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Review

Piperine: Chemical, biological and nanotechnological applications

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Piperine (PIP) is an alkaloid present in several species of piper, mainly *Piper nigrum* Linn. and *P. longum*, among other species. The present article provides a comprehensive review of PIP research in the last years concerning its chemical properties, synthesis, absorption, metabolism, bioavailability and toxicity. The reviewed PIP literature has shown many pharmacological properties, such as antidiabetic, antidiarrheal, antioxidant, antibacterial, and antiparasitic activity of PIP. However, its low solubility and absorption make its application challenging. This review also includes advances in the development of nanosystems containing PIP, including liposomes, micelles, metal nanoparticles, nanofibers, polymeric nanoparticles, and solid-lipid nanoparticles. Finally, we discuss different *in vitro* and *in vivo* studies to evaluate the biological activity of this drug, as well as some methods for the synthesis of nanosystems and their physical characteristics.

Keywords: absorption, metabolism, pharmacological activity, nanosystems

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INTRODUCTION

Piperine (1-piperoylpiperidine), by its IUPAC name (2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one, is found in various piper species, such as black pepper *(Piper nigrum* Linn.) (97.25 to 98.57 %), long pepper (*P. longum)* (96.50 to 97.50 %), *P. retrofractum* (0.03 %), *P. crussi* and *P. geniculatum* (1). It can also be found in the leaves of *Rhododendron faurie* (Ericaceae) (2), dry rhizomes of *Zingiber officinale* (ginger) (3), *Vicoa Indica* (Asteraceae), *Fructus piperis longi* (4), the bark of *Careya arborea* (Lecythidaceae) (5) and the seeds of *Anethum sowa* (Apiaceae) (6). This alkaloid is weakly basic with the molecular formula $C_{17}H_{19}NO_3$, M_r 285.34 (Fig. 1). It is slightly soluble in water (0.04 g L^{-1} at 18 °C) and highly soluble in ether (0.027 g mL^{-1}), chloroform (0.58 g mL^{-1}) and ethanol (0.06 g mL^{-1}). Its melting range is from 128 to 130 °C. PIP was first isolated by Hans Christian Oersted in 1819 from black pepper (*P. nigrum*), in addition to its isomeric alkaloids such as isochavicine (*trans-cis* isomers), chavicine (*cis-cis* isomers) and isopiperine (*cis-trans* isomers) (Fig. 2) (7, 132). Despite its wide biological potential, such as modulating the bioavailability of

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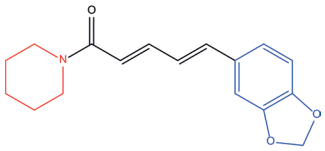

Fig. 1. Chemical structure of piperine. Three sub-units: butadiene chain (sub-unit in black), an amide function formed by piperidine and α,β-unsaturated carbonyl (subunit in red), a piperonal nucleus (sub-unit in blue).

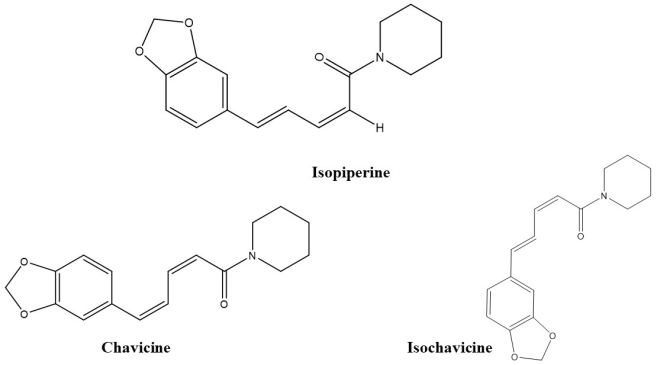

Fig. 2. Alkaloids obtained from black pepper (*P. nigrum* L.): chavicine, isopiperine and isochavicine (*7*, 132).

drugs, antimutagenic action, antitumor effects, antioxidant and anti-inflammatory effects, the lipophilic character of PIP makes it difficult to dissolve, limiting its access to the site of action and its bioavailability in the body.

A lot of research in the field of pharmaceutical technology has been focused on optimizing the dissolution of drugs and bioactive molecules with lipophilic properties. Among the developed strategies, the nano-delivery of such molecules has been used to increase their solubilization, as well as to promote their controlled release (8).

The objective of this review is to provide information on the synthesis, absorption, metabolism, bioavailability and toxicity of PIP, and to analyze various pharmacological activities and nanosystems containing PIP, which improve its bioavailability and pharmacological effects.

SYNTHESIS OF PIPERINE

PIP synthesis starts with the biosynthesis of L-lysine (Fig. 3). In the presence of pyridoxal phosphate (PLP), L-lysine is subjected to decarboxylation into cadaverine, and then the oxidative enzyme diamine oxidase causes its deamination into an amino aldehyde. This amine undergoes a cyclization forming the imine, Δ1-piperideine, which is reduced to piperidine and reacts with the piperonyl-CoA (piperic acid-coenzyme A ester) to finally form PIP (6).

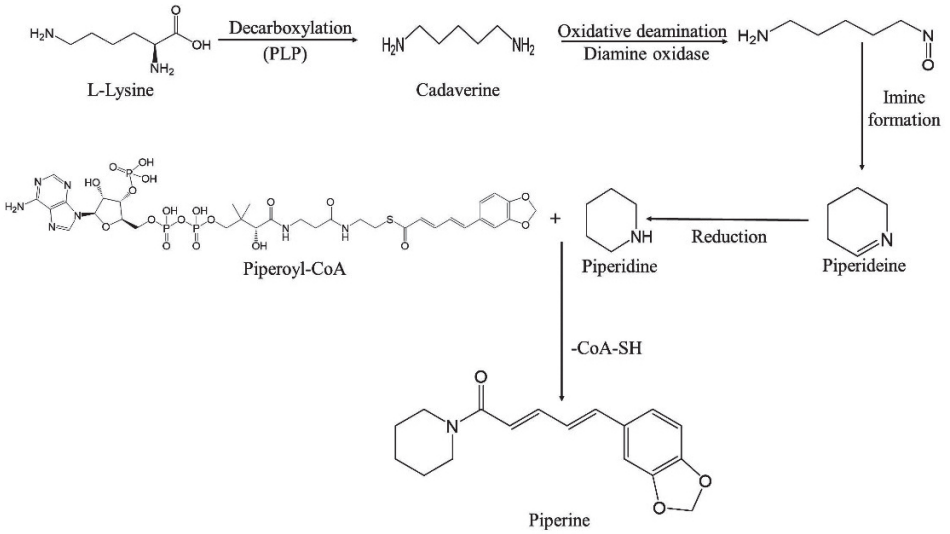

Fig. 3. Schematic representation of PIP biosynthesis from L-lysine (6).

