

# Research progress on evodiamine, a bioactive alkaloid of *Evodiae fructus*: Focus on its anti-cancer activity and bioavailability (Review)

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**Abstract.** *Evodiae fructus* (Wu-Zhu-Yu in Chinese) can be isolated from the dried, unripe fruits of *Tetradium ruticarpum* and is a well-known traditional Chinese medicine that is applied extensively in China, Japan and Korea. *Evodiae fructus* has been traditionally used to treat headaches, abdominal pain and menorrhagia. In addition, it is widely used as a dietary supplement to provide carboxylic acids, essential oils and flavonoids. Evodiamine (EVO) is one of the major bioactive components contained within *Evodiae fructus* and is considered to be a potential candidate anti-cancer agent. EVO has been reported to exert anti-cancer effects by inhibiting cell proliferation, invasion and metastasis, whilst inducing apoptosis in numerous types of cancer cells. However, EVO is susceptible to metabolism and may inhibit the activities of metabolizing enzymes, such as cytochrome P450. Clinical application of EVO in the treatment of cancers may prove difficult due to poor bioavailability and potential toxicity due to metabolism. Currently, novel drug carriers involving the use of solid dispersion techniques, phospholipids and nano-complexes to deliver EVO to improve its bioavailability and mitigate side effects have been tested. The present review aims to summarize the reported anti-cancer effects of EVO whilst discussing the pharmacokinetic behaviors, characteristics and effective delivery systems of EVO.

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## 1. Introduction

*Evodiae fructus* (Wu-Zhu-Yu in Chinese) is the dried, unripe fruit of *Tetradium ruticarpum*, also known as *Euodia rutaecarpa* (1). *Evodiae fructus* is traditionally used as a medicinal herb in China, Japan and Korea (2). *Evodiae fructus* combined with *Coptidis rhizome* at a ratio of 6:1 (w/w) forms the well-known Chinese medicinal formula, Zuo-Jin-Wan, which is commonly used to treat different types of cancer including gastric and multidrug-resistant colorectal cancer cells (3-7). The anti-tumor effects of *Evodiae fructus* has been reported by a number of pharmacological studies (8,9), where in one such study treatment with a 70% ethanol extract of *Evodiae fructus* inhibited the proliferation of prostatic hyperplasia-1 epithelial cells (9). *Evodiae fructus* is widely used as a dietary supplement to provide carboxylic acids, essential oils, flavonoids (10) and has been recorded in multiple versions of the *Chinese Pharmacopoeia* for its wide range of pharmacological activities (11).

Evodiamine (EVO; Fig. 1) is an alkaloid with a quinazolinocarbolone skeleton (12). It is one of the major bioactive components that can be isolated from *Evodiae fructus* and has been reported to exert several pharmacological properties (13-17), including its ability to treat cancer, autoimmune and inflammatory disorders. In particular, the anti-tumor capacity of EVO has attracted the interest of researchers. EVO acts as a dual catalytic inhibitor of the nuclear enzyme topoisomerases I and II, which are vital anti-cancer drug targets (18). In addition, accumulating evidence suggests that EVO exhibits potential anti-cancer activities both *in vivo* and *in vitro* (19-21).

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EVO is considered to be a novel class of multi-target compounds that can be used to treat different types of cancer (16). In addition to topoisomerases, transient receptor potential cation channel subfamily V member 1 (TRPV1) and the aryl hydrocarbon receptor (AhR) are considered direct protein targets of EVO (16). TRPV1 has been linked to processes mediating inflammation, cancer and cardiovascular and other disease (22,23). AhR serves a functional role in physiology and toxicology, especially in cell proliferation and differentiation, the adaptive and toxic responses and immunomodulation (24,25). However, the potential application of EVO in the clinic is hindered by its poor bioavailability due to limited absorption (26). Furthermore, similar to several anticancer drugs, such as doxorubicin, erlotinib, sunitinib and sorafenib, that can induce cardiotoxicity (27-29), EVO has been reported to induce cardiovascular side effects, including oxidative stress, both *in vitro* and *in vivo* (30). The cardiotoxicity of EVO has been investigated on primary cultured neonatal rat cardiomyocytes *in vitro*, and on zebrafish *in vivo*; EVO induced cardiac malfunction, which was evidenced by a decrease in the heart rate, as well as in circulation and pericardial malformations (30). In addition to cardiotoxicity, the issue of hepatotoxicity has also been raised with regards to the application and safety of EVO. It has been reported that the alkaloid-rich extract of *Evodiae fructus* is likely to cause hepatic injury in mice (31). As a major alkaloid compound contained within *Evodiae fructus*, hepatotoxicity mediated by EVO is induced by enhancing the activity of aspartate aminotransferase, alanine transaminase, lactate dehydrogenase and alkaline phosphatase (32).

