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Review

Piperlongumine, a potent anticancer phytotherapeutic: Perspectives on contemporary status and future possibilities as an anticancer agent

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ABSTRACT

Piperlongumine, a white to beige biologically active alkaloid/amide phytochemical, has high pharmacological relevance as an anticancer agent. Piperlongumine has several biological activities, including selective cytotoxicity against multiple cancer cells of different origins at a preclinical level. Several preclinical studies have documented the anticancer potential of piperlongumine through its targeting of multiple molecular mechanisms, such as cell cycle arrest, anti-angiogenesis, anti- invasive and anti-metastasis pathways, autophagy pathways, and intrinsic apoptotic pathways *in vitro* and *in vivo*. Mechanistically, piperlongumine inhibits cancer growth by resulting in the accumulation of intracellular reactive oxygen species, decreasing glutathione and chromosomal damage, or modulating key regulatory proteins, including PI3K, AKT, mTOR, NF-kβ, STATs, and cyclin D1. Furthermore, combined treatment with piperlongumine potentiates the anticancer activity of conventional chemotherapeutics and overcomes resistance to chemo- and radio- therapy. Nanoformulation of piperlongumine has been associated with increased aqueous solubility and bioavailability and lower toxicity, thus enhancing therapeutic efficacy in both preclinical and clinical settings. The current review highlights anticancer studies on the occurrence, chemical properties, chemopreventive mechanisms, toxicity, bioavailability, and pharmaceutical relevance of piperlongumine *in vitro* and *in vivo*.

1. Introduction

Cancer remains the most devastating and life-threating disease worldwide [1]. In recent years, the number of new cancer cases with high mortality has drastically increased globally, including in India, mainly because of the high population rate, lifestyle changes, lower physical activity, obesity, and aging [2]. Approximately 1.4 million cancer cases were reported in 2015 in India, and this figure is expected to increase to 1.74 million in the year 2020 [3]. Current treatment modalities for cancer include chemotherapy, radiotherapy, surgery, and immunotherapy. However, all treatment modalities (except surgery) are associated with serious side effects and resistance within several months of treatment. Therefore, there is an increasing need to discover novel anticancer agents with the potential to target multiple factors in cancer with nominal side effects.

To date, many plant-based products, including alkaloids, have shown promising therapeutic anti-cancer potential alone or in combination with conventional anticancer therapeutics [4]. Most of the phytochemicals have shown chemo- and radio- sensitizing effects in chemo- and radio-resistant cancer cells [5]. Interestingly, most of the phytochemicals have selective toxicity for human tumor cells rather than normal cells and have minimal or negligible side effects [6]. Piperlongumine (piplartine) is a well-known biologically active alkaloid/amide found in the roots of the plant *Piper longum* (long pepper) [7].

Abbreviations: ROS, reactive oxygen species; PI3K, phosphatidylino sitol-3-kinase; AKT, protein kinase B; Gsk3β, glycogen synthase kinase 3 beta; mTOR, mammalian target of rapamycin; RAS, rat sarcoma; RAF, v-raf murine sarcoma viral oncogene homolog B1; MEK, mitogen- activated protein kinase; ERK, extracellular signal-regulated kinase; Keap1, Kelch-like ECH-associated protein 1; Nrf- 2, nuclear factor erythroid 2 (NFE2)-related factor 2; TNFα, tumor necrosis factor alpha; IKKβ, small molecule inhibitors of IκB kinase β; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; CDK, cyclin-dependent kinase; STAT3, signal transducer and activator of transcription 3; HK2, hexokinases2; SETDB1, SET domain bifurcated histone lysine methyltransferase 1; FosB, FBJ murine osteosarcoma viral oncogene homolog B; PARP, poly ADP ribose polymerase; JNK, c-Jun N-terminal kinase; Cyto C, cytochrome C; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2 associated X; HO1, heme oxygenase; CIAP-1/2, cellular inhibitor of apoptosis protein-1/2; VEGF, vascular endothelial growth factor; TWIST, twist-related protein 1; MMP-9, matrix metallopeptidase 9; CXCR-4, C-X-C chemokine receptor type 4; IL-6, interleukin 6; ABCG1, ATP binding cassette subfamily G member 1; P-gp1, permeability glycoprotein 1; MRP1, multidrug resistance-associated protein 1; GSH, glutathione

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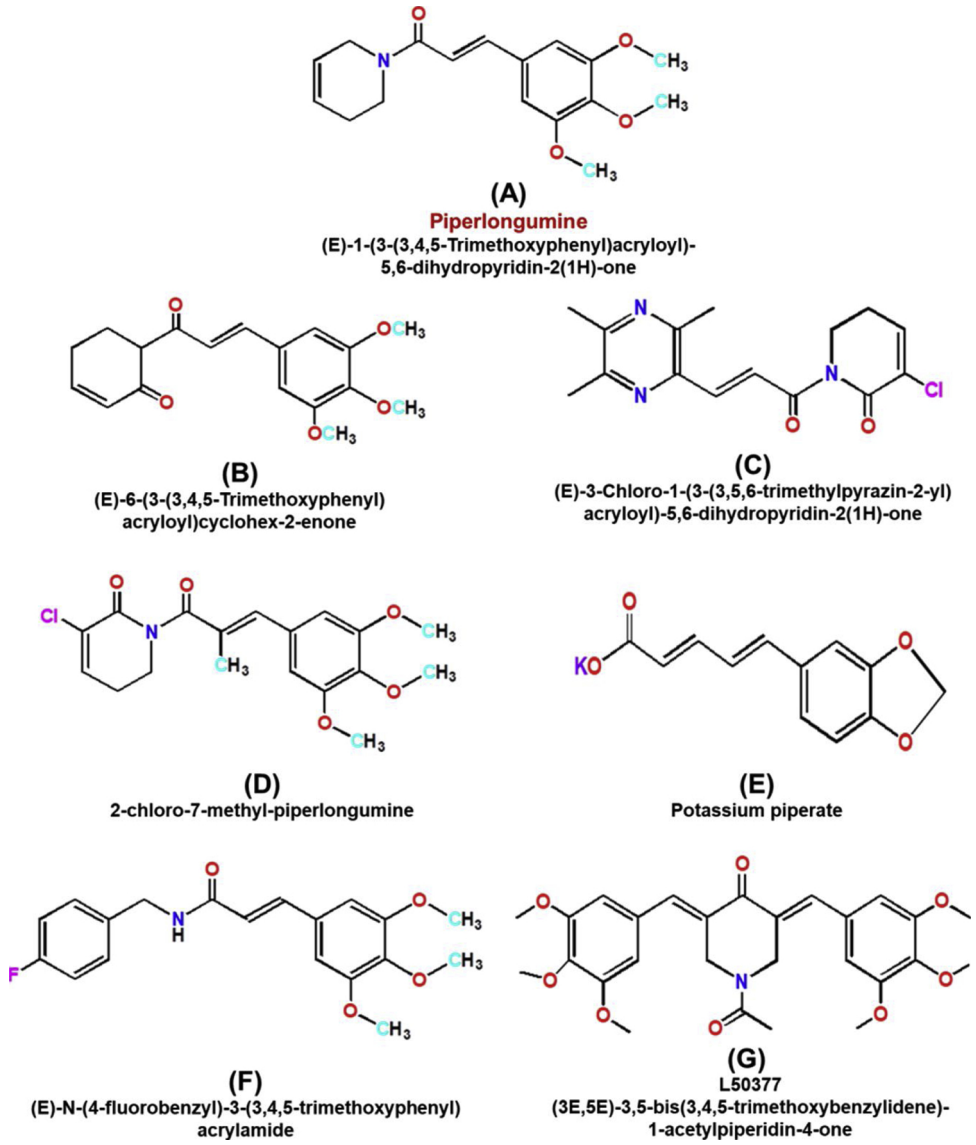

Fig. 1. Chemical structures of piperlongumine (A) and its synthetic analogs (B–G).

Interestingly, piperlongumine shows cytotoxicity against a variety of cancer cells but causes minimal harm to normal human cells, thus confirming its selectivity toward cancer cells over normal human cells [8]. Many *in vitro* and *in vivo* studies have shown that piperlongumine exhibits anxiolytic, antiangiogenic, antidepressant, anti-metastatic, anti-tumoral, cytotoxic, genotoxic, antibacterial, antifungal, and anti-diabetic activity [9]. An effective decrease in tumor growth without considerable side effects has been observed in tumor xenograft models treated with piperlongumine [10]. Available studies suggested that piperlongumine induces cytotoxicity in cancer cells mainly through the accumulation of intracellular reactive oxygen species (ROS) [11]. Piperlongumine has modulatory effects on various aberrant mechanisms involved in inflammation and proliferation (*e.g.*, IL-6, TNF-α, and COX-2), angiogenesis and metastasis (*e.g.*, VEGF, MMP9, TWIST, CXCR4, NF-κB, and STAT), cell survival (*e.g.*, Bcl-2, Bcl-xL, survivin, and CIAP1/2), and cell cycle regulation (*e.g.*, cyclin A, cyclinD1, Cdks, p21, and p53) [7]. Keywords used during the online literature survey in electronic databases including PubMed are phytochemicals, cancer, piperlongumine, anticancer potential of piperlongumine, piperlongumine in cancer drug resistance, anticancer potential of piperlongumine as combinatorial drug, solubility and bioavailability of piperlongumine, pharmaceutical relevance of piperlongumine, and current clinical status of piperlongumine. Datasets outline in the manuscripts are mainly covered recent studies published from 2011 to 2020. However, some highly relevant studies to our manuscript published before 2011 have also been included. This review summarizes and discusses the chemical properties, pharmacokinetics, anticancer potential, toxicity, and pharmaceutical relevance of piperlongumine. It also discusses the molecular mechanisms targeted in piperlongumine's anticancer activity, the combinatorial therapeutic potential, and the chemo- and radio-sensitizing effects of piperlongumine in cancer treatment. With this review on the phytochemical piperlongumine, we aim to provide useful insights as well as a basis for clinical studies to develop piperlongumine as an anticancer drug.

2. Sources and chemical properties of piperlongumine

Piperlongumine (alkaloid/amide) is a naturally occurring compound in herbs and spices of the *Piper* species. Among 5166 molecules, piperlongumine was screened as a potent anticancer agent during an anticancer drug screening program (2000–2007) at the Laboratorio de Oncologia Experimental, Universidade Federal do Ceara [12]. It is also occurred in some other *Piper* species, such as *Piper alatabaccum* Trel. & Yunck [13], *Piper arborescens* Roxb. [14,15], *Piper chaba* Hunter [16],

Piper cenocladum C. DC. [17], *Piper puberulum* Benth. [18], *Piper sylvaticum* Roxb. [19] and *Piper tuberculatum* Jacq. [20]. Piperlongumine is a white to beige, crystalline solid phyto-compound with strong biological activity. Its IUPAC name is 1-[(2*E*)-3-(3, 4, 5-trimethoxyphenyl) prop-2-enoyl]-5, 6- dihydropyridin-2(1*H*)-one [7]. The chemical formula of piperlongumine is $C_{17}H_{19}NO_5$, its molar mass is 317.34 g/mol, and its melting point is 124 °C. Piperlongumine has a solubility approximately 26 μg/ml in water, and the addition of 10 % Tween 80 increases the water solubility by 27 fold. However, piperlongumine has better solubility in organic solvents, such as ethanol (0.150 mg/ml), dimethyl sulfoxide (20 mg/ml), and dimethylformamide (20 mg/ml). Finally, piperlongumine can be stored at −20 °C for up to 2 years.

3. Chemical structures of piperlongumine and its synthetic analogs

Poor aqueous solubility is a major concern for phyto-based drugs, because it decreases absorption of drugs across intestinal cell membranes. Therefore, the pharmacokinetic profiles may need to be optimized by preparing synthetic analogs to enhance the bioavailability of phytochemicals. To date, various synthetic analogs of piperlongumine have been synthesized to improve its aqueous solubility, bioavailability, and pharmacological activity [21–26]. The chemical structures of piperlongumine and its main synthetic analogs are shown in Fig. 1.