ABSORPTION AND METABOLISM

Despite the current use of PIP, there is little information about its metabolism and absorption. However, a study undertaken in 1986 by Bhat *et al*. (9) analyzed the absorption of PIP in male albino mice at a dose of 15 mg (85 mg kg^{-1}) administered intraperitoneally or 30 mg (170 mg kg^{-1}) by the oral route. The results showed that the intraperitoneal administration of PIP was detected in the liver at a concentration of 1–2.5 % and 15 % was found in the spleen, kidney and serum, in contrast to the oral administration, which yielded only 0.1–0.25 % in the same organs. Suresh *et al.* (10) administered PIP orally in rats (170 mg kg^{-1}) and found that the maximum level of PIP reached after 6 h in the intestine was 8 % of the total amount of PIP administered. Also, Liu *et al.* (11) detected PIP concentration in different tissues of the rat (100 mg kg^{-1}), such as liver, heart, spleen, lungs and kidneys, the highest concentration being 50 % in the liver.

Several types of metabolites similar to PIP have been found in human urine. These contained the methoxy group (OCH_3) instead of the hydroxyl group (OH) as a substituent

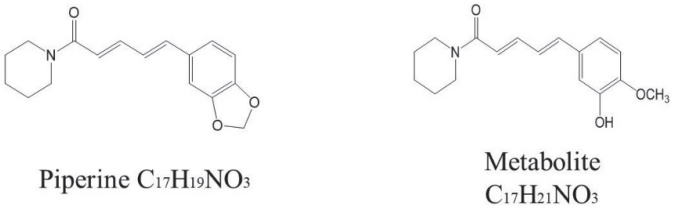

Fig. 4. Piperine and similar metabolites in human urine (4,5-dihydroxypiperine) (12).

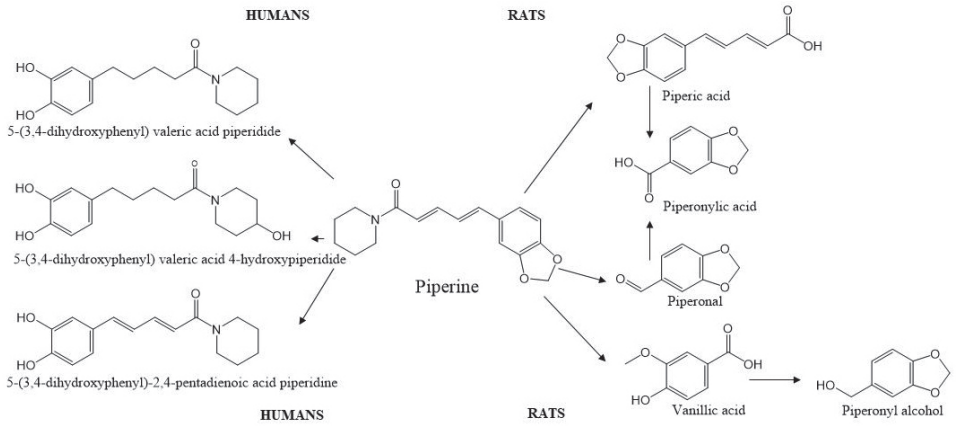

Fig. 5. Structure of piperine and metabolites identified in human and rat urine (12).

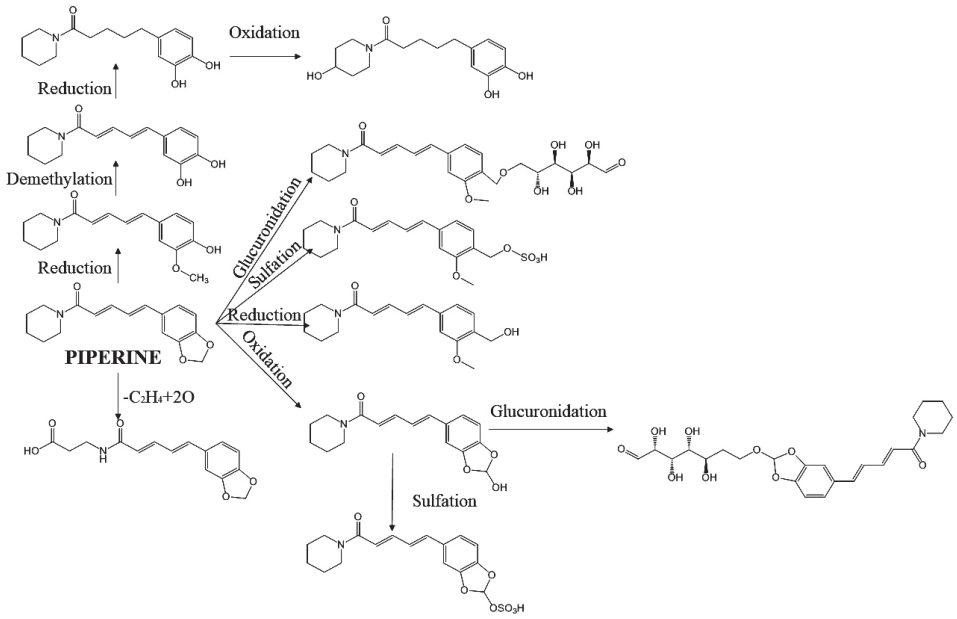

Fig. 6. Metabolic pathways of piperine identified after *in vivo* experiments with rats (133).

in the 3-position in the benzene ring (Fig. 4). Some examples are valeric acid piperidide [5-(3,4-dihydroxyphenyl)], valeric acid pipididine [5-(3,4-dihydroxyphenyl)] and [5-(3,4-dihydroxyphenyl)-2,4-pentadienoic acid] piperidine (Fig. 5). This study also showed that PIP can be converted to peptic acid by amidase and then metabolized by oxidation as

piperonyl alcohol, piperonal, vanillic acid and piperonyl acid. Also, it was observed that piperic acid is excreted by bile, along with vanillic acid (4.3 %), piperonal acid (1.3 %) and piperonyl alcohol (3.6 %) (12). Other studies carried out by Shang *et al*. (13) identified a total of 148 metabolites of PIP of different structures during a study in mice, and they showed that PIP was mainly subjected to dehydrogenation, hydrogenation, methylation, glucuronic conjugation, ring cleavage, demethylation, hydroxylation, methoxylation, sulfate conjugation, oxidation, glucuronidation, and their complex metabolic reactions (Fig. 6).

BIOENHANCER

The oral administration of drugs is subjected to a variety of adverse conditions, such as gastrointestinal content, the action of intestinal wall enzymes, the action of the microflora, and, mainly, enzymatic action in the liver. The first-pass metabolism can be a desired step for prodrug activation, but in other drugs, it may promote bioavailability reduction and rapid excretion, thus preventing the therapeutic effect. PIP has been widely reported in the literature for its ability to increase the effect and bioavailability of orally administered drugs, and different mechanisms are involved in promoting these effects. This alkaloid may increase the permeability of the cell membrane by interfering with the lipid environment and consequently increasing drug absorption. Another well-known mechanism of PIP is enzymatic inhibition (14). In general, PIP can inhibit enzymes involved in the biotransformation of drugs, preventing its inactivation. Such enzymes include cytochrome nicotinamide adenine dinucleotide phosphate (NADPH) (15), cytochrome B_5, uridine diphosphate glucose dehydrogenase (UDP-GDH) (16), aryl hydrocarbon hydroxylase (AAH), ethylmorphine-*N*--demethylase, 7-ethoxycoumarin-*O*-deethylase, UDP-glucuronyl transferase (17), 5-lipoxygenase (18), cyclooxygenase-1 and cytochrome P450 (17). The inhibition of these enzymes results in better bioavailability of drugs and nutrients such as fatty acids, β-carotene, resveratrol, norfloxacin, ampicillin, metronidazole, ampicillin, aflatoxin B1, and some vaccines (19), phenytoin, pentobarbitone, propranolol/theophylline, curcumin, coenzyme Q10, rifampicin, oxytetracycline, losartan potassium, ibuprofen, atenolol, gatifloxacin, ampicillin trihydrate, metronidazole and sparteine. Another mechanism is the inhibition of flux transport mediated by glycoprotein P (P-gp), such as digoxin and cyclosporin A; this effect was observed in Caco-2 cell line (human colon carcinoma cell line). The inhibition consists of increased intestinal glycoprotein, which reduces the hepatic P-gp and the P-gp of the kidney and causes an increase in the sub-cytotoxic concentration of the drugs through the cell membranes (20). Other effects have been described in the literature, such as binding to the DNA receptor (21), modulation of cell signal transduction, and inhibition of the medication output pump, allowing longer circulation time of the active drug (22).