Drug metabolism serves crucial roles in the bioavailability and subsequent pharmacological activities of poorly soluble, naturally occurring therapeutic agents, which significantly impacts their toxicity profile and side effects (33). Previous studies have reported that the toxicity of EVO may be associated with its mechanism of metabolism *in vivo* and pharmacokinetics (34,35). EVO is readily susceptible to metabolism, P450 enzymes-mediated dehydrogenation reactions may cause toxicity via formation of electrophilic intermediates, then produce glutathione (GSH)-conjugated metabolites to exert more potent cytotoxic effects than EVO itself (34). In addition, 10-hydroxyevodiamine and 11-hydroxyevodiamine are two primary toxic metabolites of EVO (36). Therefore, there is a demand to identify suitable EVO delivery systems for enhancing its bioavailability and bioactivity, in addition to alleviating the toxicity of EVO by regulating its metabolism and pharmacokinetics. The present review aims to summarize the potential mechanism of EVO in cancer therapeutics and discuss its pharmacokinetic characteristics. Finally, strategies designed to improve its oral bioavailability and alleviating its side effects are also mentioned.

## 2. Anticancer activity

Accumulating evidence suggests that EVO can exert anticancer effects both *in vitro* and *in vivo*. Numerous types of cancer cell lines derived from different organs have been reported to have their physiological characteristics inhibited by EVO (Fig. 2; Table I). As shown in Table I, the antitumor effects of EVO have been reported mainly in colon cancer, lung

cancer, hepatocellular carcinoma and melanoma, suggesting that EVO may exert more favorable effects in the treatment of these tumors. EVO can exert a range of different physiological mechanisms, including inhibition of proliferation, induction of apoptosis, reduction of migration/invasion and inhibition of metastasis (37). The alkyl group on position N-14 and the configuration of hydrogen at position C-13b of EVO has been reported to mediate its antitumor effects (38,39).

**Induction of apoptosis.** Apoptosis is a programmed mechanism of cell death that can eliminate foreign or malignant cells to protect organisms against cancer, in addition to being important for normal development (40). EVO is a potent inducer of apoptosis in human non-small-cell lung cancer A549 cells (41), where EVO-induced apoptosis has been observed to occur downstream of mitotic arrest and subsequent mitotic slippage (42). The potential underlying mechanism of EVO on the induction of tumor cell apoptosis is presented in Fig. 3. EVO has been reported to induce apoptosis through both caspase-dependent and caspase-independent pathways (43). Apoptotic induction by EVO in human cervical cancer cells can be completely blocked by Pan-caspase inhibitors, suggesting that EVO induces cell apoptosis via the mitochondrial caspase-dependent pathway (44). In another study, EVO-induced apoptosis in human melanoma cells was not completely reversed by caspase inhibitor z-VAD-fmk (45). EVO has also been found to induce apoptosis in gastric cancer cells by inhibiting the mTOR signaling pathway, both in the presence and absence of z-VAD-fmk (46), suggesting that the caspase-independent cell death pathway is also involved in the proapoptotic mechanism of EVO. EVO can decrease the activity of the apoptosis suppressor Bcl-2 whilst increasing that of the apoptosis inducer Bax in tumor cells (47,48), thereby activating initiator caspases (caspase-8 and 9) and the effector caspase (caspase-3) (49,50). Activation of the caspase-independent apoptotic pathway has been observed to be mediated by translocation of the apoptosis-inducing factor into the nuclei of human leukemia cells pretreated with EVO (43). Taken together, these findings suggest the role of the caspase-independent pathway in EVO against certain types of cancer cell line. Recently, EVO has been reported to induce human cholangiocarcinoma cell apoptosis by blocking the STAT3 signaling pathway, which is mediated by upregulating shatterproof 2 expression (51). In addition, generation of reactive oxygen species, nitric oxide (52,53) and inactivation of the PI3K/AKT (19,47,54-57) or PI3K/Protein Kinase C (PKC) (58) signaling pathways have been reported to serve promoting roles in EVO-induced apoptosis.

