

REVIEW

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# Molecular and pharmacological aspects of piperine as a potential molecule for disease prevention and management: evidence from clinical trials

Amit Kumar Tripathi<sup>1,2†</sup> , Anup Kumar Ray<sup>3,4†</sup> and Sunil Kumar Mishra<sup>3\*</sup>

## Abstract

**Background:** Piperine is a type of amide alkaloid that exhibits pleiotropic properties like antioxidant, anticancer, anti-inflammatory, antihypertensive, hepatoprotective, neuroprotective and enhancing bioavailability and fertility-related activities. Piperine has the ability to alter gastrointestinal disorders, drug-metabolizing enzymes, and bioavailability of several drugs. The present review explores the available clinical and preclinical data, nanoformulations, extraction process, structure–activity relationships, molecular docking, bioavailability enhancement of phytochemicals and drugs, and brain penetration properties of piperine in the prevention, management, and treatment of various diseases and disorders.

**Main body:** Piperine provides therapeutic benefits in patients suffering from diabetes, obesity, arthritis, oral cancer, breast cancer, multiple myeloma, metabolic syndrome, hypertension, Parkinson's disease, Alzheimer's disease, cerebral stroke, cardiovascular diseases, kidney diseases, inflammatory diseases, and rhinopharyngitis. The molecular basis for the pleiotropic activities of piperine is based on its ability to regulate multiple signaling molecules such as cell cycle proteins, anti-apoptotic proteins, P-glycoprotein, cytochrome P450 3A4, multidrug resistance protein 1, breast cancer resistance protein, transient receptor potential vanilloid 1 proinflammatory cytokine, nuclear factor- $\kappa$ B, c-Fos, cAMP response element-binding protein, activation transcription factor-2, peroxisome proliferator-activated receptor- $\gamma$ , Human G-quadruplex DNA, Cyclooxygenase-2, Nitric oxide synthases-2, MicroRNA, and coronaviruses. Piperine also regulates multiple signaling pathways such as Akt/mTOR/MMP-9, 5'-AMP-activated protein kinase-activated NLR family pyrin domain containing-3 inflammasome, voltage-gated K<sup>+</sup> current, PKC $\alpha$ /ERK1/2, NF- $\kappa$ B/AP-1/MMP-9, Wnt/ $\beta$ -catenin, JNK/P38 MAPK, and gut microbiota.

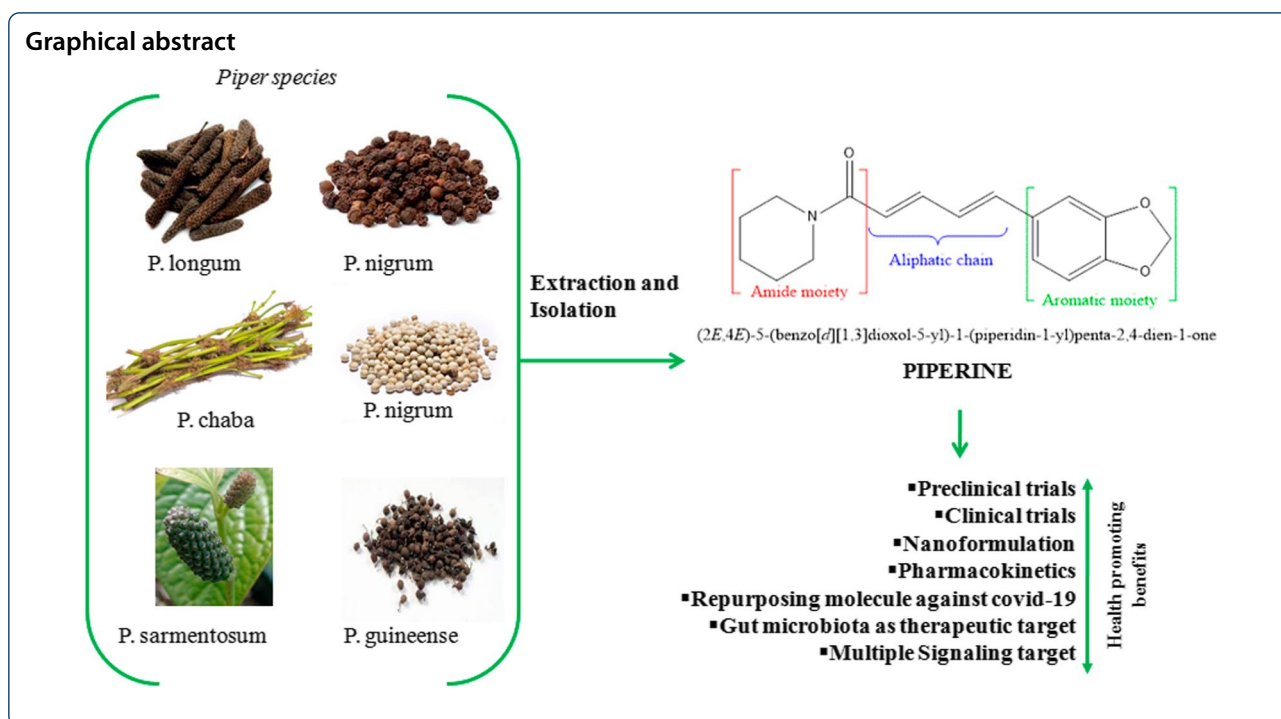
**Short conclusion:** Based on the current evidence, piperine can be the potential molecule for treatment of disease, and its significance of this molecule in the clinic is discussed.

**Keywords:** Anticancer, Piperine, Pharmacokinetics, Extractions, Clinical trials, COVID-19, Gut microbiota

\*Correspondence: skmishra.phe@itbhu.ac.in

<sup>†</sup>Amit Kumar Tripathi and Anup Kumar Ray have equal contribution

<sup>3</sup> Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University, Varanasi 221005, India  
Full list of author information is available at the end of the article



## 1 Background

Piperine (1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadienyl]piperidine) is a nitrogen-containing alkaloid molecule, first isolated in the form of yellow crystalline solid (MW 285.33 g.mol<sup>-1</sup>, mp = 128–130 °C) by Danish chemist Hans Christian Orstedt in 1820 from the dried fruit extract of *pepper* [1]. Chemically, piperine molecules consist of conjugated aliphatic chains, which act as a connecting structure between piperidine and 5-(3,4-methylenedioxypheyl) moiety. Piperine occurs naturally in black, green, and white *pepper* (Table 1) [2–4]. Other alkaloids are also present in black pepper extracts such as piperanine, piperettine, piperylin A, piperolein B, and pipericine [5]. During the last two decades, piperine has received considerable attention for its beneficial health effects [6–8].

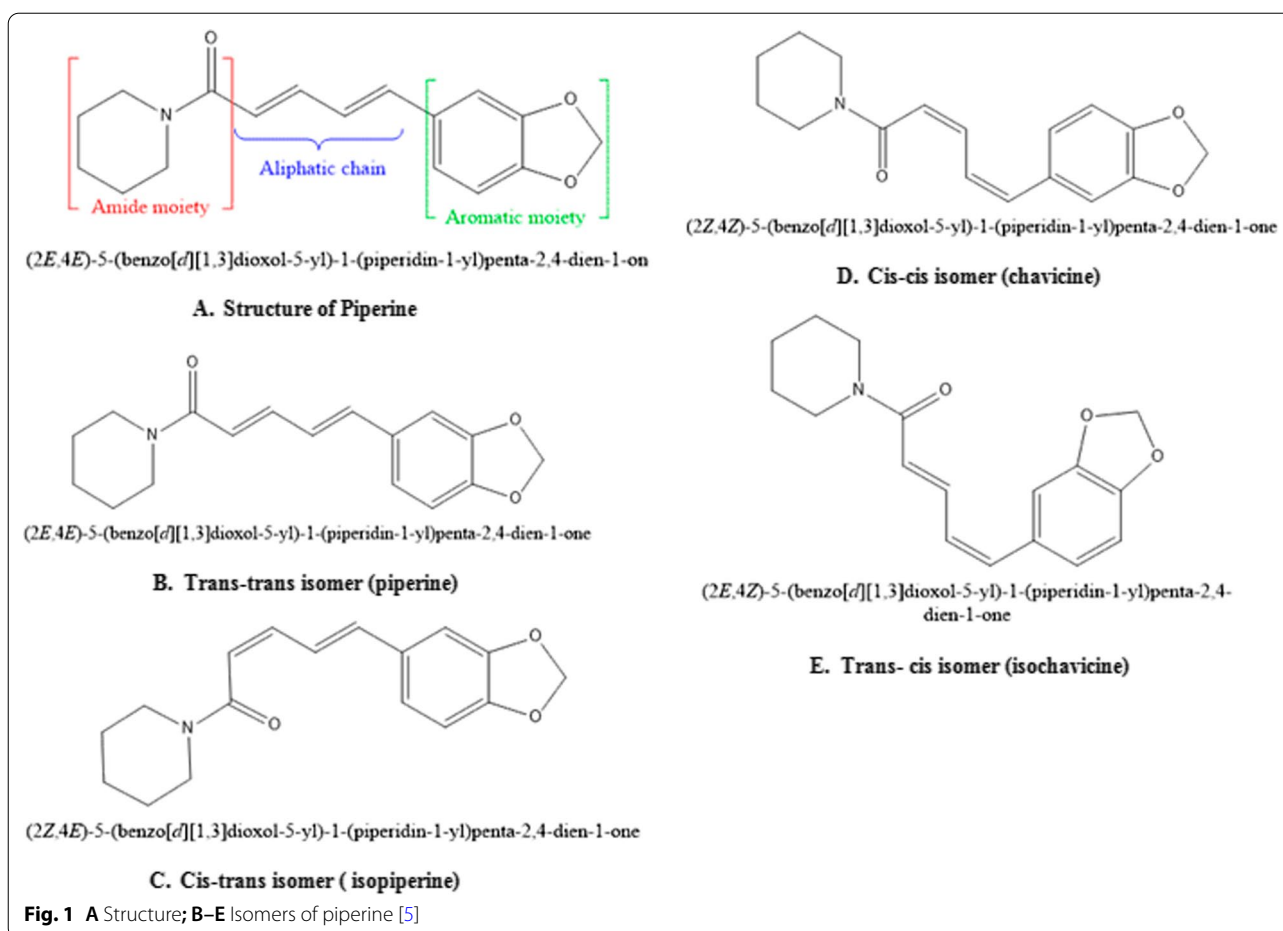
Naturally, piperine exists in four isomeric forms (Fig. 1) [9, 10]. However, only piperine isomers have pungency and biological activity compared to the other three. Other studies showed *cis* and *trans*-isomer of piperine possess significant anti-hepatotoxic as well as antioxidant effects [1]. Light-induced isomerization of piperine increases with light intensity and its exposure time [11]. Chemical synthesis of piperine was done by Ladenburg and Scholtz in 1894, by reaction of the piperic acid chloride with piperidine. The multiple biological activities of piperine have been demonstrated in both preclinical and clinical studies. The clinical trials completed are 11 and in addition that are currently ongoing are 5; a total of

1002 articles have been published on piperine in the last 10 years (Table 2 and Fig. 2) [12–15]. However, some of the clinical trial data is published but not registered.

Exploring the broad-spectrum bioactivities of piperine has been demonstrated over a decade that can be harnessed in agriculture as pesticide and medicinal use. The insecticidal properties of piperine have been first observed in 1924 [16]. The LD<sub>50</sub> for piperine is 330 and 200 mg/kg for single intra-gastric and subcutaneous injections, respectively [16]. Piperine is also reported to inhibit enzymes (cytochrome P450, UDP-glucuronyl-transferase) that catalyze the biotransformation of nutrients and drugs, thereby enhancing their bioavailability and *in vivo* efficacies [11].

**Table 1** Members of *Piperaceae* family containing piperine [9]

Name of plant	Part of plant	Piperine content (%)
<i>Piper nigrum</i>	Fruit	1.7–7.4
<i>Piper longum</i>	Spike and root	5–9
	Fruit	0.03
<i>Piper chaba</i>	Fruit	0.95–1.32
<i>Piper guineense</i>	Fruit	0.23–1.1
<i>Piper sarmentosum</i>	Root	0.20
	Stem	1.59
	Leaf	0.104
	Fruit	2.75



Clinical trials have looked into the protective and therapeutic effect of piperine against many diseases and disorders including hypertension, diabetes, cancer, neurological, cardiovascular, and reproductive as well as against microbial infections such as viral, bacterial, and fungal infections. Both clinical and preclinical data have shown that piperine has many targets (Figs. 3 and 4) and that it can modulate the various signaling molecules such as Wnt, NF- $\kappa$ B, cAMP response element-binding protein, activation transcription factor-2, peroxisome proliferator-activated receptor- $\gamma$ , human G-quadruplex DNA, cyclooxygenase-2, nitric oxide synthase-2, MicroRNA, SARS CoV-2, Akt/mTOR/MMP-9, AMPK-activated NLRP3 inflammasome, IK, ERK1/2, nuclear factor erythroid 2 like 2 (Nrf2) and r (CGG) exp RNA. The pleiotropic mechanistic action of piperine is therefore attributed to its ability to interact with a broad spectrum of molecular targets that include kinases, transcription factors, cell cycle proteins, inflammatory cytokines, receptors, and signaling molecules.

oxide synthase-2, MicroRNA, SARS CoV-2, Akt/mTOR/MMP-9, AMPK-activated NLRP3 inflammasome, IK, ERK1/2, nuclear factor erythroid 2 like 2 (Nrf2) and r (CGG) exp RNA. The pleiotropic mechanistic action of piperine is therefore attributed to its ability to interact with a broad spectrum of molecular targets that include kinases, transcription factors, cell cycle proteins, inflammatory cytokines, receptors, and signaling molecules.

## 2 Main text

### 2.1 Chemical modification, structure–activity relationships, and synthetic analogs of piperine

Chemically, piperine is an alkaloid and the structure is composed of three subunits: An amide function constituted by a piperidine ring with  $\alpha$ - $\beta$ -unsaturated carbonyl moiety, a 1,3-benzodioxole group, also called piperonal nucleus and a butadiene chain (Fig. 1A). All four isomers of piperine showed inhibitory activity against *Leishmania donovani* pteridine reductase 1 (LdPTR1), while the maximum inhibitory effect was demonstrated by isochavicine. It was reported that piperine, isopiperine, isochavicine, activated both TRPV1 and TRPA1. Many studies have reported different types of derivatives and analogues of piperine (Table 3) along with their structure–activity relationship (SAR) and biological activities. The efficiency of piperine derivatives increases by replacing the piperidine moiety with *N,N*-dipropyl, *N,N*-diisopropyl, *N,N*-dibutyl, *p*-methyl piperidine, or *N,N* bis(trifluoromethyl) groups. Potency enhancers exchange the piperidine moiety with

**Table 2** Clinical trials on piperine\*

Condition & (number of patients)	Phases I, II, III or IV & (Status)	Dose, duration	Principal Investigator affiliation	Duration (months)	References
KOA (60)	I (Completed)	7.5 mg/day, 4 wks	Motahar Heidari-Beni, IUMS	Jan 2018–May 2018 (5)	Heidari-Beni et al. [154]
TCS (12)	I (Completed)	20 mg/day, 10 days	S. K. Bedada, UCPSKU	2016–2016	Bedada et al. [155, 156]
MS (12)	I (Completed)	20 mg/day, 2 days	HMO & A. Hoffman and A. Domb, DRBCPHU	Aug 2013–Jan 2015 (17)	Cherniakov et al. [157]
AIDS (08)	I (Completed)	20 mg/day, 7 days	Ravisekhar Kasibhatta, BCRPL, Hyderabad	2007–2008	Kasibhatta and Naidu [158]
NAFLD (79)	III (Completed)	5 mg/day, 8 weeks	Dr. Abasalt Borji, NUMS	Jan 2017–Nov 2017 (10)	Mirhafez et al. [159]
T2DM (100)	III (Ongoing)	5 mg/day, 12 weeks		Jun 2015–Present (12)	*
NAFLD (70)	II (Ongoing)	5 mg/day, 12 weeks		Jan 2018–Present	
HIVS (60)	I (Completed)	–	Philip C Smith, School of Pharmacy, UNC Chapel Hill	Sep 2003–Mar 2006 (30)	
MN, Pain, BS, Urinary Urgency (09)	I (Active, not recruiting)	–	Aminah Jatoy, Mayo Clinic, Rochester, Minnesota, United States	Mar 2016–Mar 2021 (60)	
CKD (30)	NA (Recruiting)	500 mg of curcumin and piperine, 3 capsules/day, 12 weeks	Denise Mafrá, Federal University Fluminense, Rio de Janeiro, Brazil	Oct 2020–Oct 2021 (12)	
Hair Thinning (70)	NA Recruiting)	95% piperine extract in formulation, 4 capsules/day, 180 days	Glynis Ablon, ABSIRC, Manhattan Beach, California, United States	Jun 2019–Jan 2021 (18)	
Epilepsy (10)	I (Completed)	20 mg/day, 2 days	Smita Pattanaik, NOD-PGIMER, Chandigarh, India	2017–2017	Pattanaik et al. [160]
OD (40)	I&II (Completed)	Group 1 = 150 µM Group 2 = 1 mM	Laia Rofes, GPLRU, Hospital de Mataró, Spain	Jun 2011–Feb 2012 (9)	Rofes et al. [161]
MS (12)	I (Completed)	20 mg/day, 10 days	S K Bedada, UCPSKU	2016–2016	Bedada and Boga [162]
OA (53)	III (Completed)	15 mg/day, 6 weeks	Dr. Yunes Panahi, CIRC-BUC, CRDU-BH, BUMS, Iran	Jan 2011–Jan 2012 (12)	Panahi et al. [163–165]
Vitiligo (63)	II&III (Completed)	1% Topical solution, 12 weeks	Anoosh Shafiee, SRC-SBUM, Iran	Jun 2016–Sep 2016 (3)	Shafiee et al. [15]

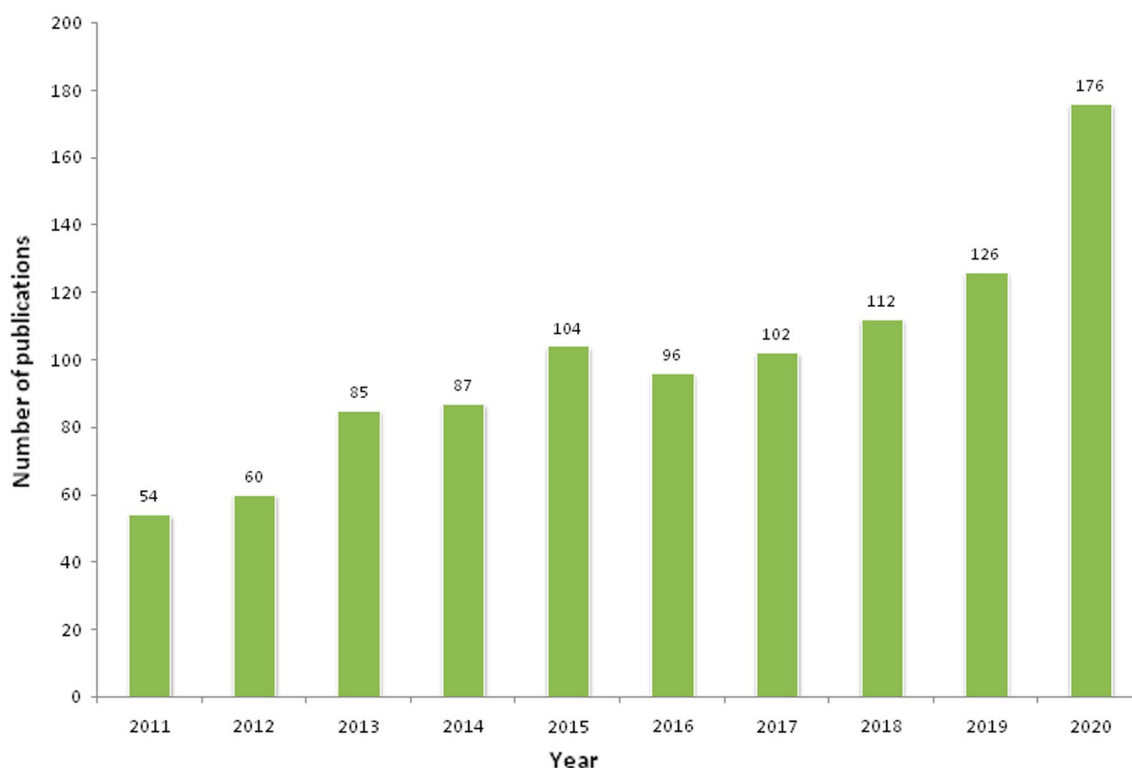
\* Referenced from:—US National Library of Medicine: <https://www.clinicaltrials.gov/ct2/results?cond=&term=piperine&cntry=&state=&city=&dist=>; University hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR): <https://www.umin.ac.jp/ctr/index.htm> & Iranian Registry of Clinical Trials: <https://www.ircr.ir/>

*N,N*-dibutyl, *N,N*-diisobutyl, or *N,N*-bis trifluoromethyl groups [17]. The most active piper amides are the *N*-isobutyl-substituted ones that resemble pesticidal activity. For the activity of piper amides, the lipophilic chain must contain at least four carbons and a conjugated bond adjacent to amide carbonyl with a bulky amine is necessary for binding, which makes piperine a model compound for the bioactive amides. Activity among the piperidine amides increases with increasing substitution on the piperidine ring carbons, with ethyl substituted being more active than the methyl analogues. Saturation of the side chain in piperine resulted in enhanced inhibition of Cytochrome P450 (CYP450), while modifications in the phenyl and basic moieties in the

analogues produced maximal selectivity in inhibiting either constitutive or inducible CYP450 [18]. Several piperine derivatives with modifications at the piperidine moiety and the aliphatic chain have been reported to inhibit survivin protein, a small target in the inhibitor of apoptosis (IAP) family and regulator of cell division in cancer [19]. Few modified analogues of piperine showed promising activity on the TRPV1 and GABAA receptors [17, 20–24].

## 2.2 Extraction methods of piperine from black and white pepper

Piperine content varies in plants from the *Piperaceae* family from 2 to 7.4% in both black and white pepper [1].



**Fig. 2** Total number of publications of piperine in previous ten years. Source: [https://pubmed.ncbi.nlm.nih.gov/?term=PIPERINE&filter=datesearch\\_y\\_10&timeline=expanded](https://pubmed.ncbi.nlm.nih.gov/?term=PIPERINE&filter=datesearch_y_10&timeline=expanded)

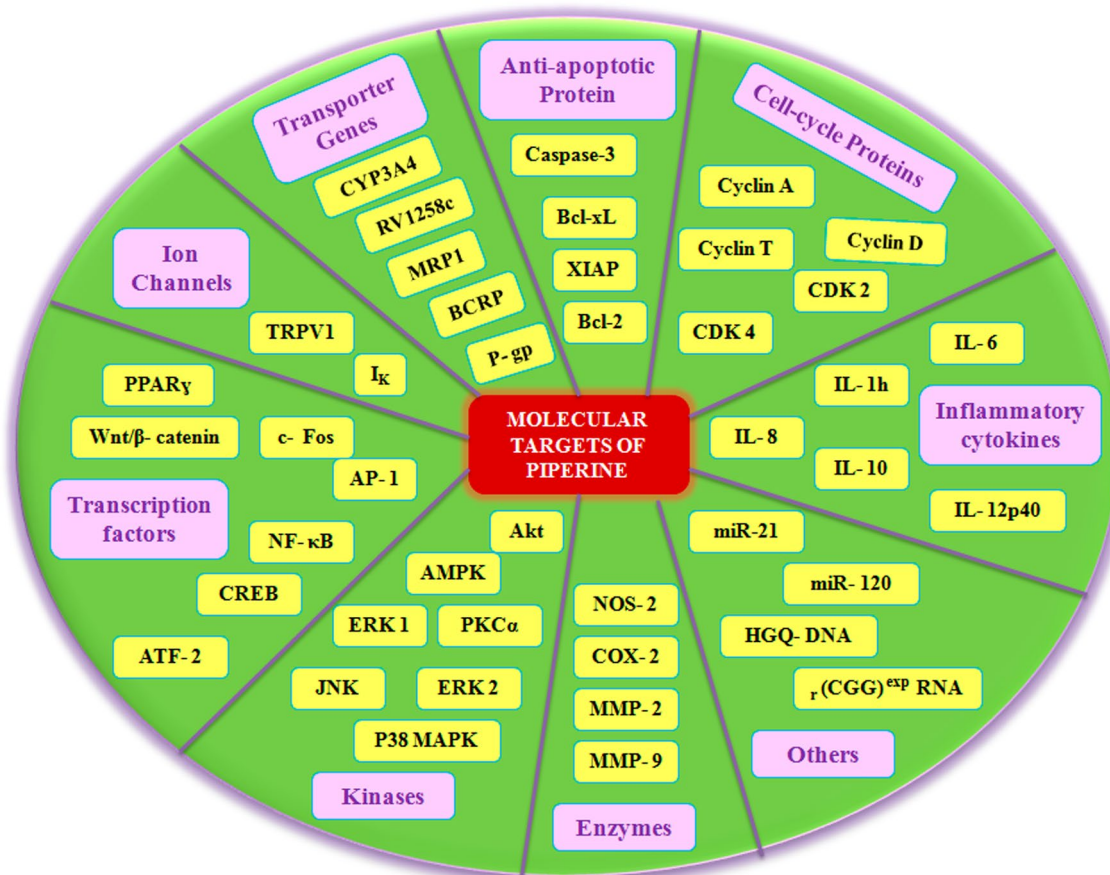
Different methods are used to extract piperine (Table 4); these methods often suffer demerits such as inadequate extraction efficiency, photodegradation, tedious and expensive isolation methodology. It is therefore needed in the first place to determine the best factors and conditions to optimize these shortcomings [25]. Increasing the surface area of the pepper improved the efficiency of extraction by 109.02% [26]. The nonpolar solvents like petroleum ether brought the highest extraction efficiency of 94% with a purity of 85% [1]. The double bypasses Soxhlet apparatus (DBSA) for the extraction of piperine was found to be more efficient than conventional Soxhlet apparatus (SA) based on extraction time, which is  $12 \pm 1$  h for DBSA and  $22 \pm 1$  h for SA [27]. In the hydro-tropic extraction of piperine, hydrotropes are adsorbed on the cell wall to destroy it and then the extractant gets penetrated the cell membrane, which later helps to dis-organize the amphiphilic lipid bilayer and enable easy release of piperine. Extraction temperature is increased causing more lysis of the cell, and as a result, the permeability of the cell wall is enhanced for the hydrotrope solution to act on it [1]. It demonstrated selective and rapid extraction of piperine from black pepper and the recovered piperine was approximately 90% pure [28]. The enzymolysis facilitates the breakage of the Piper nigrum

L cells. This accelerates the extraction, while the addition of the surfactant promotes enzymatic hydrolysis by affecting the process of adsorption and desorption of enzymes from the substrate. This could reduce the inefficient adsorption of the enzyme, leading to its inactivation due to which an increase in the yield of piperine from 0.14 to 4.42% through HPLC is observed in surfactant-assisted enzymatic extraction of piperine [29]. In microwave-assisted extraction (MAE), the microwave power and extraction temperature are two important factors to be considered seriously as the extraction yield increases proportionally to the power increase until the increase becomes insignificant or the yield declines. Through MAE, an 85% pure piperine with a yield of 45% in 4 h was observed [1, 30].