TOXICITY

Consecutive oral administration of PIP in Swiss albino mice (2.25–4.5 mg kg^{-1}) for five days caused a decrease in the myogenic response of B lymphocytes and an increase in neutrophils, resulting in a reduction in leukocytes. However, at a lower dose of 1.12 mg kg^{-1}, PIP is considered immunologically safe (23).

BIOLOGICAL PROPERTIES OF PIPERINE

PIP has a wide variety of biological properties, which have been studied both *in vivo* and *in vitro*. Due to its chemical structure (Fig. 1), PIP contains three subunits: (*i*) a butadiene chain, (*ii*) an amide function formed by piperidine and an α,β-unsaturated carbonyl, (*iii*) a piperonal nucleus. These subunits are responsible for several bioactivities (24). A brief presentation of the data gathered in the last years is shown in Table I.

Antibacterial properties

Prokaryotic cells can inhibit the clinical efficacy of antibiotics. These cells have a tool called the efflux pump, carried out by membrane proteins. This mechanism helps to eliminate toxins inside the cell and protect it from hostile environments, thus contributing to the resistance to multi-drugs. Pharmaceutical companies are currently searching for combinations of antimicrobial drugs and PIP because this alkaloid can inhibit the efflux pump (25, 26). For example, *Helicobacter pylori* can induce serious gastric upset and PIP has the ideal characteristics of a chemopreventive agent against this bacterium. A study by Toyoda *et al*. (27) showed that PIP inhibits the *in vitro* proliferation of *H. pylori*. Also, *in vivo* studies (gerbils infected with *H. pylori*) showed that PIP suppressed the infiltration of neutrophils and mononuclear cells and the expression of tumor necrosis factor (TNF-α phospho-IκB-α), interleukin (Il-1β), interferons (IFN-γ), Il-6 and inducible nitric oxide synthase (iNOS). In conclusion, PIP may have potential use in the chemoprevention of gastric carcinogenesis associated with *H. pylori*.

Anticancer and antimutagenic properties

It has been reported that PIP has anticancer and antimutagenic activities of various types of cancer cells. (*i*) It can cause apoptosis in cancer cells which is usually executed through two major pathways: death receptor-mediated extrinsic pathway and mitochondria-mediated intrinsic pathway (28). Also, at the molecular level, it can influence many proteins in the apoptotic process, suppressing the development of metastasis and tumor (29). (*ii*) It can alter the redox homeostasis, thus slowing down the development of cancer. It is known that the final reactive forms produced by the metabolic activation of pro-carcinogens, free radicals, and reactive oxygen species (ROS) play a critical role in the development of cancer (30). Therefore, PIP can influence cellular physiology in redox changes, causing cell death. (*iii*) It can cause the arrest of cell cycle regulation, decreasing fundamental protein regulators such as cyclins, cyclin-dependent kinases (CDK), CDK inhibitors (CDKi), matrix metalloproteinase 9 (MMP-9), nuclear factor *k*-light-chain-enhancer of activated B cells (NF-*K*B), caspases, cAMP response element-binding (CREB), activated transcription factor 2 (ATF-2) (Fig. 7) (31). (*iv*) It can inhibit angiogenesis (the formation of a new blood vasculature) (32). (*v*) It was able to inhibit the activity of glycoprotein P (also known as MDR1 or ABCB1), a protein known to cause the resistance to chemotherapeutic agents, due to the effusion pump that depends on adenosine triphosphate (ATP) (33). Also, PIP can restrain cancer by modulating multiple signaling pathways, resulting in the inhibition of chemoprevention, antimetastatic, differentiation, autophagy and apoptosis (2). A recent study by Han *et al*. (34) investigated cervical cancer cells and analyzed the co-treatment between PIP and MitoMycin-c (MMc). They demonstrated growth inhibitory effects

Table I. Biological activities of piperine

Biological activity	Type of biological activity/ disease (reference)	Observed effects
Anticancer	Anticancer activities (96)	PIP inhibited the growth *in vitro* of triple-negative breast cancer cells (TNBC), without affecting the normal growth of the cells. The expression of the protein associated with the G1 and G2 phase and the expression of p21 decreased. PIP inhibited Akt activation, which promotes survival in TNBC cells. PIP caused caspase-dependent apoptosis through the mitochondrial pathway. Intratumoral administration of PIP inhibited the growth of TNBC xenografts in immuno-deficient mice.
	Anticancer activities (97)	PIP inhibited cell proliferation of HT-29 colon carcinoma. PIP caused the arrest of the cell cycle in the G1 phase with decreased expression of cyclin D1 and D3. PIP showed a loss of mitochondrial membrane integrity and cleavage of poly (ADP-ribose) polymerase-1.
	Cytotoxic and genotoxic carcinogenesis (98)	PIP as a protective agent against damage to DNA and genotoxic agent induced by aflatoxin B_1.
	Breast cancer (99)	PIP decreased protein levels of E-cadherin, c-Myc and vascular endothelial growth factor in MCF-7 cells. PIP can suppress tumor cell invasion, migration, and angiogenesis. PIP generated reactive oxygen species (ROS) for increased cellular stress of cancer cells.
	Anticancer activity (100)	PIP caused inhibition of tyrosine kinase receptors and the mitigation of the progression of hepatocellular carcinoma.
	Anticancer activity (34)	PIP decreased phosphorylated signal transducer and transcriptional activator (p-STAT3) in cervical cancer cells.
	Tumor suppressor (101)	PIP caused decreased phosphorylation of JNK and p38 MAPK in A2780 cells. PIP inhibited caspase-3, caspase-9, and JNK.