**Inhibition of proliferation and cell cycle progression.** EVO exerts potent antiproliferative effects on the cervical cancer cell line HeLa at the same concentration as those mediated by 2,4-dihydroxy-5-fluoropyrimidine (44). The reported underlying mechanism of action of EVO on the inhibition of proliferation and the cell cycle progression is summarized in Fig. 4. EVO suppresses proliferation by cell cycle arrest at the G<sub>2</sub>/M phase (50,59-62). EVO can block the cell cycle at the G<sub>2</sub>/M phase of human gastric adenocarcinoma SGC-7901 and breast cancer NCI/ADR-RES cells in a dose-dependent

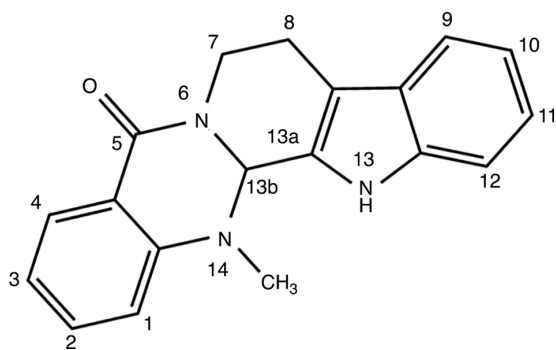


Figure 1. Structure of evodiamine.

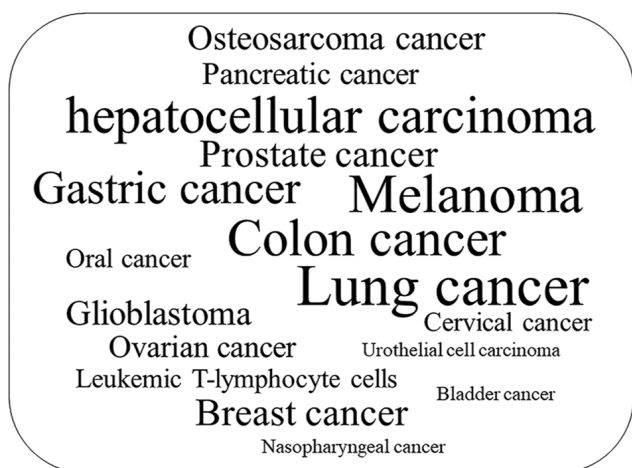


Figure 2. Different types of cancer cells that have been reported to be inhibited by evodiamine. These tumor cells are referred to as a 'word cloud', where the size of each name is proportional to the number of publications that reported inhibitory effects in their respective the cancer type (9,25-100).

manner, with prominent arrest at both the sub- $G_1$  and  $G_2/M$  phases observed in NCI/ADR-RES cells with longer incubation times (62,63). The phosphorylation of Cdc2 occurs on three regulatory sites: Threonine 14 (Thr14), tyrosine 15 (Try15, the inactive form) and threonine 161 (Thr161, the active form) (64). Phosphorylation of Cdc2 on Thr161 results from the concurrent inhibition of Wee-1 and Myt-1 and activation of Cdc25C phosphatase, leading to activation of Cdc2/cyclin B complex (60,65). The  $G_2/M$  arrest is accompanied by an increase in the protein expression of cyclin B1 and phosphorylated form of Cdc2 (Thr161) (60). EVO has been shown to arrest cell cycle at the  $G_2/M$  phase in prostate cancer cells by significantly increasing the protein expression levels of activated Cdc2 (Thr161) and cyclin B1 whilst diminishing the activity of Myt-1 and unphosphorylated Cdc25C (59-61). The inhibitory effect of EVO on the proliferation of colon cancer LoVo cells occurred by S-phase arrest by decreasing the protein expression levels of cyclin A and Cdc25C (49). In addition, the peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) signaling pathway may be involved in the EVO-induced inhibition of the proliferation of leukemia cells. EVO exerts an inhibitory effect on proliferation of leukemia cells by enhancing expression of PPAR $\gamma$  via stimulating p21 and inhibiting cyclin D1 (66).

**Decreased invasion and metastasis.** Metastasis is a major cause of cancer-associated mortality, where EVO may be a promising candidate for antitumor therapy by inhibiting metastasis (38). The roles of EVO in suppressing the invasion and metastasis of cancer cells are presented in Fig. 5. Hepatocyte growth factor (HGF) can promote the invasion and migration of different types of tumor cells (67). EVO has been reported to reverse HGF-stimulated invasion of colon 26-L5 carcinoma, B16-F10 melanoma and Lewis lung carcinoma cells in a dose-dependent manner, whereby 100% inhibition of HGF activity was achieved using 30  $\mu$ M EVO (68). Inhibition of MMP-2 expression and attenuation of ERK1/2 activation have also been documented to contribute to the anti-metastasis and anti-invasion effects of EVO on tumor cells (69). Previous studies found that EVO can inhibit the migration and invasion of colorectal cancer cells both *in vitro* and *in vivo* by decreasing MMP-9 expression and suppressing NF- $\kappa$ B/p65 nuclear translocation and acetylation (69,70).