4. Toxicity of piperlongumine

To date, medicinal plants have been of great pharmacological interest, including in chemoprevention, owing to their high availability and affordability worldwide. Numerous studies have suggested the therapeutic potential of natural products with minimal side effects, although safety concerns have been raised and must be addressed before they are used as anticancer drugs. The plant purified compound piperlongumine shows several biological activities, including cytotoxicity against various types of cancers from different origins. However, none of the health hazard reports associated with piperlongumine exposure have examined its production and processing. No epidemiological studies or other evidence is available on the cancer risk from piperlongumine use. Regarding the *in vivo* toxicological aspects, piperlongumine shows almost negligible toxicity in treated model organisms [27]. *In silico* toxicity analysis by Zeng et al. has demonstrated that piperlongumine has little toxicity; however, *in vivo* experiments have shown significant antitumor activity of piperlongumine with no considerable side effects in a mouse model [10]. A summary of preclinical studies suggesting the anti-tumoral effects along with observed side effects of piperlongumine in *in vivo* xenograft models is provided in Table 1.

Cytochrome 450 (CYP450) is a well-recognized superfamily of proteins responsible for drug metabolism and the removal of xenobiotic compounds from the human body. Piperlongumine shows competitive inhibition toward CYP450 isoform CYP1A2- catalyzed phenacetin *O*-deethylase activity *via* an NADPH-independent mechanism in HLMs *in vitro* [28]. Many studies have analyzed the genotoxic potential of piperlongumine in model organisms. Piperlongumine has shown negative Ames test results in *P. tuberculatum*, thus suggesting that the compound has no mutagenic effects in prokaryotic model systems [29]. In contrast, piperlongumine has been found to have a mutagenic effect through DNA damage *in vitro* and *in vivo* in a eukaryotic system [30,31]. Another study by Bezerra et al. has suggested that piperlongumine induces mutagenic effects in V79 cells with micronucleus formation [30]. However, administration of 50 mg/kg piperlongumine has not been found to result in micronucleus formation, although micronucleus formation has been observed after 100 mg/kg administration of piperlongumine in a model system [30]. Overall, studies have suggested that piperlongumine does not show any considerable toxicity *in vitro* and *in vivo*, and thus may be introduced as an anticancer drug after rigorous clinical studies.

Table 1
Anti-tumor potential and side effects of piperlongumine in different tumor models.

Xenograft model	Cell type	Treatment Dose (mg/kg)	Observations	Ref.
Seven weeks old male BALB/C nudemice	A549 cells	2.5 mg/kg and 5 mg/kg of body weight twice per week for 3 weeks	Suppressed lung tumor growth, weight, and volume as per the increasing dose of piperlongumine. Also, induced apoptosis of lung tumor cells without any side effects in mice.	[10]
Five to six weeks old nude mice	MDA-MB-468 cells	15 mg/kg/day of body weight for 21 days	Suppressed breast tumor weight by > 50 % with 40 % decrease in phosphorylation level of STAT3 in tumor cells as compared to control. No side effects were seen in piperlongumine treated mice.	[32]
Female Nu/Nu mice	HT29 cells	5 mg/kg/day and 10 mg/kg/day of body weight for 20 days	Delayed colon tumor growth in a dose-dependent manner and initiated apoptosis in tumor cells by decreasing mutant p53 protein levels with increasing cleaved PARP and cleaved caspase-3 levels. Furthermore, combined treatment of piperlongumine (7.5 mg/kg/day) and cisplatin or doxorubicin shows better tumor regression activity as compared with either compound alone. No side effects were seen as appeared with chemotherapy in piperlongumine treated mice either alone or in combination.	[33]
Nude mice	HGC27 cells	3.6 mg/kg/day of body weight for 16 days	Suppressed growth, weight, and volume of gastric tumor. Anti-tumor activity was found *via* decreased expression of proliferation marker gene Ki67 and enhanced expression GADD45α as compared to tumors of control mice. No side effects were seen in piperlongumine treated mice.	[34]
Adult male Kunming mice	H22 hepatoma cells	1.5 mg/kg/day, 2.5 mg/kg/day, and 3.5 mg/kg/day of body weight for 14 days	Reduced hepatoma tumor growth, weight, and volume as per the increasing dose of piperlongumine. Activated p-PERK and MAPKs (such as p38, JNK, and Erk) with up-regulated Ire 1α, PDI, and CHOP were observed in piperlongumine treated H22 tumor xenografts.	[35]
Four to five weeks Balb/c nude female mice	A2780cells	20 mg/kg/day of body weight for 15 days	Reduced ovarian tumor weight and volume as compared to control. Piperlongumine treated tumor tissue were shown low expression of survivin. Well tolerable and no severe weight loss was observed.	[36]
Five to six weeks female nude mice	NCI-H929 cells	50 mg/kg of body weight after 5 consecutive days in a week for 3 weeks	Delayed myeloma tumor growth was observed as compared to control. Piperlongumine treated tumor cells showed increased cleaved caspase-3 and decreased phosphorylation of STAT3. No weight loss and side effects were seen in mice.	[27]

5. Pharmacokinetic studies and bioavailability of piperlongumine

Rapid metabolism, early excretion, and low bioavailability are major drawbacks with biological agents, which decrease their potential as anticancer agents [37]. For a compound, bioavailability data are very important to determine the minimum dose of a drug required to treat a disease. Data from preclinical studies have indicated satisfactory bioavailability of piperlongumine [38]. The analysis of the plasma of piperlongumine treated mice (50 mg/kg) after intraperitoneal administration, 1511.9 ng/ml, 418.2 ng/ml, and 41.9 ng/ml concentrations of plasma piperlongumine were found at 30 minutes, 3 hours, and 24 hours, respectively [39]. Fofaria et al. study has suggested that 2 mg/kg intravenous injection of piperlongumine in mice shows very low Cmax values approximately 4339.608 ng/mL with high volume distribution (140.449 mL) of piperlongumine at steady state [40]. Therefore, it could conclude that due to high volume distribution; low plasma exposure of piperlongumine has been observed as compared with the administrative dose. Moreover, the bioavailability is significantly improved after oral administration of piperlongumine [40]. Unfortunately, there is no preclinical and clinical data associated with calculated oral bioavailability of piperlongumine have been available till date. In 2017, Liu et al. investigated the binding effect between piperlongumine and human serum albumin (HSA) protein under virtual physiological conditions [41]. Their study has revealed that piperlongumine promotes the unfolding of HSA polypeptide and formation of HSA–piperlongumi ne complexes *via* hydrophobic forces, with a binding distance (r) 3.199 nm. Furthermore, the results suggest efficient binding between HSA and piperlongumine. HSA- piperlongumine binding increases with increasing temperature, thus indicating that temperature increases may facilitate delivery of piperlongumine *via* an increase in binding with HSA [41]. An *in silico* (molecular docking) study has also revealed that piperlongumine and HSA interact *via* hydrophobic interactions, such as hydrogen bonds and van der Waals interactions [42]. The metabolic fate of piperlongumine at the clinical level remains unexplored. Moreira and colleagues have demonstrated the metabolic profile of piperlongumine in human liver microsomes (HLMs) *in vitro* and *in vivo* [43]. CYP450 family enzymes are well known for their roles in drug metabolism in the human body; therefore, investigating the effects of these enzymes on novel drugs is very important. Four novel compounds have been recovered after piperlongumine metabolism from HLMs. The metabolic profiles after piperlongumine metabolism from HLMs show low hepatic extraction, thus indicating negligible CYP450 enzyme based first-pass metabolism [43]. However, the consistent small clearance (CL) value with liver blood flow indicated that piperlongumine elimination is strictly controlled by CYP450 enzymes. The low hepatic extraction ratio indicated piperlongumine hepatic metabolism and elimination *in vivo* [43]. Studies have suggested that CYP 1A2 contributes to bio- activation of secondary cancer-causing agents, which may cause toxicity to cells. Song et al. have revealed that piperlongumine competitively inhibits CYP1A2 in HLMs [28]. In addition, combined treatment with piperlongumine and docetaxel (DTX) effectively increases the oral bioavailability of docetaxel, to levels 1.68 fold higher than those of docetaxel alone in Sprague Dawley rats [38]. Moreover, co- treatment with piperlongumine effectively inhibits the metabolism of DTX by cytochrome enzymes and P-glycoprotein (P-gp) efflux (from 2.37 to 1.52), and increases $T_{1/2}$ by 2 fold. These results indicate that piperlongumine can improve the bioavailability of other existing anticancer drugs, including DTX [38]. Attempts to enhance the bioavailability of piperlongumine by modifying its structure or synthesizing polymeric nanoparticles have been undertaken in many *in vitro* and *in vivo* studies. A brief discussion on those studies is given in the piperlongumine pharmaceutical relevance section of this review.

6. The pharmaceutical relevance of piperlongumine

To date, phytotherapy provides a unique and potent therapeutic strategy to treat cancer. It shows selectivity toward human cancer cells over normal cells and has minimal side effects. Piperlongumine derived from plants shows multivalent effects, including selective cytotoxicity against tumor cells with low or no adverse effects in model organisms. However, its low aqueous solubility affects its anti-cancer activity by limiting its bioavailability during oral administration [44]. Hence, nano- encapsulation of phytochemicals, including piperlongumine by using water-based formulations may improve the water solubility and delivery *via* overcoming poor bioavailability related issues in cancer therapy [45]. Micelle formulations of piperlongumine provide high encapsulation efficiency within very small particle sizes, thus improving its solubility and stability in water [46]. *In vitro* assessment of polymeric micelle encapsulated piperlongumine has indicated that polymeric encapsulation significantly improves cytotoxicity, cellular uptake, ROS, oxidized glutathione disulfide, and reduced glutathione (GSH) levels in mouse colon cancer cells (CT26). Furthermore, *in vivo* study of these polymeric piperlongumine micelles has shown significant improvement in many anticancer activities, including the anti-proliferative, anti- migratory, anti-invasive, anti-tube formation activities in human umbilical vein endothelial cells (HUVECs), and tumor growth-inhibiting activity with lower toxicity than that of piperlongumine [46]. Encapsulation of piperlongumine in another biocompatible natural polymer, chitosan, has been found to result in pH-dependent piperlongumine release and to enhance cytotoxicity *via* efficient intracellular ROS accumulation against human gastric carcinoma cells [47]. Choi and colleagues have also formulated chitosan and fucoidan combination (CS–F) encapsulated piperlongumine nanoparticles [48] and observed that CS-F encapsulation effectively improves the water solubility and bioavailability of piperlongumine. Moreover, CS-F encapsulated piperlongumine significantly induces higher cell death of prostate cancer cells than normal human dermal fibroblast cells *via* intracellular ROS accumulation [48]. However, methoxy poly (ethylene glycol)-grafted chitosan (ChitoPEG) encapsulated piperlongumine nanoparticles show similar anticancer activity to that of piperlongumine against A549 and CT26 cells *in vitro* [49]. ChitoPEG encapsulated piperlongumine nanoparticles have been effectively delivered to the tumor mass and found to induce better anti-metastatic and anticancer activity in a CT26 cell pulmonary metastasis mouse model [49]. Considering the delivery related issue of piperlongumine, Carvalho et al. have found that chitosan or hyaluronic acid-based nano-encapsulation of piperlongumine has the same bio-adhesive potential *in vitro*, prolonging mammary tissue retention (to 120 h) without damaging the tissue [50]. Furthermore, the study indicated stability of piperlongumine in both nano-formulations for more than 60 days, thus indicating strong effects of both nano- encapsulations on the delivery and stability of piperlongumine. Moreover, Fofaria et al. have developed piperlongumine loaded nano- emulsions by using capmul PG8 in combination with Tween 80 and PEG 400 [40]. Nano- emulsions of piperlongumine effectively improved its solubility (>2 mg/mL), with higher stability and a low polydispersity index. Cellular permeability and anti-tumor activity have been found to be significantly increased in melanoma tumor-bearing mice (10 mg/kg) without any sign of toxicity after long-term administration [40]. Interestingly, pharmacokinetic analysis has revealed 1.5 fold increases in the oral bioavailability of piperlongumine encapsulated nano-emulsions as compared with that of free piperlongumine [40]. Sharkey et al. have examined a very interesting combination therapy for cancer treatment, in which piperlongumine and TRAIL encapsulated poly (lactic-co-glycolic acid) nanoparticles were synthesized [51]. Combined drug encapsulated nanoparticle treatment showed higher cytotoxicity against PC3 and HCT116 cells than did single drug encapsulated nanoparticles [51]. Similarly, combined nano-encapsulation of piperlongumine with paclitaxel in poly (lactic-co- glycolic acid) and D-α- tocopheryl