Biological activity	Type of biological activity/ disease (reference)	Observed effects
Anti-inflammatory	Allergic rhinitis (102)	PIP reduced the levels of cell loss and activation of astrocytes in the chemical model of epilepsy.
	Periodontitis (103)	PIP inhibited the loss of alveolar bone and reformed the microstructures of the trabeculae in a dose-dependent manner.
		PIP significantly reduced the infiltration of inflammation in the soft tissues.
		PIP limited the fractions of degraded areas in the collagen fibers.
		PIP significantly reduced the expression of IL-1β, MMP-8, and MMP-13 in periodontitis, but not that of TNF-α.
	Ulcerative colitis (104)	PIP inhibited the abnormal secretion of proinflammatory mediators, nitric oxide, the cytokines TNF-α and reduced inflammation mediated by TLR4 induced by a free fatty acid.
	Acute lung injury (105)	PIP attenuated the activity of myeloperoxidase induced by lipopolysaccharides, pulmonary edema and inflammatory cytokines TNF-α, IL-6, and IL-1β production.
	Hyperglycemia (106)	PIP significantly decreased the expression of thioredoxin renal (TXNIP) and NLRP3 inflammasome in the diabetic kidneys.
		PIP showed significant inhibition of NF-κB together with decreased levels of IL-1β and TNF-α in diabetic rats.
		Diabetic rats showed a significant decrease in creatinine clearance and an increase in blood glucose, serum creatinine, blood urea nitrogen, malondialdehyde, and proteinuria.
	Helicobacter pylori-induced chronic gastritis (27)	PIP suppressed the expression of Il-1β, IFN-γ, Il-6, and INOs.
		PIP caused the infiltration of neutrophils and mononuclear cells.
	Lung tissue damage (46)	PIP reduced glutathione content and significantly limited the elevation of tumor necrosis factor TNF-α, interleukin-1β, and interleukin-6 levels.
		The lung tissues showed minimal injury using PIP.
Antidepressant	Chronically stresses (79, 107)	PIP significantly improved the behavioral deficits of mice treated with stress in the sucrose preference test and the forced swimming test.
		Capsaicin and piperine synergistically increased the cytotoxicity of doxorubicin in Caco-2 cells.
		Capsaicin and piperine increased the intracellular accumulation of P-glycoprotein substrates and inhibited their exit from cell lines and resistance to multiple drugs.

Biological activity	Type of biological activity/ disease (reference)	Observed effects
Antiparasitic	Leishmaniasis (53, 108)	PIP caused anti-promastigote activity.
		The bioactive fractions of PIP increased the secretion of Th1 cytokines (INF-γ, TNF-α, and IL-2) and decreased IL-4 and IL-10.
		PIP increased the production of IgG2a.
		PIP increased the expression of the costimulatory molecules CD80 and CD86.
		PIP increased the population of splenic $CD4^+$ and $CD8^+$ T cells.
		Capsaicin and piperine in combination with meglumine antimonate showed anti-leishmanial activity against the promastigote and amastigote forms.
Hepatoprotector	(109)	The mixed formulation of micellar lipids with piperine was useful in solving the problem of the low bioavailability of phyllanthin in the liver.
Insecticidal and larvicidal	*Aedes aegypti* (110)	PIP showed maximum residual larvicidal activity for 10 days.
	Anopheles malaria (67)	Insecticide-resistant and susceptible strains by species proved equally susceptible to black pepper and piperine.
Antiapoptotic	Deltamethrin (111)	PIP mitigated the phenotypic changes induced by deltamethrin.
		PIP restored the levels of cytokines, which were suppressed by the treatment with deltamethrin.
		PIP had a good binding affinity towards CD4 and CD8 receptors.
Antioxidant	Cataract model (112)	PIP decreased oxidative stress.
Anticonvulsant	Serotonin, norepinephrine, and GABA (113)	PIP decreased mortality in mice with epilepsy induced by maximal electroshock.
		PIP delayed the appearance of tonic-clonic convulsion seizures in the pentylenetetrazol test and reduced the associated mortality.
		PIP caused complete protection against mortality observed in seizures induced by BAYK-8644.
	Epilepsy (77)	PIP reduced the epileptic status and prevented memory deterioration.
		PIP decreased inflammation and oxidative stress.
Antidiabetic	PPAR-γ agonists (36)	PIP exhibited its effect by enhancing PPAR-γ gene expression.

Biological activity	Type of biological activity/ disease (reference)	Observed effects
Antidiarrheal	Not informed (40)	PIP showed antidiarrheal and antispasmodic activities, mediated possibly by the blockage of calcium channels.
Spermatogenic	(61)	PIP significantly altered the epididymal sperm count, motility, viability, weight of the epididymis, cauda, corpus, and seminal vesicles.
Neuroprotective	Cerebral ischemic injury (114)	PIP treatment showed a markedly decreased neurological deficit, less ischemia-induced cell damage, as well as smaller areas of cerebral infarction, with less severe macro and microcellular brain structural changes.
Antiviral and bacterial	Vaccine (115)	PIP proved to be effectively aiding the transepithelial passage of vaccine particles.

on cancer cells, showing a dose-dependent suppression of cell proliferation, transducer signals (phosphorylation) and transcriptional activators (p-STAT3). It also induced apoptosis in the inhibition of Bcl-2.

Antidiabetic properties

Concerning the biological potential of PIP, studies have also begun to analyze its mechanism of action in various biological targets. Park *et al*. (35) observed that PIP showed antiadipogenesis activity through the activation of peroxisome proliferator-activated receptors (PPAR-γ), which is also linked to insulin sensitization. Considering these points, Kharbanda *et al*. (36) evaluated and determined the antidiabetic potential of these benzothiazoles using the glucose tolerance test followed by the evaluation of the active derivatives in the diabetic model induced by streptozotocin (45 mg kg^{-1} in healthy male Wistar mice). It was observed that these PIP derivatives showed a significantly higher antidiabetic activity than rosiglitazone (standard drug). They also showed fewer side-effects on body mass, lipid peroxidation and hepatotoxicity.

Antidiarrheal properties

Miyako *et al*. (37) demonstrated that PIP reduced intestinal mobility in guinea-pig ileum, causing fewer diarrheal events. Different mechanisms of action were identified in the literature, involving capsaicin-sensitive neurons (38) or the vanilloid receptor (39). Considering the ambiguous mechanisms reported in the literature, Taqvi *et al*. (40) decided to investigate in more detail the mechanism involved in the antispasmodic and antidiarrheal properties of PIP. This alkaloid showed antidiarrheal and antispasmodic activities, mediated by the blockage of calcium channels, due to sustained contractions induced by high K^+ (80 mmol). PIP inhibited such contractions with an EC_{50} value of 80.86 μmol, which suggested a blocking effect of calcium channels.