**Other effects.** Autophagy serves a synchronized role with apoptosis in the cytotoxic activities mediated by EVO (63). Blockade of angiogenesis also contributes to the anticancer activity of EVO (71,72). EVO exerts antitumor effects by inhibiting the activation of NF- $\kappa$ B and therefore inhibits the transcription of NF- $\kappa$ B-regulated gene products, including those that can mediate proliferation (cyclin D1 and c-Myc), inhibit apoptosis (survivin and TNF receptor associated factor 1), immunomodulation (chemokines and IL) and metastasis (intracellular adhesion molecule-1 and MMP-9) (73). Previously, EVO has been reported to promote hepatocellular carcinoma cell death by downregulating hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) expression (74). In addition, bone morphogenetic protein 9 has been found to mediate the anticancer effects of EVO by upregulation of HIF-1 $\alpha$ /p53 in colon cancer cells (75). Suppressing the Wnt and Notch signaling pathways, which serve crucial roles in cancer stem cell signaling, may also be involved in the anticancer activity of EVO (76-79).

### 3. Pharmacokinetic characteristics

**Absorption and distribution.** EVO is highly soluble in acetone, but barely soluble in ether or diluted alcohol and is insoluble in water, benzene or chloroform (80). Poor solubility and absorption means that EVO has low bioavailability (81). Shyr *et al* (26) reported that the bioavailability of EVO was only 0.1%, with a maximum plasma concentration ( $C_{max}$ ) of 49.0  $\pm$  19.0 ng/ml following oral administration in rats (500 mg/kg) (26). A pharmacokinetic study of EVO previously performed in rabbits demonstrated that 5 min after intravenous administration of 4 mg/kg EVO, the plasma concentration of EVO was 877.0  $\pm$  96.6 ng/ml (82). Pharmacokinetic parameters simulated in beagle dog models demonstrated that the area under the curve (AUC)<sub>0-24 h</sub> and the  $C_{max}$  of EVO were 45.85  $\pm$  29.17 ng h/ml and 30.94  $\pm$  12.16 ng/ml, respectively, after administration with capsules encapsulated with 10 mg/kg EVO (83). The pharmacokinetics of EVO were also assessed following the oral administration of [ $^3$ H] EVO in rats (84). The radioactivity levels in the plasma reached their maximum level within 1 h of oral administration, which declined in a biphasic manner, with half-times of 1.6 and 78.4 h (84). EVO

Table I. Reported anti-cancer activities of evodiamine.

System involved	Cancer cells	(Refs.)
Digestive system	Colon cell lines (Iovo cells, colon cancer stem cell, HCT116 cells, colon 26-L5 cells, colon COLO205 and HT-29 cells)	(19,39,49,75,76, 111-113)
	Gastric cancer cells (SGC7901 cells, BGC-823 cells)	(46,50,63,77,114-116)
	Oral cancer cells (MC3 cells, HSC4 cells, CAL-27 cell line)	(117,118)
	Pancreatic cancer cells (SW1990 cells, PANC-1 and SW1990 PC cell lines)	(54,55,119)
	Hepatocellular carcinoma cells (HepG2, Bel-7402, QGY-7701 cells, Hepa1-6 cells, SMMC-7721)	(21,47,48,71,74, 120-122)
Respiratory system	Lung cancer cell lines (Lewis lung carcinoma, NCI-H446 and NCI-H1688 cell lines, A549 cells, H1299 cells)	(38,41,78,79,122-129)
	Nasopharyngeal carcinoma cells (HONE1 and CNE1 cells)	(69)
Circulatory system	Leukemic T-lymphocyte cells (CCRF-CEM cells, U937 cells, K562 cells, THP-1 cells)	(43,66,130,131)
Nervous system	Glioblastoma cells (U87 cells, C6 glioma cells)	(56,132-134)
Motor system	Osteosarcoma cells (143B cells, MG63 Cells)	(135-138)
Urinary system	Prostate cancer cells (BPH-1 cells, PC-3 cells, DU145 cells, LNCaP cells)	(9,59-61,139)
	Bladder cancer cells (253J cells and T24 cells)	(140)
Genital system	Urothelial cell carcinoma	(141)
	Breast cancer cells (NCI/ADR-RES cells, MCF-7 cells and ADR cells, MDA-MB-231 cells)	(62,107,142-144)
	Cervical cancer cells (HeLa cells)	(44,45)
Others	Ovarian cancer cells (A2780 cell lines, ES-2 cells, SKOV-3 cells)	(20,145,146)
	Melanoma cells (A375-S2 cells)	(45,52,57,58,147-150)