### 2.3 Pharmacokinetics and brain uptake distribution of piperine

Piperine (30 mg/kg, p.o.) showed a high degree of brain exposure with a  $K_p$ , brain of 0.95 and  $K_p$ , uu, brain of 1.10 it also showed high-BBB penetration potential with no interaction with efflux transporter and suggested that efficient brain uptake of piperine is due to its very limited liver metabolism evidenced by its much lower intrinsic clearance in the liver. The maximum brain concentration





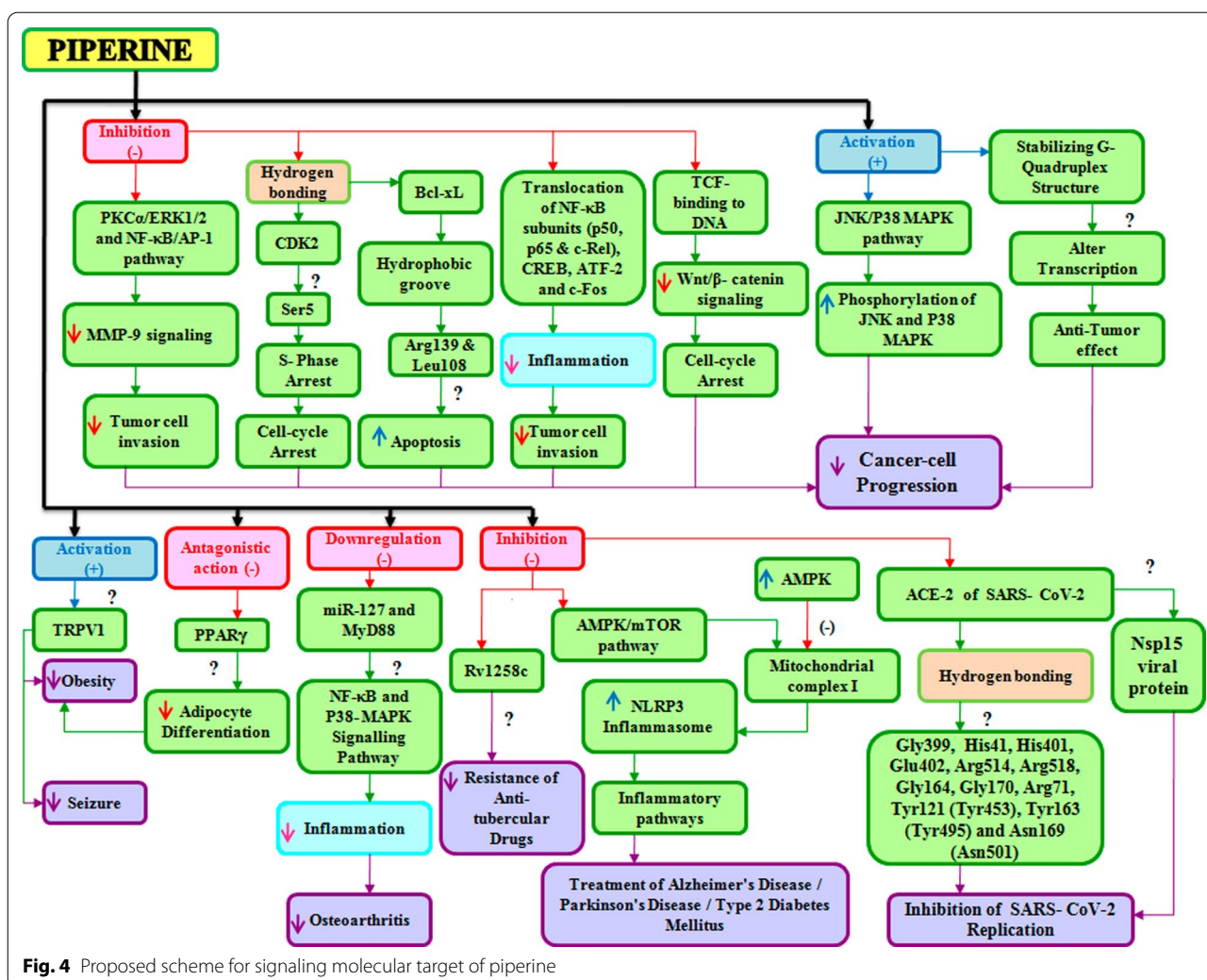
**Fig. 3** Targets of Piperine

of piperine (20 mg/kg, i.p.) was found to be  $51 \pm 9$  ng/g after 3 h, which could later be increased to  $121 \pm 7$  ng/g after formulating piperine (18 mg/kg, i.p.) into solid lipid particles [31, 32]. Half-life ( $t_{1/2}$ ) of piperine in humans is about 13.2–15.8 h, suggesting that it has a long elimination time in the human body [31, 32]. To extrapolate the molecular mechanism of piperine, researchers are trying to explore the pharmacokinetics profile and brain uptake of piperine as a single drug and in combination with other (Table 5) [33]. Tables 6 and 7 list pharmacokinetic parameters of piperine in the human body and rodents. It was demonstrated that piperine (20 mg/kg, p.o.), when administered in conscious rats, gets absorbed rapidly through the g.i.t and could be detected in plasma within 15 min after administration. However, its metabolites were not excreted in the biliary excretion, which will be the topic of future research. In another study, it was found that  $C_{max}$  in plasma assay of piperine in Wistar rats at a dose of 10 mg/kg to be about 59 ng/mL and  $t_{1/2}$  to be about 6 h [34]. Piperine demonstrated an unexplored effect on the oral bioavailability and intestinal

permeability of cyclosporine A by modulating the P-gp (T. [31, 32]. Piperine also induces acidity by stimulating the histamine  $H_2$  receptors [35]. Piperine can enhance cannabinoid absorption even in chronic consumption [36]. The plasma concentration of sodium valproate (SVP) was enhanced to 14.8-fold when SVP was administered with piperine, and a 4.6-fold increase in the AUC of SVP + piperine was also seen [37]. Piperine combined with oxyresveratrol led to an 1.5-fold increase in the  $C_{max}$  & AUC, with a shorter  $T_{max}$  from 2.08 to 1.30 h; it is excreted in an unchanged form through the urinary route [38].

#### 2.4 Enhancement of bioavailability by nanoformulations of piperine

Pure piperine, despite multiple biological actions, has poor water solubility and low bioavailability; thus, a modified drug-delivery system is utilized to deliver piperine in inappropriate amounts. Despite this, there are few possible explanations for the bio-enhancing property of piperine (Fig. 5). The relative bioavailability of piperine-SR-pellets



**Fig. 4** Proposed scheme for signaling molecular target of piperine

is 2.70-fold higher than that of the pure piperine and a 1.62-fold compared with that of piperine solid dispersion and a 3.65-fold higher oral bioavailability as a nanosuspension than its coarse suspension [39], Y. [40, 41]. The studies provide evidence that piperine enhances the bioavailability of many compounds; the serum response of  $\beta$ -carotene is increased by 60% when supplemented with piperine through the oral route [42]. Piperine also increased the bioavailability of silybin by 146–181% and contributed to enhance the therapeutic effect in  $\text{CCl}_4$ -induced acute liver-injury rat model [43]. For raloxifene in pro-nano lipospheric form with piperine, it provides a twofold increase in the oral bioavailability [44].

Piperine in combination with curcumin loaded in the emulosome is reported to achieve a sixfold increase in caspase-3 activity and is found to be most effective in the inhibition of cell proliferation [45]. A new mechanism has been introduced by which piperine enhances the bioavailability of curcumin by selectively reducing

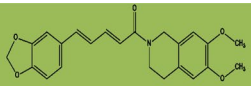
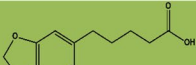
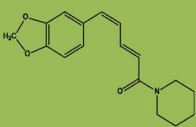
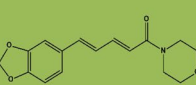
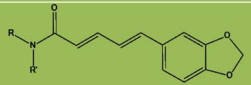
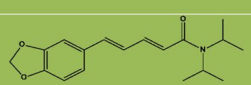
the expression of uridine diphosphate glucuronosyltransferase (UGT) and sulfotransferase (SULT) [46].

Supplementation of iron (Fe) with piperine orally benefitted the absorption of Fe and could be potentially helpful in the treatment of anemia, but an investigation is needed in this regard [47]. (-)-Epigallocatechin-3-gallate (EG) obtained from *Camellia sinensis* (green tea) is reported for its chemopreventive activity in animal models of carcinogenesis, piperine was also reported to enhance its bioavailability by 1.3-fold as compared to EG alone [48]. The combination of paclitaxel and piperine was found to improve the bioavailability of paclitaxel for triple-negative breast cancer by targeting epidermal growth factor receptor (EGFR) [49].

## 2.5 Molecular targets of piperine in human

Piperine being a bioavailability enhancer increases plasma concentration of various drugs. It inhibits the drug-metabolizing enzymes by acting on P-gp substrate

**Table 3** Analogues and derivatives of piperine with their biological activity and mechanism of action

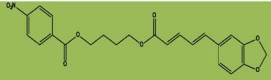
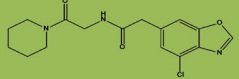
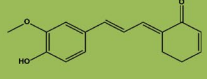
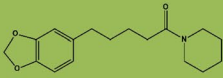
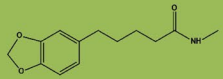
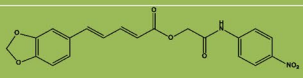
Analogue and derivatives of piperine	Biological activity reported after modification of piperine to analogue	Mechanism of action of the analogue	References
 <p>(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)penta-2,4-dien-1-one (Pip 1)</p>	Anticancer activity	Inhibition of P-glycoprotein function	Syed et al. [50]
 <p>5-(benzo[d][1,3]dioxol-5-yl)pentanoic acid (Piperinic acid)</p>  <p>(2E,4Z)-5-(benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)penta-2,4-dien-1-one (8c)</p>  <p>(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-1-morpholinopenta-2,4-dien-1-one (morpholynil amide) and (THP) Faas et al. [168]</p>	Leishmanicidal activity	Reduction of triglyceride, diacylglycerol, and monoacylglycerol contents	Ferreira et al. [94], Sahi et al. [95]
 <p>Compound 5b (R=R'= Ethyl) Compound 5c (R=H; R'= Isopropyl)</p>	Insecticidal activity	—	Paula et al. [97]
 <p>(2E,4E)-5-(1,3-benzodioxol-5-yl)-N,N-dipropyl-2,4-pentadienamide (Compound 23)</p>	Neurological activity	Induction of strong anxiolysis by TRPV1 (transient receptor potential vanilloid type 1 receptor) activation and modulation of $\gamma$ -aminobutyric acid type A (GABA <sub>A</sub> ) receptors	Schöffmann et al. [17]

[50]. Although piperine has demonstrated its health benefits in human, its underlying mechanism remains unknown; information corroborated from the clinical trials often suffers the limitation of small-sized racial variations, typical considerations and geographical variations, which compromise to explore the molecular mechanism.

However, cell-cycle proteins, P-gp, Rv1258c, PRPV1, Akt/mTOR signaling, AMPK-mediated activation of NLRP3 inflammasome, voltage-gated K<sup>+</sup> current, IL 10, miR21, and PKC $\alpha$ /ERK1/2 and NF- $\kappa$ B/AP-1-dependent MMP-9 expression are the main targets altered by piperine (Figs. 3, 4 and 5).



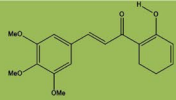
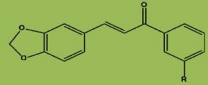
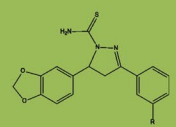
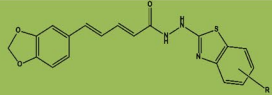
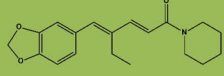
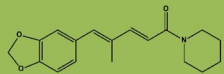
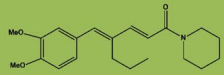
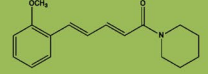
**Table 3** (continued)

 <p><b>Butyl 4-(4-nitrobenzoate)-piperinoate (DE-07)</b></p>	Antitumor effect	Oxidative stress induced cell death	Ferreira et al. [166]
 <p><b>2-(4-chlorobenzo[d]oxazol-6-yl)-N-(2-oxo-2-(piperidin-1-yl)ethyl)acetamide (HJ22)</b></p>	Activity in Alzheimer's disease	Inhibition of Kelch-like ECH-associated protein (Keap1)-nuclear factor erythroid-2-related factor 2 (Nrf2) protein-protein interaction	Yang et al. [167]
 <p><b>(E)-6-((E)-3-(4-hydroxy-3-methoxyphenyl)allylidene)cyclohex-2-en-1-one (9m)</b></p>	Cytotoxic activity	Inhibits TrxR that induces ROS and autophagy, activates p38 signaling and suppresses Akt/mTOR signaling	Zhu et al. [40, 41]
 <p><b>5-(3,4-methylenedioxyphenyl)-pentano-1-yl piperidine (THP)</b></p>	Activity against depigmenting disease (Vitiligo treatment)	Darker pigmentation of skin in treated areas corresponds with an increase in the number of DOPA+ melanocytes	Faas et al. [168]
 <p><b>5-(3,4-methylenedioxyphenyl)-2,4-pentano-1-yl cyclohexylamine (rCHP)</b></p>			
 <p><b>2-((4-nitrophenyl)amino)-2-oxoethyl (2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)penta-2,4-dienoate (HE-02)</b></p>	Antitumor effect	Th1-Biased immuno-modulation by increase of Th1 helper T lymphocytes cytokine profile levels (IL-1 $\beta$ , TNF- $\alpha$ , IL-12) and a decrease of Th2 cytokine profile (IL-4, IL-10)	Santos et al. [169]

The preclinical studies suggested piperine acting on various cell cycle proteins (Cyclin D, Cyclin T, CDK2 and CDK4) became a future point of intense investigation. The molecular docking analysis confirmed that piperine binds cell cycle proteins via hydrogen bonding and impaired the cell cycle progression [51]. Piperine exhibited an antitumor effect by inhibiting the S-phase by

forming a hydrogen bond with Ser5 at the ATP-binding site on CDK2 protein. It interacts with the Lys8 residue in cyclin A and inhibits apoptosis by interacting with the hydrophobic groove of the Bcl-xL protein [52]. Piperine accelerates the bioavailability of phenytoin and rifampin by inhibiting the drug transporter P-gp and CYP3A4 [53]. In addition to this, ABC transporter genes are also

**Table 3** (continued)

 <p>(E)-1-(2-hydroxycyclohexa-1,3-dien-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one <b>10</b></p>  <p>R=H= (E)-3-(benzo[d][1,3]dioxol-5-yl)-1-phenylprop-2-en-1-one (<b>3a</b>); R=NH<sub>2</sub>= (E)-1-(3-aminophenyl)-3-(benzo[d][1,3]dioxol-5-yl)prop-2-en-1-one (<b>3c</b>)</p>  <p>R=H= 5-(benzo[d][1,3]dioxol-5-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (<b>4a</b>); R=NH<sub>2</sub>= 3-(3-aminophenyl)-5-(benzo[d][1,3]dioxol-5-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (<b>4c</b>)</p>	Anti-inflammatory, anti-carcinogenic activity	Inhibition of the IL-1 $\beta$ and NF- $\kappa$ B pathway	Zazeri et al. [170]
 <p>Compound <b>6c</b> (R= 6- Cl) Compound <b>6d</b> (R=6- Br)</p>	Anti-diabetic	Enhancing the PPAR- $\gamma$ gene expression	Kharbanda et al. [171]
13 analogues of Ethyl 3',4',5'-trimethoxycinnamate and piperine, from the combined hexane and chloroform extracts of <i>Piper longum</i>	Anti-inflammatory and anticancer	Inhibition of TNF- $\alpha$ -induced expression of cell adhesion molecules like ICAM-1, VCAM-1, and E-selectin,	Kumar et al. [172]
 <p>(2E,4E)-4-(benzo[d][1,3]dioxol-5-ylmethylene)-1-(piperidin-1-yl)hex-2-en-1-one (<b>SK-20</b>)</p>  <p>(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-4-methyl-1-(piperidin-1-yl)penta-2,4-dien-1-one (<b>SK-29</b>)</p>  <p>(E)-4-((E)-3,4-dimethoxybenzylidene)-1-(piperidin-1-yl)hept-2-en-1-one (<b>SK-56</b>)</p>  <p>(2E,4E)-5-(2-methoxyphenyl)-1-(piperidin-1-yl)penta-2,4-dien-1-one (<b>3b</b>)</p>	Anti-bacterial activity	Inhibition of NorA efflux pump	Kumar et al. [173]
	Neuroprotective action in the treatment of Parkinson's disease	Activation of Nrf2/keap1 pathway	Wang et al. [174]

**Table 4** Methods of piperine extraction from black and white pepper

Methods	Apparatus used	Extraction yield & (extraction time)	Solvent used	Advantage	Disadvantage	References
CHE	Soxhlet apparatus	3.2% 2 h	95% Ethanol, 10% aq. Potassium hydroxide	Simple, low cost, no filtration is required after leaching	A long time of extraction and a large amount of extractant is required	Luque de Castro and Priego-Capote [175], Shingate et al. [25]
Reflux Extraction	RBF, condenser, Buchner funnel, etc.	5% 20 min	Dichloro-methane, Acetone and Hexane	More efficient than percolation or maceration and requires less extraction time and solvent	Cannot be used for thermolabile natural products	Shingate et al. [25], Zhang et al. [176]
Cold Maceration	Separating funnel, Rota evaporator, etc.	4.6% –	GAA, chloroform, 10% sodium bicarbonate, toluene, ethyl acetate, sodium hydroxide, diethyl ether	Yield is high, pure, and crystallizable as compared to the above two methods	Complex and time-consuming	Shingate et al. [25]
EASC-CO <sub>2</sub> Extraction	SPEED SFE 2, Ice-bath	0.88–1.38 mg/g dry black pepper 2.25 h	α-Amylase, CO <sub>2</sub> , methanol	Efficient	Expensive	Dutta and Bhattacharjee [177, 178]
ILUA Extraction	KQ-100DA and KQ-500 ultrasonic water baths (Kunshan, Jiangsu, China), Acuity <sup>TM</sup> UPLC (Waters, Milford, MA, USA)	3.57% 30 min	1-Alkyl-3-methylimidazolium ionic liquids, deionized water, methanol	High extraction efficiency, and less extraction time	Expensive	Cao et al. [179]
SLDE	Naviglio Extractor <sup>®</sup>	317.7 mg/g 3 h	96% Ethanol	Simple application, exhaustion in a short period, production of high-quality extracts	Expensive	Gigliarelli et al. [180], Naviglio et al. [181]
SMUAE	Microwave oven (CQ4250, Samsung) Ultrasonic bath (Elmasonic S10H)	46.6 mg/g 31 min	Ethanol, Methanol, Acetone, Dichloromethane Potassium hydroxide, Hexane, Acetonitrile	Increased extraction efficiency	Not suitable for thermolabile natural products	Gorgani et al. [1, 182], Zhang et al. [176]
MAE	IFBdomestic microwave oven (model Neutron)	45% 4 h	Petroleum ether, water	Simple, rapid, and reliable	Not suitable for thermolabile natural products	Raman and Gaikar [30]
UAE	–	0.58% w/w 18 min	Ethanol, hexane, and acetone	Short running time, higher extractive yield, controllable parameters	Small particle size, more filtration steps	Shityakov et al. [183]
SFE	–	90–96% w/w 2–5 h	Liquid carbon dioxide	Efficient, selective, clean, fast	High cost, less pressure-resistant	Shityakov et al. [183]

**Table 5** Pharmacokinetics effect of piperine on different drugs

Drug	Dose (Piperine + Drug, duration)	ROA	Methods of detection	Plasma level	References
Propranolol	20 mg + 40 mg, 7 days	oral	Spectrofluorimetric method	1000–1200 ng mL <sup>-1</sup> h	Bano et al. [184]
Theophylline	20 mg + 150 mg, 7 days	Oral	EMIT	*80–90 µg mL <sup>-1</sup> h	Bano et al. [184]
Diclofenac	20 mg + 100 mg, 10 days	Oral	NCAM, Phoenix WinNonlin 6.2 software	7.09–11.81 µg mL <sup>-1</sup> h	Satish Kumar Bedada et al. [155, 156]
CBZ	20 mg + 200 mg, 10 days	Oral	NCAM, Phoenix®, WinNonlin 6.4® software	40–70 µg mL <sup>-1</sup> h	Bedada et al. [155, 156]
Emodin	20 mg/kg + 20 mg/kg, 1 day	Oral	LC–MS/MS	1913–2555 ng mL <sup>-1</sup> h	Di et al. [185]
Linarin	20 mg/kg + 50 mg/kg, 1 day	Oral	NCAM, DAS 2.1.1 Software, ANOVA	240–934 ng mL <sup>-1</sup> h	Feng et al. [186]
Curcumin	In rats-20 mg/kg + 2 g/kg, 1 day In humans- 5 mg + 500 mg, 1 day	Oral	MIM, PHARMKIT computer programme with SIMPLEX algorithm	3.33–3.95 µg mL <sup>-1</sup> h 0.07–0.09 µg mL <sup>-1</sup> h	Shoba et al. [187]
Cannabidiol	10 mg/kg + 15 mg/kg, 10 days	Oral	NCAM, WinNonlin® (version 5.2, Pharsight, Mountain View, CA)	Acute- 576–610 Ng mL <sup>-1</sup> h Chronic- 722–896 ng mL <sup>-1</sup> h	Izgelov et al. [36]
Fexofenadin	10 mg/kg + 10 mg/kg 10 mg/kg + 5 mg/kg, 1 day	Oral oral + IV	NCAM, WinNonlin® (version 5.2, Pharsight, Mountain View, CA)	687–1353 ng mL <sup>-1</sup> h 5670–9830 ng mL <sup>-1</sup> h	Jin and Han [188]
Sodium valproate	5 mg/kg + 150 mg/kg, 1 day	Oral	NCAM, trapezoidal method	1024 µg mL <sup>-1</sup> h	Parveen et al. [37]
OXR	10 mg/kg + 100 mg/kg 1 mg/kg + 10 mg/kg, 1 day	Oral IV	NCAM, PK Solution 2.0 software (Summit Research Service)	9375.27 ± 1974.32 µg h/L 1471.00 ± 1945.62 µg h/L	Junsaeng et al. [38]

\* Serum concentration

**Table 6** Pharmacokinetic parameters of piperine in human body

Route of administration	Dose (mg)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>0–∞</sub> (µg h/mL)	t <sub>1/2</sub> (h)	References
Oral	24	3.77 ± 1.63	2.15 ± 1.21	58.41 ± 23.50	8.74 ± 8.95	Itharat et al. [189]
Oral	20	290.00 ± 42.47 595.4 ± 108.6	–	59.32 ± 10.82 15.79 ± 50.50	13.26 ± 1.91 15.82 ± 4.95	Wen-xing [190]
Oral	20	290 ± 40	3.50 ± 1.78	5.93 ± 1.08	13.3 ± 1.9	Ren and Zuo [191]

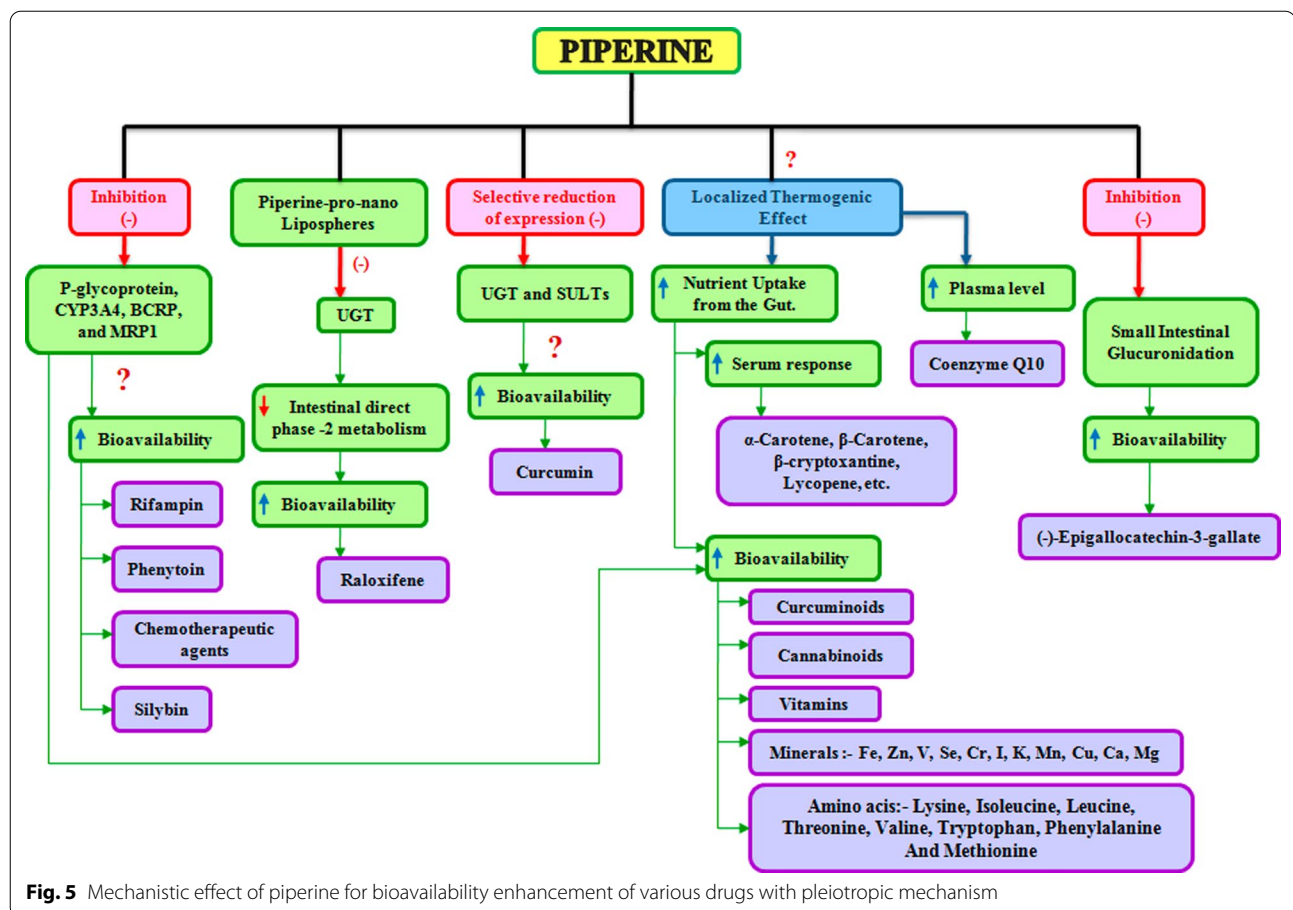
**Table 7** Pharmacokinetic parameters of piperine in rodents

Route of administration	Dose (mg/kg)	C <sub>max</sub> (µg/mL)	T <sub>max</sub> (h)	t <sub>1/2</sub> (h)	AUC <sub>0–∞</sub> (µg h/mL)	References
Oral	20	1.10	2.00	1.27	7.20	Ren and Zuo [191]
Oral	54.4	4.29 ± 0.97	2.45 ± 2.12	4.10 ± 0.94	23.1 ± 0.1	
Intravenous	10	2.90	–	8	15.6	
Intravenous	3.5	5.90 ± 1.76	–	1.68 ± 0.40	3.80 ± 0.84	
Intra-peritoneal	20	0.051 ± 0.009	3.00 ± 0.17	–	1.22	

inhibited by piperine, which pumps out several chemotherapeutic agents [54–56].