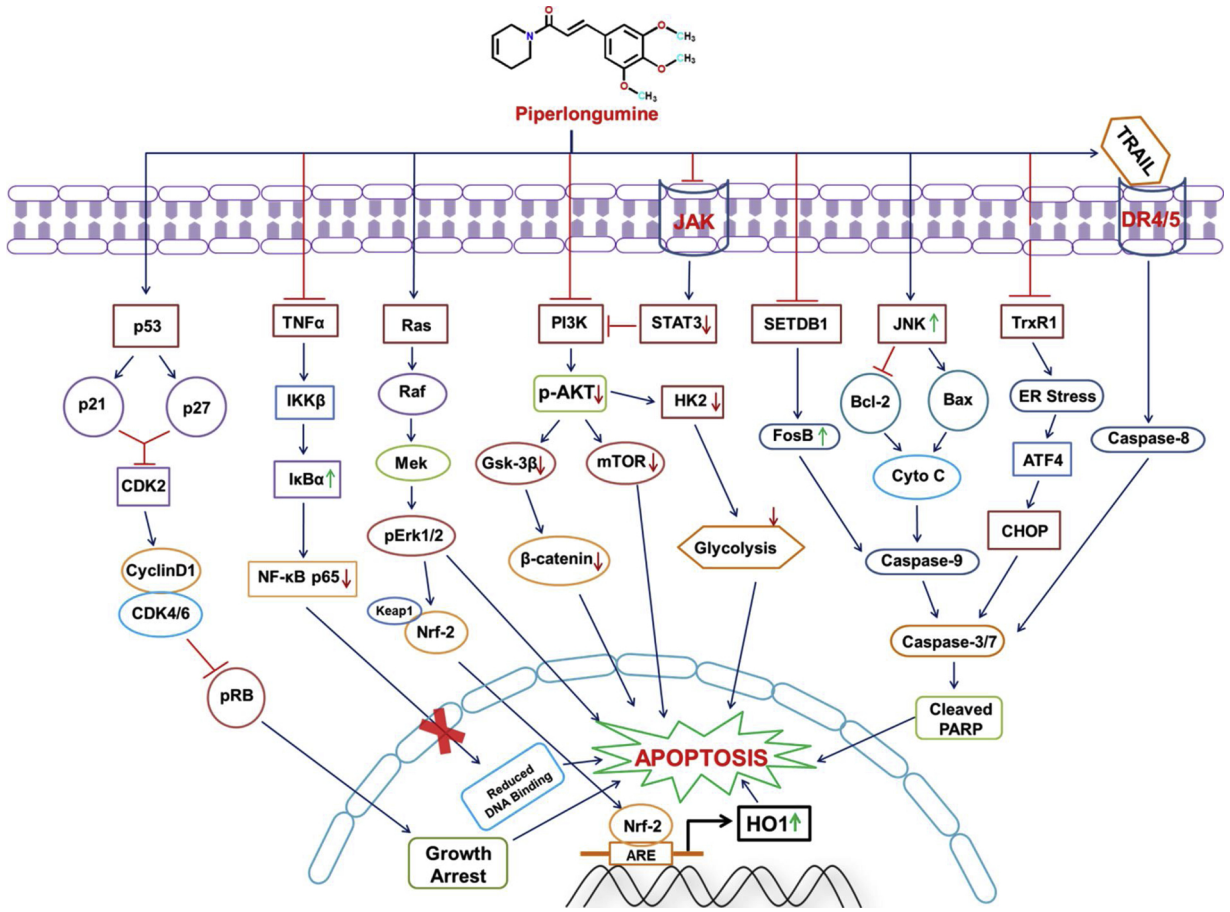

Fig. 2. Schematic depiction of piperlongumine regulated aberrant signalling pathways involved in cancer initiation and progression.

polyethylene glycol succinate facilitated more intracellular uptake of paclitaxel with higher cytotoxicity toward HepG2 cells than free paclitaxel [52]. *In vivo* analysis of the same nanoparticle has shown improved antitumor activity with less toxicity than that of free paclitaxel in the HepG2 xenograft tumor model [52]. Given these results, nanoformulation strategies may overcome this solubility and bioavailability related issues with the phyto-drug piperlongumine. However, rigorous clinical trials are needed for further validation.

7. Anticancer potential along with molecular mechanisms and targets of piperlongumine toward different cancer types

Piperlongumine has been documented to have efficient pharmacological activities, including anticancer activity *in vitro* and *in vivo*. The compound can selectively target and kill cancer cells by activating multiple molecular and cellular mechanisms involved in apoptosis. Recent studies on the anticancer activity of piperlongumine have described its modulatory effect on several aberrant signaling pathways involved in cancer initiation and progression, including phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), nuclear factor kappa B (NF- κB), and cyclooxygenase-2 (COX-2) (Fig. 2). The piperlongumine *in vitro* cytotoxicity and targeted and modulatory aberrant mechanisms are summarized in Table 2.

7.1. Piperlongumine and lung cancer

Activation of the NF-κB signaling pathway has been reported as a main factor causing the initiation and progression of many cancers, including lung cancer. Zheng et al. have established a link between piperlongumine and NF-κB through a docking model and pull-down assays, and have found that piperlongumine binds with the p50 subunit of NF-κB in a concentration- dependent manner [10]. The researchers have suggested that piperlongumine decreases the DNA binding ability of NF-κB, thereby promoting apoptosis in lung cancer cells. In addition, treatment with piperlongumine with phenylarsine oxide (an NF-κB inhibitor) or p50 inhibition reverses the apoptotic effect of piperlongumine *via* Fas and DR4 activation. Furthermore, treatment with piperlongumine in p50 mutant plasmid transfected lung cancer cells inhibits the piperlongumine induced cell growth inhibition *via* down-regulation of Fas and DR4. In addition, an *in vivo* study has confirmed that piperlongumine treatment (2.5–5 mg/ kg body weight) decreases the growth of lung tumors *via* inhibition of NF-κB signaling in a xenograft mouse model [10]. However, another study has reported that piperlongumine treatment has an anti-proliferative effect by inhibiting the phosphorylation of Akt and nuclear translocation of NF-κB p65 in lung cancer cells [53]. Additionally, piperlongumine decreases the proliferation rate in a dose-dependent manner *via* ROS mediated G1 phase cell cycle arrest; decreases expression of cyclin D1, cyclin- dependent kinase (CDK)-4, CDK-6, p- retinoblastoma (p-Rb); and increases phosphorylation of extracellular signal receptor- activated kinase 1/2 (ERK1/2) in A549 NSCLC cells [53].

Earlier studies have reported the aberrant activation of STAT3 in non-small cell lung carcinoma (NSCLC). Therefore, targeting aberrant STAT3 may provide a possible therapeutic strategy to treat NSCLC. A recent study has reported elevated expression of pSTAT3, STAT3α, and STAT3β in many NSCLC cell lines [54]. Treatment with compounds either piperlongumine or C188-9 in NSCLC cells has been found to result in an approximately 50 % decrease in levels of pSTAT3 (which tightly regulates the expression of two other STAT3 proteins) and to decrease the mRNA levels of anti-apoptotic proteins by 25–60 %.

Table 2
Piperlongumine *in vitro* cytotoxic activity and targeted molecular mechanisms in different cancer types.

Cancer	Cell line	Treatment concentration (IC_{50}) 24 h	Targeted molecular mechanisms	Ref.
Lung cancer	A549	27.3 μM	Cell proliferation ↓ G1 phase cell cycle arrest ↑ ROS ↓ Oxidative stress ↑ Cyclin D1 ↓ CDK4 ↓ CDK6 ↓ p-Rb ↓ p-Akt ↓ p-ERK1/2 ↑ Nuclear translocation of NF-κB p65 ↓	[53]
Breast cancer	MCF-7	Approx. 25 μM	Cell proliferation ↓ G0/G1 phase cell cycle arrest ↑	[83]
	MDA-MB- 231	Approx. 20 μM	Cyclin D1 ↓ Apoptosis ↑ Caspase-3 ↑ Caspase-9 ↑	
	MDA-MB- 453	Approx. 20 μM	Mitochondrial membrane potential ↑ Bax ↑ Bcl-2 ↓ p21 ↑	
	BT-549	Approx. 45 μM	Mutant p53 ↓ NF-κB p65 ↓	
Colorectal cancer	HCT 116	13.9 μM	Cancer cell death ↑ Clonogenic capacity ↓ Apoptosis ↑ Damaged DNA ↑ G2/M phase cell cycle arrest ↑	[58]
Gastric cancer	MKN45	Approx. 20–25 μM	Cell proliferation ↓ Ki-67 ↓ G2/M phase cell cycle arrest ↑	[68]
	AGS		Invasion and migration ↓ MMP-9 ↓ p-JAK1, p- JAK2, and p-STAT3 ↓ Cyclin D1 ↓ Twist ↓	
Gliomas	LN229 U87 8 MG	10–20 μM	Cancer cell death ↑ ROS ↑ p-JNK ↑ p-p38 ↑ Sub-G1 phase cell cycle arrest ↑	[72]
Leukemia	U937	–	Cell proliferation ↓ Apoptosis ↑ Autophagy ↑ p-Akt/Akt ↓ mTOR ↓ p-p38 ↑ Caspase-3 ↑	[76]
Melanoma	A375 A875 B16-F10	2.5–5 μM	Cell growth ↓ Apoptosis ↑ Colony- forming ability ↓ ROS ↑ G2/M phase cell cycle arrest ↑ Mitochondrial membrane potential ↑ p21 ↑ p27 ↑ Bax ↑ Bcl-2 ↓ p-JNK ↑ Cleaved caspases-3 ↑	[99]
Prostate cancer	PC-3	4.9 μM	Cell proliferation ↓ NF-κB ↓ ROS ↑ Degradation of IkBα ↑ Nuclear translocation of NF-κB ↓	[96]
	DU-145	3.4 μM	DNA-binding activity of NF-κB ↓ Nuclear accumulation of p50 and p65 ↓ IL-6 ↓ IL-8 ↓ MMP-9 ↓ Invasiveness ↓ Adhesion ↓ ICAM-1 ↓	
Oral cancer	OCSL OC2	11.3 μM 7.4 μM	Cell proliferation ↓ ROS ↑ Apoptosis ↑ G1 phase cell cycle arrest ↑ p21 ↑ Cleaved caspases-3 ↑ PARP ↑	[87]

Furthermore, either piperlongumine (30 mg/kg; 5 days/week for 3 weeks) or C188-9 (50 mg/kg twice daily for 3 weeks) both decreases the growth, weight, and volume of A549 cell tumors in nude mice *via* decreasing the phosphorylation of STAT3 and the mRNA expression of anti-apoptotic proteins [54]. Overexpression of actin-binding proteins, such as profilin-1 (PFN1), causes a loss of sensitivity in endothelial cancer cells toward alkaloids treatment. Gagat et al. have examined the effect of piperlongumine on PFN1 expression in A549 NSCLC cells [55] and suggested the anti-proliferative and apoptotic potential of piperlongumine in the EA.hy926 and A549 cell lines. In addition, PFN1 overexpression stabilizes the endothelial lung cancer cell junction and results in a loss of sensitivity toward alkaloids including piperlongumine. In turn, knockdown of PFN1 re-sensitizes A549 cells to piperlongumine, thereby decreasing cell migration and metastasis and finally causing cell death [55]. The rate- limiting enzyme in glycolysis, hexokinase 2 (HK2), is also known for its roles in initiation and maintenance of tumors in many cancers. Zhou and collaborators have found that piperlongumine treatment decreases cell proliferation, single-cell colony-formation ability, and HK2-mediated glycolysis in NSCLC cells *via* inhibiting the interaction between HK2 and voltage-dependent anion channel 1 (VDAC1) [56]. In addition, piperlongumine induces activation of the intrinsic apoptotic pathway and inhibits Akt signaling in HCC827 and H1975 NSCLC cells. Furthermore, *in vivo* results from the same study have confirmed the antitumor potential of piperlongumine *via* inhibition of Akt signaling and HK2 mediated glycolysis in a xenograft lung tumor mouse model [56].