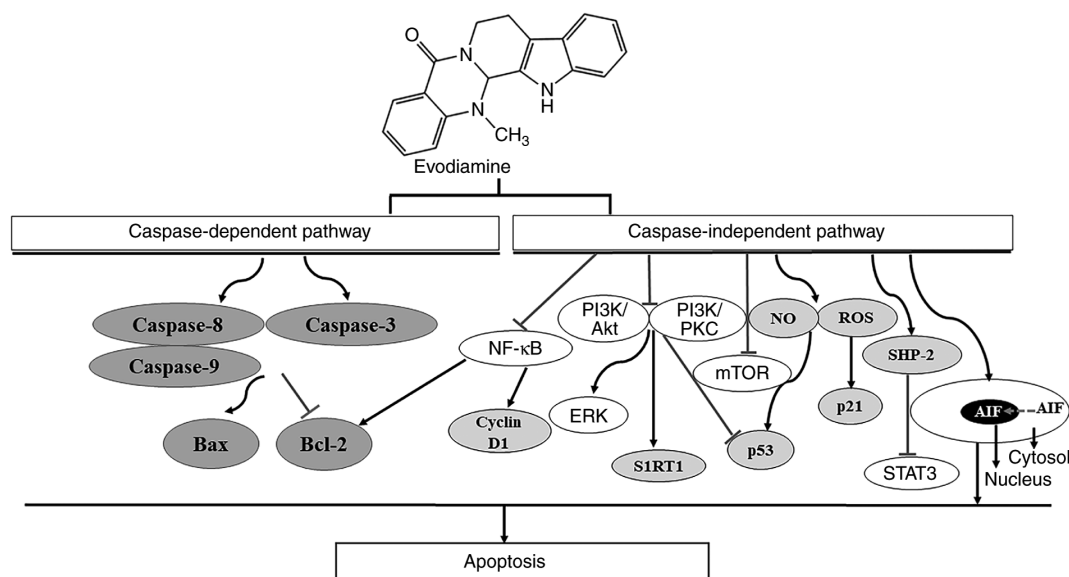


Figure 3. Potential underlying mechanism of EVO on the induction of tumor cell apoptosis. EVO can induce apoptosis through caspase-dependent pathways by activating either caspase-8, 9 or 3, which decreases the expression of apoptosis suppressor Bcl-2 in tumor cells, whilst increasing the expression of the apoptosis inducer Bax. Activation of the caspase-independent apoptotic pathway is mediated by translocation of apoptosis-inducing factor into the nucleus. EVO exhibits apoptotic activity by inhibiting NF-κB activation, which inhibits the expression of downstream gene products cyclin D1 and Bcl-2. In addition, inactivation of the PI3K/AKT or PI3K/PKC signaling pathways decreases expression of the anti-apoptotic proteins SIRT1 and ERKs whilst increasing p53 expression. Generation of reactive oxygen species and nitric oxide also potentiates the function of p53 and p21, which can in turn mediate EVO-induced apoptosis. Arrows represent stimulatory effects, whilst dotted arrows represent translocation and T-lines represent inhibitory effects. EVO, evodiamine; PKC, protein kinase C; SIRT1, sirtuin 1.

has also been reported to reach a maximum plasma concentration in rat 3.38 h ( $T_{max}$ ) after oral administration (35). EVO is

predominantly distributed in the liver, kidney, heart and lungs, where 19 and 63% of orally administered EVO is eliminated