The natural product analogue Pip1 (Table 3) is found superior to piperine as for its inhibition of the P-gp function and resistance reversal in a cancer cell [50]. Rv1258c is a transporter protein that confers resistance to anti-tubercular agents like isoniazid, rifampicin, ethambutol,

pyrazinamide, and p-aminosalicylic acid, approved by *in silico* studies. However, piperine in combination with these agents increases bioavailability by inhibiting the Rv1258c pump. Non-selective cation channel TRPV1 gets mildly activated by piperine, thereby inhibiting the seizure and obesity. Studies corroborated that piperine downregulated the PI3K/Akt/mTOR signaling pathway



**Fig. 5** Mechanistic effect of piperine for bioavailability enhancement of various drugs with pleiotropic mechanism

[57]. However, it reduces the MMP-9 expression in DU-145 cells. The AMPK signaling pathway plays a key role in regulating the immunological disease progression [58].

The increasing dose-dependent concentration of piperine effectively downregulates the increased NLRP3 inflammasome; however, pro-IL-18 and serum levels of IL-18 were excluded in the study [59]. Piperine induces G1 cell-cycle arrest and induces apoptosis in androgen-sensitive LNCaP and androgen-insensitive PC-3 cells by inhibiting the  $I_K$  [60]. Piperine in combination with curcumin and taurine decreases the plasma level of IL-10 and miR-21; however, the exact molecular mechanism of interaction needs to be investigated [61, 62].

MMP-9 is expressed abundantly in malignant tumors and contributes to cancer invasion and metastasis [63]. PKC $\alpha$ /ERK1/2 and NF- $\kappa$ B/AP-1 pathways are among the major signaling pathway that regulates tumor cell invasion. Piperine downregulated the MMP-9 expression by inhibiting PKC $\alpha$ /ERK1/2 and NF- $\kappa$ B/AP-1 pathway in PMA-induced *in vitro* tumor model [64]. It also inhibits the invasion and migration of HT-1080 cells.

PPAR $\gamma$  is an adipogenic transcription factor and is associated with several diseases [65]. Piperine inhibits adipocyte differentiation via an antagonistic effect on PPAR $\gamma$  [66]. GM-CSF, TNF- $\alpha$ , MMP-2, MMP-9, and proinflammatory cytokines like IL-1 $\beta$  and IL-6 are involved in cancer progression mediated by NF- $\kappa$ B and AP-1. Piperine inhibited the translocation of NF- $\kappa$ B subunits like p50, p65, and c-Rel as well as CREB, ATF-2, and c-Fos [67]. MiR-127 up-regulation is correlated with worsening of LPS-induced inflammation [31, 32]. Piperine has been showing anti-inflammatory action in the LPS-induced *in vitro* model of osteoarthritis by down-regulating miR-127 and MyD88 expression [68]. The wnt/ $\beta$ -catenin signaling is a molecular target for colorectal cancer, ovarian cancer, and HCC [69–71]. Piperine inhibits the wnt/ $\beta$ -catenin signaling by impairing the TCF binding to the DNA and alters the cell-cycle progression. It also decreases the metastasis in intestinal tumor cells [72]. Altered pathways are involved in many tumor developments. Piperine increases the JNK and p38 MAPK phosphorylation, thereby activating the JNK/P38 MAPK pathway and inducing apoptosis in ovarian cancer cells [73]. The anti-tumor effect of piperine is associated



with stabilizing the G-quadruplex structure formed at the c-myc promoter region, which alters the transcription mechanism [74]. Piperine improves CIRI-induced injury of the ischemic penumbra region by downregulating the COX-2, NOS-2, and NF- $\kappa$ B [75, 76].

Piperine interacts with the  $_{\text{r}}(\text{CGG})^{\text{exp}}$  RNA with high selectivity to the G-rich RNA motif whose expansion in 5' UTR of *FMR1* gene causes the Fragile X-associated tremor/ataxia syndrome. The transcripts of these expanded repeats  $_{\text{r}}(\text{CGG})^{\text{exp}}$  either form RNA foci or undergo the RAN translation, which in turn produces toxic proteins in the neuronal cells. Piperine is found to improve the  $_{\text{r}}(\text{CGG})^{\text{exp}}$ -related splicing defects and RAN translation in the FXTAS cell model system [77].

## 2.6 Different biological activities reported on piperine

### 2.6.1 Anticancer activity

Piperine alone and in combination with other natural or synthetic drugs has shown potential for anti-cancer activity [78]. In an *in vitro* model, piperine showed synergistic antiproliferative effects in MCF7 cell line, and it synergizes tamoxifen in combination with hesperidin and bee venom in MCF7 and T47D cell lines [79]. It lowered the LC50 value of paclitaxel (from 50 to 25  $\mu\text{M}$ ) and decreased the lag phase mostly during the paclitaxel action-time in an *in vitro* MDA MB-231 cell-line model. It also increased the cytotoxic and anti-proliferative effect of paclitaxel and doxorubicin when used in combination (Kanthaiah Original Research et al. [80]). In an *in vivo* model (EMT6/P cells were inoculated in Balb/C mice), piperine along with thymoquinone inhibited angiogenesis, induced apoptosis, and shifted the immune response toward T helper1, and further study is needed in this context [81]. *In vitro* stem cell model for breast cancer was utilized to evaluate the cancer-preventive effects of piperine and curcumin in combination therapy and the inhibition of mammosphere formation, serial passaging, aldehyde dehydrogenase (ALDH+) breast stem cells in both normal and malignant breast cells, and inhibition of Wnt signaling was observed [82]. Proliferation and induced apoptosis through caspase-3 activation and PARP (*Poly (ADP-ribose) polymerase*) cleavage were strongly inhibited by piperine, thereby inhibiting the HER2 gene expression at the transcriptional level. Pretreatment with piperine also accelerated sensitization to paclitaxel killing in HER2-overexpressing breast cancer cells [83]. Piperine causes G1 phase cell cycle arrest and apoptosis in SK-MEL 28 and B16-F0 cell lines via the activation of checkpoint kinase 1 followed by downregulation of XIAP, full-length Bid (FL-Bid), and cleavage of Caspase-3 and PARP [84]. Multidrug-resistant cancers were targeted and treated by curcumin–piperine dual drug-loaded nanoparticles [85]. Guar gum microvehicle

loaded with thymoquinone and piperine exhibited low median lethal dose (LD50) value against human hepatocellular carcinoma cell lines [86]. **Piperine-free extract of *Piper nigrum* exhibited anticancer effects on cholangiocarcinoma cell lines** [87]. Piperine exhibited cytoprotective. The proliferation of prostate cancer cell lines was inhibited by piperine by reducing the expression of phosphorylated STAT-3 and nuclear factor- $\kappa$ B (NF- $\kappa$ B) transcription factors [88]. Piperine-loaded core-shell nanoparticles caused a substantial change in cytotoxicity compared to free drugs, with a rise in G2/M-phase and pre-G1-phase population, CDK2a inhibition, and apoptotic/necrotic rates in human brain cancer cell line (Hs683) [89]. Piperine inhibited cell-cycle progression in rectal cancer cells by causing ROS-mediated apoptosis [90].

### 2.6.2 Antimicrobial activity

Piperine exhibited potential inhibitory activity against Ebola and Dengue viruses by suppressing the targeted enzymes such as *Methyltransferase* of Dengue and *VP35 interferon inhibitory domain* of the Ebola virus [91]. It also showed more affinity toward viral proteins in comparison with Ribavirin. Piperine (12.5 and 25  $\mu\text{g/ml}$ ) showed a twofold reduction in the MIC of ciprofloxacin (0.25–0.12  $\mu\text{g/ml}$ ) for *Staphylococcus aureus* (ATCC 29213), the underlying mechanism for which is stated as that piperine inhibits the ciprofloxacin efflux from bacterial cells by inhibiting the P-glycoprotein [92]. Twenty-five analogues of piperine were also found to inhibit the *Staphylococcus aureus* NorA efflux pump [93]. Piperine, along with its derivatives and analogues, exhibited Leishmanicidal activity against *Leishmania amazonensis* and *Leishmania donovani* [94, 95]. Piperine (15  $\mu\text{g/ml}$ ) was found to inhibit the planktonic growth and shows a stage-dependent activity against biofilm growth of *Candida albicans* (ATCC10231) by affecting its membrane integrity [96]. Amide derivatives of piperine have also emerged as potential insecticides, among which the compounds 5b and 5d are the most toxic against Brazilian insect *Ascia monuste orseis* with a mortality percentage of 97.5% and 95%, respectively [97].

### 2.6.3 Action on metabolic diseases

The use of piperine for reversing metabolic disease usually involves a bioavailability enhancer. Greater consumption of energy leads to adiposity and fat cell enlargement producing the pathology of obesity, which is the most significant medical problem [98, 99]. Increased fat mass is associated with risk conditions such as stroke, coronary heart disease, and type 2 diabetes mellitus known as excessive fat-related metabolic disorders (EFRMD) [99, 100]. Melanocortin-4(MC-4),

a hypothalamic neuropeptide, regulates obesity by controlling the feeding mechanism via binding to the MC-4 receptor [101–103]. Increased MC-4 receptor activity leads to a decrease in appetite, increased energy expenditure, and insulin sensitivity. Studies reported that piperine (40 mg/kg) can be used as an MC-4 agonist and has potential use in improving the lipid profile [104]. In addition, piperine (50 mg/kg bw) improves insulin signaling in HFD-induced hepatic steatosis by reversing the plasma adiponectin, insulin, and glucose concentration [105]. Another study suggested that supplementation of piperine (30 mg/kg) is helpful for normalizing the blood pressure, plasma parameters of oxidative stress, and inflammation [106]. However, in a randomized controlled trial to improve the bioavailability, the curcuminoids were administered with piperine (Bioperine®) in the ratio of 100:1, an efficacious adjunct therapy for patients with metabolic diseases [14].

#### 2.6.4 Action on neurological diseases

The most common neurological disorders where piperine has shown experimental neuroprotective potential are Alzheimer's disease (AD), Parkinson's disease (PD), and cognitive impairment [107–109]. Various signaling molecular pathways such as oxidative stress, ER stress, inflammation, MicroRNA, mitochondrial damage, and gut microbiota have been implicated in these diseases [107–113]. Piperine with 50 mg oral dose given to human volunteers shows plasma concentration of 5 ng/mL [10]. Therefore, piperine is likely to cross the BBB [114], and the development of its potential analogue explores the application in treating neurological disorders. Piperine analogue interacts with potential CNS target like GABAA, TRPV1 and adenosine A2A receptors and MAO-B involved in neurodegenerative disease. Other studies have shown that combinational treatment of piperine with other phytochemicals like curcumin improves cognitive impairment by decreasing oxidative stress [111, 112]. Piperines play a pivotal role in neuroprotection by reducing the inflammatory cytokine, oxidative stress, and mitochondrial impairment.

Cerebral stroke is the leading cause of death and physical disability worldwide; still, only one FDA-approved drug recombinant tissue plasminogen activator (r-tPA) is working with a low therapeutic window [115]. Co-administration of r-tPA and curcumin with piperine (20 mg) can be used to increase the therapeutic window of treatment by boosting the bioavailability of curcumin by 2000% [116]. An elevated level of proinflammatory cytokine IL-1 $\beta$ , IL-6, and TNF- $\alpha$  manifests in inflammation. Piperine is able to reduce neuronal cell death in the ischemic penumbral zone by anti-inflammatory effect [76]. Piperine is a natural bioenhancer to increase the

bioavailability of phytochemicals including curcumin and resveratrol [38].

Piperine neuroprotective efficacy on neurological and cognitive disorders has been examined in the rodent model of Alzheimer, Parkinson, and epilepsy diseases [108, 109, 114, 117]. Piperine (2–5–10 mg/day body weight) may also exert neuroprotective potential by examining the locomotor activity, cognitive performance, and biochemical and neurochemical manifestation of the hippocampus [108, 118]. The oral treatment of piperine (10 mg/day bwt) enhanced the cognitive learning ability in MPTP- and 6-OHDA-induced Parkinson's mouse model [109, 114]. The antioxidant property of piperine is demonstrated by its anti-apoptotic and anti-inflammatory mechanism of the 6-OHDA-induced PD model [114]. Piperine exerted in vitro neuroprotective effects against corticosterone-induced neurotoxicity in PC12 cells via antioxidant and mRNA expression of BDNF [119, 120]. Therefore, these results suggested that piperine crosses the BBB [121]. However, these results of pre-clinical studies remain to be validated for translational effect on human subjects.

#### 2.6.5 Action on cardiovascular disease

Piperine exhibited the cardioprotective effect by regulating lipid metabolism, inflammation, and oxidative stress. Piperdardine and piperine in equal amounts lower hypotension and heart rate [122]. Intravenous administration of piperine (1.5, 2.5, and 5.0 mg/kg) decreased the increased blood pressure in rats [123]. The *Sahat-satara* (a herbal formulation) contains piperine (1.29% w/w) caused relaxation in the thoracic aorta and showed potential for vasculoprotective effect in hypertensive and nitric oxide-impaired condition in rats [124]. Piperine (20 mg/kg) exhibited significant cardioprotective ability in combination with curcumin (50 mg/kg) [125]. Piperine exhibited a vasomodulatory and blood pressure-lowering effect that could be mediated via the Ca<sup>2+</sup> channel [126]. Piperine upregulates the ABCA1 and aids in promoting the cholesterol efflux in THP-1-derived macrophages, which later inhibits calpain activity, which indicates that piperine is a good candidate for further exploration in atherosclerosis and cardiovascular diseases [127].

#### 2.6.6 Anti-inflammatory action

Piperine has been employed in various animal models like carrageenan-induced rat paw edema, cotton pellet granuloma, croton oil-induced granuloma pouch, formalin-induced arthritis, high fat diet-induced inflammation in subcutaneous adipose tissue, and another model like IL-1 $\beta$  induced expression of inflammatory mediators and ultraviolet B (UV-B)-induced inflammatory responses in the human skin for anti-inflammatory activities

[128–133]. The suppression of activated phosphorylated p38, JNK, and AP-1 as well as the levels of COX-2/PGE2 and iNOS synthesis was seen after pretreating the HaCaT keratinocyte cells with piperine prior to UV-B treatment [129]. A recent study showed that bioperine improved the bioaccessibility and in vivo anti-inflammatory activity of carrageenan-complexed piperine in Wistar rats by revealing a better bioaccessibility ( $C_{\max}=0.34 \mu\text{g/ml}$ ;  $T_{\max}$  at 30 min) of the carrageenan-complexed piperine than that of the isolated piperine ( $C_{\max}=0.12 \mu\text{g/ml}$ ,  $T_{\max}$  at 60 min) [132]. The percentage inhibition of inflammation was considerable at 56% for the carrageenan-induced paw edema model and 40% for the formalin-induced arthritis model; however, in the cotton pellet-induced granuloma model, it was only 10% [131]. Piperine in combination with curcumin at nutritional doses was able to reduce the expression of the inflammatory cytokine in the adipose tissue, indicating that it could be utilized in the treatment of inflammatory conditions in metabolic disorders related to obesity [130]. It has promising activity in the reversal of hepatotoxicity in combination with *Aegle marmelos* leaf extract; it potentiates the antioxidant and anti-inflammatory properties of *A. marmelos* [134]. It effectively abrogated the IL-1 $\beta$ -induced overexpression of inflammatory mediators by inhibiting the production of PGE2 and nitric oxide induced by IL-1 $\beta$ ; in addition, it decreased the IL-1 $\beta$ -stimulated gene expression and production of MMP-3, MMP-13, iNOS, and COX-2 in human osteoarthritis chondrocytes; it also inhibited the IL-1 $\beta$ -mediated activation of NF- $\kappa$ B by suppressing the I $\kappa$ B $\alpha$  degradation in the cytoplasm [133]. Apart from its own anti-inflammatory activity, it is also found to enhance the anti-inflammatory activities of Thymoquinone [135]. Piperine is in combination with resveratrol decreases morbidity to some extent with little or no effect on mortality associated with lupus in Systemic Lupus Erythematosus (SLE) [136].

#### 2.6.7 Action on reproductive organs

Piperine showed inhibitory action in the inflammation of inner lining of uterus mainly caused by *Staphylococcus aureus* [137]. Through the ERK1/2 and AKT pathways, piperine mediates the stimulation of pubertal Leydig cellular development; however, it inhibits spermatogenesis in rodents [138]. However, at a dose of 10 mg/kg, the serum gonadotropin concentration increases, whereas testosterone concentration decreases [139]. It impaired reproductive function via altered oxidative stress by increased expression of Caspase-3 and Fas protein in testicular germ cells [140]. It is reported to decrease the antioxidant activity of enzymes and sialic acid levels in the epididymis, and thus, reactive oxygen species (ROS) level increases that could potentially harm the epididymal

environment and sperm function [141]. Piperine could be a lead molecule to develop reversible oral male contraceptive; however, further evidences are needed to be investigated.

#### 2.6.8 Role of piperine on gut microbiota

Microbiota and host form complex super organism in which a symbiotic relationship confers the benefits of the host in many key aspects of life. Understanding the healthy microbiome (totality of microbes) in the human microbiome project has the major challenge and needs to decipher after the oral administration of certain phytochemicals such as piperine, lycopene, and curcumin. Piperine was tested against various culture media like *Prevotella bryantii* (B14), *Acetoanaerobium sticklandii* (SR), *Bacteroides fragilis* (ATCC 25285), *Clostridioides difficile* (ATCC 9689) among which piperine showed inhibitory action against only *B. fragilis* at concentrations  $\geq 0.10 \text{ mg mL}^{-1}$  ( $105 \text{ cells mL}^{-1}$ ) [142]. Piperine with curcumin displayed an average of 69% increase in the species detected in gut microbiota [143]. There is an unmet need to explore the potential interaction of piperine with another nutrient by using LC–MS/MS [144]. LC–MS/MS is a technique available for simultaneous detection of degraded microbial metabolites of piperine. It was revealed by HPLC analysis that tetrahydro curcumin ( $235 \pm 78 \text{ ng/100 mg tissue}$ ) was present in the adipose tissue after supplementing Curcuma-P<sup>®</sup> (extract rich in curcumin and associated with white pepper) for 4 weeks [130].

#### 2.7 Toxicological effect of piperine

Spices and herbs have been consumed for centuries either as food or remedial necessity. The potential health benefits of the phytochemicals from these herbs could become toxic depending on the dose of exposure and may exhibit toxic effects [145]. Piperine, when administered IV, is more toxic as compared to IG, SC, and IM. The less toxicity of piperine through the IG route is suggested as for its insolubility or chemical instability in the stomach. Thereby, piperine induces hemorrhagic ulceration in the stomach and mild-to-moderate enteritis in the SI and histopathologic lesions in the g.i.t., suggesting that piperine has a local and direct effect on the gastrointestinal lumen. The LD50 values in adult male mice for a single dose of piperine through i.v., i.p., s.c., i.g., and i.m. administration are about 15.1, 43, 200, 330, and 400 mg/kg body wt, respectively [146]. Piperine's toxicity affects mainly the reproductive system [147]. Piperine (10 mg/kg, p.o.) induced an increase in serum gonadotropins and a decrease in intratesticular testosterone in male albino rats; reports were also there that piperine interferes

with crucial reproductive events in a Swiss albino-mammalian model [148].

## 2.8 Piperine as a repurposing molecule for reversing the COVID-19 pandemic

Healthy gut microbiota helps to increase the immune system of COVID-19 patients. There is unmet need to identify the different microbial metabolites present after the degradation of piperine and other plant-derived molecules by using LC–MS/MS. Microbial metabolites have an ability to cross the BBB and provide pleiotropic effects on the brain and other organs by altering the gene expression. Healthy gut microbiome identification in stool samples of COVID-19 patients may be a better approach for precision medicine by utilizing Fecal Microbiota Transplantation (FMT) technologies for COVID-19 patients. Black pepper consumption, besides its immunomodulatory functions, may also aid in combating SARS-CoV-2 directly through possible antiviral effects [149]. It has recently been reported that piperine has demonstrated binding interactions toward the spike glycoprotein and ACE2 cellular receptor for SARS-CoV-2. The interactions of hydrogen bonds with Gly399, His401, Glu402, Arg514, Arg518 were found significant by forming one predictable hydrogen bond with each amino acid residue [150]. Piperine interacts with the main protease at the docking score of -90.95 and binding energy score of -78.10 kcal mol<sup>-1</sup>, forming one hydrogen bond with His41; other stabilizing interactions include  $\pi$ -sulfur,  $\pi$ - $\sigma$ ,  $\pi$ - $\pi$  T-shaped, and alkyl interactions. Piperine with a binding affinity of -6.4 kcal mol<sup>-1</sup> forms hydrogen bond interaction with GLY164 and GLY170; its binding process is also governed by van der Waals interactions with ARG71, TYR121 (TYR453), TYR163 (TYR495), and ASN169 (ASN501) of SARS-CoV-2 receptor-binding domain spike protein (RBD Spro). The major stabilizing interactions of piperine with SARS-CoV-2 RBD Spro were by covalent hydrogen bonding,  $\pi$ - $\pi$  T-shaped, and van der Waals force of interactions [151]. Piperine acts on the Nsp15 viral protein and inhibits SARS-CoV-2 replication [152, 153]. Furthermore, binding chemistry of piperine and curcumin via  $\pi$ - $\pi$  intermolecular interactions enhances curcumin's bioavailability, which facilitates curcumin to bind RBD Spro and ACE-2 receptors of host cell, thereby inhibiting the entry of virus inside the host [152, 153].

## 3 Conclusion

Since its identification in 1820, piperine pleiotropic activities have been reported in many studies. However, most of the discussions are based on preclinical as well as in vitro model systems. As summarized

in this review, piperine exhibits significant preclinical activities against a number of human diseases including cancer and inflammatory disorders. A few potential molecular targets were explored in the context of different diseases. However, some targets remain unexplored for the DAB-2 gene in the TGF- $\beta$  pathway in chronic kidney disease. The underlying mechanism of its efficacy against different ailments and chronic illnesses seems to be due to its ability to modulate many different signaling pathways. Bioavailability enhancement by retarding the glucuronidation reactions, affecting certain proteins and enzymes, and increasing the nutrient uptake from the gut is among the few explanatory findings in the scope of its bioenhancer properties. Future research is needed to explore the different metabolic products produced from the gut microbiota after the microbial degradation of piperine and its related isomers. These microbe-mediated products may play a contributing factor for the toxicity of different organs.

Among all the clinical trials done on piperine, it was used either alone or in combination with other drugs, and the safe dose reported for action was 5 mg/day. A threshold of toxicity of 50 mg/kg bw/day is proposed for piperine. It is also used as a repurposed medicine to explore the inhibitory action on new molecular targets in the context of COVID-19, and only a few computational studies have been able to produce satisfactory results; however, *in vivo* models should be designed to provide thorough evidence. Further studies are needed to explore the role of other isomers isolated from black and white pepper against different targets of COVID-19 pathophysiology.

Since piperine has been consumed for centuries; the immunomodulatory action and lipid-lowering effect on metabolic diseases including cardiovascular diseases were discussed in this review. In Langendorff's rabbit heart preparation, piperine caused partial inhibition and verapamil caused complete inhibition of ventricular contractions and coronary flow.

Piperine stimulates the digestive capacity by activating the release of digestive enzymes from the pancreas. However, the effect of piperine on the gut microbiota has been explored on a very limited scope, and therefore, it is suggested that rigorous exploration is needed in this context. The effects of piperine on kidney-related diseases need to be studied since it has a very little published establishment in this scope. The synergistic effects, as well as the combinatorial combination of piperine and other phytochemicals, should be explored for other diseases.

Piperine treatment has also been evidenced to decrease lipid peroxidation and beneficially influence the



cellular thiol status, antioxidant molecule, and antioxidant enzymes. Work has been done on a computational scope for a nanoformulation incorporated in combination with piperine for human neuroblastoma SH-SY5Y cells; the conclusive results were satisfactory to have an augmented antioxidant effect on an Alzheimer's model in vitro; however, animal-based models are needed to provide further evidence.

Regardless of all these reports, it is not yet prescribed for human use as for its limited number of clinical trials. In combination, piperine alters the metabolism and bioavailability of co-administered drugs. The number of publications on this molecule continues to increase with few clinical trials that are still ongoing. As we gather more information on the health benefits of piperine, it is more likely that the medicinal utility will be widely accepted.

#### Abbreviations

ABSIRC: Ablon Skin Institute and Research Center; AIDS: Acquired immunodeficiency syndrome; Akt: Protein kinase B; AMPK: AMP-activated protein kinase; AP-1: Activated protein-1; AP-1: Activator protein 1; ATF-2: Activated transcription factor-2; ATP: Adenosine triphosphate; Bcl-xL: B cell lymphoma-extra large; BCRP: Breast Cancer Resistance Protein; BCRPL: Bioserve Clinical Research Pvt Ltd; BH: Baqiyatallah Hospital; BS: Bladder Spasm; BUC: Baqiyatallah University Clinic; BUMS: Baqiyatallah University of Medical Sciences; CA: California America; CBZ: Carbamazepine; CDK2: Cyclin-dependent Kinase 2; CDK4: Cyclin-dependent Kinase 4; CHE: Continuous Hot Extraction; CIRIC: Chemical Injuries Research Center; CIRI: Cerebral ischemic reperfusion induced; CKD: Chronic kidney disease; c-myc: Cellular myelocytomatosis; COX-2: Cyclooxygenase-2; CREB: CAMP-response element binding protein; CYP3A4: Cytochrome P450 3A4; DNA: Deoxyribonucleic acid; DRBCPHU: David R. Bloom center for pharmacy at The Hebrew University; EASC-CO<sub>2</sub>: Enzyme-assisted supercritical carbon dioxide; EMIT: Enzyme-multiplied immunoassay technique; ERK1/2: Extracellular-signal-regulated kinase 1/2; FMR1: Fragile X mental retardation 1; FXTAS: Fragile X-associated tremor/ataxia syndrome; GAA: Glacial acetic acid; GM-CSF: Granulocyte-macrophage colony-stimulating factor; GPLRU: Gastrointestinal Physiology Laboratory and in the Radiology Unit; HCC: Hepatocellular carcinoma; HIVS: Human Immunodeficiency Virus Seronegativity; HMO: Hadassah Medical Organization; HPLC: High-performance liquid chromatography; HSE: Hydrotropic Solubilization Extraction; IL-10: Interleukin-10; IL-12p40: Interleukin-12p40; IL-18: Interleukin-18; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-8: Interleukin-8; ILUA: Ionic liquid-based ultrasonic-assisted; IUIMS: Isfahan University of Medical Sciences; IV: Intravenous; JNK: C-Jun N-terminal kinase; KOA: Knee Osteoarthritis; LC-MS/MS: Liquid chromatography with tandem mass spectrometry; LNCaP: Lymph Node Carcinoma of the Prostate; LPS: Lipopolysaccharide; Lys8: Lysine8; MAE: Microwave-assisted extraction; MDR: Multi-drug resistance; MIM: Model-independent method; MiR-127: MicroRNA-127; miR-21: MicroRNA-21; MMP2: Matrix metalloproteinase 2; MMP-9: Matrix metalloproteinase-9; MN: Malignant neoplasm; MRP1: Multidrug resistance protein 1; MS: Multiple sclerosis; MS: Muscular spasticity; mTOR: P-mammalian target of rapamycin; MyD88: Myeloid differentiation primary response 88; NA: Not Available; NAFLD: Non-alcoholic fatty liver disease; NCAM: Non-compartmental analysis method; NF-κB: Nuclear factor kappa B; NLRP3: (NOD)-like receptor protein 3; NOD: Neurology Outpatient Department; NOS-2: Nitric oxide synthase-2; NUMS: Neyshabour University of Medical Sciences; OA: Osteoarthritis; OD: Oropharyngeal dysphagia; OXR: Oxyresveratrol; P38 MAPK: P38 mitogen-activated protein kinase; p38MAPK: P38 mitogen-activated protein kinases; PC-3: Prostate cancer cell-3; PGIMER: Postgraduate Institute of Medical Education and Research; P-gp: P-glycoprotein; PKCα: Protein kinase Cα; PMA: Phorbol-12-myristate-13-acetate; PNS: Peripheral nervous system; PPARγ: Peroxisome Proliferator-Activated Receptor Gamma; RAN: Repeat-associated non-ATG; RBF: Round bottom flask; ROA: Route of administration; Ser5: Serine5; SFE: Supercritical fluid extraction; SLDE: Solid-liquid dynamic extraction; SMUAE:

Sequential microwave-ultrasound-assisted extraction; SRC-SBUM: Skin Research Center, Shahid Beheshti University of Medical Sciences; T2DM: Type 2 diabetes mellitus; TAP-2: Transporter associated with antigen-presenting 2; TCF: T cell factor; TCS: Tonic-clonic seizures; TNF-α: Tumor necrosis factor alpha; TRPV1: Transient Receptor Potential Vanilloid 1; UAE: Ultrasound-assisted extraction; UCPSKU: University College of Pharmaceutical Sciences Kakatiya University; UPLC: Ultra-performance liquid chromatography; UTR: Untranslated region; Wnt: Wingless-related integration site.