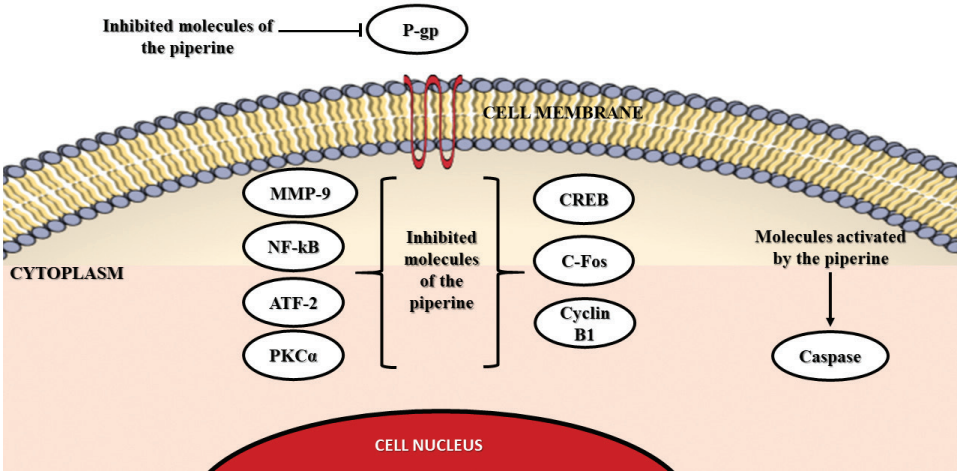

Fig. 7. The schematic diagram of the molecular machinery and possible targets for the antineoplastic properties of piperine. ATF-2 – activated transcription factor 2, CREB – cAMP response element-binding, MMP-9 – matrix metalloproteinase 9, NF-κB – nuclear factor κ-light-chain-enhancer of activated B cells, PKC – protein kinase C.

Antihypertensive properties

Antihypertensive drugs are substances that help reduce blood pressure. Their mechanism of action depends on the treatment – diuretics, ß-adrenergic blockers, calcium channel blockers, angiotensin-converting enzyme blockers, angiotensin receptor blockers and central adrenergic inhibitors (41). Booranasubkajorna *et al*. (42) analyzed the antihypertensive effects of PIP in rats with endothelial dysfunction induced by Nω-nitro-L-arginine methyl ester hydrochloride. The purpose of this study was to investigate the pharmacokinetics, mechanism of action, hemodynamic and vasoactive effects, and toxicity of PIP in rats with spontaneous hypertension (SHR) and normal Wistar rats (NWR). They showed that PIP did not affect blood pressure and heart rate in SHR and NWR. However, PIP increased the vasorelaxation induced by acetylcholine of the thoracic aorta and had a vasculoprotective effect in rats with the deterioration of nitric oxide. Also, they found no liver or kidney toxicities. They concluded that although PIP did not affect blood pressure in SHR and NWR, it was able to relax the isolated thoracic aorta, showing the potential of a vasculoprotective effect in hypertensive conditions.

Antiinflammatory properties

The inflammatory process occurs for different reasons, in response to infection by microorganisms (bacteria, viruses, or fungi), tissue damage, carcinogenesis, tumor growth, or ischemia (43, 44). The innate and adaptive immune systems are involved in the inflammatory process, and the complexity of mediators is involved in the inflammatory response (43–45). This process proves beneficial to the body, but in some cases, it is deleterious and presents uncomfortable symptoms to patients (44). The medicinal use of plants is widespread in a large

Table II. Nanosystems for encapsulation of piperine

Applications of nanotechnology with piperine	Nanosystem	Nanosystem characterization						Biological tests		Reference
		Physical and/or chemical methodology	Active compound	Hydrodynamic diameter or size (nm)	Zeta potential (mV)	PDI	Encapsulation efficiency (%)	*In vitro*/*in vivo*		
Food	Liposomes	Sonication	Piperine	29.75 ± 0.84	–25.2	<0.3	78.6	NI		84
Antibacterial activity	Liposomes	Dehydration-rehydration	Piperine and gentamicin	159.1 ± 1.44	–9.11 ± 1.24	0.31 ± 0.06	66 ± 3	Staphylococcus aureus	NI	116
Anti-cancer	Micelles	Solvent evaporation	Resveratrol piperine	195.00 ± 1.00	–17.99 ± 0.65	NI	76.50 ± 2.01	NI	Wistar albino rats	117
Anti-cancer	Nanofibers	Electrospinning	Piperine	300–400	NI	NI	NI	HeLa and MCF-7	NI	90
Alzheimer's disease	Chitosan nanoparticles	Ionic gelation	Piperine	248.50	+56.30	0.24	81.70	NI	Wistar rats	118
Breast cancers	Polymeric nanoparticle	Emulsification and solvent evaporation	Piperine	132	–18.5 to –4.8	0.1	NI	MCF-7	NI	119
Breast cancer	Polymeric nanoparticles	Nanoprecipitation	Piperine	139.9	NI	0.1	NI	Breast cancer cell line (MDA-MB-231)	NI	120
Bioenhancer	Polymeric nanoparticles	Precipitation	Piperine	132 ± 7.21	–26.5	NI	89.77 ± 1.06	NI	Wistar rats	121

Applications of nanotechnology with piperine	Nanosystem	Nanosystem characterization						Biological tests		Reference
		Physical and/or chemical methodology	Active compound	Hydrodynamic diameter or size (nm)	Zeta potential (mV)	PDI	Encapsulation efficiency (%)	*In vitro*/*in vivo*		
Epilepsy	Chitosan nanoparticles	Ionic gelation	Piperine	5.46–54.6	NI		53	NI	NMRI mice	102
Anti-cancer	Chitosan nanoparticles	Electrospray	Piperine and curcumin	500	NI		87	Neuroblastoma cell line (SH-SY5Y)	NI	93
Bioavailability	Micellar lipid	NI	Piperine and phyllanthin	304.52 ± 5.97	NI				Albino Wistar rats	109
Bioavailability	Solid lipid nanoparticles	Emulsification evaporation-low temperature solidification	Piperine and curcumin	130.8	20	0.152	14.7 ± 0.2	Taxol cells (A2780)	NI	95
Analgesic and anti-inflammatory	Polymeric nanoparticles	Double emulsion (w/o/w)	Piperine	192 ± 46.97	–5.1 ± 8.39	0.028	99	Salmonella	NI	122
Anti-cancer	Micelles	Thin-film hydration method	Piperine and docetaxel	63.73 ± 1.07	–1.26	0.049 ± 0.001	95.8 ± 0.04	HepG2 cell1277	Sprague-Dawley rats	123
Anti-cancer	Lecithin-chitosan nanoparticles	NI	Piperine and doxorubicin	157.67 ± 2.65	28.14 ± 0.84	NI	52.91 ± 3.56	NI	NI	124

Applications of nanotechnology with piperine	Nanosystem	Nanosystem characterization						Biological tests		Reference
		Physical and/or chemical methodology	Active compound	Hydrodynamic diameter or size (nm)	Zeta potential (mV)	PDI	Encapsulation efficiency (%)	*In vitro*/*in vivo*		
Gastrointestinal digestion	Zein-carrageenan core-shell nanoparticles	Constant stirring	Curcumin and piperine	2268 ± 53	−46.0 ± 1.4	NI	NI	NI	NI	125
Epilepsy	Nanoparticles	Nanoprecipitation	Piperine	130.20 ± 1.57	−10.27 ± 0.52	0.195 ± 0.002	92.2 ± 2.5	NI	Sprague–Dawley rats	126
Antileishmanial	Polymeric nanoparticle	Constant stirring	Amphotericin B and piperin	< 200	NI		71 ± 0.6	*L. donovani*	Golden hamsters (inbred strain)	127
Food	Polymeric nanoparticle	Constant stirring	Coenzyme Q10 and piperine	128	–37.9	NI				128
Epilepsy	Polymeric nanoparticle	Emulsification-solvent-evaporation	Piperine	2–5	NI		85.43 ± 5.0	NI	Wistar rats	129
Anti-cancer	Nanoemulsions	Emulsification-solvent-evaporation	Curcumin and piperine	248.76 ± 50.8	20.46 ± 6.85	0.250 ± 0.12	3.6	Human colon carcinoma cell line (HCT116)		130
Anti-cancer	Polymeric nanoparticle	Nanoprecipitation	Curcumin and piperine	81.82	NI	+27	96.2	Caco-2 cells	Albino Balb/c mice	131

NI – not informed

number of cultures, and ethnomedicine is responsible for investigating their efficacy and safety. There are currently several investigations on the anti-inflammatory effects of natural products with PIP being one of the biomolecules investigated for this purpose (43).

