor (VEGF) level to 177.5 pg / mL to the control group (890.4 pg / mL). VEGF is a protein that is responsible for the angiogenesis process. Expressed VEGF will attach to the VEGF-1 and VEGF-2 receptors that are attached.
Immu-no-modulatory Effect
The immune system is one factor that plays a role in cancer progression, especially breast cancer. In general, the cancer condition will encourage a shift in the immune system’s balance to be more inclined towards a Th2 response which is dominated by the cytokine IL-4. Several previous studies have shown that Piperine could act as a modulator of the immune response against breast cancer cells by increasing several types of cytokines. Talib et al. showed that Piperine administration increased the expression of cytokines typical of the Th1 immune response, which was marked by an increase in the expression of the cytokines IFN-γ and IL-2. This suggests Piperine has the potential to increase the anticancer Th1 immune response.62

Cancer Stemness Inhibitor
The hypothesis of stem cells in the pathogenesis of breast cancer evolved in the tissue stem cells that are subject to regulatory disorders. Regulatory disorders occur in the self-renewal process or progenitor cells. Research by Kakarala et al. showed that Piperine administration could inhibit the ability of self-renewal breast tissue stem cells. Piperine was able to inhibit the formation of mammosphere in breast stem cells. The inhibition of mammosphere formation is very important in preventing cancer because the formation of the mammosphere is a strong indication of stem cells’ self-renewal capability. The self-renewal inhibition capability of Piperine was also confirmed by reducing the expression of breast stem cell marker, ALDH1.66 Piperine inhibits breast tissue self-renewal ability by inhibiting the Wnt signaling pathway. As is well known, Wnt signaling pathways are often dysregulated in breast cancer. The Wnt signaling pathway induces breast tissue tumor cells originating from stem cells or progenitor cells through the LRP5/6-mediated beta-catenin activation pathway and the mTOR pathway.66 So, the suppression effect of Piperine works specifically on stem cells/progenitor cells of breast tissue so that it does not affect differentiated breast tissue cells.66

CONCLUSIONS
Piperine is a potential anticancer agent in breast cancer because it has anti-proliferative effects, induces apoptosis, anti-migration or anti-metastasis, chemosensitizers, cytotoxic agents, anti-angiogenesis, immune response modifiers, and inhibits self-renewal abilities. The potential delivery agents for Piperine administration include PLGA, PEG-PLGA and liposomes.

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AUTHOR CONTRIBUTION
All authors have contributed from concepting, literature searching and preparing this article.

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