Subsequently, Wu et al. examined the structure-activity relationship of piperlongumine by synthesizing 16 novel structural analogs [21], two of which have shown potent cytotoxicity against lung cancer cells and modest activity against normal lung cells. Additionally, among two selected piperlongumine analogs, 2-chloro-7-methyl- piperlongumine has shown excellent antitumor potency by decreasing the tumor growth approximately 48.58 % at a dose of 2 mg/kg in a human lung cancer cell xenograft model. Interestingly, there were no side effects observed at the treatment dose (2 mg/kg) in animal models [21]. Studies have reported that the presence of a 3, 4, 5- trimethoxyphenyl ring confers anticancer potential to biologically active drugs. Structural analysis of piperlongumine confirmed that piperlongumine also contains an active 3, 4, 5- trimethoxyphenyl ring moiety, which provides anticancer potential. A recent study has reported that a novel derivative of piperlongumine, L50377, derived from a 3, 4, 5-trimethoxybenzyl moiety shows more potent anticancer activity than piperlongumine in NSCLC cells [22]. Similarly to piperlongumine, L50377 shows cytotoxicity and causes pyroptosis *via* ROS mediated NF-κB inhibition in NSCLC cells [22].

7.2. Piperlongumine and colorectal cancer

Colorectal cancer or colon cancer affect the digestive tract and are increasing in incidence worldwide [1]. Chemoprevention is one of the best treatment options for colorectal cancer because of its slow-growing nature. Treatment with the natural compound piperlongumine in the human colorectal cancer cell line HCT116 induces apoptosis and decreases cell viability by promoting the p-Jun N-terminal kinase (JNK) phosphorylation mechanism [57]. In multiple studies, piperlongumine has been found to increase the expression of p53, p21, and Bax in many cancers, including colorectal cancer [33]. In contrast, Machado et al. have reported that piperlongumine induces apoptosis of the human colorectal cancer cell line HCT116 in a manner independent of the expression of p53, p21, and Bax [58]. Furthermore, the same study did not observe a direct relationship between the treated cell's DNA and piperlongumine [58]. Regarding the effect of piperlongumine on MEK/ERK signaling in colorectal cancer, the available data suggest that piperlongumine induces cell death in the human colon cancer cell line HT-29 *via* increasing ERK phosphorylation [59]. To confirm this result, the researchers treated HT-29 cells with piperlongumine combined with the MEK inhibitor U0126 and found that U0126 significantly attenuated piperlongumine mediated colon cancer cell death [59].

An *in vivo* study has documented the antineoplastic potential of piperlongumine against 1, 2 –dimethyl hydrazine (DMH) and dextran sulfate sodium salt (DSS) induced colon cancer [60]. Mechanistically, piperlongumine downregulates the expression of Ras protein and thus PI3K signaling, thereby inhibiting the activity of other related proteins, such as Akt/NF-κB, c-Myc, and cyclin D1 in DMH + DSS induced colon tumor cells without causing any toxicity in selected model organisms [60]. Additionally, piperlongumine activates the mitochondrial apoptotic pathway by arresting colon tumor cells in the G2/M phase of the cell cycle and inhibiting Bcl-2, thereby decreasing tumor size and growth [60]. Many earlier studies indicated that piperlongumine is a good ROS inducer in cells, thus promoting and increasing the radiation response during cancer treatment. Wang et al. have examined the effect of piperlongumine (2.4 kg/mg/day for consecutive 13 days) on radiotherapy in CT26 and DLD-1 colorectal cancer cells and a CT26 tumor xenograft mouse model [61]. The results suggest that piperlongumine treatment enhances ROS production *via* decreasing GSH levels and causing thioredoxin reductase inhibition in CT26 and DLD-1 cells. Furthermore, piperlongumine causes ROS mediated G2/M phase cell cycle arrest, DNA damage, and inhibition of cellular respiration, thereby increasing the intrinsic and hypoxic radio-sensitivity of tumor cells [61]. Treatment with piperlongumine did not show tumor inhibitory effect in a CT26 tumor xenograft mouse model, while the compound has been found to increase the tumor response to radiation therapy and significantly increase the survival rate of mice without causing side effects such as weight loss. The study concluded that piperlongumine might have potential as a radio- sensitizer in colon cancer [61].

Poor water solubility limits the medicinal use of purified plant based compounds, including piperlongumine. Therefore, synthesis of analogs of these compounds by replacing some of its moieties with water-soluble moieties may overcome this solubility related issue. To address this limitation, Zou and collaborators have synthesized a piperlongumine analog by replacing its trimethoxyphenyl moiety with a ligustrazine moiety and introducing halogen chlorine (Cl) at the 3rd position [26]. Assessment of the derived compound suggested that the modified compound has 14 times greater aqueous solubility and causes greater ROS production than piperlongumine. In addition, the compound suppresses the proliferation, invasion, and migration of HCT-116 cells *via* inhibiting Wnt/β-catenin activation and Akt and GSK-3β phosphorylation mechanisms. In addition, the compound (5 mg/kg/day) treatment for 21 days decreases tumor growth of implanted colon cancer cells and lung metastasis and increases the survival rate of tumor-bearing mice [26]. Similarly, the N-heteroaromatic ring based analog (Cl at 2nd position) of piperlongumine has also shown greater aqueous solubility and more potent *in vitro* and *in vivo* activity against colon cancer than piperlongumine [62]. In another study, piperlongumine derivatives synthesized by using ruthenium have shown more potent cytotoxicity than piperlongumine in HCT-116 cells. These piperlongumine containing ruthenium complexes induce cell death in HCT- 116 cells, and this is followed by ROS mediated activation of intrinsic mitochondrial apoptotic pathways [63]. Luo et al. have synthesized compounds by mixing two natural piperamides (piperlonguminine and refrofractamide A) and examined their histone deacetylase (HDAC) inhibition activity in HCT-116 cells [64]. In preliminary studies, the compounds with a hydroxamic acid moiety and a long carbon chain were found to be good HDAC inhibitors and to show cytotoxicity in HCT-116 cells [64]. These results indicate that piperlongumine and its derivatives might be possible effective therapeutic options against colorectal cancer.

7.3. Piperlongumine and gastric cancer

The incidence of the deadliest malignant diseases such as gastric cancer is increasing worldwide; therefore, developing novel therapeutics for better curative effects is of major concern [65]. Many studies have provided evidence of the ROS mediated anticancer activity of piperlongumine. However, piperlongumine treatment inhibits gastric cancer cell proliferation with G2/M phase arrest *via* increased expression of GADD45α and decreased expression of the telomerase reverse transcriptase (TERT) gene [34]. Furthermore, the *in vivo* experiments in the same study suggest that continuous 16 day piperlongumine treatment (3.6 mg/kg/day) results in a significant decrease in gastric tumors, as compared with those in the control group, without any significant toxicity [34]. Targeting thioredoxin reductase 1 (TrxR1), a selenocysteine-containing antioxidant enzyme, is known to have ROS mediated apoptosis induction activity in many cancers. Zou and colleagues have revealed that knockdown of TrxR1 results in an increase in intracellular ROS levels in human gastric cancer cells [66]. Treatment of piperlongumine in TrxR1 knockdown gastric cancer cells increases intracellular ROS accumulation, which in turn stimulates endoplasmic reticulum stress and mitochondrial dysfunction mediated cancer cell death *in vitro*. Interestingly, piperlongumine treatment with GSH inhibitors (BSO and Erastin) shows synergistic lethality in TrxR1 knockdown gastric cancer cells [66]. Moreover, piperlongumine (4 or 12 mg/kg/day for 15 days) administration significantly inhibits increase in tumor weight and volume with less TrxR1 activity in SGC-7901 cell xenograft mouse model compared with control treated group. Interestingly, piperlongumine has well tolerable in treated group for 15 days without showing any toxicity on vital organs, such as kidney, liver, and heart [66]. The upregulated protein regulator of cytokinesis 1 (PRC1) may improve poor prognosis and overall survival in gastric cancer patients. Zhang et al. have revealed that piperlongumine targets and inhibits PRC1 *via* a p53 dependent mechanism in gastric cancer cells [67]. Therefore, the authors conclude that treatment with piperlongumine improves prognosis and overall survival in gastric cancer by inhibiting PRC1 [67]. Another study by Song and colleagues has indicated that piperlongumine treatment in gastric cancer cells decreases the phosphorylation of JNK1,2/STAT3, thereby inhibiting their effects on invasion, proliferation, and migration [68]. Diminished levels of gastrokine2 (GKN2), also known as gastric dramatic down-related gene (GDDR), have been reported in gastric cancer. Zhang et al. have found that GKN2 overexpression facilitates exposure of gastric cancer cells to drugs and enhances their cell death [69]. The authors also suggest that piperlongumine has antitumor activity *via* a GKN2 dependent mechanism. To confirm this observation, the authors subcutaneously injected piperlongumine (4 mg/kg once every other day) in mice with gastric tumors induced by GKN2 overexpressing MGC cells and GKN2 silenced SGC cells. After continued four week subcutaneous piperlongumine treatment, GKN2 silenced SGC cell induced xenografts

had larger tumors than those induced by GKN2 overexpressing MGC cells [69]. Therefore, this study concluded that piperlongumine induced anti-gastric cancer activity is related to GKN2 expression. Overall, these results strongly support the anti- tumorigenic role of piperlongumine in gastric cancer *via* targeting of deregulated signaling pathways *in vitro* and *in vivo*.

7.4. Piperlongumine and gliomas

Gliomas are associated with the most aggressive type of tumor initiates in glial cells of the brain, and have high metastatic properties in the surrounding area including the spine. The World Health Organization (WHO) has classified glioma as a grade IV brain tumor with a median survival rate of approximately 14.6 months [70,71]. Similarly to other cancers, in gliomas, piperlongumine treatment enhances the intracellular ROS levels. Piperlongumine has been found to be selectively cytotoxic toward glioblastoma multiforme (GBM) cells (LN229, U87, and 8 MG) *via* ROS dependent JNK and p38 protein activation [72]. However, activation of the Erk and Akt pathways was not observed after piperlongumine treatment in the same study. Further use of an antioxidant (NAC) with JNK and p38 specific inhibitors SB203580 and SP600125 did not show cytotoxic effects of piperlongumine, thus confirming that the anti- GBM property of the compound is mediated by intracellular ROS accumulation [72]. In a subsequent study, the researchers further confirmed the selective suppression of human glioma (LN229 or U87 MG) cell invasion and metastasis by piperlongumine *via* a ROS mediated JNK and p38 activation mechanism [73]. Overexpression of the ROS-degrading enzyme peroxiredoxin 4 (PRDX4) is known to be associated with the progression of human glioblastomas, whereas its silencing promotes apoptosis of glioblastoma cells *in vitro* and *in vivo* by increasing ROS levels. Kim et al. have reported the cytotoxic potential of piperlongumine in high-grade glioma cells by inhibiting the proper folding of overexpressed PRDX4 protein in the endoplasmic reticulum (ER) [74]. Thus, piperlongumine kills high-grade glioma cells *via* oxidative inactivation of PRDX4 mediated ROS induction, thereby inducing intracellular ER stress. Piperlongumine analogs have also been found to be very effective against many cancers. Turkez and collaborators have synthesized a synthetic derivative of piperlongumine named (*E*)- *N*-(4-fluorobenzyl)-3-(3, 4, 5- trimethoxyphenyl) acrylamide (NFBTA) and determined its cytotoxicity against the U87MG cell line [23]. *In vitro* and computational approaches have suggested the potent cytotoxic nature of NFBTA against glioma cells *via* regulation of the BRAF/MAPK and PI3K/Akt signaling pathways [23].