#### Acknowledgements

The authors would like to thank the reviewer for thoroughly reading the articles. AKT acknowledges the financial support from Science and Engineering Research Board, Government of India.

#### Authors' contributions

AKR has written the subtitles assigned, drawn the figure, molecular structures and tables. AKT developed the subtitle, initiated the project, polished the article and wrote the abstract. SKM provided the extensive suggestion for designing the title and subtitle, and motivation for project to conduct. SKM Lab is provided with Funding of Indian Institute of Technology. All authors read and approved the final manuscript.

#### Funding

Department of Science and Technology-Science and Engineering Research Board (PDF/2016/002996/LS) and Indian Institute of Technology (Banaras Hindu University).

#### Availability of data and materials

None.

#### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

The authors agree to the publication of this review.

#### Competing interests

The authors declare that they have no competing interests.

#### Author details

<sup>1</sup>Molecular Biology Unit, Institute of Medical Science, Banaras Hindu University, Varanasi 221005, India. <sup>2</sup>Clinical Research Division, School of Basic and Applied Science, Galgotias University, Gautam Buddha Nagar, UP, India. <sup>3</sup>Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University, Varanasi 221005, India. <sup>4</sup>Department of Pharmacognosy, I.T.S College of Pharmacy, Ghaziabad, UP 201206, India.

Received: 2 September 2021 Accepted: 11 January 2022

Published online: 28 January 2022

#### References

- Gorgani L, Mohammadi M, Najafpour GD, Nikzad M (2017) Piperine—the bioactive compound of black pepper: from isolation to medicinal formulations. *Compr Rev Food Sci Food Saf* 16:124–140. <https://doi.org/10.1111/1541-4337.12246>
- Aziz NS, Sofian-Seng N, Mohd Razali NS, Lim SJ, Mustapha WA (2019) A review on conventional and biotechnological approaches in white pepper production. *J Sci Food Agric* 99:2665–2676. <https://doi.org/10.1002/jsfa.9481>
- Buckle KA, Rathnawathie M, Brophy JJ (2007) Compositional differences of black, green and white pepper (*Piper nigrum* L.) oil from three cultivars. *Int J Food Sci Technol* 20:599–613. <https://doi.org/10.1111/j.1365-2621.1985.tb01819.x>
- Takooree H, Aumeeruddy MZ, Rengasamy KRR, Venugopala KN, Jeewon R, Zengin G, Mahomoodally MF (2019) A systematic review on black pepper (*Piper nigrum* L.): from folk uses to pharmacological applications.



- Crit Rev Food Sci Nutr 59:S210–S243. <https://doi.org/10.1080/10408398.2019.1565489>
5. Tiwari A, Mahadik KR, Gabhe SY (2020) Piperine: a comprehensive review of methods of isolation, purification, and biological properties. *Med Drug Discov* 7:100027. <https://doi.org/10.1016/j.medidd.2020.100027>
  6. Derosa G, Maffioli P, Sahebkar A (2016) Piperine and its role in chronic diseases. pp 173–184. [https://doi.org/10.1007/978-3-319-41334-1\\_8](https://doi.org/10.1007/978-3-319-41334-1_8)
  7. Meghwal M, Goswami TK (2013) Piper nigrum and piperine: an update. *Phyther Res* 27:1121–1130. <https://doi.org/10.1002/ptr.4972>
  8. Singletary K (2010) Black pepper. *Nutr Today* 45: 43–47. <https://doi.org/10.1097/NT.0b013e3181cb4539>
  9. Haq IU, Imran M, Nadeem M, Tufail T, Gondal TA, Mubarak MS (2020) Piperine: a review of its biological effects. *Phyther Res*. <https://doi.org/10.1002/ptr.6855>
  10. Kakarala M, Dubey SK, Tarnowski M, Cheng C, Liyanage S, Strawder T, Tazi K, Sen A, Djuric Z, Brenner DE (2010) Ultra-low flow liquid chromatography assay with ultraviolet (UV) detection for piperine quantitation in human plasma. *J Agric Food Chem* 58:6594–6599. <https://doi.org/10.1021/jf100657r>
  11. Kozukue N, Park M-SS, Choi S-HH, Lee S-UU, Ohnishi-Kameyama M, Levin CE, Friedman M (2007) Kinetics of light-induced cis-trans isomerization of four piperines and their levels in ground black peppers as determined by HPLC and LC/MS. *J Agric Food Chem* 55:7131–7139. <https://doi.org/10.1021/jf070831p>
  12. Panahi Y, Ghanei M, Hajhashemi A, Sahebkar A (2016) Effects of curcuminoids-piperine combination on systemic oxidative stress, clinical symptoms and quality of life in subjects with chronic pulmonary complications due to sulfur mustard: a randomized controlled trial. *J Diet Suppl* 13:93–105. <https://doi.org/10.3109/19390211.2014.952865>
  13. Panahi Y, Hosseini MS, Khalili N, Naimi E, Majeed M, Sahebkar A (2015) Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: a randomized controlled trial and an updated meta-analysis. *Clin Nutr* 34:1101–1108. <https://doi.org/10.1016/j.clnu.2014.12.019>
  14. Panahi Y, Khalili N, Hosseini MS, Abbasnazar M, Sahebkar A (2014) Lipid-modifying effects of adjunctive therapy with curcuminoids-piperine combination in patients with metabolic syndrome: results of a randomized controlled trial. *Complement Ther Med* 22:851–857. <https://doi.org/10.1016/j.ctim.2014.07.006>
  15. Shafiee A, Hoormand M, Shahidi-Dadras M, Abadi A (2018) The effect of topical piperine combined with narrowband UVB on vitiligo treatment: a clinical trial study. *Phyther Res* 32:1812–1817. <https://doi.org/10.1002/ptr.6116>
  16. Han ES, goleman, daniel; boyatzis, Richard; Mckee A (2019) 濟無 No Title No Title. *J Chem Inf Model* 53: 1689–1699
  17. Schöffmann A, Wimmer L, Goldmann D, Khom S, Hintersteiner J, Baburin I, Schwarz T, Hintersteiner M, Pakfeifer P, Oufir M, Hamburger M, Erker T, Ecker GF, Mihovilovic MD, Hering S (2014) Efficient modulation of  $\gamma$ -aminobutyric acid type A receptors by piperine derivatives. *J Med Chem* 57:5602–5619. <https://doi.org/10.1021/jm5002277>
  18. Koul S, Koul JL, Taneja SC, Dhar KL, Jamwal DS, Singh K, Reen RK, Singh J (2000) Structure-activity relationship of piperine and its synthetic analogues for their inhibitory potentials of rat hepatic microsomal constitutive and inducible cytochrome P450 activities. *Bioorganic Med Chem* 8:251–268. [https://doi.org/10.1016/S0968-0896\(99\)00273-4](https://doi.org/10.1016/S0968-0896(99)00273-4)
  19. Sattarinezhad E, Bordbar AK, Fani N (2015) Piperine derivatives as potential inhibitors of Survivin: an in silico molecular docking. *Comput Biol Med* 63:219–227. <https://doi.org/10.1016/j.combiomed.2015.05.016>
  20. Correa EA, Högestätt ED, Sterner O, Echeverri F, Zygmunt PM (2010) In vitro TRPV1 activity of piperine derived amides. *Bioorganic Med Chem* 18:3299–3306. <https://doi.org/10.1016/j.bmc.2010.03.013>
  21. Dong Y, Yin Y, Vu S, Yang F, Yarov-Yarovoy V, Tian Y, Zheng J (2019) A distinct structural mechanism underlies TRPV1 activation by piperine. *Biochem Biophys Res Commun* 516:365–372. <https://doi.org/10.1016/j.bbrc.2019.06.039>
  22. Eigenmann DE, Dürig C, Jähne EA, Smieško M, Culot M, Gosselet F, Cecchelli R, Cederberg Helms HC, Brodin B, Wimmer L, Mihovilovic MD, Hamburger M, Oufir M (2016) In vitro blood-brain barrier permeability predictions for GABAA receptor modulating piperine analogs. *Eur J Pharm Biopharm* 103:118–126. <https://doi.org/10.1016/j.ejpb.2016.03.029>
  23. Wimmer L, Schönbauer D, Pakfeifer P, Schöffmann A, Khom S, Hering S, Mihovilovic MD (2015) Developing piperine towards TRPV1 and GABAA receptor ligands - synthesis of piperine analogs via Heck-coupling of conjugated dienes. *Org Biomol Chem* 13:990–994. <https://doi.org/10.1039/c4ob02242d>
  24. Zabela V, Hettich T, Schlotterbeck G, Wimmer L, Mihovilovic MD, Guillet F, Bouaita B, Shevchenko B, Hamburger M, Oufir M (2018) GABAA receptor activity modulating piperine analogs: In vitro metabolic stability, metabolite identification, CYP450 reaction phenotyping, and protein binding. *J Chromatogr B Anal Technol Biomed Life Sci* 1072:379–389. <https://doi.org/10.1016/j.jchromb.2017.11.036>
  25. Shingate PN, Dongre PP, Kannur DM (2013) New method development for extraction and isolation of piperine from black pepper. *Int J Pharm Sci Res* 4:3165–3170
  26. Olalere OA, Abdurahman HN, Yunus RM, Alara OR, Thraisingam J (2017) Comparative study of pulsed microwave and hydrodistillation extraction of piperine oil from black pepper. *IUM Eng J* 18:87–93. Doi:<https://doi.org/10.31436/iiumej.v18i2.802>
  27. Subramanian R, Subbramaniyan P, Noorul Ameen J, Raj V (2016) Double bypasses soxhlet apparatus for extraction of piperine from Piper nigrum. *Arab J Chem* 9:S537–S540. <https://doi.org/10.1016/j.arabjc.2011.06.022>
  28. Raman G, Gaikar VG (2002) Extraction of piperine from Piper nigrum (black pepper) by hydrotropic solubilization. *Ind Eng Chem Res* 41:2966–2976. <https://doi.org/10.1021/ie0107845>
  29. Yu Y, Hu S, Fu D, Zhang X, Liu H, Xu B, Huang M (2020) Surfactant-assisted enzymatic extraction of piperine from Piper nigrum L. *Int J Food Prop* 23:52–62. <https://doi.org/10.1080/10942912.2019.1707221>
  30. Raman G, Gaikar VG (2002) Microwave-assisted extraction of piperine from Piper nigrum. *Ind Eng Chem Res* 41:2521–2528. <https://doi.org/10.1021/ie010359b>
  31. Ren Q, Zhao S, Ren C, Ma Z (2018) Astragalus polysaccharide alleviates LPS-induced inflammation injury by regulating miR-127 in H9c2 cardiomyoblasts. *Int J Immunopathol Pharmacol* 31:1–11. <https://doi.org/10.1177/2058738418759180>
  32. Ren T, Wang Q, Li C, Yang M, Zuo Z (2018) Efficient brain uptake of piperine and its pharmacokinetics characterization after oral administration. *Xenobiotica* 48:1249–1257. <https://doi.org/10.1080/00498254.2017.1405293>
  33. Bajad S, Singla AK, Bedi KL (2002) Liquid chromatographic method for determination of piperine in rat plasma: application to pharmacokinetics. *J Chromatogr B Anal Technol Biomed Life Sci* 776:245–249. [https://doi.org/10.1016/S1570-0232\(02\)00352-5](https://doi.org/10.1016/S1570-0232(02)00352-5)
  34. Ramesh B, Rao Vadaparathi PR, Sukumar G, Manjula N, Suresh Babu K, Sita Devi P (2016) LC-HRMS determination of piperine on rat dried blood spots: a pharmacokinetic study. *J Pharm Anal* 6:18–23. <https://doi.org/10.1016/j.jpha.2015.07.002>
  35. Han HK (2011) The effects of black pepper on the intestinal absorption and hepatic metabolism of drugs. *Expert Opin Drug Metab Toxicol* 7:721–729. <https://doi.org/10.1517/17425255.2011.570332>
  36. Izgelov D, Domb AJ, Hoffman A (2020) The effect of piperine on oral absorption of cannabidiol following acute vs. chronic administration. *Eur J Pharm Sci* 148:13–16. <https://doi.org/10.1016/j.ejps.2020.105313>
  37. Parveen B, Pillai KK, Tamboli ET, Ahmad S (2015) Effect of piperine on pharmacokinetics of sodium valproate in plasma samples of rats using gas chromatography-mass spectrometry method. *J Pharm Bioallied Sci* 7:317–320. <https://doi.org/10.4103/0975-7406.168036>
  38. Junsang D, Anukunwithaya T, Songvut P, Sritularak B, Likhitwitayawuid K, Khemawoot P (2019) Comparative pharmacokinetics of oxyresveratrol alone and in combination with piperine as a bioenhancer in rats. *BMC Complement Altern Med* 19:1–10. <https://doi.org/10.1186/s12906-019-2653-y>
  39. Zafar F, Jahan N, Khalil-Ur-Rahman, Bhatti HN (2019) Increased oral bioavailability of piperine from an optimized piper nigrum nanosuspension. *Planta Med* 85:249–257. <https://doi.org/10.1055/a-0759-2208>
  40. Zhu P, Qian J, Xu Z, Meng C, Liu J, Shan W, Zhu W, Wang Y, Yang Y, Zhang W, Zhang Y, Ling Y (2020) Piperlonguminine and piperine analogues as

- TrxR inhibitors that promote ROS and autophagy and regulate p38 and Akt/mTOR signaling. *J Nat Prod* 83:3041–3049. <https://doi.org/10.1021/acs.jnatprod.0c00599>
41. Zhu Y, Yu J, Zhou G, Gu Z, Adu-Frimpong M, Deng W, Yu J, Xu X (2020) Piperine fast disintegrating tablets comprising sustained-release matrix pellets with enhanced bioavailability: formulation, in vitro and in vivo evaluation. *Pharm Dev Technol* 25:617–624. <https://doi.org/10.1080/10837450.2020.1725892>
  42. Badmaev V, Majeed M, Norkus EP (1999) Piperine, an alkaloid derived from black pepper increases serum response of beta-carotene during 14-days of oral beta-carotene supplementation. *Nutr Res* 19:381–388. [https://doi.org/10.1016/S0271-5317\(99\)00007-X](https://doi.org/10.1016/S0271-5317(99)00007-X)
  43. Bi X, Yuan Z, Qu B, Zhou H, Liu Z, Xie Y (2019) Piperine enhances the bioavailability of silybin via inhibition of efflux transporters BCRP and MRP2. *Phytomedicine* 54:98–108. <https://doi.org/10.1016/j.phymed.2018.09.217>
  44. Izgelov D, Cherniakov I, Aldouby Bier G, Domb AJ, Hoffman A (2018) The effect of piperine pro-nano lipospheres on direct intestinal phase II metabolism: the raloxifene paradigm of enhanced oral bioavailability. *Mol Pharm* 15:1548–1555. <https://doi.org/10.1021/acs.molpharmaceut.7b01090>
  45. Bolat ZB, Islek Z, Demir BN, Yilmaz EN, Sahin F, Ucisik MH (2020) Curcumin- and piperine-loaded emulsomes as combinational treatment approach enhance the anticancer activity of curcumin on HCT116 colorectal cancer model. *Front Bioeng Biotechnol* 8:1–21. <https://doi.org/10.3389/fbioe.2020.00050>
  46. Zeng X, Cai D, Zeng Q, Chen Z, Zhong G, Zhuo J, Gan H, Huang X, Zhao Z, Yao N, Huang D, Zhang C, Sun D, Chen Y (2017) Selective reduction in the expression of UGTs and SULTs, a novel mechanism by which piperine enhances the bioavailability of curcumin in rat. *Biopharm Drug Dispos* 38:3–19. <https://doi.org/10.1002/bdd.2049>
  47. Fernández-Lázaro D, Mielgo-Ayuso J, Martínez AC, Seco-Calvo J (2020) Iron and physical activity: bioavailability enhancers, properties of black pepper (bioperine®) and potential applications. *Nutrients* 12:1–12. <https://doi.org/10.3390/nu12061886>
  48. Lambert JD, Hong J, Kim DH, Mishin VM, Yang CS (2004) Piperine enhances the bioavailability of the tea polyphenol (–)-epigallocatechin-3-gallate in mice. *J Nutr* 134:1948–1952. <https://doi.org/10.1093/jn/134.8.1948>
  49. Burande AS, Viswanadh MK, Jha A, Mehata AK, Shaik A, Agrawal N, Poddar S, Mahto SK, Muthu MS (2020) EGFR targeted paclitaxel and piperine co-loaded liposomes for the treatment of triple negative breast cancer. *AAPS PharmSciTech* 21:1–12. <https://doi.org/10.1208/s12249-020-01671-7>
  50. Syed SB, Arya H, Fu IH, Yeh TK, Periyasamy L, Hsieh HP, Coumar MS (2017) Targeting P-glycoprotein: investigation of piperine analogs for overcoming drug resistance in cancer. *Sci Rep* 7:1–18. <https://doi.org/10.1038/s41598-017-08062-2>
  51. Selvaraj J (2020) Molecular docking analysis of piperine with CDK2, CDK4, Cyclin D and Cyclin T proteins. *Bioinformation* 16:359–362. <https://doi.org/10.6026/97320630016359>
  52. Grinevicius VMAS, Andrade KS, Mota NSRS, Bretanha LC, Felipe KB, Ferreira SRS, Pedrosa RC (2019) CDK2 and Bcl-xL inhibitory mechanisms by docking simulations and anti-tumor activity from piperine enriched supercritical extract. *Food Chem Toxicol* 132:110644. <https://doi.org/10.1016/j.fct.2019.110644>
  53. Bhardwaj RK, Glaeser H, Becquemont L, Klotz U, Gupta SK, Fromm MF (2002) Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. *J Pharmacol Exp Ther* 302:645–650. <https://doi.org/10.1124/jpet.102.034728>
  54. Choi Y, Yu A-M (2014) ABC transporters in multidrug resistance and pharmacokinetics, and strategies for drug development. *Curr Pharm Des* 20:1–34. <https://doi.org/10.2174/138161282005140214165212>
  55. Gameiro M, Silva R, Rocha-Pereira C, Carmo H, Carvalho F, Bastos MDL, Remião F (2017) Cellular models and in vitro assays for the screening of modulators of P-gp, MRP1 and BCRP. *Molecules* 22:4–6. <https://doi.org/10.3390/molecules22040600>
  56. Li S, Lei Y, Jia Y, Li N, Wink M, Ma Y (2011) Piperine, a piperidine alkaloid from *Piper nigrum* re-sensitizes P-gp, MRP1 and BCRP dependent multi-drug resistant cancer cells. *Phytomedicine* 19:83–87. <https://doi.org/10.1016/j.phymed.2011.06.031>
  57. Zeng Y, Yang Y (2018) Piperine depresses the migration progression via downregulating the Akt/mTOR/MMP-9 signaling pathway in DU145 cells. *Mol Med Rep* 17:6363–6370. <https://doi.org/10.3892/mmr.2018.8653>
  58. Mihaylova MM, Shaw RJ (2011) The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol* 13:1016–1023. <https://doi.org/10.1038/ncb2329>
  59. Peng X, Yang T, Liu G, Liu H, Peng Y, He L (2018) Piperine ameliorated lupus nephritis by targeting AMPK-mediated activation of NLRP3 inflammasome. *Int Immunopharmacol* 65:448–457. <https://doi.org/10.1016/j.intimp.2018.10.025>
  60. George K, Thomas NS, Malathi R (2019) Piperine blocks voltage gated K<sup>+</sup> current and inhibits proliferation in androgen sensitive and insensitive human prostate cancer cell lines. *Arch Biochem Biophys* 667:36–48. <https://doi.org/10.1016/j.abb.2019.04.007>
  61. Sadri Nahand J, Bokharai-Salim F, Salaminejad A, Nesaei A, Mohajeri F, Moshtanzan A, Tabibzadeh A, Karimzadeh M, Moghooei M, Marjani A, Yaghoubi S, Keyvani H (2019) microRNAs: key players in virus-associated hepatocellular carcinoma. *J Cell Physiol* 234:12188–12225. <https://doi.org/10.1002/jcp.27956>
  62. Yang R, Gao N, Chang Q, Meng X, Wang W (2019) The role of IDO, IL-10, and TGF- $\beta$  in the HCV-associated chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. *J Med Virol* 91:265–271. <https://doi.org/10.1002/jmv.25083>
  63. Huang H (2018) Matrix metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: REcent advances. *Sensors (Switzerland)* 18:5–7. <https://doi.org/10.3390/s18103249>
  64. Hwang YP, Yun HJ, Kim HG, Han EH, Choi JH, Chung YC, Jeong HG (2011) Suppression of phorbol-12-myristate-13-acetate-induced tumor cell invasion by piperine via the inhibition of PKC $\alpha$ /ERK1/2-dependent matrix metalloproteinase-9 expression. *Toxicol Lett* 203:9–19. <https://doi.org/10.1016/j.toxlet.2011.02.013>
  65. Janani C, Ranjitha Kumari BD (2015) PPAR gamma gene: a review. *Diabetes Metab Syndr Clin Res Rev* 9:46–50. <https://doi.org/10.1016/j.dsx.2014.09.015>
  66. Park UH, Jeong HS, Jo EY, Park T, Yoon SK, Kim EJ, Jeong JC, Um SJ (2012) Piperine, a component of black pepper, inhibits adipogenesis by antagonizing PPAR $\gamma$  activity in 3T3-L1 cells. *J Agric Food Chem* 60:3853–3860. <https://doi.org/10.1021/jf204514a>
  67. Pradeep CR, Kuttan G (2004) Piperine is a potent inhibitor of nuclear factor- $\kappa$ B (NF- $\kappa$ B), c-Fos, CREB, ATF-2 and proinflammatory cytokine gene expression in B16F-10 melanoma cells. *Int Immunopharmacol* 4:1795–1803. <https://doi.org/10.1016/j.intimp.2004.08.005>
  68. Ren C, Liang Z (2018) Piperine alleviates lipopolysaccharide-induced inflammatory injury by down-regulating microRNA-127 in murine chondrogenic ATDC5 cells. *Biomed Pharmacother* 103:947–954. <https://doi.org/10.1016/j.biopha.2018.04.108>
  69. Arend RC, Londoño-Joshi AI, Straughn JM, Buchsbaum DJ (2013) The Wnt/ $\beta$ -catenin pathway in ovarian cancer: a review. *Gynecol Oncol* 131:772–779. <https://doi.org/10.1016/j.ygyno.2013.09.034>
  70. Krishnamurthy N, Kurzrock R (2018) Targeting the Wnt/ $\beta$ -catenin pathway in cancer: update on effectors and inhibitors. *Cancer Treat Rev* 62:50–60. <https://doi.org/10.1016/j.ctrv.2017.11.002>
  71. Vilchez V, Turcios L, Marti F, Gedaly R (2016) Targeting Wnt/ $\beta$ -catenin pathway in hepatocellular carcinoma treatment. *World J Gastroenterol* 22:823–832. <https://doi.org/10.3748/wjg.v22.i2.823>
  72. de Almeida GC, Oliveira LFS, Predes D, Fokoue HH, Kuster RM, Oliveira FL, Mendes FA, Abreu JG (2020) Piperine suppresses the Wnt/ $\beta$ -catenin pathway and has anti-cancer effects on colorectal cancer cells. *Sci Rep* 10:1–12. <https://doi.org/10.1038/s41598-020-68574-2>
  73. Si L, Yang R, Lin R, Yang S (2018) Piperine functions as a tumor suppressor for human ovarian tumor growth via activation of JNK/p38 MAPK-mediated intrinsic apoptotic pathway. *Biosci Rep*. <https://doi.org/10.1042/BSR20180503>
  74. Tawani A, Amanullah A, Mishra A, Kumar A (2016) Evidences for Piperine inhibiting cancer by targeting human G-quadruplex DNA sequences. *Sci Rep* 6:1–12. <https://doi.org/10.1038/srep39239>
  75. International A, Reviewed P, Tripathi AK, Ali S, Mishra DP (2013) G- Journal of Environmental Science and Technology attenuated antioxidant