The inflammation induced by radiotherapy and the production of reactive oxygen species (ROS) cause adverse effects during the treatment of cancer. Elkady *et al.* (46) applied γ-ray radiation therapy and the administration of PIP in lung tissue. They showed that PIP acted as a potent free radical scavenger, showing a decrease in the activities of pulmonary catalase, glutathione peroxidase, tumor necrosis factor in serum-α, interleukin-1β and interleukin-6 levels, compared to an irradiated control group. Besides, there were minimal injuries with or without a few degenerative changes.

Inflammatory disorders are caused by enzymes such as cyclooxygenase types 1 and 2 (COX-1 and COX-2), lipooxygenase, prostaglandin receptor (DP_1) and prostaglandin D2 receptor (2CRTH2). Zakerali *et al.* (47) selected different spices commonly used in the food industry to observe their ability to inhibit the enzymes that cause inflammatory processes. PIP demonstrated an inhibitory effect of COX-2 and a better affinity value (–7.8 kcal mol^{-1}) and energy binding (–85.08 kcal mol^{-1}) than aspirin and celecoxib. In summary, they suggest incorporating this alkaloid into the daily diet, as it is a potent anti-inflammatory and anticancer agent.

Antioxidant properties

Antioxidant compounds are those capable of preventing the oxidation of other compounds (48). Oxidative stress is defined as an imbalance between oxidative and antioxidant compounds, promoting molecular damage (49) and leading to pathological and aging processes. Several studies were conducted in search of new molecules with antioxidant power for therapeutic and aesthetic purposes. Among the natural antioxidants, flavonoids have been the subject of studies due to their ability to sequester reactive oxygen species and their high natural availability (48). In this sense, it is of interest to evaluate the antioxidant potential of PIP.

Vijayakumar *et al.* (50) analyzed tissue lipid peroxidation using PIP as an enzymatic and non-enzymatic antioxidant in male Wistar mice, fed a high-fat diet for 10 weeks. They concluded that PIP can reduce the oxidative stress induced by the high-fat diet in the cells, showing that it reduced the levels of thiobarbituric acid reactive substances (TBARS), conjugated dienes and glutathione, besides maintaining the levels of catalase, glutathione-*S*--transferase, superoxide dismutase and glutathione peroxidase.

Antiparasitic properties

Parasitic diseases affect people all over the world and cause high morbidity and mortality. Antiparasitic agents are drugs used to treat diseases such as malaria, trichomoniasis, and leishmaniasis. These are caused by protozoa, flat and roundworms, as well as ectoparasites such as ticks, fleas, lice and mites. The ideal antiparasitic drugs must have a broad spectrum of action in all stages of parasite development and must be safe (non-toxic) and low cost (51). These characteristics make PIP a promising candidate for some parasitic diseases. For example, its antiparasitic activity is determined by its capacity to produce nitric oxide (NO), resulting in various activities such as immunomodulatory

effects and cell cycle detection (epimastigote forms of *Trypanosoma cruzi*), and affecting the mitochondria of the parasites at the biochemical and intracellular levels (52). Previous studies have shown that capsaicin has anti-leishmaniasis activity, but it has undesirable side-effects on the body. Therefore, a study by Vieira-Araujo *et al*. (53) combined PIP with capsaicin to improve the activity against *Leishmaniasis infantum* in both the promastigote and the amastigote. They showed a better activity with the half-maximal effective concentration (EC_{50}) of 4.31 ± 0.44 and 7.25 ± 4.84 μg mL^{-1}, resp., and demonstrated that PIP improved the pharmacological action of capsaicin against leishmaniasis.

Kumar *et al*. (54) aimed to evaluate the antiparasitic effect of PIP against *Argulus* spp. – one of the main concerns in aquaculture – in *Carassius auratus*. They obtained a median lethal concentration after 96 h for 52.64 mg L^{-1} of PIP. Besides, the *in vitro* effect led to a 100 % mortality of *Argulus* with a concentration of 9.0 mg L^{-1} after 3 h, while, in an *in vivo* test, the antiparasitic efficacy of 100 % of the PIP solution was found with 9.0 mg L^{-1} after 48 h. The concentration of 9.0 mg L^{-1} can be used as a potential natural agent to control the *Argulus* parasite.

Spermatogenic properties

The infertility of the human population is caused by various chemical, hormonal, and immunological agents. Currently, some secondary metabolites extracted from plants have been examined to increase fertility in men (55–60). Therefore, PIP has been shown to interact with the androgen receptor and androgen binding protein. Based on this knowledge, Chinta *et al*. (61) evaluated the fertility effect of PIP in male albino rats after a 60-day treatment. They showed that this alkaloid increases the epididymal sperm count, such as motility and viability. They also found a decrease in the levels of epididymal acid and antioxidant enzymes (superoxide dismutase and catalase). The results of this study concluded that PIP can be used to increase fertility.

Hepatoprotective properties

Due to the biological importance of liver, research is being conducted to identify ways of reducing hepatotoxicity, whether caused by pathological processes or by extrinsic toxicity. The use of phytochemicals by traditional medicines has also been the object of investigation in ethnomedicine, which yielded potential hepatoprotective findings in different species (62). Given the antioxidant potential of PIP, researchers have explored this property as a possible hepatoprotective agent. Rather *et al*. (63) combined leave extract of *Aegle marmelos* with PIP to improve this effect. They showed that the treatment with *A. marmelos* reduced the severity of the toxicity in a dose-dependent manner, after administration in mice with carbon tetrachloride (CCl_4). However, the low dose of *A. marmelos* extract (25 mg kg^{-1}) did not significantly reverse the hepatotoxicity, but the low dose of *A. marmelos* in combination with PIP showed a significant reversal of hepatotoxicity. They concluded that PIP improves the hepaprotective treatment of *A. marmelos* through its antioxidant and anti-inflammatory properties.

Sabina *et al*. (64) evaluated the potential hepatoprotective property of PIP in mice with acetaminophen-induced hepatotoxicity. They also analyzed hepatic marker enzymes and inflammatory mediators and determined the antioxidant and pre-oxidation status. After

PIP administration, acetaminophen-exposed mice showed a reduction in liver marker enzyme activity and levels of lipid peroxidation, as well as an increase in antioxidant status, suggesting that PIP has a higher potential hepatoprotective property than silymarin, the drug chosen as the standard for the study.