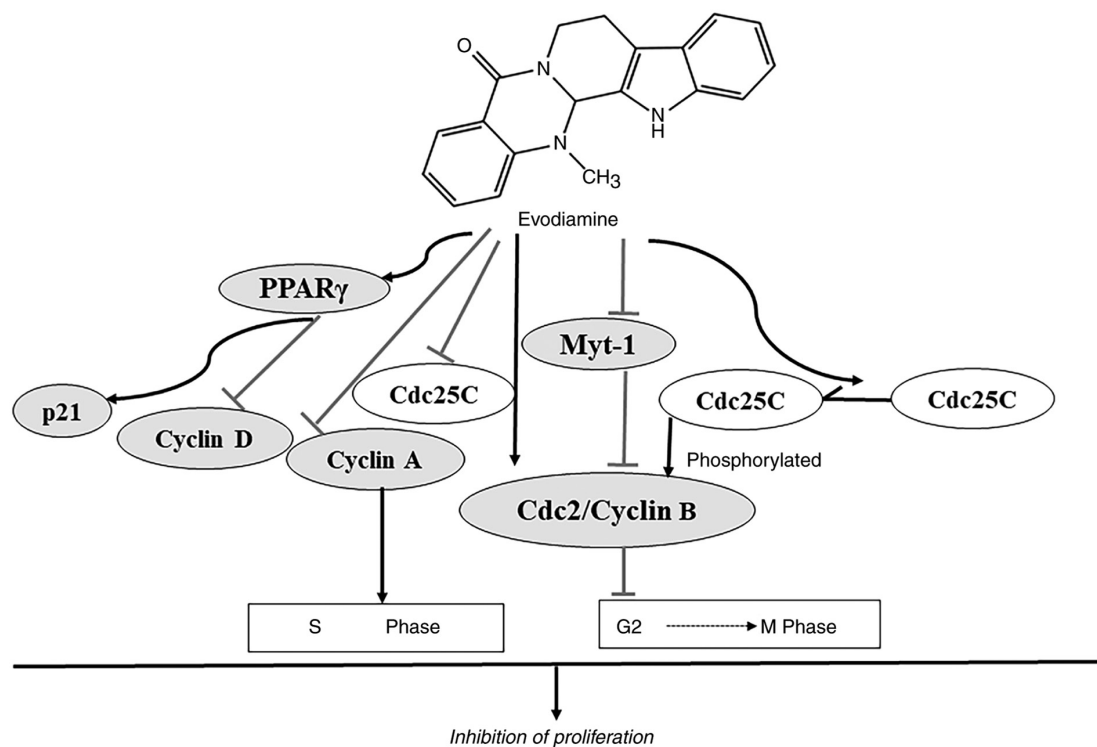


Figure 4. Potential underlying mechanism of EVO on the inhibition of proliferation and cell cycle progression. EVO suppresses cell proliferation by blocking cell cycle progression at G<sub>2</sub>/M phase by concurrently inhibiting Myt-1 and activating Cdc25C phosphatase, which significantly increases the activation of the Cdc2/cyclin B complex. In addition, EVO downregulates cyclin A expression, which induces S-phase arrest in cancer cells. EVO can also inhibit cancer cell proliferation by activating the PPAR $\gamma$  signaling pathway by downregulating cyclin D1 whilst upregulating the expression of the cyclin-dependent kinase inhibitor p21. Arrows represent stimulatory effects and T-lines represent inhibitory effects. EVO, evodiamine; Cdc, cell division cycle; PPAR $\gamma$ , peroxisome proliferators-activated receptor  $\gamma$ ; myt-1, membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase.

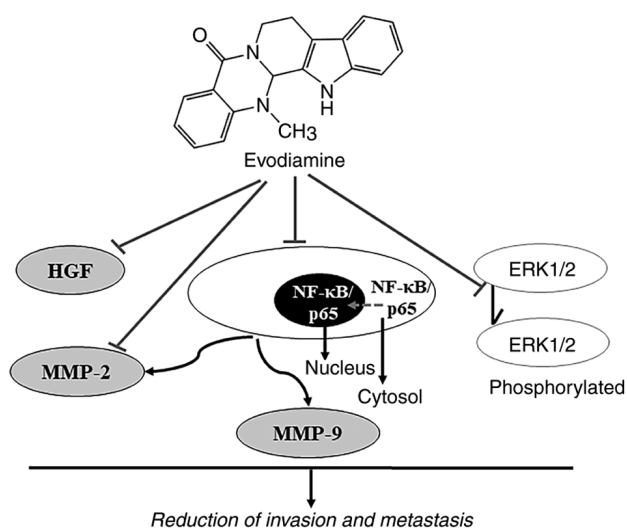


Figure 5. Key roles of EVO in inhibiting the invasion and metastasis of cancer cells. EVO inhibits the invasion and metastasis of tumor cells by suppressing HGF activity, suppressing MMP-2 expression or attenuating the activation of ERK1/2. Furthermore, EVO inhibits the migration and invasion of cancer cells by decreasing MMP-9 expression by suppressing NF- $\kappa$ B/p65 nuclear translocation and NF- $\kappa$ B/p65 acetylation. Arrows represent stimulatory effects; dotted arrows indicates translocation; half arrows represent phosphorylation and T-lines indicate inhibitory effects. EVO, evodiamine; HGF, hepatocyte growth factor.

in the urine and bile after 24 h, respectively (84). The pharmacokinetic process following the intravenous administration of

EVO was characterized by a two-compartment model, which appears to be a biphasic phenomenon with a rapid distribution phase followed by a slower elimination phase (85). The absorption of EVO by rats with headaches induced by Nitroglycerin was significantly higher compared with that in the healthy group (86). Compared with the crude drug, the Wu-Zhu-Yu extract appeared to increase the bioavailability of EVO, with increased purities (16-80%), suggesting that some ingredients in the Wu-Zhu-Yu extract may promote the efficacy of EVO *in vivo* (81). At present, the low bioavailability of EVO has been confirmed in different animal models. These differences in the pharmacokinetic parameters may be due to the diversity of measurements and estimation methods. However, further studies are required to understand the distribution in the body and excretion of EVO.