7.5. Piperlongumine and leukemia

Acute myeloid leukemia (AML) remains the most devastating type of blood cancer worldwide. Because of its heterogeneous nature, AML shows many types of cancer-causing mutations, which vary among patients. Pei and collaborators have shown that piperlongumine treatment in CD34+ primary human AML cells with aberrant GSH metabolism induces 60–95 % toxicity at a 10 μM concentration *via* a GSH depletion mechanism [8]. Interestingly, piperlongumine has shown limited toxicity in CD34+ normal bone marrow cells, thus suggesting the selective toxic nature of piperlongumine toward AML cells over normal human cells [8]. Another study on bone marrow mononuclear cells (BMMNCs) isolated from two patients with myelodysplastic syndrome and nine patients with acute or chronic myeloid leukemia has shown decreased viability *via* markedly increased ROS levels in BMMNCs isolated from the patients with myeloid leukemia after treatment with piperlongumine [75]. In addition, after piperlongumine treatment, p38 and JNK phosphorylation, and expression apoptotic proteins (Bax, Bcl-2, and caspase-3) and autophagic proteins (Beclin-1 and LC3B) significantly increased in BMMNCs isolated from patients with myeloid leukemia. However, piperlongumine showed no apoptotic and autophagic death effects on BMMNCs isolated from a patient with myelodysplastic syndrome [75]. Piperlongumine treatment also resulted in a significant decrease in cell proliferation, thus leading to apoptotic and autophagic death of the leukemic cell line U937 *via* activation of p38 signaling and inhibition of PI3K/Akt/mTOR signaling [76]. Although piperlongumine (piplartine) shows potent cytotoxicity against many cancers, including leukemia, Oliveira and colleagues have attempted to enhance the cytotoxic potential of piperlongumine by synthesizing its novel platinum complex derivative cis-[PtCl(PIP-OH)(PPh3)2]PF6 [77]. Treatment with this platinum-based derivative of piperlongumine in human acute promyelocytic leukemia HL-60 cells has indicated that platinum-based structural modification of piperlongumine enhances its cytotoxic potential without any changes in the apoptotic mechanism. Nonetheless, the platinum-based derivative of piperlongumine is able to target and induce ROS/ERK/p38-mediated apoptosis in HL-60 cells, a result similar to the piperlongumine mediated cytotoxicity observed in cancer cells [77]. These findings suggest that piperlongumine could be used to treat leukemia cells.

7.6. Piperlongumine and liver cancer

Liver cancer is the fifth most common cancer in males and the seventh most common cancer in females worldwide [1]. Because of its high malignancy and aggressiveness, effective treatment strategies are lacking. Therefore, development of better therapeutics to treat liver cancer is urgently needed. The plant product piperlongumine selectively kills hepatocellular carcinoma (HCC) cells without harming normal hepatocytes. *In vitro* treatment with low concentrations of piperlongumine in HCC cells shows anti-invasive and anti-migratory activity *via* elevated ROS, thus further activating the downstream ER-MAPK-C/EBP homologous protein (CHOP) signaling pathway [35]. Furthermore, administration of piperlongumine (1.5–3.5 mg /kg/day for 14 days) significantly reduces the tumor growth, weight, and volume in H22 hepatoma cell xenograft mouse model compared with DMSO treated control group [35]. Another *in vivo* and *in vitro* study by Zhang et al. has confirmed the selectivity of piperlongumine toward HCC tumor cells over normal hepatocytes [78]. *In vivo* piperlongumine (10 mg/kg every three days for 14 days) administration reduces tumor weight and volume in HUH-7 cell xenograft mouse model than control group. The researchers suggest that piperlongumine induces ROS mediated apoptosis in HCC cells *via* reduced TrxR1 activity *in vitro* and *in vivo*. There is no any sign of side effects and toxicity in vital organs (heart, liver, and kidney) of treated group appears compared with untreated mice [78]. Cancer of the bile duct system (which connects the gallbladder to the liver and small intestine) is a rare but highly malignant disease. An *in vitro* study by Chen et al. has demonstrated the potential therapeutic efficacy of piperlongumine in intrahepatic cholangiocarcinoma cells (HuCC T-1) and biliary cancer cells (OCUG-1) [79]. Piperlongumine treatment in both cell lines enhances ROS production and causes arrest of cells in distinct cell cycle stages. HuCC T-1 and OCUG-1 cancer cells are arrested in the G2/M and G0/G1 phase of the cell cycle, respectively. Furthermore, piperlongumine treatment-induces autophagy through the ROS activated Erk signaling pathway, thus resulting in the accumulation of LC3-II in biliary cancer cells [79]. Despite the potent *in vitro* anti- carcinogenic effect of piperlongumine in biliary cancer cells, the *in vivo* therapeutic potency against biliary cancer requires further exploration.

7.7. Piperlongumine and breast cancer

Breast cancer is another frequent malignancy in females in India and worldwide [1]. Breast cancer is frequently associated with the overexpression of the HER family members HER1, HER2, and HER3, which induce resistance to many drugs [80]. Piperlongumine induced ROS mediates downregulation of these receptors in breast cancer cells. However, HER2-overexpressing BT474 and SkBr3 breast cancer cells show slightly more sensitivity toward piperlongumine than do MCF-7

cells with low HER2 expression [80]. In addition, piperlongumine shows more selective cytotoxicity against human breast cancer MCF-7 cells than human breast epithelial MCF-10A cells [81]. In a mechanistic analysis, nuclear factor erythroid-2- related factor-2 (Nrf2) activation has been found to mediate the upregulation of heme oxygenase-1 (HO-1) in piperlongumine treated MCF-7 and MCF-10A cells [81]. HO-1 downregulation induces resistance to piperlongumine in human breast cancer MCF-7 cells but enhances enhanced apoptosis in human breast epithelial MCF-10A cells after piperlongumine treatment. Thus, HO-1 expression promotes piperlongumine's anticancer activity against breast cancer cells but is cytoprotective in normal cells [81]. Another study by Jeong et al. has described ROS mediated anti- proliferative and anti-migratory effects in the estrogen receptor (ER)-positive breast cancer cell line MCF-7 [82]. Moreover, piperlongumine significantly modulates the mRNA expression of p21, cyclins B1/D1, cyclin- dependent kinases 1/4/6, and proliferating cell nuclear antigen (PCNA). Furthermore, piperlongumine has an anticancer effect by inhibiting the nuclear translocation of NF- κB *via* blocking IKKβ in MCF-7 cells [82]. Similarly to findings in the previous study, piperlongumine has been found to result in cell growth inhibition, G1 phase cell cycle arrest, and NF-κB pathway inhibition, thereby inducing mitochondrial apoptotic pathway mediated cell death in human triple-negative breast cancer cells [83]. Additionally, insulin-induced upregulation of p- Akt, Bcl-2, cyclinD1, p53, 4E-BP1, and p70S6K1, and downregulation of Bax and cytochrome c, are abolished by piperlongumine in human triple- negative breast cancer cells [83]. Makhov et al. have shown that piperlongumine stimulates autophagy-mediated breast cancer cell death *via* inhibiting Akt phosphorylation, thus further suppressing the expression of downstream mTORC1 *in vitro* and *in vivo* [84]. Administration of piperlongumine (20 mg/kg) or chloroquine (40 mg/kg) in PC-3 cells xenograft mouse model revealed that chloroquine alone cannot reduce the tumor growth, while piperlongumine alone significantly reduces the tumor growth. Furthermore, co-treatment of piperlongumine and chloroquine results synergistic increase in tumor growth regression property of piperlongumine than alone treated control group [84]. Deregulation of the histone methyltransferase SET Domain Bifurcated Histone Lysine Methyltransferase 1 (SETDB1) contributes to tumor growth and metastasis of a variety of cancers, including breast cancer. Park et al. have reported that piperlongumine decreases cell growth and induces apoptosis *via* downregulating SETDB1 expression in MCF-7 cells [85]. Moreover, piperlongumine induced SETDB1 downregulation further induces the transcriptional activity of FosB, thus enhancing caspase-9 dependent PARP cleavage in MCF-7 cells [85]. Another mechanism by which piperlongumine may induce apoptosis in breast cancer cells has been reported by Bharadwaj and collaborators, who have found that the compound inhibits the ligand-induced and constitutive phosphorylation and nuclear translocation of STAT3 by inhibiting the interaction between STAT3 and its peptide ligand phosphotyrosyl [32]. The same study further confirmed the anti-mammosphere formation potential (derived from tumor cells of breast cancer patients) of piperlongumine *via* STAT3 inhibition. Interestingly, of piperlongumine (30 mg/kg/day for 14 days) administration decreased tumor growth *via* 40 % decrease in pStat3 level in a MDA-MB-468 cell xenograft mouse model than control group. There is no toxicity and side effects observed in piperlongumine treated mouse model [32]. Many studies have suggested that piperlongumine analogs can overcome the limitations of piperlongumine as an anticancer agent in breast cancer treatment. In a recent study, a potassium derivative of piperate (piperlongumine) has shown potent anti- proliferative and cell growth inhibition activity by arresting breast cancer cells in G1 phase and inducing expression of p27 (an inhibitor of cyclin-dependent kinases) [24]. In addition, potassium piperate induces apoptosis in breast cancer cells by activating the mitochondrial apoptotic pathway and downregulating cyclin A, cyclin B, cyclin E, and mini-chromosome maintenance (MCM) protein expression [24]. These results suggest that piperlongumine induces anticancer activity in human triple-negative breast cancer by modulating NF-κB, PI3K/Akt/mTOR, SETDB1, and STAT3 activity. Overall, these studies suggest the therapeutic potential of piperlongumine against breast cancer.

7.8. Piperlongumine and multiple myeloma

Usually, treatment for multiple myeloma (Kahler's disease), a disease of plasma cells, has limited success, owing to advanced stage diagnosis. Many reports suggest that DNA damage is a common feature in epithelial cancer. Cottini et al. have found that overexpression of the oncogene MYC causes DNA replicative stress, thus leading to extensive chromosomal instability and DNA damage in multiple myeloma [86]. By relying on ataxia telangiectasia mutated (ATM), multiple myeloma cells compensate for the replicative stress and chromosomal instability. However, the plant product piperlongumine is also known for its DNA damaging nature *via* enhanced ROS accumulation in cancer cells. On the basis of these observations, Cottini and co- workers have inhibited expression of ataxia telangiectasia and Rad3-related protein (ATR) expression and then treated multiple myeloma cells with piperlongumine [86]. Interestingly, the combination of ATR inhibition followed by piperlongumine treatment synergistically caused significant cytotoxicity in multiple myeloma cells *via* an oxidative stress-inducing mechanism [86]. Another study has shown that piperlongumine treatment in multiple myeloma cells could potentially cause an anti-proliferative effect and suppress osteoclastogenesis. After treatment, piperlongumine directly interacted with the STAT3 Cys712 residue, thus inducing inhibition of the STAT3 signaling pathway in multiple myeloma cells [27]. Moreover, *in vivo* experiments in the same study revealed that piperlongumine (50 mg/kg five days in a week for 3 weeks) decreases tumor growth and size in NCI-H929 cells xenograft mouse model compared with a vehicle-treated xenograft mouse model. Importantly, piperlongumine enhances the survival of multiple myeloma tumor bearing mice and causes no clear toxicity [27]. These findings thus support the clinical importance of piperlongumine as a new phyto-based anticancer drug in multiple myeloma.

7.9. Piperlongumine and oral cancer

Oral cancer is one of the most common types of malignancy in head and neck carcinoma and is the eighth leading cause of mortality worldwide [1]. Treatment with piperlongumine in human oral squamous cell carcinoma (OSCC) cells (OC2 and OCSL) induces apoptosis *via* excess ROS production [87]. Mechanistically, piperlongumine elevates the expression of cyclin-dependent kinase inhibitor p21, thus leading to OSCC cell arrest in G0/G1 phase. In addition, piperlongumine enhances ROS production, thereby activating caspase-dependent pathways and initiating apoptosis in OSSC cells [87]. Development of cancer stem cell phenotypes in tumor cells promotes invasion and migration of cancer cells, thereby resulting in resistance to anticancer therapies. A recent study has suggested that piperlongumine treatment in oral cancer cells upregulates the expression of cell differentiation markers such as cytokeratin 18 and downregulates the expression of stemness marker genes, such as Nanog homeobox, POU class 5 homeobox 1, and SRY Box 2 [88]. Piperlongumine (2.4 mg/kg/day) treatment for 42 constitutive days reduces xenograft SAS tumor by 66 % than control group. The study concluded that piperlongumine mediates upregulation of differentiation markers and downregulation of stemness markers, thus indicating anti-stem cell-forming and anti-epithelial to mesenchymal transition abilities of the compound in oral cancer [88]. Overall, these studies support the potent anti-carcinogenic nature of piperlongumine against oral cancer.