- property of UV-B irradiated piperine in ischemia-reperfusion injury in SD rat 1: 29–36
76. Vaibhav K, Shrivastava P, Javed H, Khan A, Ahmed ME, Tabassum R, Khan MM, Khuwaja G, Islam F, Saeed Siddiqui M, Safhi MM, Islam F (2012) Piperine suppresses cerebral ischemia-reperfusion-induced inflammation through the repression of COX-2, NOS-2, and NF- $\kappa$ B in middle cerebral artery occlusion rat model. *Mol Cell Biochem* 367:73–84. <https://doi.org/10.1007/s11010-012-1321-z>
  77. Verma AK, Khan E, Mishra SK, Jain N, Kumar A (2019) Piperine modulates protein mediated toxicity in fragile X-associated tremor/ataxia syndrome through interacting expanded CGG repeat (r(CGG)exp) RNA. *ACS Chem Neurosci* 10:3778–3788. <https://doi.org/10.1021/acschemneuro.9b00282>
  78. Aumeeruddy MZ, Mahomoodally MF (2019) Combating breast cancer using combination therapy with 3 phytochemicals: piperine, sulforaphane, and thymoquinone. *Cancer* 125:1600–1611. <https://doi.org/10.1002/cncr.32022>
  79. Khamis AAA, Ali EMM, El-Moneim MAA, Abd-Alhaseeb MM, El-Maghd MA, Salim EI (2018) Hesperidin, piperine and bee venom synergistically potentiate the anticancer effect of tamoxifen against breast cancer cells. *Biomed Pharmacother* 105:1335–1343. <https://doi.org/10.1016/j.biopha.2018.06.105>
  80. Kanthiah Original Research Y, Ragini P, Av D, Ch A, Yv K, Kanthiah Y (2014) Enhancement of paclitaxel and doxorubicin cytotoxicity in breast cancer cell lines in combination with piperine treatment and analysis of expression of autophagy and apoptosis genes. *J Med Sci Res* 2:62–672
  81. Talib WH (2017) Regressions of breast carcinoma syngraft following treatment with piperine in combination with thymoquinone. *Sci Pharm* 85:1–11. <https://doi.org/10.3390/scipharm85030027>
  82. Kakarala M, Brenner DE, Korkaya H, Cheng C, Tazi K, Ginestier C, Liu S, Dontu G, Wicha MS (2010) Targeting breast stem cells with the cancer preventive compounds curcumin and piperine. *Breast Cancer Res Treat* 122:777–785. <https://doi.org/10.1007/s10549-009-0612-x>
  83. Do MT, Kim HG, Choi JH, Khanal T, Park BH, Tran TP, Jeong TC, Jeong HG (2013) Antitumor efficacy of Piperine in the treatment of human HER2-overexpressing breast cancer cells. *Food Chem* 141:2591–2599. <https://doi.org/10.1016/j.foodchem.2013.04.125>
  84. Fofaria NM, Kim SH, Srivastava SK (2014) Piperine causes G1 phase cell cycle arrest and apoptosis in melanoma cells through checkpoint kinase-1 activation. *PLoS ONE* 9:1–10. <https://doi.org/10.1371/journal.pone.0094298>
  85. Moorthi C, Kathiresan K (2013) Curcumin-piperine/curcumin-quercetin/curcumin-silibinin dual drug-loaded nanoparticulate combination therapy: a novel approach to target and treat multidrug-resistant cancers. *J Med Hypotheses Ideas* 7:15–20. <https://doi.org/10.1016/j.jmhi.2012.10.005>
  86. Das S, Bera D, Pal K, Mondal D, Karmakar P, Das S, Dey A (2020) Guar gum micro-vehicle mediated delivery strategy and synergistic activity of thymoquinone and piperine: an in vitro study on bacterial and hepatocellular carcinoma cells. *J Drug Deliv Sci Technol* 60:101994. <https://doi.org/10.1016/j.jddst.2020.101994>
  87. Tedasen A, Khoka A, Madla S, Sriwiriyan S, Graidist P (2019) Anticancer effects of piperine-free piper nigrum extract on cholangiocarcinoma cell lines. *Pharmacogn Mag* 15:S38–S46. [https://doi.org/10.4103/pm.pm\\_288\\_19](https://doi.org/10.4103/pm.pm_288_19)
  88. Samykutty A, Shetty AV, Dakshinamoorthy G, Bartik MM, Johnson GL, Webb B, Zheng G, Chen A, Kalyanasundaram R, Munirathinam G (2013) Piperine, a bioactive component of pepper spice exerts therapeutic effects on androgen dependent and androgen independent prostate cancer cells. *PLoS ONE* 8:1–11. <https://doi.org/10.1371/journal.pone.0065889>
  89. Sedeky AS, Khalil IA, Hefnawy A, El-Sherbiny IM (2018) Development of core-shell nanocarrier system for augmenting piperine cytotoxic activity against human brain cancer cell line. *Eur J Pharm Sci* 118:103–112. <https://doi.org/10.1016/j.ejps.2018.03.030>
  90. Yaffe PB, Doucette CD, Walsh M, Hoskin DW (2013) Piperine impairs cell cycle progression and causes reactive oxygen species-dependent apoptosis in rectal cancer cells. *Exp Mol Pathol* 94:109–114. <https://doi.org/10.1016/j.yexmp.2012.10.008>
  91. Nag A, Chowdhury RR (2020) Piperine, an alkaloid of black pepper seeds can effectively inhibit the antiviral enzymes of Dengue and Ebola viruses, an in silico molecular docking study. *VirusDisease* 31:308–315. <https://doi.org/10.1007/s13337-020-00619-6>
  92. Khan IA, Mirza ZM, Kumar A, Verma V, Qazi GN (2006) Piperine, a phytochemical potentiator of ciprofloxacin against *Staphylococcus aureus*. *Antimicrob Agents Chemother* 50:810–812. <https://doi.org/10.1128/AAC.50.2.810-812.2006>
  93. Sangwan PL, Koul JL, Koul S, Reddy MV, Thota N, Khan IA, Kumar A, Kalia NP, Qazi GN (2008) Piperine analogs as potent *Staphylococcus aureus* NorA efflux pump inhibitors. *Bioorganic Med Chem* 16:9847–9857. <https://doi.org/10.1016/j.bmc.2008.09.042>
  94. Ferreira C, Soares DC, Barreto-Junior CB, Nascimento MT, Freire-De-Lima L, Delorenzi JC, Lima MEF, Atella GC, Folly E, Carvalho TMU, Saraiva EM, Pinto-Da-Silva LH (2011) Leishmanicidal effects of piperine, its derivatives, and analogues on *Leishmania amazonensis*. *Phytochemistry* 72:2155–2164. <https://doi.org/10.1016/j.phytochem.2011.08.006>
  95. Sahi S, Tewatia P, Ghosal S (2012) *Leishmania donovani* pteridine reductase 1: Comparative protein modeling and protein-ligand interaction studies of the leishmanicidal constituents isolated from the fruits of *Piper longum*. *J Mol Model* 18:5065–5073. <https://doi.org/10.1007/s00894-012-1508-y>
  96. Thakre A, Jadhav V, Kazi R, Shelar A, Patil R, Kharat K, Zore G, Karuppaiyl SM (2020) Oxidative stress induced by piperine leads to apoptosis in *Candida albicans*. *Med Mycol*. <https://doi.org/10.1093/mmy/myaa058>
  97. de Paula VF, de Barbosa LCA, Demuner AJ, Piló-Veloso D, Picanço MC (2000) Synthesis and insecticidal activity of new amide derivatives of piperine. *Pest Manag Sci* 56:168–174. [https://doi.org/10.1002/\(sici\)1526-4998\(200002\)56:2%3c168::aid-ps110%3e3.3.co;2-8](https://doi.org/10.1002/(sici)1526-4998(200002)56:2%3c168::aid-ps110%3e3.3.co;2-8)
  98. Fontaine KR, Redden DT, Wang C, Westfall AO, Allison DB (2003) Years of life lost due to obesity. *J Am Med Assoc* 289:187–193. <https://doi.org/10.1001/jama.289.2.187>
  99. Kushner RF, Roth JL (2003) Assessment of the obese patient. *Endocrinol Metab Clin North Am* 32:915–933. [https://doi.org/10.1016/S0889-8529\(03\)00068-9](https://doi.org/10.1016/S0889-8529(03)00068-9)
  100. Han TS, Sattar N, Lean M (2006) ABC of obesity: assessment of obesity and its clinical implications. *Br Med J* 333:695–698
  101. Foster-Schubert KE, Cummings DE (2006) Emerging therapeutic strategies for obesity. *Endocr Rev* 27:779–793. <https://doi.org/10.1210/er.2006-0041>
  102. Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen W, Woychik RP, Wilkison WO, Cone RD (1994) Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature*. <https://doi.org/10.1038/371799a0>
  103. Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. *Nature* 404:661–671. <https://doi.org/10.1038/35007534>
  104. Shah SS, Shah GB, Singh SD, Gohil PV, Chauhan K, Shah KA, Chorawala M (2011) Effect of piperine in the regulation of obesity-induced dyslipidemia in high-fat diet rats. *Indian J Pharmacol* 43:296–299. <https://doi.org/10.4103/0253-7613.81516>
  105. Choi S, Choi Y, Choi Y, Kim S, Jang J, Park T (2013) Piperine reverses high fat diet-induced hepatic steatosis and insulin resistance in mice. *Food Chem* 141:3627–3635. <https://doi.org/10.1016/j.foodchem.2013.06.028>
  106. Diwan V, Poudyal H, Brown L (2013) Piperine attenuates cardiovascular, liver and metabolic changes in high carbohydrate. *High Fat-Fed Rats Cell Biochem Biophys* 67:297–304. <https://doi.org/10.1007/s12013-011-9306-1>
  107. Chonpathompikunlert P, Wattananthorn J, Muchimapura S (2010) Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of Alzheimer's disease. *Food Chem Toxicol* 48:798–802. <https://doi.org/10.1016/j.fct.2009.12.009>
  108. Wang C, Cai Z, Wang W, Wei M, Kou D, Li T, Yang Z, Guo H, Le W, Li S (2019) Piperine attenuates cognitive impairment in an experimental mouse model of sporadic Alzheimer's disease. *J Nutr Biochem* 70:147–155. <https://doi.org/10.1016/j.jnubio.2019.05.009>
  109. Yang W, Chen Y-H, Liu H, Qu H-D (2015) Neuroprotective effects of piperine on the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced

- Parkinson's disease mouse model. *Int J Mol Med* 36:1369–1376. <https://doi.org/10.3892/ijmm.2015.2356>
110. Guo J, Cui Y, Liu Q, Yang Y, Li Y, Weng L, Tang B, Jin P, Li X-J, Yang S, Li S (2018) Piperine ameliorates SCA17 neuropathology by reducing ER stress. *Mol Neurodegener* 13:4. <https://doi.org/10.1186/s13024-018-0236-x>
  111. Singh S, Kumar P (2018) Piperine in combination with quercetin halt 6-OHDA induced neurodegeneration in experimental rats: Biochemical and neurochemical evidences. *Neurosci Res* 133:38–47. <https://doi.org/10.1016/j.neures.2017.10.006>
  112. Singh S, Kumar P (2016) Neuroprotective activity of curcumin in combination with piperine against quinolinic acid induced neurodegeneration in rats. *Pharmacology* 97:151–160. <https://doi.org/10.1159/000443896>
  113. Yarbeygi H, Panahi Y, Javadi B, Sahebkar A (2018) The Underlying role of oxidative stress in neurodegeneration: a mechanistic review. *CNS Neurol Disord - Drug Targets* 17:207–215. <https://doi.org/10.2174/187152731766618042512557>
  114. Sacks D, Baxter B, Campbell BCV, Carpenter JS, Cognard C, Dippel D, Eesa M, Fischer U, Hauegger K, Hirsch JA, Shazam Hussain M, Jansen O, Jayaraman MV, Khalessi AA, Kluck BW, Lavine S, Meyers PM, Ramee S, Rüfenacht DA, Schirmer CM, Vorwerk D (2018) Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke. *Int J Stroke* 13:612–632. <https://doi.org/10.1177/1747493018778713>
  115. Wardlaw JM, Murray V, Berge E, Del Zoppo G, Sandercock P, Lindley RL, Cohen G (2012) Recombinant tissue plasminogen activator for acute ischaemic stroke: an updated systematic review and meta-analysis. *Lancet* 379:2364–2372. [https://doi.org/10.1016/S0140-6736\(12\)60738-7](https://doi.org/10.1016/S0140-6736(12)60738-7)
  116. Strimpakos AS, Sharma RA (2008) Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxidants Redox Signal* 10:511–545. <https://doi.org/10.1089/ars.2007.1769>
  117. Da Cruz GMP, Felipe CFB, Scorza FA, Da Costa MAC, Tavares AF, Menezes MLF, De Andrade GM, Leal LKAM, Brito GAC, Da Graça Naffah-Mazzacoratti M, Cavalheiro EA, De Barros Viana GS (2013) Piperine decreases pilocarpine-induced convulsions by GABAergic mechanisms. *Pharmacol Biochem Behav* 104:144–153. <https://doi.org/10.1016/j.pbb.2013.01.002>
  118. Kumar Tripathi A, Dwivedi A, Kumar Pal M, Ali S, Singh Ray R, Prasad Mishra D (2014) Involvement of attenuated antioxidant and Bcl2 signaling property in UV-R/ sunlight irradiated piperine treated ischemia/reperfusion rat model. *Highlights Free Radicals Antioxidants* 4:47–54. <https://doi.org/10.5530/fra.2014.1.8>
  119. Mao QQ, Huang Z, Ip SP, Xian YF, Che CT (2012) Protective effects of piperine against corticosterone-induced neurotoxicity in PC12 cells. *Cell Mol Neurobiol* 32:531–537. <https://doi.org/10.1007/s10571-011-9786-y>
  120. Mao QQ, Huang Z, Zhong XM, Xian YF, Ip SP (2014) Piperine reverses the effects of corticosterone on behavior and hippocampal BDNF expression in mice. *Neurochem Int* 74:36–41. <https://doi.org/10.1016/j.neuint.2014.04.017>
  121. Al-Baghdadi OB, Prater NI, Van Der Schyf CJ, Geldenhuys WJ (2012) Inhibition of monoamine oxidase by derivatives of piperine, an alkaloid from the pepper plant *Piper nigrum*, for possible use in Parkinson's disease. *Bioorganic Med Chem Lett* 22:7183–7188. <https://doi.org/10.1016/j.bmcl.2012.09.056>
  122. de Araújo-Júnior JX, Ribeiro ÉAN, da Silva SAS, da Costa CDF, Alexandre-Moreira MS, Santos BVO (2011) Cardiovascular effects of two amides (piperine and piperdardine) isolated from *piper tuberculatum* JACQ. *Emir J Food Agric*, 265–274
  123. Okwute SK, Egharevba HO (2013) Piperine-type amides: review of the chemical and biological characteristics. *Int J Chem* 5:99–122. <https://doi.org/10.5539/ijc.v5n3p99>
  124. Booranasubkajorn S, Huabprasert S, Wattananarangsang J, Chotitham P, Jutasompakorn P, Laohapand T, Akarasereenont P, Tripatara P (2017) Vasculoprotective and vasodilatation effects of herbal formula (Sahatsatara) and piperine in spontaneously hypertensive rats. *Phytomedicine* 24:148–156. <https://doi.org/10.1016/j.phymed.2016.11.013>
  125. Chakraborty M, Bhattacharjee A, Kamath JV (2017) Cardioprotective effect of curcumin and piperine combination against cyclophosphamide-induced cardiotoxicity. *Indian J Pharmacol* 49:65–70. <https://doi.org/10.4103/0253-7613.201015>
  126. Taqvi SIH, Shah AJ, Gilani AH (2008) Blood pressure lowering and vasomodulator effects of piperine. *J Cardiovasc Pharmacol* 52:452–458. <https://doi.org/10.1097/FJC.0b013e31818d07c0>
  127. Wang L, Palme V, Rotter S, Schilcher N, Cukaj M, Wang D, Ladurner A, Heiss EH, Stangl H, Dirsch VM, Atanasov AG (2017) Piperine inhibits ABCA1 degradation and promotes cholesterol efflux from THP-1-derived macrophages. *Mol Nutr Food Res* 61:1–31. <https://doi.org/10.1002/mnfr.201500960>
  128. Biol S (1990) OF SUMMARY : was isolated from *Piper nigrum* Linn for the evaluation of anti-inflammatory activity in rats. Different acute and chronic experimental models like carrageenin-induced rat paw edema biochemical estimations were made to elucidate the underlying, 95–100
  129. Jaisin Y, Ratanachamnong P, Wongsawatkul O, Watthammawut A, Malaniyom K, Natewong S (2020) Antioxidant and anti-inflammatory effects of piperine on UV-B-irradiated human HaCAT keratinocyte cells. *Life Sci*. <https://doi.org/10.1016/j.lfs.2020.118607>
  130. Neyrinck AM, Alligier M, Memvanga PB, Névraumont E, Larondelle Y, Pr  at V, Cani PD, Delzenne NM (2013) Curcuma longa extract associated with white pepper lessens high fat diet-induced inflammation in subcutaneous adipose tissue. *PLoS ONE* 8:1–10. <https://doi.org/10.1371/journal.pone.0081252>
  131. Dhargawe N, Mahakalkar S, Bhagyashree Mohod JPR (2018) Article in pharmacognosy research - October 2017. *Pharmacognosy Res* 10:24–30. <https://doi.org/10.4103/pr.pr>
  132. Simanjuntak N, Levita J, Subarnas A (2020) Inclusion of biopiperine in the kappa-carrageenan complex might improve its bioaccessibility and in vivo anti-inflammatory activity in edema-induced wistar rats. *J Appl Pharm Sci* 10:39–43. <https://doi.org/10.7324/JAPS.2020.10606>
  133. Ying X, Chen X, Cheng S, Shen Y, Peng L, Xu H (2013) Piperine inhibits IL-   induced expression of inflammatory mediators in human osteoarthritis chondrocyte. *Int Immunopharmacol* 17:293–299. <https://doi.org/10.1016/j.intimp.2013.06.025>
  134. Rathee D, Kamboj A, Sidhu S (2018) Augmentation of hepatoprotective potential of Aegle marmelos in combination with piperine in carbon tetrachloride model in wistar rats. *Chem Cent J* 12:1–13. <https://doi.org/10.1186/s13065-018-0463-9>
  135. Abdel-daim MM, Sayed AA, Abdeen A, Aleya L, Ali D, Alkahtane AA, Alarifi S, Alkahtani S (2019) Piperine enhances the antioxidant and anti-inflammatory activities of thymoquinone against microcystin-LR-induced hepatotoxicity and neurotoxicity in mice
  136. Pannu N, Bhatnagar A (2020) Combinatorial therapeutic effect of resveratrol and piperine on murine model of systemic lupus erythematosus. *Inflammopharmacology* 28:401–424. <https://doi.org/10.1007/s10787-019-00662-w>
  137. Zhai W, Zhang Z, Xu N, Guo Y, Qiu C, Li C, Deng G, Guo M (2016) Piperine plays an anti-inflammatory role in staphylococcus aureus endometritis by inhibiting activation of NF-  B and MAPK pathways in mice. *Evid-Based Complement Altern Med* 2016:1–10. <https://doi.org/10.1155/2016/8597208>
  138. Chen X, Ge F, Liu J, Bao S, Chen Y, Li D, Li Y, Huang T, Chen X, Zhu Q, Lian Q, Ge RS (2018) Diverged effects of piperine on testicular development: stimulating leydig cell development but inhibiting spermatogenesis in rats. *Front Pharmacol* 9:1–13. <https://doi.org/10.3389/fphar.2018.00244>
  139. Malini T, Manimaran RR, Arunakaran J, Aruldas MM, Govindarajulu P (1999) Effects of piperine on testis of albino rats. *J Ethnopharmacol* 64:219–225. [https://doi.org/10.1016/S0378-8741\(98\)00128-7](https://doi.org/10.1016/S0378-8741(98)00128-7)
  140. D'Cruz SC, Vaithinathan S, Saradha B, Mathur PP (2008) Piperine activates testicular apoptosis in adult rats. *J Biochem Mol Toxicol* 22:382–388. <https://doi.org/10.1002/jbt.20251>
  141. D'Cruz SC, Mathur PP (2005) Effect of piperine on the epididymis of adult male rats. *Asian J Androl* 7:363–368. <https://doi.org/10.1111/j.1745-7262.2005.00059.x>
  142. Lakes JE, Richards CI, Flythe MD (2020) Inhibition of Bacteroidetes and Firmicutes by select phytochemicals. *Anaerobe* 61:102145. <https://doi.org/10.1016/j.anaerobe.2019.102145>
  143. Peterson CT, Vaughn AR, Sharma V, Chopra D, Mills PJ, Peterson SN, Sivamani RK (2018) Effects of turmeric and curcumin dietary supplementation on human gut microbiota: a double-blind, randomized, placebo-controlled pilot study. *J Evid-Based Integr Med* 23:1–8. <https://doi.org/10.1177/2515690X18790725>



144. Rama Raju KS, Taneja I, Singh SP, Tripathi A, Mishra DP, Hussain KM, Gayen JR, Singh SK, Wahajuddin M (2015) Simultaneous determination of centchroman and tamoxifen along with their metabolites in rat plasma using LC–MS/MS. 7: 967–979. doi:<https://doi.org/10.4155/BIO.14.253>
145. Guldiken B, Ozkan G, Catalkaya G, Ceylan FD, Ekin Yalcinkaya I, Capanoglu E (2018) Phytochemicals of herbs and spices: Health versus toxicological effects. *Food Chem Toxicol* 119:37–49. <https://doi.org/10.1016/j.fct.2018.05.050>
146. Piyachaturawat P, Glinsukon T, Toskulkao C (1983) Acute and subacute toxicity of piperine in mice, rats and hamsters. *Toxicol Lett* 16:351–359. [https://doi.org/10.1016/0378-4274\(83\)90198-4](https://doi.org/10.1016/0378-4274(83)90198-4)
147. Chonpathompikunlert P, Yoshitomi T, Han J, Isoda H, Nagasaki Y (2011) The use of nitroxide radical-containing nanoparticles coupled with piperine to protect neuroblastoma SH-SY5Y cells from A $\beta$ -induced oxidative stress. *Biomaterials* 32:8605–8612. <https://doi.org/10.1016/j.biomaterials.2011.07.024>
148. Daware MB, Mujumdar AM, Ghaskadbi S (2000) Reproductive toxicity of piperine in Swiss albino mice. *Planta Med* 66:231–236. <https://doi.org/10.1055/s-2000-8560>
149. Choudhary P, Chakdar H, Singh D, Selvaraj C, Singh SK, Kumar S, Saxena AK (2020) Computational studies reveal piperine, the predominant oleoresin of black pepper (*Piper nigrum*) as a potential inhibitor of SARS-CoV-2 (COVID-19). *Curr Sci* 119:1333–1342
150. Maurya VK, Kumar S, Prasad AK, Bhatt MLB, Saxena SK (2020) Structure-based drug designing for potential antiviral activity of selected natural products from Ayurveda against SARS-CoV-2 spike glycoprotein and its cellular receptor. *Virus Disease* 31:179–193. <https://doi.org/10.1007/s13337-020-00598-8>
151. Alagu Lakshmi S, Shafreen RMB, Priya A, Shunmugiah KP (2020) Ethnomedicines of Indian origin for combating COVID-19 infection by hampering the viral replication: using structure-based drug discovery approach. *J Biomol Struct Dyn*. <https://doi.org/10.1080/07391102.2020.1778537>
152. Kumar G, Kumar D, Singh NP (2020) Therapeutic approach against 2019-nCoV by inhibition of ACE-2 receptor. *Drug Res (Stuttg)*. <https://doi.org/10.1055/a-1275-0228>
153. Kumar S, Kashyap P, Chowdhury S, Kumar S, Panwar A, Kumar A (2020) Identification of phytochemicals as potential therapeutic agents that binds to Nsp15 protein target of coronavirus (SARS-CoV-2) that are capable of inhibiting virus replication. *Phytomedicine*. <https://doi.org/10.1016/j.phymed.2020.153317>
154. Heidari-Beni M, Moravejolahkami AR, Gorgian P, Askari G, Tarrahi MJ, Bahreini-Esfahani N (2020) Herbal formulation “turmeric extract, black pepper, and ginger” versus Naproxen for chronic knee osteoarthritis: a randomized, double-blind, controlled clinical trial. *Phyther Res* 34:2067–2073. <https://doi.org/10.1002/ptr.6671>
155. Bedada SK, Appani R, Boga PK (2017) Effect of piperine on the metabolism and pharmacokinetics of carbamazepine in healthy volunteers. *Drug Res (Stuttg)* 67:46–51. <https://doi.org/10.1055/s-0042-118173>
156. Bedada SK, Boga PK, Kotakonda HK (2017) Study on influence of piperine treatment on the pharmacokinetics of diclofenac in healthy volunteers. *Xenobiotica* 47:127–132. <https://doi.org/10.3109/00498254.2016.1163752>
157. Cherniakov I, Izgelov D, Barasch D, Davidson E, Domb AJ, Hoffman A (2017) Piperine-pro-nanolipospheres as a novel oral delivery system of cannabinoids: pharmacokinetic evaluation in healthy volunteers in comparison to buccal spray administration. *J Control Release* 266:1–7. <https://doi.org/10.1016/j.jconrel.2017.09.011>
158. Kasibhatta R, Naidu MUR (2007) Influence of piperine on the pharmacokinetics of nevirapine under fasting conditions: a randomised, crossover, placebo-controlled study. *Drugs R D* 8:383–391. <https://doi.org/10.2165/00126839-200708060-00006>
159. Mirhafez SR, Farimani AR, Gholami A, Hooshmand E, Tavallaie S, Gh BFNM (2019) The effect of curcumin with piperine supplementation on pro-oxidant and antioxidant balance in patients with non-alcoholic fatty liver disease: a randomized, double-blind, placebo-controlled trial. *Drug Metab Pers Ther* 34:1–7. <https://doi.org/10.1515/dmpt-2018-0040>
160. Pattanaik S, Hota D, Prabhakar S, Kharbanda P, Pandhi P (2009) Pharmacokinetic interaction of single dose of piperine with steady-state carbamazepine in epilepsy patients. *Phytother Res* 23:1281–1286. <https://doi.org/10.1002/ptr.2676>
161. Rofes L, Arreola V, Martin A, Clavé P (2014) Effect of oral piperine on the swallow response of patients with oropharyngeal dysphagia. *J Gastroenterol* 49:1517–1523. <https://doi.org/10.1007/s00535-013-0920-0>
162. Bedada SK, Boga PK (2017) Effect of piperine on CYP2E1 enzyme activity of chlorzoxazone in healthy volunteers. *Xenobiotica* 47:1035–1041. <https://doi.org/10.1080/00498254.2016.1241450>
163. Panahi Y, Alishiri GH, Parvin S, Sahebkar A (2016) Mitigation of systemic oxidative stress by curcuminoids in osteoarthritis: results of a randomized controlled trial. *J Diet Suppl* 13:209–220. <https://doi.org/10.3109/19390211.2015.1008611>
164. Panahi Y, Khalili N, Sahebi E, Namazi S, Simental-Mendía LE, Majeed M, Sahebkar A (2018) Effects of curcuminoids plus piperine on glycemic, hepatic and inflammatory biomarkers in patients with type 2 diabetes mellitus: a randomized double-blind placebo-controlled trial. *Drug Res (Stuttg)* 68:403–409. <https://doi.org/10.1055/s-0044-101752>
165. Panahi Y, Valizadegan G, Ahamdi N, Ganjali S, Majeed M, Sahebkar A (2019) Curcuminoids plus piperine improve nonalcoholic fatty liver disease: a clinical trial. *J Cell Biochem* 120:15989–15996. <https://doi.org/10.1002/jcb.28877>
166. Ferreira RC, Batista TM, Duarte SS, Silva DKF, Lisboa TMH, Cavalcanti RFP, Leite FC, Manguera VM, de Sousa TKG, de Abrantes RA, da Trindade EO, de Athayde-Filho PF, Brandão MCR, de Medeiros KC P, Farias DF, Sobral MV (2020) A novel piperine analogue exerts in vivo antitumor effect by inducing oxidative, antiangiogenic and immunomodulatory actions. *Biomed Pharmacother* 128, 110247. <https://doi.org/10.1016/j.biopha.2020.110247>
167. Yang X, Ji J, Liu C, Zhou M, Li H, Ye S, Hu Q (2020) HJ22, a Novel derivative of piperine, Attenuates ibotenic acid-induced cognitive impairment, oxidative stress, apoptosis and inflammation via inhibiting the protein-protein interaction of Keap1-Nrf2. *Int Immunopharmacol* 83:106383. <https://doi.org/10.1016/j.intimp.2020.106383>
168. Faas L, Venkatasamy R, Hider RC, Young AR, Soumyanath A (2008) In vivo evaluation of piperine and synthetic analogues as potential treatments for vitiligo using a sparsely pigmented mouse model. *Br J Dermatol* 158:941–950. <https://doi.org/10.1111/j.1365-2133.2008.08464.x>
169. Santos J, Brito M, Ferreira R, Moura AP, Sousa T, Batista T, Manguera V, Leite F, Cruz R, Vieira G, Lira B, Athayde-Filho P, Souza H, Costa N, Veras R, Barbosa-Filho JM, Magalhães H, Sobral M (2018) Th1-biased immunomodulation and in vivo antitumor effect of a novel piperine analogue. *Int J Mol Sci* 19:1–22. <https://doi.org/10.3390/ijms19092594>
170. Zazeri G, Povinelli APR, Le Duff CS, Tang B, Cornelio ML, Jones AM (2020) Synthesis and spectroscopic analysis of piperine- and piperlongumine-inspired natural product scaffolds and their molecular docking with IL-1 $\beta$  and NF- $\kappa$ B proteins. *Molecules* 25:1–17. <https://doi.org/10.3390/molecules25122841>
171. Kharbanda C, Alam MS, Hamid H, Javed K, Bano S, Ali Y, Dhulap A, Alam P, Pasha MAQ (2016) Novel piperine derivatives with antidiabetic effect as PPAR- $\gamma$  agonists. *Chem Biol Drug Des*. <https://doi.org/10.1111/cbdd.12760>
172. Kumar S, Arya P, Mukherjee C, Singh BK, Singh N, Parmar VS, Prasad AK, Ghosh B (2005) Novel aromatic ester from *Piper longum* and its analogues inhibit expression of cell adhesion molecules on endothelial cells. *Biochemistry* 44:15944–15952. <https://doi.org/10.1021/bi050941u>
173. Kumar A, Khan IA, Koul S, Koul JL, Taneja SC, Ali I, Ali F, Sharma S, Mirza ZM, Kumar M, Sangwan PL, Gupta P, Thota N, Qazi GN (2008) Novel structural analogues of piperine as inhibitors of the NorA efflux pump of *Staphylococcus aureus*. *J Antimicrob Chemother* 61:1270–1276. <https://doi.org/10.1093/jac/dkn088>
174. Wang L, Cai X, Shi M, Xue L, Kuang S, Xu R, Qi W, Li Y, Ma X, Zhang R, Hong F, Ye H, Chen L (2020) Identification and optimization of piperine analogues as neuroprotective agents for the treatment of Parkinson's disease via the activation of Nrf2/keap1 pathway. *Eur J Med Chem* 199:1–21. <https://doi.org/10.1016/j.ejmech.2020.112385>
175. Luque de Castro MD, Priego-Capote F (2010) Soxhlet extraction: Past and present panacea. *J Chromatogr A* 1217:2383–2389. <https://doi.org/10.1016/j.chroma.2009.11.027>