**Metabolism.** Komatsu *et al* (84) demonstrated that [<sup>3</sup>H] EVO can be converted into metabolites in rats. A previous study demonstrated that EVO is readily susceptible to metabolism, forming hydroxylation metabolites, which exhibit potent cytotoxicity compared with that mediated by EVO (35). Furthermore, another study suggested that the 3-alkylindole moiety on EVO is a potential toxicophore in P450-mediated dehydrogenation reactions (34). Therefore, the metabolic bioactivation of EVO may be closely associated with its pharmacological effects and toxicity (35).

Aromatic, aliphatic hydroxylation, N-demethylation, oxygenation, dehydrogenation, glucuronidation and GSH conjugation are involved in the metabolic pathways of EVO,

where a methyl group in position 14 of EVO may contribute to its metabolic properties (87,88). *In vitro* incubation of EVO with human and rat liver microsomes, in the presence of NADPH, resulted in the formation of four mono-hydroxylated metabolites and one N-demethylated metabolite (87). Cytochrome (CYP)3A4, CYP2C9 and CYP1A2 have been identified to be the main CYP isoforms involved in the metabolism of EVO in human liver microsomes (87). Several GSH conjugates of EVO were also found after the addition of sulfhydryl nucleophiles to EVO (34). Recently, a total of 12 phase I metabolites of EVO were found in human liver microsomes, whilst 12 phase I metabolites and seven phase II conjugated metabolites were identified and quantified in human hepatocytes (88). *In vivo* experiments demonstrated that the metabolites of EVO, 10-hydroxyevodiamine and 18-hydroxyevodiamine, were rapidly converted from EVO within 0.167 h after oral administration in rats, which further conjugated with glucuronide to form 10-hydroxyevodiamine-glucuronide and 18-hydroxy-evodiamine-glucuronide (35). A cocktail method was used to investigate the metabolism of EVO involving the P450 family of metabolizing enzymes (89). The results demonstrated that EVO inhibited the activities of the metabolizing enzymes, CYP1A2, CYP2C9 and CYP2D6, which increases the half-life ( $t_{1/2}$ ),  $C_{max}$  and  $AUC_{(0-\infty)}$ , whilst decreasing the clearance of the corresponding enzymatic substrates (89).

#### 4. Approaches to improve the oral bioavailability

The metabolism of low-solubility natural medicines partly depend on drug-drug interactions and the delivery systems (33). Appropriate drug delivery systems can contribute to increased absorption, improved bioavailability, prolonged residence time and minimized side effects of low-solubility natural medicines (33).

The solid dispersion technique has been used to enhance the dissolution rate and solubility of EVO. A previous study demonstrated that solid dispersion of EVO in hard capsules has a greater absorption rate compared with that of enriched samples of EVO in physical hard capsules (90). Following oral administration of EVO (57.5 mg), the  $C_{max}$  of solid dispersion ( $27.85 \pm 13.78$  mg/l) was notably higher compared with that of physical mixtures ( $10.48 \pm 7.28$  mg/l). Notably, solid dispersion also contributed to an advanced  $T_{max}$  and a shorter  $T_{1/2}$  of EVO in beagle dogs (90). Phospholipids also possess the potential for improving oral bioavailability and biological efficacy of drugs with low aqueous solubility, by forming noncovalently bonded drug-phospholipid complexes (91,92). Tan *et al* (91) previously designed a novel EVO-phospholipid complex (EPLC), with a higher bioavailability than free EVO. Compared with free EVO, the relative bioavailability of EPLC was significantly increased to 218.82% (91).

Cytotoxic drug-carrying nano-carriers are a viable strategy for enhancing cancer cell cytotoxicity and minimizing side effects. Nano-emulsion, which includes lipid nano-emulsion, inclusion complexes, nanoparticles and niosomes, is a vital component of nano-carriers (33). A novel type of water-in-oil nano-emulsion containing EVO-phospholipid nanocomplexes (NEEPN) was considered to be a good carrier for the oral delivery of EVO due to its favorable *in vivo* kinetic characteristics in rat (93). It markedly enhanced the oral bioavailability of

EVO to 630.35% by increasing gastrointestinal absorption and the effective permeability of NEEPN in the colon was increased 8.64-fold compared with free EVO (93). Woody oil-based emulsive nanosystem was also used to increase the sensitivity of lung cancer cells to EVO (93,94). In addition, the bioavailability of EVO was increases following formation of inclusion complexes (95,96). EVO hydroxypropyl- $\beta$ -cyclodextrin complexes have been reported to improve the oral bioavailability of EVO by 2.56-fold, increase the  $C_{max}$  and extend the  $T_{max}$  by 1.57- and 1.01-folds, respectively (95).