7.10. Piperlongumine and ovarian cancer

Ovarian cancer is the fifth most common cancer in females globally and has a 5 year survival rate less than 45 % [1]. In ovarian cancer,

similarly to other cancers, piperlongumine treatment selectively suppresses the growth of cancer cells through a ROS dependent mechanism. Gong and collaborators have documented that piperlongumine initiates apoptosis and G2/M phase arrest, and increases ROS accumulation in human ovarian cancer cells (A2780, OVCAR3, and SKOV3) [89]. However, piperlongumine treatment with *N*- acetyl glucosamine (a ROS scavenger) drastically reverses these effects, thus indicating a ROS mediated anti-carcinogenic effect of the compound against ovarian cancer. Furthermore, combined treatment with piperlongumine (range from 0.1 to 1 μM) and existing anticancer drugs, such as cisplatin (0.1–1 &#1 81;M) or paclitaxel (0.01–0.1 μM), synergistically improves the therapeutic efficacy in human ovarian cancer cells [89]. Han et al. have prepared piperlongumine analogs by using a novel phosphonoacetamide reagent through the Horner–Wadsworth-Emmons coupling approach [90]. Their study has revealed that piperlongumine and its analogues effectively cause ROS mediated growth inhibition and cytotoxicity against colorectal (HCT 116) and ovarian (IGROV-1) carcinoma cell lines [90]. Overexpression of the well-known anti-apoptotic protein survivin results in poor survival and chemoresistance in many cancers, including ovarian cancer. Nan et al. have investigated piperlongumine induced ROS dependent depletion of survivin protein and observed a decreased growth rate of ovarian cancer *in vitro* and *in vivo* [36]. The same study further revealed that ovarian cancer cells overexpressing survivin show resistance to piperlongumine. However, the activated ROS- mediated proteasome-dependent pathway is a possible mechanism to overcome piperlongumine resistance mediated by aberrant survivin expression in ovarian cancer. *In vivo* part of the study have suggested that piperlongumine (20 mg/kg/day for 15 days) treatment reduces tumor weight and volume in A2780 cell xenograft mice without weight loss as compared with vehicle control *via* decreased survivin [36].

7.11. Piperlongumine and pancreatic cancer

Pancreatic cancer is the fourth most commonly occurring cancer in both males and females worldwide [1]. It is highly aggressive among cancers, and resistance rapidly develops against conventional therapeutics, thus resulting in the poor prognosis of this disease. *In vivo* and *in vitro* studies have suggested that piperlongumine inhibits pancreatic tumor cell proliferation by elevating ROS levels, thus leading to pancreatic cancer cell DNA damage and enhancing oxidative stress [11]. Another study has suggested that the intracellular hydrolysis product of piperlongumine (hPL) forms a bond with GSH and forms an hPL-GSH conjugate [91]. Furthermore, bond formation between the hPL-GSH conjugate and glutathione S-transferase pi 1 (GSTP1) might be a possible mechanism inhibiting GSTP1 activity, thus increasing ROS and inhibiting pancreatic cancer cell proliferation [91]. Moreover, Dhillon and co-workers have generated transcriptome analysis data through RNA- sequencing, revealing that piperlongumine shows anti-pancreatic cancer effects by altering the expression of regulatory genes in oxidative stress pathways and ER stress pathways [92]. Furthermore, the same study has suggested that piperlongumine (2.4 mg/kg/day for 30 days) intraperitoneal administration in PANC-1 cell tumor xenograft mouse causes 50 % reduction in tumor mass as compared with control treated animals without obvious side effects [92]. Activation of JNK occurs during oxidative stress, which negatively influences cancer initiation and progression. In a recent study, piperlongumine treatment has been found to activate JNK pathways *via* the dissociation of the JNK-GSTP1 interaction in pancreatic cancer cells [93]. Piperlongumine induces JNK dissociation, thus further supporting JNK, c-Jun, and early ERK activation, which are followed by suppression, activation of intrinsic pathway proteins, and nuclear translocation of c-Myc and Nrf2 in pancreatic cancer cells, thereby leading to cancer cell death [93]. Collectively, these results suggest that elevating oxidative stress is a possible mechanism through which piperlongumine shows anticancer effects toward pancreatic cancer cells.

7.12. Piperlongumine and prostate cancer

According to available data, prostate cancer is the second-most commonly occurring cancer in men worldwide [1]. Early- stage prostate cancer is curable by surgery or radiation therapy. However, in the advanced metastatic stage, prostate cancer is completely untreatable. Studies have suggested that modulation of androgen receptor (AR) signaling drives the initiation and progression of prostate cancer. Therefore, targeting AR signaling may offer a new therapeutic option for the treatment of prostate cancer. Piperlongumine rapidly decreases the expression of AR protein in prostate cancer cells at particular concentrations [94]. Treatment with piperlongumine in prostate cancer cells may induce AR protein degradation *via* the activation of the ubiquitin- proteasome pathway and a significant increase in ROS levels [94]. Furthermore, Maund et al. have developed a tissue slice culture model that bears structural, cellular, and genetic resemblance to human prostate tumors and has similar potential as a new therapeutic in prostate cancer cells [95]. The authors have observed that treatment with piperlongumine in tissue slice cultures significantly decreases the activity of AR receptor and promotes apoptosis. Another *in vitro* study has suggested that treatment with increasing concentrations of piperlongumine suppresses the rapid growth of prostate cancer cells [96]. Mechanistically, piperlongumine inhibits the NF- κB DNA-binding activity and decreases p50 and p65 subunit nuclear translocation in prostate cancer cells. The same study has also suggested that piperlongumine inhibits the cell-to-matrix adhesion and invasiveness properties *via* decreased expression of IL-6, IL-8, ICAM-1, and MMP-9 proteins in prostate cancer cells [96]. Kim and colleagues have synthesized the piperlongumine analog CG-06 (replacing cyclic amide with aliphatic amide), which has high cytotoxicity against human prostate cancer cells (DU-145) [97]. In this study, piperlongumine was shown to inhibit STAT3 phosphorylation at tyrosine 705 as well as the expression levels of its target genes, such as Bcl- 2, cyclin-A, and survivin. The study also suggests that CG-06 directly interacts with STAT3 and promotes ROS mediated inhibition of STAT3 phosphorylation [97]. Together, these findings suggest that piperlongumine and its analog may be a possible therapeutic for prostate cancer.

7.13. Piperlongumine and renal carcinoma

Aberrant expression of the proto-oncogene cMet (a transmembrane receptor tyrosine kinase) is associated with the initiation and progression of many cancers, including renal carcinoma. Studies have suggested that inhibition of c-Met may induce a significant decrease in the viability of cancer cells. Golovine et al. have examined the functional effect of piperlongumine on renal carcinoma cells [98] and found that piperlongumine and its analogs suppress the expression of cMet at both the RNA and protein levels in renal carcinoma cells (786-O and PNX0010) *via* a ROS-dependent mechanism [98]. In addition, piperlongumine induces downregulation of cMet and further inhibits the expression of downstream cMet signaling proteins, such as Akt/mTOR, Erk/MAPK, NF-κB, and STAT3. Moreover, *in vivo* experiments indicated the significant tumor growth inhibition potential of piperlongumine (20 mg/kg intraperitoneally, 3 times/week) and its analogs without any signs of side effects in PNX0010 cell xenografts [98]. On the basis of these results, piperlongumine may have the potential to treat renal carcinoma.

7.14. Piperlongumine and skin cancer (melanoma)

Melanoma, commonly known as cancer of the skin, affects millions of people worldwide, especially those with pale skin [1]. Only several treatment options are available for the treatment of melanoma. However, they show several side effects, and resistance develops within several months of treatment. Several studies have suggested potent anticancer and tumor- suppressing effects of piperlongumine against

human skin cancer cells. Song and collaborators have described the effect of piperlongumine on human melanoma cells (A375, A875, and B16-F10) and revealed that piperlongumine inhibits the viability and growth of melanoma cells [99]. Piperlongumine treatment also arrests melanoma cells in the G2/M phase of the cell cycle, mainly through distinct phosphorylation of JNK and a ROS mediated decrease in the mitochondrial membrane potential [99]. The study concludes that piperlongumine induced cytotoxicity on melanoma cells is associated with ROS production and mitochondrial disruption *via* activation of the JNK pathway [99]. However, many additional studies will be needed before piperlongumine can be introduced as an anti-melanoma agent at the clinical level.

7.15. Piperlongumine and bladder cancer

Bladder cancer, a cancer of the urinary tract, is the eighth most deadly disease in both men and women globally [1]. However, males are four times more frequently diagnosed with bladder cancer than females. Liu et al. have examined the effects of piperlongumine on bladder cancer *in vitro* and *in vivo* [100]. Treatment with piperlongumine in bladder cancer BCa cells decreases cell proliferation, invasion and migration rates and arrests the cells at the G2/M to S phase transition, thus causing bladder cancer cell death [100]. Similarly to earlier studies in other cancers, in this study, piperlongumine increased the ROS accumulation, and treatment with ROS scavengers inhibited the piperlongumine induced effects on bladder cancer cells. The same study has also reported that piperlongumine destroys lamellipodia by inhibiting F-actin in BCa cells. However, intraperitoneal injection of piperlongumine (3.5 mg/kg/day) in a BCa xenografts for 14 days showed suppression of tumor growth and volume by reversing the epithelial to mesenchymal transition *via* inhibition of the expression of mesenchymal markers, such as β-catenin, N-cadherin, Slug, and ZEB1 as compared with control [100]. Interestingly, the researchers have also suggested that piperlongumine (3.5 mg/kg/day) exerts almost similar antitumor activity as cisplatin (2 mg/kg/day), but piperlongumine has preference due to its selectivity for BCa tumor cells and lesser side effects [100]. These results indicate that piperlongumine induced inhibition of bladder cancer cell invasion and migration follows the inhibition and disorganization of F- actin *in vitro* and *in vivo* [100].

7.16. Piperlongumine and cervical cancer

Cervical cancer, arising mainly in the cervix, is the fourth most devastating cancer in females worldwide. According to Niu et al. piperlongumine induces anticancer activity *via* inhibiting the nuclear export mechanism in HeLa cervical cancer cells [101]. Their results suggest that piperlongumine causes tumor suppressor proteins to accumulate inside the nucleus by inhibiting their interaction with chromosomal maintenance 1 (CRM1), also known as exportin 1 (XPO1), in HeLa cells. Importantly, the study has also reported that piperlongumine itself directly interacts with the conserved residue Cys528 in CRM1 [101]. To confirm this result, the authors examined the effect of piperlongumine on CRM1 mutant HeLa cells and found that CRM1 mutant HeLa cells are resistant to piperlongumine [101]. Therefore, piperlongumine mediated inhibition of nuclear export of tumor suppressor proteins may be used to develop new therapeutic strategies to treat cancers, including cervical cancer.

7.17. Piperlongumine and osteosarcoma

Osteosarcoma is an aggressive bone tumor, which is diagnosed primarily in adolescence. Therefore, the search for novel therapeutics for the treatment of osteosarcomas is a major goal to save the lives of teenagers and young adults. Many studies support that intracellular ROS accumulation is the most common mechanism by which piperlongumine induces apoptosis in cancer cells. A study that screened for small-molecule enhancers of ROS with non-toxic or genotype- selective cell death properties has suggested that among several small-molecules, piperlongumine is one of the best small ROS enhancers for osteosarcoma cells [102]. Piperlongumine induces autophagic cell death in apoptosis-resistant osteosarcoma cells *via* activation of p38 in the presence of a pan-caspase inhibitor [103]. Zhou et al. have examined the anticancer potential of piperlongumine against MG63 and U2OS osteosarcoma cells *in vitro* [104] and found that piperlongumine significantly inhibits the viability and growth of osteosarcoma cells. Additionally, the plant derived compound also arrests MG63 osteosarcoma cells in the G2/M phase of the cell cycle, thus leading to apoptosis *via* activation of the intrinsic caspase-9 dependent apoptotic pathway [104]. Mechanistically, the piperlongumine induced cell cycle arrest and apoptosis of osteosarcoma cells is mediated by modulation of aberrant ROS/PI3K/Akt signaling. However, *in vivo* studies are still needed to explore the anticancer potential of piperlongumine against osteosarcoma.