176. Zhang QW, Lin LG, Ye WC (2018) Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Med (UK)* 13:1–26. <https://doi.org/10.1186/s13020-018-0177-x>
177. Dutta S, Bhattacharjee P (2016) Modeling of supercritical carbon dioxide extraction of piperine from Malabar black pepper. *Mater Today Proc* 3:3238–3252. <https://doi.org/10.1016/j.matpr.2016.10.005>
178. Dutta S, Bhattacharjee P (2015) Enzyme-assisted supercritical carbon dioxide extraction of black pepper oleoresin for enhanced yield of piperine-rich extract. *J Biosci Bioeng* 120:17–23. <https://doi.org/10.1016/j.jbiosc.2014.12.004>
179. Cao X, Ye X, Lu Y, Yu Y, Mo W (2009) Ionic liquid-based ultrasonic-assisted extraction of piperine from white pepper. *Anal Chim Acta* 640:47–51. <https://doi.org/10.1016/j.aca.2009.03.029>
180. Gigliarelli G, Pagiotti R, Persia D, Marcotullio MC (2017) Optimisation of a Naviglio-assisted extraction followed by determination of piperine content in *Piper longum* extracts. *Nat Prod Res* 31:214–217. <https://doi.org/10.1080/14786419.2016.1217209>
181. Naviglio D, Scarano P, Ciaravolo M, Gallo M (2019) Rapid solid-liquid dynamic extraction (RSLDE): a powerful and greener alternative to the latest solid-liquid extraction techniques. *Foods* 8:1–22. <https://doi.org/10.3390/foods8070245>
182. Gorgani L, Mohammadi M, Najafpour GD, Nikzad M (2017) Sequential microwave-ultrasound-assisted extraction for isolation of piperine from black pepper (*Piper nigrum* L.). *Food Bioprocess Technol* 10:2199–2207. <https://doi.org/10.1007/s11947-017-1994-0>
183. Shityakov S, Bigdelian E, Hussein AA, Hussain MB, Tripathi YC, Khan MU, Shariati MA (2019) Phytochemical and pharmacological attributes of piperine: a bioactive ingredient of black pepper. *Eur J Med Chem* 176:149–161. <https://doi.org/10.1016/j.ejmech.2019.04.002>
184. Bano G, Raina RK, Zutshi U, Bedi KL, Johri RK, Sharma SC (1991) Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. *Eur J Clin Pharmacol* 41:615–617. <https://doi.org/10.1007/BF00314996>
185. Di X, Wang X, Liu Y (2015) Effect of piperine on the bioavailability and pharmacokinetics of emodin in rats. *J Pharm Biomed Anal* 115:144–149. <https://doi.org/10.1016/j.jpba.2015.06.027>
186. Feng X, Liu Y, Wang X, Di X (2014) Effects of piperine on the intestinal permeability and pharmacokinetics of linarin in rats. *Molecules* 19:5624–5633. <https://doi.org/10.3390/molecules19055624>
187. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PSSR (1998) Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med* 64:353–356. <https://doi.org/10.1055/s-2006-957450>
188. Jin MJ, Han HK (2010) Effect of piperine, a major component of black pepper, on the intestinal absorption of fexofenadine and its implication on food-drug interaction. *J Food Sci*. <https://doi.org/10.1111/j.1750-3841.2010.01542.x>
189. Itharat A, Kanokkangsadal P, Khemawoot P, Wanichsetakul P, Davies N (2020) Pharmacokinetics of piperine after oral administration of Sahastara remedy capsules in healthy volunteers. *Res Pharm Sci* 15:410–417. <https://doi.org/10.4103/1735-5362.297843>
190. Wen-xing WXP (2010) Pharmacokinetics of piperine capsules in healthy volunteers. *Cent South Pharm* 8:513–516
191. Ren T, Zuo Z (2019) Role of piperine in CNS diseases: pharmacodynamics, pharmacokinetics and drug interactions. *Expert Opin Drug Metab Toxicol* 15:849–867. <https://doi.org/10.1080/17425255.2019.1672658>

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# Anticancer Effects of Piperine-Free *Piper nigrum* Extract on Cholangiocarcinoma Cell Lines

Aman Tedasen<sup>1,2</sup>, Araya Khoka<sup>3</sup>, Siribhorn Madla<sup>1</sup>, Somchai Sriwiriyan<sup>3</sup>, Potchanapond Graidist<sup>1,2</sup>

<sup>1</sup>Department of Biomedical Sciences, Faculty of Medicine, Prince of Songkla University, <sup>2</sup>The Excellent Research Laboratory of Cancer Molecular Biology, Prince of Songkla University, <sup>3</sup>Department of Pharmacology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Submitted: 01-08-2019

Revised: 30-09-2019

Published: 31-03-2020

## ABSTRACT

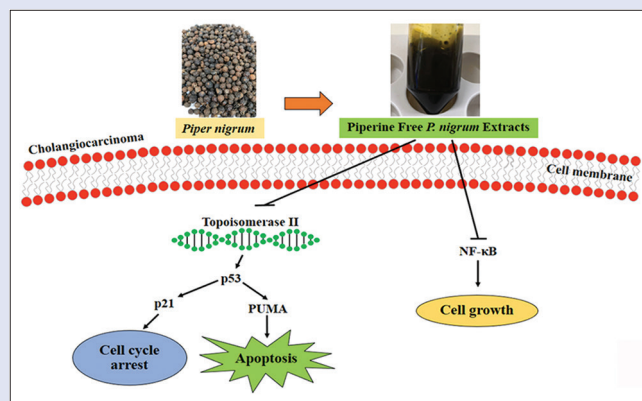
**Background:** Black pepper (*Piper nigrum* L.) is widely used as a traditional medicine, including usage for pain relief, fevers, as well as an anticancer agent. Previously, we reported that piperine-free *P. nigrum* extract (PFPE) inhibited breast cancer *in vitro* and *in vivo*.

**Objective:** In this present study, we explored the anticancer effects of PFPE on cholangiocarcinoma (CCA). **Materials and Methods:** 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was performed to analyze cytotoxic potential of PFPE whereas deoxyribonucleic acid (DNA) fragmentation followed by Western blot analysis were used. **Results:** PFPE composed of alkaloid, flavonoid, amide, lignans, opioid, and steroid. This crude extract represented cytotoxic effect against CCA cells which stronger than dichloromethane *P. nigrum* crude extract and piperine, especially on KKU-M213 (median inhibition concentration [IC<sub>50</sub>] at 13.70 µg/ml) and TFK-1 (IC<sub>50</sub> at 15.30 µg/ml). Interestingly, PFPE showed lower cytotoxicity against normal human cholangiocyte MMNK-1 cells (IC<sub>50</sub> at 19.65 µg/ml) than KKU-M213 and TFK-1 cells. Then, the molecular mechanisms of PFPE were firstly evaluated by DNA fragmentation followed by Western blot analysis. The degradation of DNA was observed on KKU-M213 and TFK-1 cells after treatment with PFPE at day 2. Then, proliferation proteins including topoisomerase II, AKT8 virus oncogene cellular homolog, avian myelocytomatosis virus oncogene cellular homolog, cyclin D1, signal transducer and activator of transcription 3, cyclooxygenase-2, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) were decreased and p21 was increased. Furthermore, apoptotic proteins, such as tumor protein p53, Bcl-2-associated X protein, and p53 upregulated modulator of apoptosis were upregulated. Meanwhile, antiapoptotic protein B-cell lymphoma 2 was down-regulated. **Conclusion:** These results indicated that PFPE inhibited CCA through the down-regulation of cell proliferation and induction of apoptosis pathway.

**Key words:** Anticancer, apoptosis, cell proliferation, cholangiocarcinoma, *Piper nigrum*

## SUMMARY

- piperine free *Piper nigrum* extract (PFPE) inhibited cholangiocarcinoma (CCA) cell lines
- PFPE induces CCA cells to undergo apoptosis and cell cycle arrest via the inhibition of topoisomerase II
- PFPE inhibit cell growth through the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells.



**Abbreviations used:** PFPE: Piperine free *Piper nigrum* extract; CCA: Cholangiocarcinoma; DPCE: dichloromethane *P. nigrum* crude extract; NMU: N-nitrosomethylurea; ER: Estrogen receptor; MMP-9: Matrix metalloproteinase-9; MMP-2: Matrix metalloproteinase-2; VEGF: Vascular endothelial growth factor; GC-MS: Gas chromatograph-mass spectrometer; MTT: 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO: Dimethylsulfoxide; IC<sub>50</sub>: Median inhibition concentration; MCLE: Methanol crude extract of *Curcuma longa*; DNA: Deoxyribonucleic acid; STAT3: Signal transducer and activator of transcription 3; COX-2: Cyclooxygenase-2; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; c-Myc: Avian myelocytomatosis virus oncogene cellular homolog; Akt: AKT8 virus oncogene cellular homolog; Bcl-2: B-cell lymphoma 2; p53: Tumor protein p53; Bax: Bcl-2-associated X protein; PUMA: p53 upregulated modulator of apoptosis.

## Correspondence:

Dr. Potchanapond Graidist,  
Department of Biomedical Sciences, Faculty of  
Medicine, Prince of Songkla University, Hat Yai,  
Songkhla, Thailand.  
E-mail: [gpotchan@medicine.psu.ac.th](mailto:gpotchan@medicine.psu.ac.th)  
DOI: 10.4103/pm.pm\_288\_19

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## INTRODUCTION

Cholangiocarcinoma (CCA) is an epithelial cancer originating from the bile ducts with features of cholangiocyte differentiation.<sup>[1]</sup> There are 2 types of CCA (based on its location) including intrahepatic and extrahepatic.<sup>[2]</sup> For over the past four decades, incidence of CCA has been increased in United States of America,<sup>[3]</sup> Australia, England,<sup>[4]</sup> and Northeastern Thailand.<sup>[5]</sup> There are several risk factors for CCA, including primary sclerosing cholangitis, liver fluke infections (*Clonorchis sinensis* and *Opisthorchis viverrini*), choledochal cysts, Caroli's disease, hepatitis B and C infection, obesity, cirrhosis and hepatolithiasis.<sup>[5,6]</sup> The therapeutic for CCA are limited and no

current effective treatment because the majority of patients present with advanced stage disease.<sup>[7]</sup> Even treatments with advances in surgical

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**Cite this article as:** Tedasen A, Khoka A, Madla S, Sriwiriyan S, Graidist P. Anticancer effects of piperine-free *Piper nigrum* extract on cholangiocarcinoma cell lines. *Phcog Mag* 2020;16:S28-38.

techniques, chemotherapy and radiotherapy, the 5-year survival rate of patients after diagnosis still remain about 10%.<sup>[8]</sup> Although surgical resection has improved in the survival of most patients, the recurrent disease was found within 2 years after tumor resection.<sup>[9]</sup> Chemotherapy and radiation therapy are ineffective and show various side effects such as harmful to normal cells and bone marrow suppression.<sup>[10]</sup> Therefore, effective therapeutic and alternative treatments with no serious side effect for CCA are urgently needed.

*P. nigrum* L. belongs to family Piperaceae and can be used as antiapoptotic, antibacterial, anticolon toxin, antidepressant, antifungal, antidiarrhoeal, antiinflammatory, antimutagenic, antimetastatic, antioxidative, antipyretic, antispasmodic, antispermato-genic, antitumor, antithyroid, ciprofloxacin potentiator, cold extremities, gastric ailments, hepatoprotective, insecticidal, intermittent fever, and larvicidal activities.<sup>[11]</sup> The chemical constituents of *P. nigrum* are aromatic essential oils, alkaloids, amides, prophenylphenols, lignans, terpenes, flavones, and steroids.<sup>[12]</sup> Ethanolic crude extract of *P. nigrum* consists of high total phenol content shows antioxidant and anti-inflammation as well as cytotoxic property against colorectal carcinoma cell lines.<sup>[13]</sup> Using ethanol and high pressure (200 bar), *P. nigrum* crude extracts exhibits cytotoxicity against MCF-7 with median inhibition concentration ( $IC_{50}$ ) of  $14.40 \pm 3.30$   $\mu$ g/ml and represents tumor inhibitory effect in mammary adenocarcinoma mouse.<sup>[14]</sup> Previously, we reported that piperine-free *P. nigrum* extract (PFPE) strongly inhibited breast cancer MCF-7 cells with  $IC_{50}$  value of 7.45  $\mu$ g/ml. Moreover, PFPE inhibited tumor growth in *N*-nitrosomethylurea-induced mammary tumorigenesis rats without liver and kidney toxicity.<sup>[15]</sup> Interestingly, PFPE upregulated tumor protein p53 (p53) and downregulated estrogen receptor, E-cadherin, matrix metalloproteinase-9 (MMP-9), MMP-2, avian myelocytomatosis virus oncogene cellular homolog (c-Myc) and vascular endothelial growth factor (VEGF) *in vitro* and *in vivo*.<sup>[16]</sup> In this present research, we further explored the phytochemical component, investigated cytotoxicity and molecular mechanisms of PFPE on CCA cell lines.

## MATERIALS AND METHODS

### Preparation of piperine free *Piper nigrum* extract

Seeds of *P. nigrum* L. were collected from Songkhla province in Thailand. The plant specimen (voucher specimen number SKP 146161401) was identified by Asst. Prof. Dr. Supreeya Yuenyongsawad and deposited in the herbarium at the Southern Centre of Thai Traditional Medicine, Department of Pharmacognosy and Pharmaceutical Botany, Prince of Songkla University, Thailand. PFPE was prepared as previously described. Briefly, grounded 250 g of dried seeds of *P. nigrum* L. were soaked in 300 mL of dichloromethane and incubated at 35°C for 3 h in a shaking incubator. After filtration with Whatman filter paper No. 1 and concentration using rotary evaporator, the dark brown oil residue of extracts was obtained and then recrystallized with cold diethyl ether in an ice bath to get rich of yellow crystals (piperine) and obtain brown oil residue (PFPE).<sup>[15]</sup> PFPE was kept in a desiccator until used.

### Phytochemical analysis and identification of bioactive constituents by gas chromatograph-mass spectrometer

The analysis of the phytochemical screening and composition of PFPE extracts were carried out using a Gas Chromatography-Agilent 7890B combination with an Agilent 5977A triple quadrupole mass spectrometer (Agilent Technologies Inc, USA). Gas chromatograph-mass spectrometer (GC-MS) analysis is a common confirmation test, which used to make an effective chemical analysis. The PFPE samples were evaluated phytochemicals such as a flavonoids, tannins, alkaloids,

steroids, phenols, glycosides, lignans, and terpenoids. An inlet temperature of 280°C with the split ratio 7:1 was employed and the helium was used as the carried gas at the constant flow rate of 7 ml/min. The oven temperature was initially maintained at 60°C for 5 min and increase at a rate of 5°C/min to 315°C for 15 min. For MS detection, an electron ionization mode was used with an ionization energy of 70 eV, ion source temperature of 230°C, and scan mass range  $m/z$  35–500. The components were identified based on a correlation of the recorded fragmentation patterns of mass spectra that provided in the GC-MS system software version Wiley10 and NIST14. All procedures were performed at Scientific Equipment Center, Prince of Songkla University, Songkhla, Thailand.

### Measuring total phenolic, tannin, flavonoid content and radical scavenging activity

The total phenolic content was determined based on Folin–ciocalteu method. Gallic acid was used as the standard and total phenolics were expressed as mg gallic acid equivalent/mg extract (mg GAE/mg extract). Total condensed tannin was measured based on HCL-vanillin method and catechin was used as the standard. The total tannin was reported as mg catechin equivalent/mg extract (mg CE/mg extract). The total flavonoid content was determined by aluminum chloride solution ( $AlCl_3$ ) colorimetric method. Quercetin was employed as the standard and expressed the total flavonoids as mg quercetin equivalent/mg extract (mg QE/mg extract). 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity was performed according to the DPPH trolox assay and reported as mg trolox equivalent antioxidant capacity/mg extract (mg TEAC/mg extract). All procedures were performed at Center of Excellence in Natural Products Innovation, Mae Fah Luang University, Chiang Rai, Thailand.

### Cell lines and culture conditions

Three CCA (KKU-100, KKU-M213 and KKU-M055) and one cholangiocyte (MMNK-1) cells were kindly donated by Dr. Mutita Junking (Faculty of Medicine, Mahidol University, Bangkok, Thailand). TFK-1 cells were obtained from RIKEN BioResource Center and HuCC-T1 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank. Mouse fibroblast, L-929 cells, were kindly donated by Associate Professor Dr. Jasadee Kaewsichan (Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand).

KKU-100, KKU-M213, KKU-M055, MMNK-1 and L-929 cells were grown in DMEM medium (Invitrogen), which contained 10% of fetal bovine serum (Invitrogen), 2 mmol/L of L-glutamine (Invitrogen), and an antibiotic mixture of 100 units/mL of penicillin and 100  $\mu$ g/mL of streptomycin (Invitrogen). TFK-1 and HuCC-T1 cells were grown in RPMI 1640 (Invitrogen) supplemented with the same supplement as for DMEM. All cells were maintained by incubating in a 5%  $CO_2$  atmosphere, at 37°C and 96% relative humidity.

### *In vitro* cytotoxicity

The cytotoxicity assay was performed in 96-well plate. KKU-100, KKU-M055, and MMNK-1 cells were seeded at a density of  $5 \times 10^3$  cells/well. KKU-M213, TFK-1, and HuCC-T1 cells were seeded at a density of  $7.5 \times 10^3$  cells/well and L-929 cells were seeded at a density of  $8 \times 10^3$  cells/well. After incubation for 24 h, cells were treated with PFPE at various concentration for 48 h. The cells were then washed with 1X PBS and incubated in 100  $\mu$ l of 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution at 37°C for 30 min. Under light protection, the purple crystals of formazan or MTT metabolites were dissolved with 100  $\mu$ l of dimethyl

sulfoxide and incubate at 37°C for 30 min. The absorbance was measured at 570 and 650 nm using a microplate reader spectrophotometer (Spectra Max M5, Molecular Devices), and the IC<sub>50</sub> values were calculated.<sup>[17]</sup> According to US NCI plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity with IC<sub>50</sub> value ≤20 µg/ml.<sup>[18]</sup>

## Deoxyribonucleic acid fragmentation analysis

KKU-M213 and TFK-1 cells in their exponential growth phase were seeded into 6 cm culture plate at a density of  $2.5 \times 10^5$  cells/plate for 24 h and then treated with PFPE at 3 folds of IC<sub>50</sub> values. After treatment for 96 h, cells were harvested by trypsinization. Cell pellets were lysed using the extraction buffer (containing 0.7 M NaCl, 17 mM SDS, 10 mM Tris-HCl (pH 8.0) and 2 mM EDTA (pH 8.0)) and fragmented deoxyribonucleic acid (DNA) in the supernatant was extracted once with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and once with chloroform: isoamyl alcohol (24:1). The DNA was precipitated with a two-thirds volume of cold isopropanol followed by centrifugation at 8,000 ×g and washed once in 70% ethanol. Finally, DNA pellet was resuspended in deionized water and analyzed by 1.5% agarose gel electrophoresis.<sup>[19]</sup>

## Western blot analysis

KKU-M213 and TFK-1 cells were seeded into 6 cm culture plate at a density of  $2.5 \times 10^5$  cells/plate for 24 h and then treated with PFPE at IC<sub>50</sub> values. After treatment, cells were harvested every day for 4 days. Then, cell pellets were lysed using the RIPA buffer (containing 150 mM NaCl, 50 mM Tris, pH 7.4, 1% (v/v) NP-40, 0.25% (w/v) sodium deoxycholate and 1 mM EDTA). Total protein samples (150 mg) were loaded on 12% of SDS-polyacrylamide gel electrophoresis and transferred onto a 0.45 mm nitrocellulose membrane (Bio-Rad, 162-0115). Membrane was blocked at room temperature for 1 h with 5% non-fat milk in 1X TBS-T and then washed with 1% non-fat milk in 1X TBS-T. Membrane was incubated with primary antibodies against topoisomerase II, Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), p53 upregulated modulator of apoptosis (PUMA), p21, AKT8 virus oncogene cellular homolog (Akt), cyclooxygenase-2 (COX-2), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), signal transducer and activator of transcription 3 (STAT-3), cyclin D1 and p53 proteins. The membrane was then incubated with secondary horseradish peroxidase-conjugated antibodies. Bound antibodies were developed by a chemiluminescence detection kit using the SuperSignal™ West Dura Extended Duration Substrate (Thermo Scientific) and detected using a Fusion FX vilber lourmat, CCD camera (Fisher Biotechnology). GAPDH was used to normalize protein loading. Protein levels were expressed as a relative ratio to GAPDH.

## Statistical analysis

The median inhibition concentration (IC<sub>50</sub>) data was acquired by SoftMax 1 Pro 5 program (MDS Analytical Technologies Inc., California, USA). Student's *t*-test was used to analyze intergroup differences. A *P* < 0.05 was considered to be statistically significant. All results were represented as the mean ± standard deviation (SD). The values were obtained from at least three independent experiments.

## RESULTS

### Total phenolic, tannin, and flavonoid contents

Phenolics, flavonoids, and tannins are one class of secondary plant metabolites which represented anticancer activity of plant. As present in Table 1, PFPE contained phenolic, tannin and flavonoid lower than methanol crude extract of *Curcuma longa* (MCLE). However, the cytotoxicity of PFPE against breast cancer MCF-7 cells (IC<sub>50</sub> value

**Table 1:** Total phenolic, tannin and flavonoid contents in piperine free *Piper nigrum* crude extract

Crude	Phenolics (mg GAE/g extract) <sup>a</sup>	Flavonoids (mg QE/mg extract) <sup>b</sup>	Tannins (mg CE/mg extract) <sup>c</sup>
PFPE	402.46±7.49	40.69±5.99	201.82±17.78
MCLE	2090.63±15.81	148.94±33.64	2373.75±92.77

<sup>a</sup>Mg of gallic acid equivalence by mg of extract; <sup>b</sup>Mg of quercetin equivalence by mg of extract; <sup>c</sup>Mg of catechin equivalence by mg of extract; *P. nigrum*: *Piper nigrum*; PFPE: Piperine free *P. nigrum* extract; *C. longa*: *Curcuma longa*; MCLE: Metanolic *C. longa* extract; GAE: Gallic acid equivalent; QE: Quercetin equivalent; CE: Catechin equivalent

at  $7.45 \pm 0.6$  µg/ml) not significantly lower than MCLE (IC<sub>50</sub> value at  $5.74 \pm 1.48$  µg/ml). Therefore, we performed GC-MS in next experiment to identify the chemical compounds in PFPE.

## Phytochemical screening

In this study, the phytochemical analysis using GC-MS was carried out. The chromatogram and predicted constituents are shown in Figure 1 and Table 2. Results showed that PFPE contained five chemical groups including alkaloids, terpenes, amides, lignans, opioid and steroid with 17, 13, 7, 3, 1, and 1 compounds, respectively. The highest percentage of peak area of each group were piperidine (21.66%, alkaloid), caryophyllene (13.28%, terpene), acrivastine (2.34%, amide), kusunokinin (1.28%, lignan), methyldihydromorphine (1.18%, opioid), and beta-stigmasterol (1.74%, steroid) which showed the anticancer activity.

### Effect of piperine free *Piper nigrum* extract on the viability of cholangiocarcinoma, cholangiocyte and normal fibroblast cell lines

The cell viability of CCA and normal cell lines was measured using the MTT assay. All cell lines were incubated with extracts for 48 h. The IC<sub>50</sub> values represented the mean ± SD of three different experiments. Among these cell lines, PFPE showed the highest cytotoxicity against KKU-M213 cells with IC<sub>50</sub> value of  $13.70 \pm 1.14$  µg/ml. Moreover, PFPE demonstrated cytotoxic effect stronger than dichloromethane *P. nigrum* crude extract (DPCE) (IC<sub>50</sub> at  $22.22 \pm 0.26$  µg/ml) and piperine (IC<sub>50</sub> at  $27.01 \pm 0.36$  µg/ml). The positive reference drug (doxorubicin) showed a very strong cytotoxic activity on normal and almost cancer cells. Surprisingly, doxorubicin showed same cytotoxic activity with PFPE against TFK-1 cells [Table 3].