Larvicide properties

Mosquitoes are vectors that can transmit various diseases such as malaria, yellow fever, dengue, chikungunya, among others. In addition to synthetic insecticides such as organochlorine and organophosphorus compounds, currently, some insecticides are specific against this species and can be obtained by extracting phytochemicals from various ornamental plants (65, 66).

PIP is a potential insecticide and larvicide because it acts as a neurotoxin-like agent paralyzing the insects. The advantage of using it as an insecticide is the low toxicity for mammals and the fact that they are not persistent in the environment, degrading quickly under sunlight. In 2016, Samuel *et al*. (67) investigated the larvicidal effects of PIP against *Anopheles arabiensis* larvae from several resistant and susceptible strains and analyzed mortality in two stages: 24 and 48 h after the application of PIP in the larvae. They concluded that the strains were susceptible to PIP.

Neuroprotection, activity against neurodegenerative diseases and other neuro-disorders

Considering the main activities of PIP as an anti-inflammatory and antioxidant agent, several studies have sought to explore such properties and to verify the neuroprotective ability and treatment of the neuropathologies that derive from oxidative and inflammatory processes.

Neuroprotector and Parkinson's disease (PD). – In view of the complexity of PD pathogenesis including oxidative stress, altered mitochondrial metabolism, and neuroinflammation (68), studies were conducted using PIP to investigate its anti-PD potential given the antioxidant and anti-inflammatory properties cited in previous sub-sections.

Shamsher *et al*. (69) combined PIP and curcumin to provide a better neuroprotective effect on behavioral, biochemical, neuroinflammatory, and neurochemical parameters in parkinsonian mice. They used 6-hydroxy dopamine (6-OHDA), a neurotoxin that causes serious motor and cognitive deficits in animals. The results showed that this combination significantly prevented behavioral, neuroinflammatory, and neurochemical changes and preserved the antioxidant potential of nigrostriatal in rats treated with 6-OHDA.

Rinwa *et al*. (70) evaluated the combination of quercetin, a bioflavonoid, and PIP to check the therapeutic potential of this combination against chronic unpredictable stress (CUS), which causes increased neuroinflammation and brain oxidative stress. In this study, these drugs were administered for 30 min daily before the CUS procedure. The results showed that the combination of quercetin and PIP promoted a reduction in the oxidative damage, neuroinflammation, and memory deficits caused by CUS.

Liu *et al*. (71) evaluated the neuroprotective effect of PIP utilizing rotenone-induced neurotoxicity in SK-N-SH cells, in rat primary cortical neurons. The researchers concluded that PIP has a neuroprotective effect through the induction of autophagy. Also, another

study conducted by Wang *et al*. (72) investigated the mechanism by which alkaloids derived from *P. longum* L. promote neuroprotection. They observed an improvement in rotenone-induced motor deficits, as well as in the reduction in ROS production and mitochondrial properties related to inhibition of ROS production. Like in the previous study, it was also possible to identify stimulated autophagy, once again proving the neuroprotective potential of PIP and its derivatives.

Considering the neuroprotective potential of PIP, some studies have evaluated its potential against Parkinson's disease. Bi *et al*. (73) evaluated the activity of *P. longum* L. extract containing alkaloids (PIP and piperlonguminine) administered orally in a mice model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The extract was administered at doses of 30, 60 and 120 mg kg^{-1}, and the metabolism analysis of the dopaminergic neurons was done using UFLC-MS/MS, immunohistochemical assays, Western blotting, and verification of enzyme level oxidants. The researchers observed that PIP and its derivatives have neuroprotective potential and optimization of dopaminergic neurons. Yang *et al*. (74) performed a similar study, administering 10 mg kg^{-1} of PIP in an MPTP model for 15 days. They observed a reduction in the number of hydroxylase cells, oxidative stress, antiapoptotic property and IL-1 beta expression. They concluded, like the previous study, that PIP has a protective effect of dopaminergic neurons through its anti-inflammatory and antioxidant properties.

Anticonvulsant and antiepileptic properties. – Antiepileptic drugs, also called anticonvulsants, are intended to combat or prevent epileptic seizures. In general, anticonvulsants are well absorbed orally, with about 80–100 % of the drug reaching the bloodstream. Anticonvulsants can be divided into five main groups: (*i*) blockers of the sodium channels of repetitive activation, (*ii*) drugs that potentiate the actions of the gamma-aminobutyric acid (GABA) neurotransmitter, (*iii*) modulators of glutamate, another neurotransmitter, *(iv*) calcium channel blockers, (*v*) carbonic anhydrase inhibitors (75, 76). A study undertaken by Mao *et al*. (77) in 2017 evaluated PIP as an anticonvulsant in epileptic mice induced by pilocarpine. The anticonvulsant effects of PIP decreased neuronal inflammation, neuronal oxidative stress in the hippocampus and memory impairment. It also suppressed the increase in caspase-3 and Bax/Bcl-2 expression levels. They concluded that PIP prevented deterioration of memory and was able to reduce epileptic status.

Antidepressant properties. – The studies undertaken by Huang *et al*. (78) evaluated the antidepressant-like potential of the combination of *trans*-resveratrol and PIP. They analyzed behavioral neurochemical and biochemical parameters *in vivo* and demonstrated synergism in the activation of the monoaminergic system in the brain. Li *et al*. (79) studied the combination of ferulic acid (FA) and PIP and evaluated the possible mechanism involved in reducing depression-like behaviors in mice. They observed a synergistic effect of the combined components, with monoaminergic system involvement.

Cognitive benefits and Alzheimer's disease. – PIP has been shown to improve cognitive performance after oral administration for a long period. Considering this benefit and the antioxidant activity, several studies have evaluated its potential use against the cognitive damage caused by Alzheimer's disease. Chonpathompikunlert *et al*. (80) investigated the memory performance and neurodegeneration in *in vivo* models of Alzheimer's disease after the oral administration of PIP. They observed that, at all doses tested, PIP demon-

strated cognitive benefits and reduced neurodegeneration, possibly due to reduced lipid peroxidation and acetylcholinesterase enzyme.

To examine the antioxidant potential as well as the cognitive benefits of PIP, Khalili-Fomeshi *et al*. (81) evaluated the lipid peroxidation and iron reduction in the cerebrospinal fluid and the dementia measure promoted by an experimental Alzheimer's disease model *in vivo*. The authors observed a reduction in oxidative stress as well as cognitive enhancement, after administration of PIP, suggesting that the antioxidant activity of PIP and the preservation of neurons in the hippocampus contributed to this result.

NANOTECHNOLOGICAL APPROACH TO INCREASE PIP SOLUBILITY AND BIOAVAILABILITY

The use of nanosystems has several advantages, such as the improvement in the dissolution rate and the bioavailability of poorly soluble drugs. However, several aspects must be considered for their application in the body, such as size and surface charge of the nanoparticle. Concerning the size, it is known that these nanosystems must be in the range of less than 200 nm to avoid the pre-systemic metabolism and to remain in the bloodstream for a long period. Besides, the charge of the nanosystem can influence efficiency. Positive charges are prone to be localized in the lungs, liver and spleen, while neutral and negative nanoparticles have a longer circulation life and less accumulation in organs (82).