8. Piperlongumine in cancer drug resistance

Unfortunately, the development of resistance to existing cancer therapies (except surgery) is a major challenge at the clinical level. Drugs lose their effectiveness when cancer cells become tolerant to therapies, and this resistance is one of the main factors responsible for cancer-related death worldwide. Thus, the search for novel chemo-sensitizing agents with greater potency and fewer side effects should be a major focus of current research. Recent cancer studies have indicated that herbal remedies may be a possible option to provide chemo-sensitization in cancer drug resistance, owing to their high therapeutic efficacy, low toxicity, and favorable selectivity for human cancer cells. Piperlongumine has a notable ability to re-sensitize the intrinsic and acquired chemo- and radio-resistance in many cancers. Kang and Yan have reported that piperlongumine effectively induces sensitivity toward doxorubicin, and promotes intracellular drug accumulation and apoptosis in doxorubicin resistant K562/A02 human leukemia cells [105]. Piperlongumine stimulates ROS accumulation; p53, p27, and PTEN overexpression; P-gp, MDR1, MRP1, survivin, p-Akt, NF-κB, and Twist downregulation; and G2/M phase cell cycle arrest, thereby causing cell death in doxorubicin resistant K562/A02 cells [105]. In contrast, piperlongumine has selective anti-proliferative and apoptotic activity against glucocorticoid resistant B-cell acute lymphoblastic leukemia (B- ALL) cells but does not sensitize these glucocorticoid resistant cells to dexamethasone [106]. Similarly to findings from other studies, in B-ALL cells, piperlongumine significantly suppresses the expression of transcription factors, such as AP-1, MYC, NF-κB, SP1, STAT1, STAT3, STAT6, and YY1. The same study has also reported that piperlongumine inhibits the transcription activation of AURKB, BIRC5, E2F1, and MYB through increased expression of p21, SOX4, and XBP in B-ALL cells [106]. Another study has reported that piperlongumine sensitizes drug resistant cells by inhibiting the expression of ABCB1, ABCG1, Bcl-2, CDK1, GST- π, MDR1, MRP1, P-gp, survivin, and Top-II. However, it enhances intracellular drug accumulation and caspase-3/8 activity in drug resistant human retinoblastoma cells (HXO- RB44/VCR and SO-Rb50/CBP) [107]. Similarly, combined use of piperlongumine with cisplatin enhances the sensitivity toward cisplatin by inhibiting Akt phosphorylation and drug efflux, and increasing ROS accumulation and apoptosis induction, in cisplatin-resistant NSCLC *in vitro* and *in vivo* [108]. Administration of piperlongumine (2.5 mg/kg/day) alone or cisplatin (5 mg/kg/day) alone for 21 days significantly inhibits the tumor growth in A549/Cis (cisplatin resistant lung cancer cells) xenograft mouse model. However, reduction in tumor weight and volume occurred only in combined treatment of piperlongumine and cisplatin in A549/Cis xenograft mouse model. There is little weight loss occurred in piperlongumine alone treated mouse model, while significant weight loss has been shown in cisplatin alone or combine cisplatin and piperlongumine treated mouse model [108]. Wang et al. have examined

the effect of piperlongumine on docetaxel resistant A549 cells (A549/DTX) [109] and found that piperlongumine promotes apoptosis in both A549 and A549/DTX cells and autophagy in A549/DTX cells, *via* inhibition of the PI3K/Akt/mTOR pathway. Mechanistically, autophagy induction protects A549/DTX cells from apoptosis. However, apoptosis is further re- induced after treatment with 3-methyladenine (autophagy-specific inhibitor) or knockdown of Beclin1 and Atg-5 expression in A549/DTX cells [109]. Administration of piperlongumine (20 and 60 mg/kg/day for 28 days) significantly reduces the tumor volume in A549/DTX xenograft mouse model without showing any toxicity such as weight loss or abnormality in behavior of treated mouse as compared with control group. Moreover, piperlongumine potentially decreases bortezomib resistance in multiple myeloma cells *via* inhibition of osteoclastogenesis and the STAT3 signaling pathway [27]. Wang and colleagues have revealed that combined treatment with piperlongumine with gemcitabine sensitizes human pancreatic cancer cells to gemcitabine *in vitro* and *in vivo* [110]. Combined treatment with piperlongumine (10 mg/kg/day) and gemcitabine (100 mg/kg twice/week) shows higher antitumor activity with reduction of micro vessel density in BxPC-3 cell tumor xenograft than either treatment alone. Interestingly, in a demonstration of the mechanism by which piperlongumine enhances sensitization toward gemcitabine in pancreatic cancer cells, the compound has been found to inhibit cell survival proteins and gemcitabine-induced NF-κB DNA-binding activity [110]. In a mechanistic study, piperlongumine has been found to inhibit TNFα mediated activation of NF-κB by directly interacting with and inhibiting the phosphorylation of IkBα kinase [111]. However, piperlongumine induced inactivation of NF- κB expression further inhibits the expression of proteins involved in proliferation, such as c-Myc and cyclin D1; inflammation (COX-2, IL6); invasion and metastasis, such as ICAM-1, MMP-9, CXCR-4, VEGF; and cell survival, such as Bcl-2, Bcl-xL, c-IAP-1/2, and survivin, thereby causing chemo-sensitization of tumor cells of different origins [111]. Additionally, the combined use of piperlongumine and radiotherapy sensitizes cells to radiation by increasing intracellular ROS accumulation, the Bax/Bcl-2 ratio, and radiation mediated apoptosis in radio-resistant MDA-MB-231 human breast adenoma cells [112]. Another study by Matschke et al. has reported that piperlongumine has the potential to target glutamine-dependent antioxidant activity, which overcomes radio-resistance in anoxia-tolerant NCI-H460 cells *in vivo* [113]. Moreover, regarding piperlongumine resistance, Nan et al. have suggested that overexpressed survivin induces resistance in ovarian cancer cells to piperlongumine treatment [36]. The same authors have also found that piperlongumine inhibits the proliferation and survival of ovarian cancer cells and overcomes apoptosis resistance *via* ROS-mediated downregulation of survivin protein. Furthermore, piperlongumine decreases tumor growth in ovarian cancer xenografts *via* suppression of survivin expression [27]. In addition, piperlongumine induces mitochondrial fission and apoptosis in cisplatin resistant ovarian cancer cells *via* activation of dynamin-related protein 1 [114]. On the basis of all the available research, piperlongumine can be concluded to have substantial chemo- and radio-resistance sensitizing potential in many cancers, either alone or in combination with resistant therapeutics (Fig. 3).

9. Piperlongumine as a combinatorial anticancer drug

The emergence of resistance against conventional chemotherapy suggests that single-agents may not be sufficient to treat cancer. In recent years, combination therapy has been focused on as a new treatment option against cancer [115]. Treatment with phytochemicals, including piperlongumine, in combination with conventional chemotherapeutics may improve the therapeutic efficacy in cancer treatment. Interestingly, many *in vitro* and *in vivo* studies have demonstrated the therapeutic potential of piperlongumine as a combinatorial anticancer drug. For example, piperlongumine alone promotes breast cancer cell death through ROS mediated CHOP activation [116]. However, combined treatment with piperlongumine and TRAIL significantly enhances the TRAIL-induced death of breast cancer cells *via* upregulation of DR5 expression [116]. Similarly, another study by Li et al. has reported that co- treatment with piperlongumine and TRAIL shows synergistic anticancer potential against many cancer types, such as human colon cancer cells (HT29), prostate cancer cells (DU145), and breast cancer cells (MDA-MB-231) [117]. The same study has described ROS accumulation, JNK and p38 kinase activation, and upregulated TRAIL receptor DR5, which thereby enhance TRAIL- induced cell death in cancer cells [117]. Furthermore, piperlongumine (2.4 mg/kg) and TRAIL (2 mg/kg) co-in human TNBC MDA-MB-231 cells NOD-SCID gamma (NSG) xenograft mice, every other day for a total of five injections shows significant reduction in tumor growth without any side effects compared as with their alone treatment [117]. TP53 variants and mutations are frequently detected in head and neck squamous cell carcinoma (HNSCC), and APR-246 reverses the wild type activity of mutant TP53 by restoring its DNA binding ability. Hang and collaborators have reported that combined treatment with piperlongumine and APR-246 induces a decrease in GSTP1 activity, thereby increasing glutathione disulfide and ROS accumulation, decreasing GSH levels, and promoting DNA damage in HNSCC cells [118]. Interestingly, piperlongumine enhances the sensitivity in HNSCC cells toward APR-246 independently of TP53. Additionally, piperlongumine (4 mg/kg/day) and APR-246 (100 mg/kg/day) alone treatment for 27 days reduces UMSCC10A cell tumor volume, but piperlongumine and APR-246 co-treatment effectively shows more tumor suppressive effect in SCID mice [118]. Studies have suggested that alpha-lipoic acid, a cofactor of multienzyme complexes, has apoptosis-inducing activity against many cancer cells. In a recent study, combined treatment with piperlongumine and alpha-lipoic acid has shown synergistic anticancer activity against acute promyelocytic leukemia *via* COX-2 inhibition and activation of cleaved caspase-3 [65]. The plant growth regulator cotylenin-A has potent anticancer activity in many different types of cancers. A very recent study by Yamaguchi et al. has reported that co-treatment with piperlongumine and cotylenin-A shows selective synergistic induction of ferroptosis in MIAPaCa-2 and PANC-1 pancreatic cancer cells, but this effect was not observed in mouse embryonic fibroblasts [119]. Use of ferroptosis inhibitors (ferrostatin-1 and liproxstatin-1) and an iron chelator (deferoxamine) significantly inhibits the co-treatment effect of piperlongumine and cotylenin-A in pancreatic cancer cells. Furthermore, treatment with the clinically approved ferroptosis inducer sulfasalazine with piperlongumine and cotylenin-A induces cell death in pancreatic cancer cells. These findings indicate the effectiveness of combined treatment with piperlongumine, cotylenin-A, and sulfasalazine against pancreatic cancer [119]. Similarly to piperlongumine, piperlongumine derivatives synergistically improve the anticancer potential of conventional chemotherapeutics. Treatment of a potassium derivative of piperate (intermediate product during chemical synthesis of piperlongumine) (0 − 100 μg/ml) in combination with etoposide phosphate (0 − 100 μg/ml) or cisplatin (0 − 20 μg/ml) for 48 hours confers synergistic improvements in the anti-proliferative and cell growth inhibition activity of etoposide phosphate or cisplatin alone [24]. Furthermore, administration of potassium derivative of piperate (10 mg/kg/day) in MCF-7 cells bearing SCID xenograft mice for 21 days significantly reduces the tumor weight and volume compared with vehicle group [24]. Xu and collaborators have designed and produced some derivatives by merging the fragments of piperlongumine and dicoumarol [120]. These derivatives show selective and potent ROS mediated cytotoxic and growth inhibition activity against different human cancer cell lines and in an A549 tumor xenograft mouse model [120]. A summary of the co-treatment effects of piperlongumine on existing chemotherapeutics is shown in Table 3. Thus, piperlongumine can be used as a combinatorial pharmacological agent to potentiate conventional chemotherapeutics.