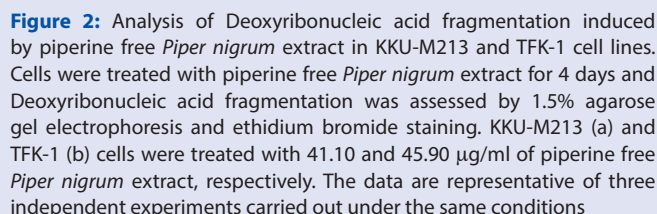
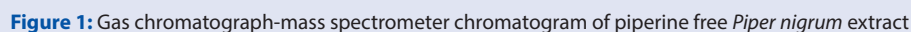
### Piperine free *Piper nigrum* extract induces deoxyribonucleic acid fragmentation on KKU-M213 and TFK-1 cells

A DNA fragmentation assay was used to determine whether the action of PFPE was associated with apoptosis or not. Apoptosis can be visualized as a ladder pattern of 180-200 base pairs due to DNA cleavage by the activation of a nuclear endonuclease enzyme. Since, PFPE demonstrated a strong cytotoxic effective on KKU-M213 and TFK-1 cells, both cell lines were used to determined DNA fragmentation. As shown in Figure 2, the DNA ladder pattern was observed at day 2 after exposure with 3 folds of IC<sub>50</sub> concentration of PFPE.

### Piperine free *Piper nigrum* extract inhibited proteins associated with inflammation that induces bile duct cancer

In this experiment, we determined proteins associated with inflammation that induced bile duct cancer including STAT-3, COX-2 and NF-κB using Western blot analysis. KKU-M213 cells were treated with 13.69 µg/ml of





### Piperine free *Piper nigrum* extract inhibited proteins involved in the cell proliferation and growth

### Piperine free *Piper nigrum* extract inhibited proteins associated with apoptosis

evaluated. After giving PFPE at IC<sub>50</sub> concentration for 48 h, death cells were observed and Bcl-2 was decreased in both cells, KKU-M213 and TFK-1 [Figure 5]. In addition, the levels of p53 and Bax proteins were significantly increased at 96 h and PUMA protein was increased from 24 to 48 h in KKU-M213 cells [Figure 5a and c]. Moreover, p53, Bax and PUMA were increased significantly at 24 h TFK-1 cells [Figure 5b and d].

The incidence of bile duct cancer or CCA has increased in Thailand and chemotherapy is not sufficient to treat the aggressive type of this cancer.<sup>[5]</sup> Therefore, medicinal plants could be an alternative treatment for bile duct cancer. There are many medicinal plants that cause cell cycle arrest and apoptosis in CCA such as *Tripterygium wilfordii*, *Atractylodes lancea* (Thunb) DC., *Zingiber officinale* Roscoe, *Phyllanthus emblica*, *Terminalia chebula* Retz., *Moringa oleifera*, and *Curcuma longa* Linn.<sup>[20,21]</sup> *Piper* species is one of medicinal plant that also shows anticancer effect, such as *Piper sarmentosum*,<sup>[22]</sup> *Piper longum*,<sup>[23]</sup> *Piper chaba*<sup>[24]</sup> and *P. nigrum*.<sup>[17]</sup> In previous study, we reported that PFPE showed anticancer activity against breast cancer in *in vitro* and *in vivo*.<sup>[15,16]</sup> Here, we further explored the biological activity of PFPE on bile duct cancer and found that PFPE exhibited anticancer activity against CCA cell lines, especially TFK-1 and KKU-M213, a moderate differentiation with p53 mutation and well differentiation CCA cells, respectively. Using GC-MS technique, many active phytochemicals were founded in PFPE including alkaloids, terpenes, amides, lignans, opioid and steroids. Piperitine, guineensine, and pipersintenamide, (an alkaloid compounds) represented percentage of peak area at 21.66, 10.17, and 5.65%, respectively. Piperitine shows toxicity against larvae of *Aedes aegypti*,<sup>[25]</sup> and guineensine has an anticancer property against the mouse lymphoma cell line L5178Y with IC<sub>50</sub> values of 17.0 µM.<sup>[26]</sup> Pipersintenamide, isolated from *Piper sintonense* Hatus, shows anticancer activity against leukemia P-388 and promyelocytic leukemia HL-60 cell lines with IC<sub>50</sub> values of 3.78 and 3.80 µg/ml.<sup>[27,28]</sup> Moreover, caryophyllene (13.28% in PFPE), a bicyclic natural sesquiterpene, exhibits antiproliferative effects against colorectal cancer cells (IC<sub>50</sub> 19 µM) though clonogenicity, migration, invasion and spheroid formation.<sup>[29]</sup> A beta-stigmasterol (1.74% in PFPE), a steroid compound, demonstrates inhibitory effects with IC<sub>50</sub> values of 11.14 and 18.28 µM against human myeloid leukemia K562 and prostate cancer PC3 cell lines, respectively.<sup>[30]</sup> In this recent study, we found a very potent compounds in the PFPE including piperlonguminine (4.77%), kusunokinin (1.28%), and cubebin (0.28%), which have been reported as anticancer agents. (-)-Kusunokinin and piperlonguminine, a natural lignan and alkaloid compounds, inhibited breast cancer cells (MCF-7 and MDA-MB-468) and colorectal cells (SW-620) through down-regulation of topoisomerase II and up-regulation of p53, p21 protein levels.<sup>[31]</sup> (-)-Cubebin, a lignan compound, represents anticancer effect against myeloid leukemia, lung and nasopharyngeal cancer.<sup>[32]</sup> Interestingly, we found that PFPE showed stronger cytotoxicity against CCA cells than DPCE and piperine [Table 3]. However, piperine, the major alkaloid compound in *P. nigrum*, still remained in the PFPE



**Table 2:** Chemical constituents in piperine free *Piper nigrum* extract

Identified compounds	Formula	Nature of compound	Molecular massb (g/mol)	Retention time	Area (%)	Biological activity
3-Carene	$C_{10}H_{16}$	Terpenes	136.24	9.0896	0.28	Antioxidant, antihyperuricemic and anti-inflammatory <sup>[33]</sup>
D-Limonene	$C_{10}H_{16}$	Terpenes	136.24	9.7228	0.39	Enhanced the antitumor effect of docetaxel against prostate cancer cells <sup>[34]</sup>
Clohexane, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans) 2,4-diisopropenyl-1-methyl-1-vinylcyclohexane (or beta-Elementene)	$C_{15}H_{24}$	Terpenes	204.36	19.2545	2.20	Cytotoxic effect on K562 (leukemic) cells by the induction of apoptosis <sup>[35]</sup>
Copaene	$C_{15}H_{24}$	Terpenes	204.36	20.2929	1.26	Antimicrobial activity against an anaerobic microorganism <i>Prevotella nigrescens</i> <sup>[36]</sup>
2,4-diisopropenyl-1-methyl-1-vinylcyclohexane (beta-Elementene)	$C_{15}H_{24}$	Terpenes	204.36	20.7150	0.73	Cytotoxic effect on K562 (leukemic) cells by the induction of apoptosis <sup>[35]</sup>
Caryophyllene	$C_{15}H_{24}$	Terpenes	204.36	21.4893	13.28	Antioxidant, preventing lipidic oxidative damage and prevention of atherosclerosis <sup>[37]</sup> ; antigenotoxic and antioxidant <sup>[38]</sup>
1,4,7,7-Cycloundecatriene, 1,5,9,9-tetra methyl-, Z, Z, Z-Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]- (or beta-helmscapene, beta-Selinene)	$C_{15}H_{24}$ $C_{15}H_{24}$	Terpenes Terpenes	204.36 204.35	22.3144 23.1348	1.15 0.60	No activity reported Antioxidant and cytotoxic activity against HT29 (colon cancer) cells <sup>[39]</sup> ; cytotoxicity against KB (oral cancer), MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer) cells <sup>[40]</sup>
2-Isopropenyl-4a, 8-dimethyl-1,2,3,4,4a, 5,6,8a-octahydronaphthalene (or 7-Epi-alpha-Selinene) delta-Cadinene	$C_{15}H_{24}$	Terpenes	204.36	23.3522	0.54	Antimicrobial activity against <i>Bacillus subtilis</i> and <i>Candida albicans</i> <sup>[41]</sup>
Caryophyllene oxide	$C_{15}H_{24}O$	Terpenes	220.36	24.0207	0.61	Induction of apoptosis and cell cycle arrest on OVACR-3 (ovarian cancer) cells <sup>[42]</sup>
Isospathulenol	$C_{15}H_{24}O$	Terpenes	220.37	25.4618	0.42	Chemosenitizing agents for doxorubicin chemotherapy <sup>[43]</sup> ; anticancer <sup>[44]</sup> increased the efficacy of DOX in MDA-MB-231 (breast cancer) cells <sup>[45]</sup> ; inhibit STAT3 signaling pathway <sup>[46]</sup>
2,4-Decadienamide, N-isobutyl-, (E, E)- (or Pellitorine) Piperidine, 1-(1-oxo-3-phenyl-2-prope nyl)- (or piperidine, 1-Cinnamoylpiperidine)	$C_{14}H_{23}NO$ $C_{14}H_{17}NO$	Amides Alkaloids	223.36 215.29	26.4932 32.8537 36.1008	0.71 2.28 0.22	Cytotoxic effects against <i>Aspergillus niger</i> , <i>Artemia salina</i> and <i>Caenorhabditis elegans</i> <sup>[47]</sup> Antibacterial, anticancer and anti-inflammatory <sup>[48]</sup> No activity reported
(2E,4E)-1-(Pyrrolidin-1-yl) deca-2,4-dien-1-one (or Iyeramide A, sarmentine)	$C_{14}H_{23}NO$	Alkaloids	221.34	36.2247	0.37	Cytotoxicity against CCRF-CEM (acute lymphoblastic leukemia), HL-60 (acute promyelocytic leukemia), PC-3 (prostate carcinoma), and HA22T (hepatoma) cells <sup>[27]</sup> inhibit lipoxigenase (5-LOX) and cyclooxygenase-1 (COX-1) <sup>[49]</sup> Inhibit allergic and inflammatory <sup>[50]</sup>
(2E,4E)-N-Isobutyldeca-2,4-dienamide (or Dodecatetraenoic acid isobutylamide)	$C_{16}H_{29}NO$	Amides	251.41	36.7524	0.48	No activity reported Hepatoprotective effect <sup>[51]</sup>
N-Benzylidene-4-fluoroaniline	$C_{13}H_{10}FN$	Alkaloids	199.23	44.1035	0.34	Anticancer against breast cancer cells <sup>[31]</sup>
(E)-5-(Benzol[d][1,3]dioxol-5-yl)-1-(pi peridin-1-yl) pent-2-en-1-one (or piperanine)	$C_{17}H_{21}NO_3$	Alkaloids	287.359	44.5123	0.88	No activity reported
Piperlonguminine	$C_{16}H_{19}NO_3$	Alkaloids	273.33	44.8101	4.77	Anticancer against Hep-G2 (hepatocellular carcinoma) <sup>[52]</sup> and Hela (cervical cancer) cells <sup>[53]</sup>
(E)-1-(Piperidin-1-yl) hexadec-2-en-1-one	$C_{21}H_{39}NO$	Alkaloids	321.54	45.3603	0.79	No activity reported
Piperine	$C_{17}H_{19}NO_3$	Alkaloids	285.34	46.3182	5.09	Anticancer against Hep-G2 (hepatocellular carcinoma) <sup>[52]</sup> and Hela (cervical cancer) cells <sup>[53]</sup>
(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide	$C_{20}H_{35}NO$	Amides	305.50	46.5162	0.48	No activity reported
(2E,4E)-N-Isobutyltodeca-2,4-dienamide (or Pipericine)	$C_{22}H_{41}NO$	Amides	335.58	46.6004	0.85	Hepatoprotective effect <sup>[54]</sup>
1-Benzyl-2-(1-ethoxycarbonyl-2-phenylethyl)-4,5-dihydroimidazole (Acrivastine)	$C_{22}H_{24}N_2O_2$	Amides	348.45	46.6023	2.34	No activity reported
(E)-7-(Benzol[d][1,3]dioxol-5-yl)-1-(pyrrolidin-1-yl) hept-6-en-1 one (or Methylidihydromorphine)	$C_{18}H_{23}NO_3$	Opioid		47.8646	1.18	No activity reported

Contid...

Table 2: Contd...

Identified compounds	Formula	Nature of compound	Molecular massb (g/mol)	Retention time	Area (%)	Biological activity
Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E, E)- (or Pyrrolidine, Trichostachine, Piperiline)	C <sub>16</sub> H <sub>17</sub> NO <sub>3</sub>	Alkaloids	271.32	47.9359	2.58	Antiproliferative effect, cycle arrest, induce apoptosis on MCF-7 cells and antitumor effect <i>in vivo</i> <sup>[55]</sup>
1H-Indene, 2-fluoro-2,3-dihydro-1-methoxy-, trans-(+)- (E)-1-(Piperidin-1-yl) octadec-2-en-1-one (or Piperitine)	C <sub>10</sub> H <sub>11</sub> FO C <sub>23</sub> H <sub>43</sub> NO C <sub>19</sub> H <sub>25</sub> NO <sub>3</sub>	Amides Alkaloids Alkaloids	349.60 315.41	48.1182 48.3679 48.5620	0.66 21.66 0.24	No activity reported Insecticidal activity <sup>[25]</sup> No activity reported
(E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) hept-6-en-1-one (or Piperolein A)	C <sub>19</sub> H <sub>23</sub> NO <sub>3</sub>	Alkaloids	313.39	49.1390	5.65	Cytotoxicity against CCRF-CEM (acute lymphoblastic leukemia), HL-60 (acute promyelocytic leukemia), PC-3 (prostate carcinoma), and HA22T (hepatoma) cells <sup>[27]</sup>
(2E,6E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) hepta-2,6-dien-1-one (or Pipersintenamide)						Cytoprotective activity on normal fibroblast L929 cells and hepatoprotective activity <sup>[54]</sup>
(2E,4E,14E)-N-Isobutylicos-2,4,14-trienamide (or 2,4,14-Eicosatrienamamide)	C <sub>24</sub> H <sub>43</sub> NO	Amides	361.61	49.3379	0.59	Antiinflammatory, <sup>[56]</sup> anticancer <sup>[32]</sup>
2-Furanol, 3,4-bis (1,3-benzodioxol-5-ylmethyl) tetrahydro- (or 2-Furanol, Cubebin)	C <sub>20</sub> H <sub>20</sub> O <sub>6</sub>	Lignan	356.37	49.6489	0.28	Antiinflammatory, <sup>[58]</sup> antioxidant <sup>[59]</sup>
Retrofractamide-A	C <sub>20</sub> H <sub>25</sub> NO <sub>3</sub>	Alkaloids	327.42	50.3585	0.34	Larvicidal activity against <i>Culex pipiens</i> pallens, <i>Aedes aegypti</i> and <i>Aedes togoi</i> ; <sup>[57]</sup> hepatoprotective effect <sup>[54]</sup>
2 (3H)-Furanone, 3,4-bis (1,3-benzodioxol-5-ylmethyl) dihydro-, (3R-trans)- (or (+)-Hinokinin, Cubebinolide)	C <sub>20</sub> H <sub>18</sub> O <sub>6</sub>	Lignan	354.36	50.5191	1.13	Antiinflammatory, <sup>[58]</sup> antioxidant <sup>[59]</sup>
(E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(pyrrolidin-1-yl) non-8-en-1-one (or Pyrrolidine, Tricholeine)	C <sub>20</sub> H <sub>27</sub> NO <sub>3</sub>	Alkaloids	329.44	50.7269	0.42	Antiproliferative activity against various cancer cells <sup>[60]</sup>
(3R,4R)-3-(Benzo[d][1,3]dioxol-5-yl methyl)-4-(3,4-dimethoxybenzyl) dihydrofuran-2 (3H) one (or Kusunokinin)	C <sub>21</sub> H <sub>22</sub> O <sub>6</sub>	Lignan	370.40	51.0435	1.28	Anticancer; <sup>[31]</sup> insecticidal activity against <i>Virola sebifera</i> and fungicidal activity against <i>Leucoagaricus gongylophorus</i> <sup>[61]</sup>
(E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) non-8-en-1-one (or Piperolein B)	C <sub>21</sub> H <sub>29</sub> NO <sub>3</sub>	Alkaloids	343.47	51.3920	1.03	Inhibitor of acyl CoA: Diacylglycerol acyltransferase for potential therapy for the treatment of obesity and type 2 diabetes <sup>[62]</sup>
(2E,4E,12E)-13-(Benzo[d][1,3]dioxol-5-yl)-N-isobutytrideca-2,4,12-trienamide (or Guineensine)	C <sub>24</sub> H <sub>33</sub> NO <sub>3</sub>	Alkaloids	383.53	51.8600	10.17	Antiinflammatory <sup>[63]</sup>
(2E,4E,6E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) hepta-2,4,6-trien-1-one (or Piperettine)	C <sub>19</sub> H <sub>21</sub> NO <sub>3</sub>	Alkaloids	311.38	52.9692	0.31	Trypanocidal effects against epimastigotes and amastigotes of <i>Trypanosoma cruzi</i> <sup>[64]</sup>
(22E)-Stigmasta-5,22-dien-3-ol (or beta-Stigmasterol, Poriferasterol)	C <sub>29</sub> H <sub>48</sub> O	Steroid	412.70	53.0319	1.74	Induce DNA damage and cell death <sup>[65]</sup>
(2E,4E,8E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) nona-2,4,8-trien-1-one (or Dehydroperipernonaline)	C <sub>21</sub> H <sub>25</sub> NO <sub>3</sub>	Alkaloids	339.47	53.5356	2.32	Coronary vasodilating activity <sup>[66]</sup>
gamma-Sitosterol (or clonasterol)	C <sub>29</sub> H <sub>50</sub> O	Terpenes	414.72	53.7147	0.48	Cytotoxicity against P388 (murine lymphocytic leukaemia) and HL60 (leukemia) cells <sup>[67]</sup>
(2E,4E,12E)-13-(Benzo[d][1,3]dioxol-5-yl)-Nisobutytrideca-2,4,12-trienamide (or Guineensine)	C <sub>24</sub> H <sub>33</sub> NO <sub>3</sub>	Alkaloids	383.53	55.6810		Antiinflammatory <sup>[63]</sup>

at 5.09% [Table 2]. Similarly, CP2 (PFPE) exhibited  $IC_{50}$  values of  $7.45 \pm 1.59 \mu\text{g/ml}$  in MCF-7 cell lines, which was better than DPCE ( $IC_{50}$  at  $23.46 \pm 1.10 \mu\text{g/ml}$ ).<sup>[17]</sup> These results indicate that PFPE, less piperine, was a potential crude extract in anticancer.

*O. viverrini* excretory/secretory products and *O. viverrini* antigen induce the expression of TLR4, IL-6, IL-8, TLR2, NF- $\kappa$ B, iNOS and COX-2 causing damage to biliary epithelium.<sup>[68]</sup> In this current study, PFPE showed down regulation of NF- $\kappa$ B, STAT-3 and COX-2 proteins [Figure 2]. In cancer cells, NF- $\kappa$ B and STAT-3 are major transcription factors that regulate proliferation, inflammatory, angiogenesis, invasive and apoptosis resistance by induction of several proteins, such as cyclin D, cyclin E1, CDK2, CDK4, CDK6, c-myc, tumor necrosis factor alpha, interleukin-1 (IL-1), IL-6, IL-8, VEGF and MMP-9.<sup>[69]</sup> NF- $\kappa$ B and STAT-3 proteins are induced by IL-6 to stimulate COX-2 expression in the inflammation process and cell cycle,<sup>[70,71]</sup> which associate to CCA progression. Therefore, suppression of NF- $\kappa$ B, STAT-3 and COX-2 proteins cause cancer growth inhibition. Piperlongumine,

an alkaloid from *P. longum* reduces NF- $\kappa$ B and c-Myc protein levels and inhibits binding of NF- $\kappa$ B with DNA at promoters in lymphoma cancer cells.<sup>[72]</sup> Moreover, piperlongumine also reduced the phosphorylation of JAK-1, JAK-2 and STAT-3 in gastric cancer cells.<sup>[73]</sup> Matrine, an alkaloid from *Sophora flavescens* Ait., significantly inhibits the viability by reduction the phosphorylation levels of JAK-2 and STAT3 proteins in CCA cells.<sup>[74]</sup> Curcumin, a natural extracted polyphenol from *C. longa*, also suppresses proliferation in human biliary cancer cells through inhibition of NF- $\kappa$ B, STAT-3 and JAK1 proteins.<sup>[75]</sup>

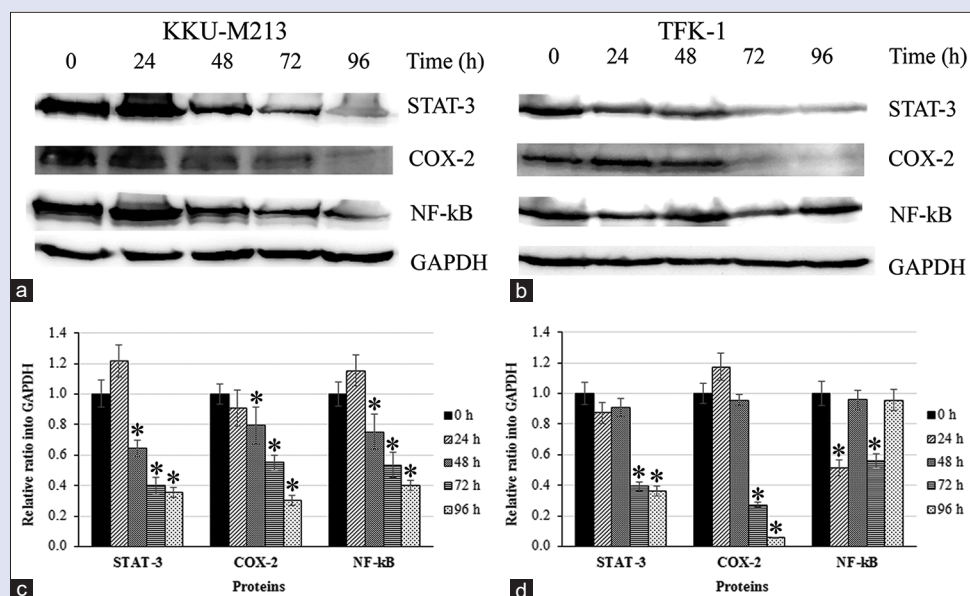
There are many evidences on genes and proteins which relate to bile duct cancer growth and progression, such as p53 mutation, inactivation of p21 and activation of Ras and MAPKs proteins.<sup>[76]</sup> Here, we found that PFPE could inhibit CCA cancer proliferation by decreasing of topoisomerase II, Akt, c-Myc, cyclin D1, and increasing of p21 protein levels [Figure 4]. Topoisomerase II is an enzyme involved in the DNA replication process that controls cell cycle with peaking at G2/M phase.<sup>[77]</sup> Therefore, down regulation of topoisomerase II by PFPE could induced DNA damage, interrupted cell growth and caused cell death on KKU-M213 and TFK-1 cells. Most of the clinically active agents, including etoposide (lignan) and doxorubicin (alkaloid) are topoisomerase inhibitors.<sup>[78]</sup> Previously andrographolide analogue 3A.1 from *Andrographis paniculata*, a diterpenoid lactone, induces cell cycle arrest by down-regulation of CDK6 and cyclin D1 in KKU-M213 cell lines.<sup>[79]</sup> Surprisingly, PFPE also exerted a significant reduction of Akt protein leading to decreasing of c-Myc and cyclin D1 and increasing of p21 levels [Figure 6]. Akt and cyclin D1 stimulate the cell cycle progression from G1/S phase to G2/M phase.<sup>[80]</sup>  $\beta$ -caryophyllene oxide, a terpene compound from *P. nigrum*, shows down-regulation of downstream of AKT pathway, including cyclin D1, COX-2 and VEGF and also up-regulation of p53 and p21 proteins in human prostate and breast cancer cells.<sup>[81]</sup>

In this study, we founded that the PFPE induced cell death by causing DNA fragmentation, increasing apoptotic proteins (p53, Bax and PUMA) and decreasing Bcl-2 protein levels [Figure 5]. p53, a tumor suppressor and transcription factor, is initially induced when DNA

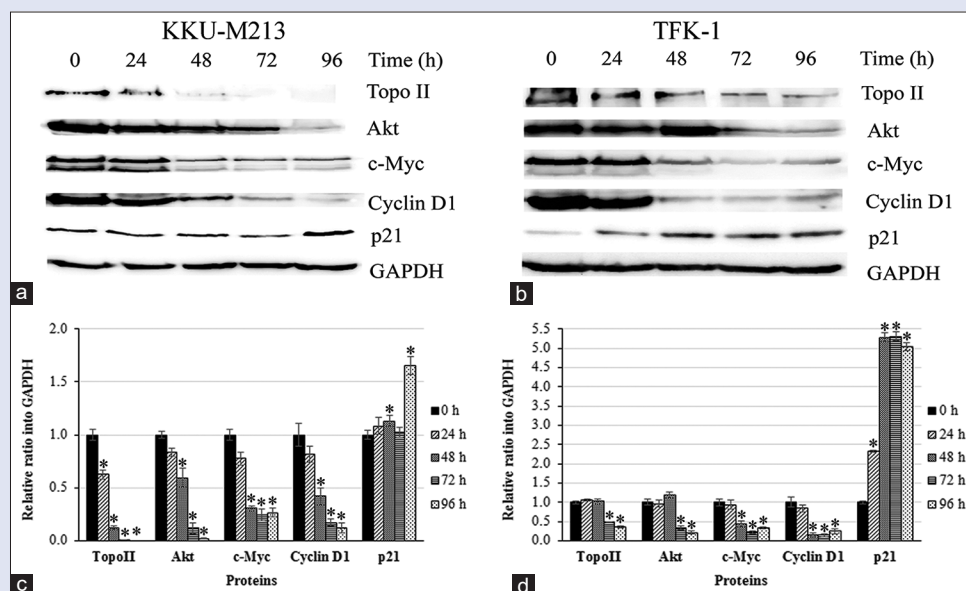
**Table 3:** Cytotoxicity of piperine free *Piper nigrum* extract against cholangiocarcinoma, cholangiocyte and normal mouse fibroblast cell lines

Cell lines	$IC_{50}$ value $\pm$ SD ( $\mu\text{g/ml}$ )			
	DPCE	Piperine	PFPE	Doxorubicin
CCA				
KKU-100	22.88 $\pm$ 0.43	46.53 $\pm$ 0.09	17.79 $\pm$ 0.88	0.78 $\pm$ 0.03
KKU-M213	22.22 $\pm$ 0.26	27.01 $\pm$ 0.36	13.70 $\pm$ 1.14	1.75 $\pm$ 0.02
KKU-M055	46.66 $\pm$ 0.48	55.32 $\pm$ 0.22	16.74 $\pm$ 0.61	0.69 $\pm$ 0.09
TFK-1	23.25 $\pm$ 0.45	29.38 $\pm$ 0.07	15.30 $\pm$ 0.18	15.19 $\pm$ 0.12
HuCC-T1	37.17 $\pm$ 0.03	35.02 $\pm$ 0.12	20.72 $\pm$ 0.75	2.53 $\pm$ 0.04
Normal cholangiocyte				
MMNK-1	33.25 $\pm$ 0.28	60.68 $\pm$ 0.72	19.65 $\pm$ 0.26	0.62 $\pm$ 0.05
Normal fibroblast				
L-929	No effect	No effect	45.53 $\pm$ 0.50	0.20 $\pm$ 0.01