Considering these parameters and the properties of PIP, to date, different types of nanosystems have been used to improve its bioavailability and increase its poor aqueous solubility, with polymer nanoparticles and liposomes being the most widely reported in the literature. The summary in Table II shows the nanosystems containing PIP developed in the last years, with their respective physical and chemical characteristics as well as some methods of their synthesis.

Types of encapsulated nanostructures with piperine

Liposomes. – Liposomes are structures formed by bilayers of phospholipids that can incorporate hydrophilic or hydrophobic molecules. They have several promising applications in the food industry because they can improve the bioavailability, stability and shelf-life of some biomolecules (83). Dutta *et al*. (84) in 2017 encapsulated PIP in liposomes developed with phosphatidylcholine to use this alkaloid as a nutraceutical. It showed an encapsulation efficiency of 78.6 %, in addition to a size of 29.75 ± 0.84 nm. The *in vitro* studies indicated higher antioxidant potency (1.1 times) and better storage stability (2.4 times higher at 4 ± 1 °C and 7.8 times higher at 70 ± 2 °C) when compared with pure PIP.

Micelles. – Polymeric micelles are core-shell structures formed by amphiphilic copolymers, used for effectively encapsulating hydrophobic and hydrophilic medications. Therefore, they are considered one of the most promising drug delivery systems to improve the solubility of highly hydrophobic molecules such as PIP (85, 86). Ding *et al*. (87) developed mixed micelles based on polyethylene glycol succinate (Soluplus®) and D-α-tocopherol (TPGS) to improve the solubility and anticarcinogenic effect of PIP. The obtained nanostructures showed an approximate diameter of 61.9 nm, the zeta potential of –1.16 ± 1.06 mV with 90.9 % of encapsulation efficiency and 4.67 % of loading efficiency of the PIP. Be-

sides, the pharmacokinetic study revealed that the area under the curve of piperine-loaded mixed micelles was 2.56 times higher than that of PIP and the mean residence time was 1.2-fold higher than PIP. In conclusion, these micelles could be a potential nanosystem for the administration of PIP for cancer chemotherapy.

Metallic nanoparticles. – Gold nanoparticles (AuNPs) have a great surface-to-volume ratio, excellent biocompatibility and low toxicity. They are a promising factor for new nanosystems, such as the functionalization of the surface with organic molecules: oligonucleotides, antibodies, proteins and drugs (88). Besides, one of the medical problems in the use of insulin is the formation of fibrils. To overcome this problem, Anand *et al*. (89) developed a nanostructure to inhibit fibril aggregation. They synthesized thermostable gold nanoparticles of uniform size encapsulating PIP (PIP AuNPs) and used this alkaloid to functionalize the surface with PIP and attack insulin residues prone to amyloid. The obtained results revealed the union of PIP with the AuNPs and the insulin. The hemolysis tests confirmed that these nanoparticles were heme group compatible. In experimental and computational studies, they concluded that this nanosystem may retain the native structure of insulin and the ability of PIP to interact with the aggregation-prone residues of insulin that are key factors for the inhibition mechanism.

Nanofibers. – The synthesis of polymeric nanofibers has a characteristic high surface-to-volume ratio and a high load capacity for various types of drugs. Jain *et al*. (90) designed a biodegradable polymeric system to release PIP for cancer treatment. The nanofibers were prepared with poly (ε-caprolactone) and gelatin by electrospinning, yielding a diameter from 300 to 400 nm. They confirmed the presence of the drug in the nanofiber mats by infrared Fourier transform spectroscopy. Besides, *in vitro* studies using HeLa and MCF-7 cancer cells determined an anticancer activity. Also, flow cytometry revealed that PIP induced the generation of reactive oxygen species (ROS) and the arrest of the cell cycle in the G2/M phase, which led to the death of cancer cells.

Polymeric nanoparticles. – Chitosan is a polymer, amino-polysaccharide, which is biocompatible and biodegradable. Another important feature is its positive charge, which makes it easy to interact with cell surfaces containing the negative charge. Therefore, it has a very promising ability to enhance drug absorption and release (91, 92). Baspinar *et al*. (93) developed zein-chitosan nanoparticles with PIP and curcumin. The authors obtained nanoparticles with an average size of 500 nm and encapsulation efficiencies of 87 % of PIP. Also, *in vitro* studies showed a reduction in the viability of approximately 50 % of the neuroblastoma cells.

Solid-lipid nanoparticles. – Solid-lipid nanoparticles (SLN) are nanosystems with great hydrophilic and hydrophobic drug loading capacity. Due to a large number of available routes of administration, these nanoparticles function differently depending on the type of formulation and the route of administration. They are formed by exchanging the liquid lipid of the emulsions for a solid lipid, the latter being solid both at room temperature and body temperature (94). Tang *et al*. (95) designed an SLN to incorporate curcumin and PIP that could sensitize MDR (multidrug resistance) tumors by inhibiting the P-gp. As a result, they showed a significant increase in the cytotoxicity and efficacy of the drugs in drug-resistant A2780/taxol cells, thus initiating a new strategy to increase the clinical management of MDR in cancer.

CONCLUSIONS

This review provides updated information on the synthesis, metabolism and uses of PIP in biomedicine. Besides, it discusses PIP's pharmacological potential in antioxidant, anticancer, anti-inflammatory activities, among others, as revealed by *in vitro* and *in vivo* studies. However, more studies are still needed on the pharmacological mechanism in most of its activities. Besides, the use of nanostructures with PIP has improved the therapeutic efficacy with a greater aqueous phase solubility and bioavailability in the target tissue. Yet, comprehensive studies are needed in the future to prepare various nanosystems to address the problem of poor bioavailability and thus improve various therapeutic PIP treatments.

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Abbreviations, acronyms, symbols. – AAH – aryl hydrocarbon hydroxylase, ATF-2 – activated transcription factor 2, ATP – adenosine triphosphate, AuNPs – gold nanoparticles, Caco-2 – human colon carcinoma cell line, CDK – cyclin-dependent kinases, CDKi – CDK inhibitors, COX – cyclooxygenase, CREB – cAMP response element-binding, 2CRTH2 – prostaglandin D2 receptor, CUS – chronic unpredictable stress, DP_1 – prostaglandin receptor, EC_{50} – half maximal effective concentration, GABA – gamma-aminobutyric acid, Il – interleukin, IFN – interferon, iNOS – inducible nitric oxide synthase, MDR – multidrug resistance, MMc – MitoMycin-c, MMP-9 – matrix metalloproteinase 9, MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, NADPH – nicotinamide adenine dinucleotide phosphate, NF-κB – nuclear factor κ-light-chain-enhancer of activated B cells, NWR – normal Wistar rats, 6-OHD – 6-hydroxy dopamine, P-gp – glycoprotein P, PIP – piperine, piperonyl-CoA – piperic acid-coenzyme A ester, PLP – pyridoxal phosphate, PPAR-γ – peroxisome proliferator-activated receptors, SHR – spontaneous hypertension, SLN – solid-lipid nanoparticle, Soluplus® – polyethylene glycol succinate, TBARS – thiobarbituric acid reactive substances, TNF – tumor necrosis factor, TPGS – D-α-tocopherol, UDP-GDH – uridine diphosphate glucose dehydrogenase.

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