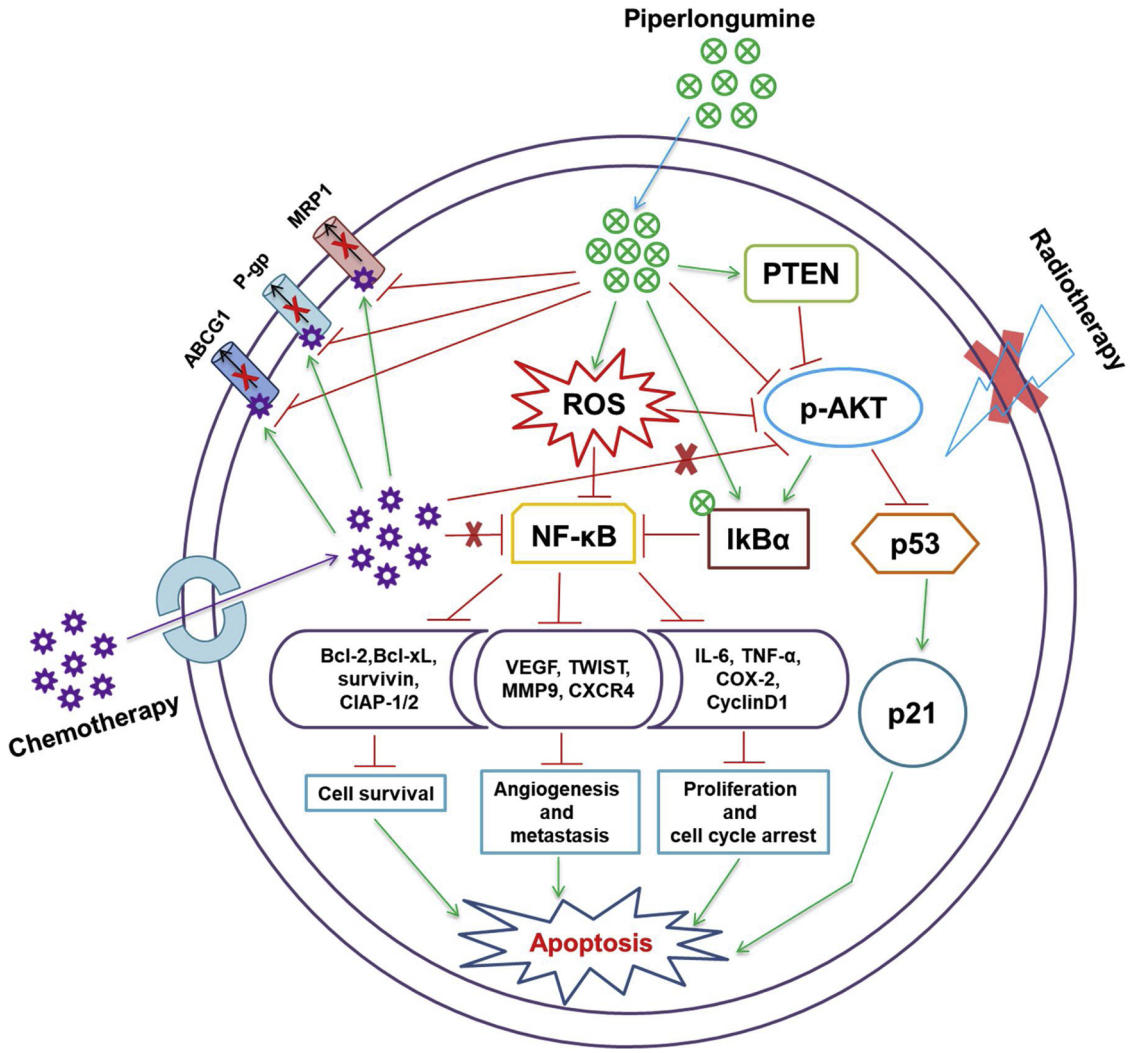

Fig. 3. Chemo- and/or radio-resistant cancer cells. Piperlongumine induces reversion of chemo- and radio-resistance in various types of cancer of different origin *via* targeting aberrant cellular signaling pathways. Piperlongumine promotes apoptosis in drug resistant cancer cells *via* intracellular ROS accumulation mediated inhibition of pAKT and NF-κB, which in turns reduces cell survival, proliferation, angiogenesis and metastasis, drug efflux, and induces cell cycle arrest. Finally, piperlongumine mediates cell death in drug resistant cancer cells.

10. Conclusion and future possibilities

To date, phytochemicals have gained interest as anticancer agents in pharmaceutical industries. Piperlongumine has various biological activities, including anticancer activity, through inhibition of various pro-cancer activities, such as angiogenesis, migration, proliferation, and invasion and metastasis. Piperlongumine also shows pro-apoptotic activity, mainly through ROS accumulation, DNA damage, and modulation of the aberrant expression of oncogenes and tumor suppressor genes. No harmful or toxicity related reports or studies of piperlongumine in humans have been identified in the literature to date. Co-treatment with piperlongumine may resensitize resistant cancer cells to conventional chemotherapeutics and radiotherapy. Although numerous preclinical studies suggest the cytotoxic potential of piperlongumine against multiple types of cancer, still piperlongumine has not attracted the attention of clinicians for its clinical utility in the treatment of cancer patients. Poor aqueous solubility and bioavailability may limit the use of piperlongumine as an anticancer agent in the biological system, although these drawbacks can be overcome through compound polymeric nano-formulations. However, available studies may not be sufficient to overcome the poor aqueous solubility and bioavailability related issues of piperlongumine. Very limited pharmacokinetic studies of piperlongumine are available to date. There is no exact calculated oral bioavailability of piperlongumine has been given in the available studies. However, knowledge of these factors about a novel drug is very crucial before starting its clinical studies. These might be some of the possible reasons behind the lack of clinical studies on the use piperlongumine as an anticancer drug. After complete consideration of these limitations, piperlongumine could be used as a novel phyto-based anticancer drug and chemosensitizing agent alone or in combination with existing conventional drugs at clinical level. Finally, to introduce piperlongumine as an anticancer drug, more preclinical and clinical studies of this small novel bioactive compound are needed for better understanding and improvement of its anticancer efficacy.

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Table 3
Co-treatment effects of piperlongumine on conventional chemotherapeutics' anticancer potential *in vitro* and *in vivo*.

Cancer (cells/tumor type)	Combination used (*in vivo* administration route)	Observation	Ref.
Lung, Acute T cell leukemia, and colon cancer (NCI-H1975, Jurkat, and HCT-116 cells)	Piperlongumine and PI3K inhibitor (pictilisib)	Pictilisib alone shows cytotoxic IC_{50} value against NCI-H1925 cells 0.16 μM, while in combination treatment, piperlongumine reduces the cytotoxic IC_{50} value of pictilisib for NCI-H1925 cells 0.015 μM. This means piperlongumine potentiates 10 fold anticancer activity of pictilisib against lung cancer cells. Similarly, piperlongumine potentiates 18 fold anticancer activity of pictilisib against leukemia cells. Moreover, co-treatment of both the drugs shows an increase in intracellular ROS accumulation with broad-spectrum synergistic anticancer activity.	[121]
Breast cancer (Triple-negative breast cancer MDA-MB-231 and MDA-MB-453 cells)/(female nude mice bearing MDA-MB-231 tumor xenografts)	Piperlongumine and doxorubicin (intraperitoneal administration every other day for 22 days)	Co-treatment of piperlongumine and doxorubicin enhances the apoptosis of breast cancer cells by inhibiting JAK2-STAT3 signaling pathway *in vitro* and *in vivo*. Piperlongumine (4 mg/kg) or doxorubicin (0.8 mg/kg) alone administration shows a significant decrease in breast tumor growth rate, while combined treatment of piperlongumine (4 mg/kg) and doxorubicin (0.8 mg/kg) synergistically reduces cell growth rate. There is no significant difference observed in body weight between treated and control groups, indicating no toxicity in treated groups towards therapy.	[122]
Pancreatic cancer (MIA PaCa-2 and PANC-1 cells)/(nude mice bearing MIA PaCa-2 orthotopic tumor xenograft)	Piperlongumine and gemcitabine (intraperitoneal administration thrice in a week 31 days)	Co-treatment of piperlongumine and gemcitabine increases ROS and G0/G1 phase cell cycle arrest compared with control *in vitro* and *in vivo*. Piperlongumine alone (5 mg/kg) shows reduction in tumor weight and volume by 37% and 67%, respectively. Gemcitabine alone (25 mg/kg) shows reduction in tumor weight and volume by 50% and 64%, respectively. Interestingly, the combined administration of piperlongumine (5 mg/kg) and gemcitabine (25 mg/kg) effectively decreases the tumor weight and volume by 68% and 83%, respectively. Furthermore, the study has not cleared about the co-treatment effects of piperlongumine and gemcitabine on normal pancreatic cells.	[123]
Prostate cancer (DU-145 cells)	Piperlongumine and doxorubicin	As compared with piperlongumine or doxorubicin treatment alone, co-treatment of piperlongumine and doxorubicin synergistically inhibits prostate cancer cell proliferation, invasion and migration with enhanced pro-apoptotic effect *via* inhibiting carbonyl reductase 1 (CBR1).	[124]
Colorectal cancer (HCT-116, LoVo, and GES-1 cells)/(female athymic BALB/c nu/nu mice bearing HCT-116 tumor xenograft)	Piperlongumine and oxaliplatin (intraperitoneal administration per day up to 24 days)	Co-treatment of piperlongumine and oxaliplatin overcomes oxaliplatin resistance and synergistically improves cytotoxic and apoptotic activity *via* elevated cellular ROS level, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress *in vitro* and *in vivo*. Piperlongumine (2.5 mg/kg) or oxaliplatin (5 mg/kg) administration alone expectedly decreases colon tumor weight, size, and volume. Meanwhile, their co-treatment shows greater tumor growth reducing effect than comparable controls. There are no changes observed in the morphology of different organs (such as heart, kidney, or liver) and body weights of treated models, indicating low or no systemic toxicity of the therapy.	[125]
Gastric cancer (SGC-7901, BGC-823, AGS, and HCT116 cells)/(female athymic BALB/c nu/nu mice bearing HCT-116 or SGC -7901 tumor xenografts)	Piperlongumine and oxaliplatin (intraperitoneal administration once every other day up to 17 days)	Combined treatment of piperlongumine and oxaliplatin enhances the anticancer effect of oxaliplatin by inhibiting TrxR1, which in turn induces intracellular ROS accumulation, DNA damage, and activation of p38 and JNK signaling pathway *in vitro* and *in vivo*. Moreover, combined treatment of piperlongumine (4 mg/kg) and oxaliplatin (5 mg/kg) shows greater tumor inhibitory effect as compared with piperlongumine or oxaliplatin treatment alone. Interestingly, piperlongumine attenuates oxaliplatin (5 mg/kg) alone induced side effects, such as weight loss in the treated animals.	[126]

(*continued on next page*)

Table 3 (*continued*)

Cancer (cells/tumor type)	Combination used (*in vivo* administration route)	Observation	Ref.
Gastric cancer (BGC-823 and SGC-7901 Cells)/(female athymic BALB/cA nu/nu mice bearing SGC-7901 tumor xenograft)	Piperlongumine and auranofin (intraperitoneal administration once every day up to 14 days)	Co-treatment of piperlongumine with auranofin enhances auranofin apoptosis inducing activity in gastric cancer cells by elevating intracellular ROS levels and ER stress *in vitro*. Moreover, combined treatment of piperlongumine (4 mg/kg) and auranofin (2 mg/kg) shows greater tumor inhibitory effect via increases intracellular ROS accumulation and activation of CHOP and caspases as compared with piperlongumine or auranofin treatment alone. There is no significant difference observed in body weights of treated and control groups, indicating no toxicity of the therapy.	[127]
Head and neck cancer (AMC-HN-2/3/4/5/6/7/8/9, SNU1041/1066/1076, HN- 30/31, UMSCC1, and 93-VU-147 T cells)/athymic BALB/c nude mice bearing AMC-HN3 or HN9 tumor xenografts	Piperlongumine and cisplatin (intraperitoneal administration piperlongumine once per day and cisplatin once per week up to 21 days)	Co-treatment of piperlongumine and cisplatin synergistically potentiates cisplatin-induced cytotoxicity by accumulating ROS and activation of cell death pathways, including JNK and PARP *in vitro* and *in vivo*. Moreover, combined treatment of piperlongumine (2.5 mg/kg) and cisplatin (5 mg/kg) shows greater tumor inhibitory effect as compared with piperlongumine or oxaliplatin treatment alone. There is no significant difference observed between body weights of treated and control groups xenograft models, indicating no obvious side effects of the therapy.	[128]
Acute Myeloid Leukemia (U937, MV4–11, HL60, MCF-10A, MOLM-13, OCI-AML3, CMK, HL-60, cytarabine resistant CMK cells)	Piperlongumine and SAHA (Vorinostat)	Co-treatment of piperlongumine and vorinostat induces higher apoptosis through reduced cellular GSH defense, DNA repair genes (Rad51 and CHK1) expression, and survival gene (XIAP) with increased expression of DNA damage gene (DSBs), and apoptotic gene (Bim) as compared with piperlongumine and vorinostat treatment alone.	[129]

Declaration of Competing Interest

The authors declare no conflict of interest regarding the publication of this review article.

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