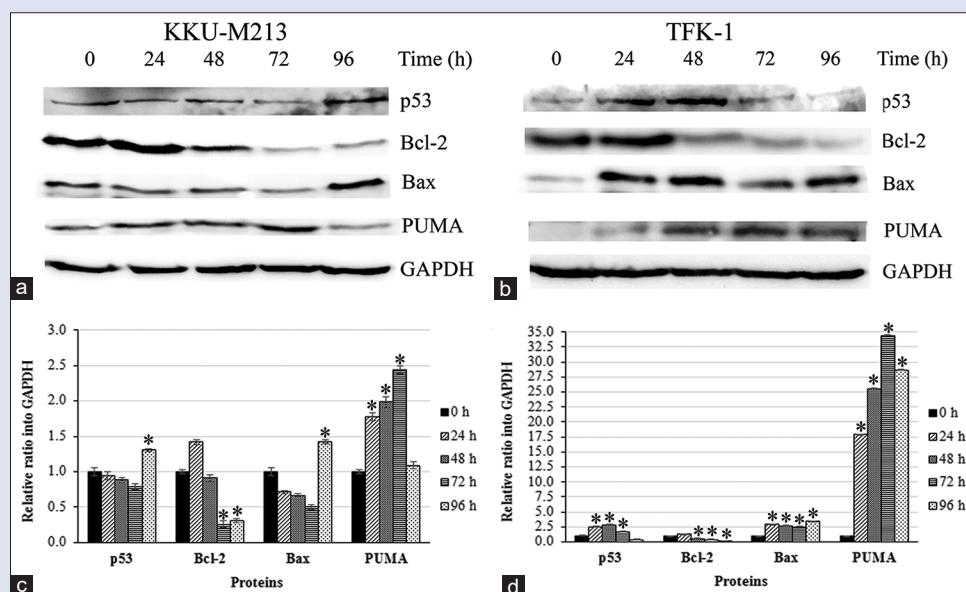
*P. nigrum*: *Piper nigrum*; DPCE: Dichloromethane *P. nigrum* crude extract; PFPE: Piperine free *P. nigrum* extract; CCA: Cholangiocarcinoma; SD: Standard deviation



**Figure 3:** Expression of inflammation-related proteins in KKU-M213 (a and c) and TFK-1 (b and d) cells treated with piperine free *Piper nigrum* extract at 24, 48, 72 and 96 h. The levels of signal transducer and activator of transcription 3, cyclooxygenase-2 and Nuclear factor kappa-light-chain-enhancer of activated B cells and GAPDH proteins were measured using the Western blot analysis. Densitometric analysis normalized to GAPDH. Data were represented as mean  $\pm$  standard deviation and three independent experiments were done. \* $P < 0.05$  compared with control group (0 h)



**Figure 4:** Effect of piperine free *Piper nigrum* extract on cell growth and cell cycle arrest. KKU-M213 (a and c) and TFK-1 (b and d) cells were treated with Median inhibition concentration concentration of piperine free *Piper nigrum* extract for 24, 48, 72 and 96 h. Then, the levels of topoisomerase II, AKT8 virus oncogene cellular homolog, avian myelocytomatosis virus oncogene cellular homolog, cyclin D1 and p21 proteins were investigated using Western blot analysis. Fold change of each protein was measured by densitometry quantitation using ImageJ software and normalized with GAPDH.  $P < 0.05$  of three independent experiments was considered to indicate a statistically significant differences compared to control group (0 h)

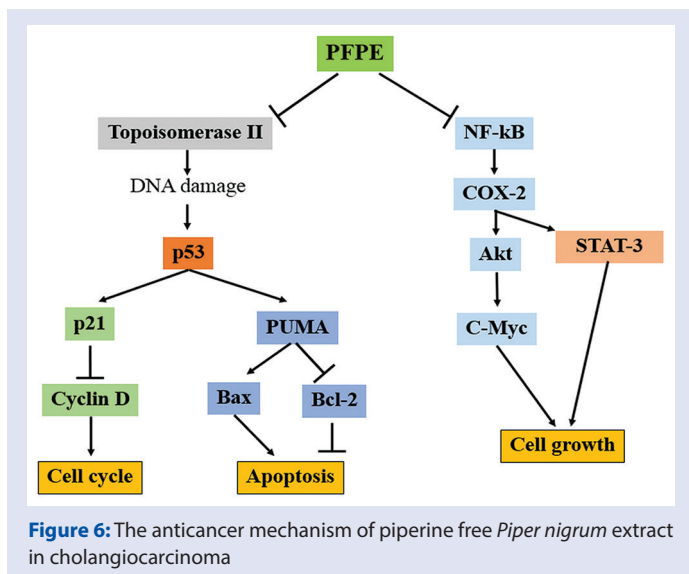


**Figure 5:** Effect of piperine free *Piper nigrum* extract on apoptosis. KKU-M213 (a and c) and TFK-1 (b and d) cells were treated with Median inhibition concentration concentration of piperine free *Piper nigrum* extract for 24, 48, 72 and 96 h. Then, the levels of tumor protein p53, B-cell lymphoma 2, Bcl-2-associated X protein and PUMA proteins were investigated using Western blot analysis. Fold change of each protein was measured by densitometry quantitation using ImageJ software and normalized with GAPDH.  $P < 0.05$  of three independent experiments was considered to indicate a statistically significant difference compared to control group (0 h)

damage and takes responsibility to activate several apoptotic genes, such as Bax, PUMA and NOXA.<sup>[82-84]</sup> Similarly, ethanolic extract of *P. nigrum* has antiproliferative effect on MCF-7 cells, antitumor effect *in vivo* and triggering apoptosis via p53 and Bax and decreasing of Bcl-2 proteins.<sup>[55]</sup> Curcumin effectively induces apoptosis in CCA (CCLP-1 and SG-231) cells by stimulation of Notch1, Hes-1 and survivin apoptotic proteins.<sup>[85]</sup> Andrographolide analog 3A.1 has cytotoxicity

with  $IC_{50}$  of 8.0  $\mu$ M on KKU-M213 cells at 24 h after treatment and induces apoptosis via induction of cleaved PARP-1, Bax, caspase-3, and p53.<sup>[79]</sup> Matrine stimulates apoptosis in CCA cells through induction of cytochrome c releasing from mitochondria and reduction of caspase-3 and-9 activity.<sup>[74]</sup> Taken together, PFPE can be a potential candidate for CCA treatment in future. However, study in CCA *in vivo* and clinical trial need to be carried out.





## CONCLUSION

PFPE showed strong cytotoxicity against KKU-M213 and TFK-1 cell lines with  $IC_{50}$  values of  $13.70 \pm 1.14$  and  $15.30 \pm 0.18$   $\mu\text{g/ml}$ , respectively. PFPE suppressed inflammation through down-regulation of NF- $\kappa$ B, STAT-3 and COX-2. Moreover, PFPE inhibited CCA cells growth and proliferation by down-regulation of topoisomerase II, Akt, c-Myc and cyclin D and up-regulation of p21. Furthermore, PFPE triggered apoptosis through inhibition of Bcl-2 and induction of p53, Bax and PUMA levels as summarized in the Figure 5. In summary, PFPE can be served as a promising crude extract for CCA treatment.

## Acknowledgement

The authors thank Prince of Songkla University for the equipment funding (the Excellent Research Laboratory (ExLab004)).

## Financial support and sponsorship

This work was supported by the research fund (REC 60-279-04-2) Faculty of Medicine and the Postdoctoral Fellowship, Prince of Songkla University.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- Blechacz B, Gores GJ. Cholangiocarcinoma: Advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008;48:308-21.
- Ghouri YA, Mian I, Blechacz B. Cancer review: Cholangiocarcinoma. *J Carcinog* 2015;14:1.
- Saha SK, Zhu AX, Fuchs CS, Brooks GA. Forty-year trends in cholangiocarcinoma incidence in the U.S.: Intrahepatic disease on the rise. *Oncologist* 2016;21:594-9.
- Khan SA, Taylor-Robinson SD, Toledano MB, Beck A, Elliott P, Thomas HC. Changing international trends in mortality rates for liver, biliary and pancreatic tumours. *J Hepatol* 2002;37:806-13.
- Sripa B, Pairojkul C. Cholangiocarcinoma: Lessons from Thailand. *Curr Opin Gastroenterol* 2008;24:349-56.
- Plentz RR, Malek NP. Clinical presentation, risk factors and staging systems of cholangiocarcinoma. *Best Pract Res Clin Gastroenterol* 2015;29:245-52.
- Gatto M, Alvaro D. New insights on cholangiocarcinoma. *World J Gastrointest Oncol* 2010;2:136-45.
- Squadroni M, Tondulli L, Gatta G, Mosconi S, Beretta G, Labianca R. Cholangiocarcinoma. *Crit Rev Oncol Hematol* 2017;116:11-31.

- van der Gaag NA, Kloek JJ, de Bakker JK, Musters B, Geskus RB, Busch OR, *et al.* Survival analysis and prognostic nomogram for patients undergoing resection of extrahepatic cholangiocarcinoma. *Ann Oncol* 2012;23:2642-9.
- Barreto JN, McCullough KB, Ice LL, Smith JA. Antineoplastic agents and the associated myelosuppressive effects: A review. *J Pharm Pract* 2014;27:440-6.
- Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Khan MA. Biological role of *Piper nigrum* L. (Black pepper): A review. *Asian Pac J Trop Biomed* 2012;2:S1945-53.
- Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, *et al.* Phytochemistry of the genus *Piper*. *Phytochemistry* 1997;46:597-673.
- Prashant A, Rangaswamy C, Yadav AK, Reddy V, Sowmya MN, Madhunapantula S. Anticancer activity of ethanolic extracts of & against colorectal carcinoma cell lines. *Int J Appl Basic Med Res* 2017;7:67-72.
- Grinevicius VM, Andrade KS, Ourique F, Micke GA, Ferreira SR, Pedrosa RC. Antitumor activity of conventional and supercritical extracts from *Piper nigrum* L. cultivar Bragantina through cell cycle arrest and apoptosis induction. *J Supercrit Fluids* 2017;128:94-101.
- Sriwiriyan S, Tedasen A, Lailerd N, Boonyaphiphat P, Nitruangjarat A, Deng Y, *et al.* Anticancer and cancer prevention effects of piperine-free *Piper nigrum* extract on n-nitrosomethylurea-induced mammary tumorigenesis in rats. *Cancer Prev Res (Phila)* 2016;9:74-82.
- Deng Y, Sriwiriyan S, Tedasen A, Hiransai P, Graidist P. Anti-cancer effects of *Piper nigrum* via inducing multiple molecular signaling *in vivo* and *in vitro*. *J Ethnopharmacol* 2016;188:87-95.
- Sriwiriyan S, Ninpesh T, Sukpondma Y, Nasomyon T, Graidist P. Cytotoxicity screening of plants of genus *Piper* in breast cancer cell lines. *Trop J Pharm Res* 2014;13:921-8.
- Geran RI, Greenberg NH, McDonald MM, Schumacher AM, Abbot BJ. Protocol for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemoth Rep* 1972;3:1-103.
- Graidist P, Martla M, Sukpondma Y. Cytotoxic activity of *Piper cubeba* extract in breast cancer cell lines. *Nutrients* 2015;7:2707-18.
- Plengsuriyakarn T, Thitapakorn V, Na-Bangchang K, Karbwang J. Thai medicinal plants: Potential sources of anticholangiocarcinoma drugs. *Int J Pharm Pharmacol* 2013;2:68-82.
- Leelawat S, Leelawat K. Molecular mechanisms of cholangiocarcinoma cell inhibition by medicinal plants. *Oncol Lett* 2017;13:961-6.
- Zainal Ariffin SH, Wan Omar WH, Zainal Ariffin Z, Safian MF, Senafi S, Megat Abdul Wahab R. Intrinsic anticarcinogenic effects of *Piper sarmentosum* ethanolic extract on a human hepatoma cell line. *Cancer Cell Int* 2009;9:6.
- OVADJE P, Ma D, Tremblay P, Roma A, Steckle M, Guerrero JA, *et al.* Evaluation of the efficacy biochemical mechanism of cell death induction by *Piper longum* extract selectively in *in vitro* and *in vivo* models of human cancer cells. *PLoS One* 2014;9:e113250.
- Mahavorasirikul W, Viyanant V, Chaijaroenkul W, Itharat A, Na-Bangchang K. Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells *in vitro*. *BMC Complement Altern Med* 2010;10:55.
- Geris R, Ribeiro PR, Brandão MD, Da Silva HH, Silva IG. Bioactive natural products as potential candidates to control *Aedes aegypti*, the vector of dengue. In: Rahman A, editor. *Studies in Natural Products Chemistry*. Vol. 37. Oxford: Elsevier; 2012. p. 227-376.
- Muharini R, Liu Z, Lin W, Proksch P. New amides from the fruits of *Piper retrofractum*. *Tetrahedron Lett* 2015;56:2521-5.
- Chen JJ, Huang YC, Chen YC, Huang YT, Wang SW, Peng CY, *et al.* Cytotoxic amides from *Piper sintonense*. *Planta Med* 2002;68:980-5.
- Chen JJ, Duh CY, Huang HY, Chen IS. Cytotoxic Constituents of *Piper sintonense*. *Helv Chim Acta* 2003;86:2058-64.
- Dahham SS, Tabana YM, Iqbal MA, Ahamed MB, Ezzat MO, Majid AS, *et al.* The Anticancer, antioxidant and antimicrobial properties of the sesquiterpene  $\beta$ -caryophyllene from the essential oil of *Aquilaria crassna*. *Molecules* 2015;20:11808-29.
- Shen T, Zhang L, Wang YY, Fan PH, Wang XN, Lin ZM, *et al.* Steroids from Commiphora mukul display antiproliferative effect against human prostate cancer PC3 cells via induction of apoptosis. *Bioorg Med Chem Lett* 2012;22:4801-6.
- Sriwiriyan S, Sukpondma Y, Srisawat T, Madla S, Graidist P. (-)-Kusunokinin and piperloguminine from *Piper nigrum*: An alternative option to treat breast cancer. *Biomed Pharmacother* 2017;92:732-43.
- Rajalekshmi DS, Kabeer FA, Madhusoodhanan AR, Bahulayan AK, Prathapan R, Prakasan N, *et al.* Anticancer activity studies of cubebin isolated from *Piper cubeba* and its synthetic derivatives. *Bioorg Med Chem Lett* 2016;26:1767-71.
- Kohoude MJ, Gbaguidi F, Agbani P, Ayedoun MA, Cazaux S, Bouajila J. Chemical composition



- and biological activities of extracts and essential oil of *Boswellia dalzielii* leaves. *Pharm Biol* 2017;55:33-42.
34. Rabi T, Bishayee A. d-Limonene sensitizes docetaxel-induced cytotoxicity in human prostate cancer cells: Generation of reactive oxygen species and induction of apoptosis. *J Carcinog* 2009;8:9.
35. Zou L, Liu W, Yu L. Beta-elemene induces apoptosis of K562 leukemia cells. *Zhonghua Zhong Liu Za Zhi* 2001;23:196-8.
36. Martins Cde M, do Nascimento EA, de Moraes SA, de Oliveira A, Chang R, Cunha LC, *et al.* Chemical constituents and evaluation of antimicrobial and cytotoxic activities of *Kielmeyera coriacea* Mart. & Zucc. essential oils. *Evid Based Complement Alternat Med* 2015;2015:1-9. doi.org/10.1155/2015/842047.
37. Baldissera MD, Souza CF, Grando TH, Stefani LM, Monteiro SG.  $\beta$ -caryophyllene reduces atherogenic index and coronary risk index in hypercholesterolemic rats: The involvement of cardiac oxidative damage. *Chem Biol Interact* 2017;270:9-14.
38. Alvarez-González I, Madrigal-Bujaidar E, Castro-García S. Antigenotoxic capacity of beta-caryophyllene in mouse, and evaluation of its antioxidant and GST induction activities. *J Toxicol Sci* 2014;39:849-59.
39. Ali NA, Wursterb M, Denkert A, Arnold N, Fadail I, Al-Didamony G, *et al.* Chemical composition, antimicrobial, antioxidant and cytotoxic activity of essential oils of *Plectranthus cyllindraceus* and *Meriandra benghalensis* from Yemen. *Nat Prod Commun* 2012;7:1099-102.
40. Keawsa-ard S, Liawruangrath B, Liawruangrath S, Teerawutgulrag A, Pyne SG. Chemical constituents and antioxidant and biological activities of the essential oil from leaves of *Solanum spirale*. *Nat Prod Commun* 2012;7:955-8.
41. Ali NAA, Chhetri BK, Dosoky NS, Shari K, Al-Fahad AJ, Wessjohann L, *et al.* Antimicrobial, antioxidant, and cytotoxic activities of *Ocimum forskolei* and *Teucrium yemense* (Lamiaceae) essential oils. *Medicines (Basel)* 2017;4:7.
42. Hui LM, Zhao GD, Zhao JJ.  $\delta$ -Cadinene inhibits the growth of ovarian cancer cells via caspase-dependent apoptosis and cell cycle arrest. *Int J Clin Exp Pathol* 2015;8:6046-56.
43. Di Giacomo S, Di Sotto A, Mazzanti G, Wink M. Chemosensitizing properties of  $\beta$ -Caryophyllene and  $\beta$ -caryophyllene oxide in combination with doxorubicin in human cancer cells. *Anticancer Res* 2017;37:1191-6.
44. Fidyk T, Fiedorowicz A, Strzadala L, Szumny A.  $\beta$ -caryophyllene and  $\beta$ -caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Med* 2016;5:3007-17.
45. Hanušová V, Caltová K, Svobodová H, Ambroz M, Skarka A, Murínová N, *et al.* The effects of  $\beta$ -caryophyllene oxide and trans-nerolidol on the efficacy of doxorubicin in breast cancer cells and breast tumor-bearing mice. *Biomed Pharmacother* 2017;95:828-36.
46. Kim C, Cho SK, Kapoor S, Kumar A, Vali S, Abbasi T, *et al.*  $\beta$ -Caryophyllene oxide inhibits constitutive and inducible STAT3 signaling pathway through induction of the SHP-1 protein tyrosine phosphatase. *Mol Carcinog* 2014;53:793-806.
47. Dosoky NS, Satyal P, Gautam TP, Setzer WN. Composition and biological activities of & *Murraya paniculata* (L.) Jack essential oil from Nepal. *Medicines (Basel)* 2016;3:7.
48. Ku SK, Lee IC, Kim JA, Bae JS. Anti-septic effects of pellitorine in HMGB1-induced inflammatory responses *in vitro* and *in vivo*. *Inflammation* 2014;37:338-48.
49. Stöhr JR, Xiao PG, Bauer R. Constituents of Chinese *Piper* species and their inhibitory activity on prostaglandin and leukotriene biosynthesis *in vitro*. *J Ethnopharmacol* 2001;75:133-9.
50. Gulledge TV, Collette NM, Mackey E, Johnstone SE, Moazami Y, Todd DA, *et al.* Mast cell degranulation and calcium influx are inhibited by an *Echinacea purpurea* extract and the alkylamide dodeca-2E,4E-dienoic acid isobutylamide. *J Ethnopharmacol* 2018;212:166-74.
51. Matsuda H, Ninomiya K, Morikawa T, Yasuda D, Yamaguchi I, Yoshikawa M. Protective effects of amide constituents from the fruit of *Piper chaba* on D-galactosamine/TNF- $\alpha$ -induced cell death in mouse hepatocytes. *Bioorg Med Chem Lett* 2008;18:2038-42.
52. Gunasekaran V, Elangovan K, Niranjali Devaraj S. Targeting hepatocellular carcinoma with piperine by radical-mediated mitochondrial pathway of apoptosis: An *in vitro* and *in vivo* study. *Food Chem Toxicol* 2017;105:106-18.
53. Han SZ, Liu HX, Yang LQ, Cui LD, Xu Y. Piperine (PP) enhanced mitomycin-C (MMC) therapy of human cervical cancer through suppressing Bcl-2 signaling pathway via inactivating STAT3/NF- $\kappa$ B. *Biomed Pharmacother* 2017;96:1403-10.
54. Matsuda H, Ninomiya K, Morikawa T, Yasuda D, Yamaguchi I, Yoshikawa M. Hepatoprotective amide constituents from the fruit of *Piper chaba*: Structural requirements, mode of action, and new amides. *Bioorg Med Chem* 2009;17:7313-23.
55. de Souza Grinevicius VM, Kwiecinski MR, Santos Mota NS, Ourique F, Porfirio Will Castro LS, Andregueti RR, *et al.* *Piper nigrum* ethanolic extract rich in piperamides causes ROS overproduction, oxidative damage in DNA leading to cell cycle arrest and apoptosis in cancer cells. *J Ethnopharmacol* 2016;189:139-47.
56. Lima TC, Lucarini R, Volpe AC, de Andrade CQJ, Souza AM, Pauletti PM, *et al.* *In vivo* and *in silico* anti-inflammatory mechanism of action of the semisynthetic (-)-cubebins derivatives (-)-hinokinin and (-)-O-benzylcubebin. *Bioorg Med Chem Lett* 2017;27:176-9.
57. Park IK, Lee SG, Shin SC, Park JD, Ahn YJ. Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. *J Agric Food Chem* 2002;50:1866-70.
58. Godoy de Lima R, Barros MT, da Silva Laurentiz R. Medicinal attributes of Lignans extracted from & *Piper cubeba*: Current developments. *ChemistryOpen* 2018;7:180-91.
59. Medola JF, Cintra VP, Pesqueira E Silva EP, de Andrade Royo V, da Silva R, Saraiva J, *et al.* (-)-Hinokinin causes antigenotoxicity but not genotoxicity in peripheral blood of Wistar rats. *Food Chem Toxicol* 2007;45:638-42.
60. Wang YH, Goto M, Wang LT, Hsieh KY, Morris-Natschke SL, Tang GH, *et al.* Multidrug resistance-selective antiproliferative activity of *Piper* amide alkaloids and synthetic analogues. *Bioorg Med Chem Lett* 2014;24:4818-21.
61. Bicalho KU, Terezan AP, Martins DC, Freitas TG, Fernandes JB, Silva MF, *et al.* Evaluation of the toxicity of *Virola sebifera* crude extracts, fractions and isolated compounds on the nest of leaf-cutting ants. *Psyche* 2012;2012:785424.
62. Lee SW, Rho MC, Park HR, Choi JH, Kang JY, Lee JW, *et al.* Inhibition of diacylglycerol acyltransferase by alkaloids isolated from the fruits of *Piper longum* and *Piper nigrum*. *J Agric Food Chem* 2006;54:9759-63.
63. Reynoso-Moreno I, Najar-Guerrero I, Escareño N, Flores-Soto ME, Gertsch J, Viveros-Paredes JM. An endocannabinoid uptake inhibitor from black pepper exerts pronounced anti-inflammatory effects in mice. *J Agric Food Chem* 2017;65:9435-42.
64. Ribeiro TS, Freire-de-Lima L, Previato JO, Mendonça-Previato L, Heise N, de Lima ME. Toxic effects of natural piperine and its derivatives on epimastigotes and amastigotes of *Trypanosoma cruzi*. *Bioorg Med Chem Lett* 2004;14:3555-8.
65. Chaturvedula VS, Gao Z, Hecht SM, Jones SH, Kingston DG. A new acylated oleanane triterpenoid from *Couepia polyandra* that inhibits the lyase activity of DNA polymerase  $\beta$ . *J Nat Prod* 2003;66:1463-65.
66. Shoji N, Umeyama A, Saito N, Takemoto T, Kajiwara A, Ohizumi Y. Dehydropiperonaline, an amide possessing coronary vasodilating activity, isolated from *Piper longum* L. *J Pharm Sci* 1986;75:1188-9.
67. Manoharan KP, Yang D, Hsu A, Huat BT. Evaluation of *Polygonum bistorta* for anticancer potential using selected cancer cell lines. *Med Chem* 2007;3:121-6.
68. Prueksapanich P, Piyaichaturawat P, Aumpansub P, Ridditid W, Chaiterakij R, Rerknimit R. Liver fluke-associated biliary tract cancer. *Gut Liver* 2018;12:236-45.
69. Fan Y, Mao R, Yang J. NF- $\kappa$ B and STAT3 signaling pathways collaboratively link inflammation to cancer. *Protein Cell* 2013;4:176-85.
70. Basu A, Das AS, Sharma M, Pathak MP, Chattopadhyay P, Biswas K, *et al.* STAT3 and NF- $\kappa$ B are common targets for kaempferol-mediated attenuation of COX-2 expression in IL-6-induced macrophages and carrageenan-induced mouse paw edema. *Biochem Biophys Res* 2017;12:54-61.
71. Chen SC, Chang YL, Wang DL, Cheng JJ. Herbal remedy magnolol suppresses IL-6-induced STAT3 activation and gene expression in endothelial cells. *Br J Pharmacol* 2006;148:226-32.
72. Han SS, Son DJ, Yun H, Kamberos NL, Janz S. Piperlongumine inhibits proliferation and survival of Burkitt lymphoma *in vitro*. *Leuk Res* 2013;37:146-54.
73. Song B, Zhan H, Bian Q, Gu J. Piperlongumine inhibits gastric cancer cells via suppression of the JAK1,2/STAT3 signaling pathway. *Mol Med Rep* 2016;13:4475-80.
74. Yang N, Han F, Cui H, Huang J, Wang T, Zhou Y, *et al.* Matrine suppresses proliferation and induces apoptosis in human cholangiocarcinoma cells through suppression of JAK2/STAT3 signaling. *Pharmacol Rep* 2015;67:388-93.
75. Prakobwong S, Gupta SC, Kim JH, Sung B, Pinlaor P, Hiraku Y, *et al.* Curcumin suppresses proliferation and induces apoptosis in human biliary cancer cells through modulation of multiple cell signaling pathways. *Carcinogenesis* 2011;32:1372-80.
76. Fava G. Molecular mechanisms of cholangiocarcinoma. *World J Gastrointest Pathophysiol* 2010;1:12-22.
77. Larsen AK, Skladanowski A, Bojanowski K. The roles of DNA topoisomerase II during the cell cycle. *Prog Cell Cycle Res* 1996;2:229-39.
78. Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. *Nat Rev Cancer* 2009;9:338-50.
79. Nateewattana J, Dutta S, Reabroi S, Saeeng R, Kasemsook S, Chairoungdua A, *et al.* Induction of apoptosis in cholangiocarcinoma by an andrographolide analogue is mediated through topoisomerase II  $\alpha$  inhibition. *Eur J Pharmacol* 2014;723:148-55.

80. Xu N, Lao Y, Zhang Y, Gillespie DA. Akt: A double-edged sword in cell proliferation and genome stability. *J Oncol* 2012;2012:1-15. doi:10.1155/2012/951724.
81. Park KR, Nam D, Yun HM, Lee SG, Jang HJ, Sethi G, *et al.*  $\beta$ -Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. *Cancer Lett* 2011;312:178-88.
82. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 1995;80:293-9.
83. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell* 2001;7:683-94.
84. Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol Cell* 2001;7:673-82.
85. Koprowski S, Sokolowski K, Kunnimalaiyaan S, Gamblin TC, Kunnimalaiyaan M. Curcumin-mediated regulation of Notch1/hairy and enhancer of split-1/survivin: molecular targeting in cholangiocarcinoma. *J Surg Res* 2015;198:434-40.