Recently, mesoporous silica nanoparticles (MSNs) have become an attractive type of carrier for hydrophobic and hydrophilic agents due to their site-specific functionalization prominent biocompatibility and large loading surface areas (97,98). EVO is loaded with berberine using a novel temperature- and pH-responsive dual drug delivery platform-coated MSN to improve efficacy and biocompatibility in the low pH and high temperature microenvironment of the tumor (99). This MSN-loaded drug pair (EVO and berberine) not only possessed optimal synergistic therapeutic effects *in vitro* (cytotoxicity, cell migration, invasion and angiogenesis) and *in vivo* (growth of tumor grafts in mice), but also exhibited low systemic toxicity (99). In another study, a delivery system based on poly (lactic-co-glycolic acid) nanoparticles was established to deliver EVO to overcome its drawbacks of limited solubility and low bioavailability (100). It persistently controlled the release of EVO for >180 h, suggesting that it can become a potential agent for improving anticancer efficacy of EVO in breast cancer therapy (100). In addition, EGFR-targeting EVO-encapsulated poly (amino acid) nanoparticles have been developed as a new class of nanotherapeutics (101). These nanoparticles exert significantly improved cytotoxicity on colon cancer cells, resulting in the downregulation of EGFR and extension of the tumor-bearing survival duration (101). However, the application of niosomes for the delivery of EVO is yet to be investigated.

Novel carriers, micelles and microspheres have been applied to load natural medicine with low solubility and high permeability. Polymeric micelles, including paclitaxel micelles (102) and tanshinone IIA micelles (103,104), exhibit high capacity for drug loading and have the ability to improve the bioavailability of drugs (4.33-fold higher than free paclitaxel and 5.60-fold higher than free tanshinone IIA). Chitosan microspheres are biocompatible and readily biodegradable, which can enhance the absolute bioavailability of paclitaxel by 1.52-fold (105) whilst increasing the oral bioavailability of capsaicin by 1.53-fold (106). However, these drug delivery systems are yet to be applied for the bioavailability of EVO.

#### 5. Conclusions and prospects

TCM is considered a promising source of potential anticancer agents and novel adjuvant therapies to improve the efficacy of chemotherapy with little to no side effects. A previous study has demonstrated that EVO can enhance doxorubicin sensitivity in doxorubicin-resistant breast cancer cells by synchronously affecting apoptosis and survival signaling transduction pathways, thereby enhancing the apoptotic action of doxorubicin (107). In addition, combined treatment of erlotinib with EVO, an oral epidermal growth factor

receptor tyrosine kinase inhibitor, successfully inhibited cell proliferation and survival in erlotinib-resistant wild-type EGFR non-small cell lung cancer cells (108). These favorable anti-cancer properties of EVO would contribute to the killing of resistant cancer cells in the clinic with minimal toxicity. Previous studies on the antitumor mechanisms of EVO have suggested an exciting future for the pursuit of anticancer therapies. Although EVO is currently used in the clinic as a TCM to treat headaches, abdominal pain, vomiting and colds in Southeast Asia (109,110), it has not been clinically approved as an anticancer agent. Considering the therapeutic potential of EVO for antitumor treatment, an approach to improve the oral bioavailability and enhance its pharmacological effects would prove beneficial, thus minimizing the possible adverse effects due to overdosing.

Since advanced delivery systems have been proposed, the oral bioavailability of EVO can be notably improved by increasing absorption whilst avoiding first-pass metabolism. Since a substantial signaling network involved in apoptosis or proliferation inside the cell can be stimulated by EVO, exploring the possible targets of EVO on cell the membrane and any ligand-receptor interactions would be a potential means of developing novel transportable compounds to address the defect in absorption. The inhibitory effects of EVO on various CYP enzymes may decrease the biotransformation of medicines primarily dependent on these pathways. The inhibition of metabolic enzymes and resultant promotion of drug potency by EVO will facilitate the investigation of potential drug-drug or herb-drug interactions and evaluation of their clinical safety.

Previous studies have focused on the anticancer activity and underlying mechanisms of EVO. The present review discussed the advances in research investigating the anticancer effects of EVO over the past two decades, highlighting the pharmacokinetics and practicality of using novel loading carriers to promote the absorption of EVO and enhance the bioavailability of drugs with low solubility. It is hoped that all of this will contribute to the clinical application of EVO and *T. ruticarpum*.

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### Availability of data and materials

Not applicable.

### Authors' contributions

CL designed and drafted the manuscript, JA collected the information and prepared the figures. ER collected the information and prepared Table I. JL and CF supervised and revised the manuscript. XLi and XLu drew the chemical structure and

prepared Fig. 2. All authors have read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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